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Effect of Vermicompost Tea on the Growth and Yield of Tomato Plants and Suppression of Root Knot Nematode in the Soil

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ABSTRACT

Vermicompost teas (VCT) are documented to increase plant growth and yield and reduce plant fungal and bacterial diseases and nematode infestation in the soil. However, the underlying mechanisms for these results remain obscure. Radioimmnoassay was used to identify and quantify phytohormones present in commercially prepared "growth-promoting" VCT. Isopentenyladenine (IPA) and indole-3-acetic acid (IAA) were detected in VCT, along with a low amount of abscisic acid (ABA). Comparison of effects of VCT applied at the recommended rate with IPA applied at an amount equivalent to that supplied in the VCT treatment provided evidence that IPA increased *Lycopersicon esculentum* vegetative biomass, whereas the VCT increased fruit number per plant and fruit size. Comparison of a commercial "nematode-suppressing" nsVCT with treatments supplying equivalent amounts of IPA and IAA present in the VCT provided evidence that the nsVCT reduced galling and increased the growth of *Lycopersicon esculentum* plants inoculated with 5,000 and 10,000 eggs of root knot nematode (*Meloidogyne incognita*). The biomass plants of plants treated with nsVCT was greater or equal to that of control plants not inoculated with nematode eggs. Fungi and bacteria in the nsVCT were identified using rRNA gene analysis. *Candida* sp., *Torulospora Sp*., *Saccharomyces sp.* and *some parasitic forms of Cladosporium sp*. were dominant. The results provide the first evidence that plant hormones are present in VCT and document the growth promoting and nematode-suppressing capacity of vermicompost teas.

Keywords: phytohormones, isopentenyladenine, indole-3-acetic acid, abscisic acid

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CHAPTER 1

Effect of vermicompost tea on growth and yield of tomato plants

Introduction

Composting is a biological decomposition process that converts organic matter to a stable, humus-like product under controlled conditions. The product formed in the process is called compost. Vermicomposts are finely divided peat-like materials with high porosity, density, aeration and water-holding capacity, and low C: N. They are produced from organic wastes that have been stabilized as a result of interactions with earthworms and microorganisms (Edwards, 1988; Dominguez, 2004). Vermicomposts have a large surface area for retention of nutrients and thus have high concentrations of nutrients, including nitrates, calcium, phosphorus and potassium that are taken up readily by plants. They also have many microsites for microbial activity (Edwards and Burrows, 1988).

Results from many studies provide evidence of a positive impact of compost and vermicompost on plant growth and plant disease suppression. They both supply plant available nutrients and maintain soil health by retaining soil moisture and influence bulk density, aggregate solubility, infiltration rate and other soil properties. Studies of the effects of vermicomposts on crops like vegetables, flowering plants, cereals, and legumes have been conducted mostly under greenhouse conditions rather than field conditions (Atiyeh et al., 2000). The addition of as little as 10% to 20% vermicompost to the growth medium, resulted in significant increases in plant biomass and yield in greenhouse grown

tomato and marigold (Atiyeh et al., 2002). The use of water-based extracts, more commonly called vermicompost tea (VCT), or vermicompost extract, have drawn increasing attention from growers and researchers. Vermicompost teas are watery extracts of vermicomposted materials made for their beneficial effects on plants (Litterick et al, 2004). Compost tea and vermicompost tea are made from compost and vermicompost, respectively, in a commercial tea brewer.

Production of vermicompost tea

Since composts and vermicomposts contain high amounts of nutrients, extracts of both have been used as foliar sprays and soil-drench fertilizers (Zaller, 2006). Originally, the term "vermicompost extract" was defined as a water based extract prepared using a wide range of different organic wastes without any aeration. It is important to understand the difference between aerated vermicompost teas and non-aerated vermicompost teas. Vermicompost extract, steepage and watery extract amendments are different terms used by the researchers for the non-aerated VCT prepared at a room temperature without stirring for defined period of time (Scheuerell and Mahaffee, 2002; Weltzein, 1989; Weltzein, 1991; Hoitink et al., 1997). Whereas aerated VCTs are produced by re-circulating water through loose vermicompost while maintaining aerobic conditions. The product formed using this method is termed "aerated vermicompost tea".

The production of aerated vermicompost teas requires a fermentation vessel and compost and includes stirring and filtration prior to application. Aerated vermicompost

teas actually take less time to produce than non-aerated VCT and provide a safer product (Weltzein, 1991). Non-aerated VCT can cause phytotoxicity. In addition, the conditions for preparation of non-aerated VCT are conducive for the growth and reproduction of human pathogens. Throughout this thesis, vermicopmost tea (VCT) refers to the aerated product.

Several additives and adjuvants may be added to VCT to increase the microbial populations either before or after the fermentation step (Brinton et al., 1996; Ingham et al., 1999). Adjuvants include sugar,fish emulsion, kelp extract, humic acid or other products (Ingham, 2000). Research results demonstrate that the composition and quantity of beneficial or pathogenic microorganisms in a VCT varies according to the raw materials and preparation procedures used. In addition, VCTs made by different methods and augmented with microbial enhancer have higher levels of total N and K and micronutrients. Despite these differences, in one study the effect of an aerated VCT with microbe enhancement on plant growth was not significantly different from that of the same VCT produced with and without aeration and without microbial enhancement. (Pant et al., 2009).

Influence of VCT on crop growth and yield

Different types of vermicompost teas have significant effects on the physiological and biochemical parameters of different crops. Research results provide evidence that both solid and liquid forms of vermicompost improve seedling germination and plant

growth (Bess et al., 2002). Vermicompost tea has measurable benefits in stimulating seed germination, flowering, crop growth, yield and quality across a range of horticultural crops, including tomatoes, cucumbers, peppers, marigold, grapes and petunia (Aroncon et al., 2007; Edwards et al., 2007; Siddiqui et al., 2008). Pant et al. 2009 reported that application of VCT to pak choi increased plant production, plant mineral nutrient content and total carotenoids under both organic and inorganic fertilization, with greater increases obtained with plants grown under organic fertilization than with chemical fertilizers.

There is evidence to suggest that VCTs increase plant growth and yield by improving plant nutrition and by a growth-stimulating effect independent of improved nutrient status (Ingham, 2003). Evidence also suggests that certain vermicompost teas naturally contain auxin-like substances (Garcia et al., 2002). Further, the interaction between earthworms and microorganisms in organic matter has been shown to produce significant amounts of plant growth regulators including auxins (IAA), cytokinins and gibberellins (Tomati et al., 1988). Similarly, Zamanov et al. (2002) showed that earthworm activity accelerates the process of humification of organic matter, which increases microbial populations and the accumulation of auxin- and gibberellin-like substances.

Certain groups of rhizobacteria found in VC and VCT have been termed plant growth-promoting rhizobacteria (PGPR). They aggressively colonize plant roots and promote plant growth (Allison and Janice, 2006). Increased root initiation, root biomass

and shoot biomass are common effects of the application of vermicompost and vermicompost tea and effects seen by application of synthetic hormones to plants (Tomati et al., 1988). Along with the presence of plant growth regulators, VCT may contain bacteria that fix nitrogen and solubilize phosphates, or directly promote plant growth independent of providing hormones. It has been speculated that the presence of arbuscular mycorrhizal fungi in VCT may be associated with growth promoting effects. Whereas the presence of hormones in vermicompost teas has been widely speculated, there is little experimental data to support this claim.

Research Objectives

The specific objectives of this research were 1) to determine the presence and concentration of hormones in a commercial source of VCT, including the cytokinin isopentenyl adenine (IPA), the auxin indole-3-acetic acid (IAA) and abscisic acid (ABA) and 2) to quantify the effect of VCT and commercial hormones on plant growth and yield of tomato (*Lycopersicon esculentum*).

MATERIALS AND METHODS

Plant Material

The hybrid, cherry-type, indeterminate tomato (*Lycopersicum esculentum*) cultivar Supersweet-100 (Ferry Morse Seed Company) was used in this research.

Vermicompost Tea

Vermicompost tea was obtained from Salton Sea Farms in Thermal, CA. It was made from garden wastes, leaf litter, vegetable residues from packing houses and rice straw acted upon by earthworms (*Eisenia fetida*), placed in 5 m x 5 m piles that were turned over weekly to ensure even aeration. At the end of 60 to 90 days the fully matured vermicompost was extracted in distilled water and then supplemented with sugar solution The solution was then placed in a standard commercial compost tea brewer (GEOTEA 250) and actively aerated for 24 hrs. The tea aliquots were immediately frozen and stored at –80° C for subsequent analysis and for use in the experiment.

Nutrient Analysis of Vermicompost Tea.

The VCT had 175 mg/L nitrogen in the form of ammonium (NH_4-N), 235 mg/L total nitrogen, 72.5 mg/L total phosphorus and 5,117 mg/L potassium.

Measurement of Endogenous IAA, ABA and IPA Concentrations in VCT

The hormones present in the VCT were identified and quantified by

radioimmunoassay (RIA) using the method of Cutting (1983), which separates acidic and neutral hormones, as modified by Bertling and Bangerth (1995).

Aliquots of VCT were defrosted in the refrigerator, and filtered through cheese cloth. ^{14}C-Indole-3-acetic acid (IAA) (~ 4000 dpm; specific activity of 57 mCi/mmole, Sigma) was added to the filtrate as an internal standard to quantify losses during purification. Samples (25 ml) (adjusted to pH > 8.5 with 0.1 M ammonium acetate) were applied to a 5.5-ml polyvinylpolypyrrolidone (PVPP) column, attached above a 5-ml [2-(diethylamino) ethyl-Sephadex] (DEAE) anion exchange column. The tandem columns were eluted with 30 ml of 0.01 M ammonium acetate (pH 8.0). Cytokinins were collected in a Sep-Pak C18 cartridge (Waters Corp., Milford, MA) attached to the end of the DEAE column. The PVPP column and Sep-Pak C18 cartridge containing the cytokinins were removed. The acidic hormones were eluted from the DEAE column with 25 ml of 1.5 M acetic acid and collected in a second Sep-Pak C18 cartridge attached to the DEAE column. All Sep-Paks were washed with 5 ml distilled water and the hormones eluted from the cartridges with 4 ml (cytokinins) or 5 ml (acidic hormones) 50% HPLC-grade methanol and dried under vacuum. The acidic fraction, containing IAA and ABA, was methylated with diazomethane (Arndt 1935) prior to radioimmunoassay (RIA) analysis.

Polyclonal antibody production, for use in RIA, includes the process of coupling each hormone to bovine serum albumin and the subsequent inoculation of rabbits with the coupled conjugate to produce antisera (Cutting et al., 1983). Antiserua was produced

by Robert Sargent, a commercial company. Tracer compounds consisted of ^{3}H-labeled IAA, ABA and isopentenyladenosine (IPA) and were obtained commercially (Amersham Life Science, Inc., Arlington Heights, IL and Sigma, St. Louis, MO). All radioimmunoassays were performed in triplicate. Hormone concentration was calculated from the measured radioactivity of the bound phase, which is inversely related to the hormone concentration of the samples, based on a standard curve using the spline approximation method. Assays were reliable from 0.01 ng/100 µl to 50 ng/100 µl for each of the three hormones analyzed. Samples were diluted to obtain concentrations within this range. Recovery of the ^{14}C-IAA internal standard was used to correct for losses during purification.

Greenhouse Experiment

Prior to the start of the experiment, 9.5-L plastic pots were filled with 5 kg University of California Soil Mix I (Baker, 1957). The pots for the VCT treatments received VCT one week before seeds were sown into the pots, whereas the pots with hormone treatments received the hormones immediately after the seeds were sown. The seeds were soaked overnight on a moistened towel, and then sown in the pots. Distilled water was applied to maintain soil moisture at field capacity. Plants were grown in the greenhouse for 80 days starting in April. Photosynthetically active radiation (PAR) at canopy level, measured with a PAR quantum sensor (Li-COR, LINCOLN, NE), averaged 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ midday under clouldless conditions between April and July. Glasshouse temperature set points were 25 °C maximum day and 18 °C minimum night.

There were seven treatments, each replicated 4 times in a completely randomized design. Each pot of 4 tomato plants was a replicate.

In the field VCT is applied at the rate of 189 L/10,000 tomato plants/ hectare three times per month during the 3-month growing season. To supply the amount of IPA in VCT at the rate it was applied in the field, it was necessary to make seven applications of 0.0054 µg IPA/plant/application every 10 days, providing total of 0.0325 µg IPA/plant. VCT was also applied at 100x the low concentration "VCT_high concentration", providing 3.25µg of IPA per plant over the 75 days of the experiment. Pots treated with VCT _high concentration and the high concentration of IPA received 70 (daily) applications of VCT. The commercial plant hormones 6-benzyladenine and compound X were applied in 70 daily applications to provide 3.25 µg of hormone per plant by the end of 70 days during the experiment. In all cases, pots contained four plants and received four times the amount hormone prescribed per plant. Thus, treatments receiving VCT low_concentration received 16.2 ml/plant of VCT every 10 days. The treatments receiving VCT high_concentration received 16.2 ml/plant every day for 70 days. All commercial plant hormones were applied in 16.2 mls every 10 days (seven applications) for low concentrations and daily for high concentrations (70 applications). Treatments included: T1 – Untreated control, T2 – VCT (low_conc.), T3 – VCT (high_conc.), T4 – IPA (low_conc.), T5 – IPA (high_conc.), T6- Benzyladenine, T7- Compound X (Proprietary compound). Compound X is another form of commercial cytokinins. All plants in the control and hormone treatments were watered with Shive's nutrient solution

(150 ml) every week. The total amount of each nutrient the plants received during the 75-day experiment is given provided in Shive's nutrient solution are given in Table 1.1.

Table 1.1 Nutrient content of Shives medium

Nutrients	VCT_low conc. (mg)	VCT_high conc. (mg)	Shive's medium (mg)
N	26.649	2668.19	0.2832
P	8.2215	823.165	1.632
K	580.2678	58,098.42	0.2088
S	-	-	0.0096
Mg	-	-	0.2952
NH_4-N	19.845	1986.95	-
Micronutrients	-	-	0.003

Determination of Plant Growth and Yield Parameters

The following biometrical parameters were measured every two weeks by sampling one plant from each pot. The root length was measured from root collar point to root tip in cm. The shoot length was measured from root collar point to shoot apex also in cm. The plants were then used to determine fresh weight in g. The number of leaves of the plant was counted and tabulated in whole number. For the dry weight, the plants were placed in oven at 70°C for 24 hrs. Dried plants were weighed.

The yield parameters were determined 55 to 75 days after sowing (DAS). This included the number of vegetative and fruiting branches, flowers, fruits and size of fruits per plant. Percent fruit set and total yield were calculated. Percent fruit set was calculated as the number of fruit remaining on each branch at the different evaluation times relative

to the initial number of flowers on the branch. The length of time to initiate flowering and fruiting in each replicate were recorded.

Data Analysis

The data obtained from the various biometrical and yield parameters were subjected to statistical analysis. Two-way analysis of variance was performed to determine treatment effects, and means separated using Fisher's protected LSD test.

RESULTS

Vermicompost tea contained 1.44 µg of IPA per 100 ml. A much greater amount of indole-3-acetic acid (IAA) was present in the VCT, 41.73 µg per 100 ml. Abscisic acid was present at only 0.022 µg per 100 ml.

Significant differences in shoot length were observed 28 days after sowing (DAS) (Table1.2). Plants treated with high and low concentrations of VCT had shorter shoots than the untreated plants, whereas the plants treated with the high concentration of IPA had shoots longer than the untreated control ($P < 0.0001$) with time, the differences became less significant.

At 28 DAS, plants treated with both the low and high concentration of VCT had shorter roots than the untreated control plants but at 40 DAS root length was greater than the untreated control for the plants treated with the high concentration of VCT and equal to the untreated control for plants receiving the low concentration of VCT (Table 1.3). Tomato plants receiving the high rate of compound X produced longer roots than plants in all other treatments at 28 DAS and 40 DAS.

Tomato plants treated with the low concentration VCT produced fewer leaves than the untreated control at 28 DAS and 40 DAS, but more leaves than the control by 57 DAS (Table 1.4). Plants treated with the high concentration of VCT produced fewer leaves than the untreated control 28 DAS, but by 40 and 57 DAS these plants had more

leaves than the untreated control. Moreover, the number of leaves was equal to or greater than that of tomato plants treated with commercial cytokinins.

At 28 DAS, the fresh weight of all plants treated with commercial cytokinins was greater than those receiving VCT and the untreated control (Table 1.5). Interestingly, by 40 DAS the fresh weight of the tomato plants receiving the low concentration of VCT was greater than all other treatments except compound X. The effect of treatments on dry weight paralleled the effects of on fresh weight. Only plants treated with the high concentration of VCT and compound X had a significantly greater dry weight than the untreated control plants (Table 1.6).

At 14 DAS, flowers were present in all treatments except the low concentration VCT. A remarkable increase in the number of flowers was seen with plants treated with low concentration VCT after 40 DAS. However, a greater number of flowers per plant was counted for plants treated with commercial IPA at low concentration than the low concentration VCT. At 57 DAS, the number of flowers was significantly higher for plants receiving the high concentration of VCT and compound X (Table 1.7).

Tomato plants receiving VCT at high concentration had a significantly greater number of vegetative branches at 55 DAS than commercial IPA applied at high concentration (Table 1.8). Whereas at 75 DAS (Table 1.9) compound X had the greatest number of vegetative branches. By 75 DAS, plants treated with the high concentration

VCT had significantly more vegetative branches than plants treated with IPA applied at the high concentration.

Tomato plants showed significant difference in fruiting branches only at 75 DAS. High concentration of VCT and compound X resulted in significantly more fruiting branches than other treatments. VCT at high concentrations significantly increased the number of fruiting braches than commercial cytokinins except for compound X. Overall, all treatments increased the number of fruiting branches significantly above that of the control (Table 1.9).

At 55 DAS, the fruit number of all plants treated with commercial cytokinins was a significantly higher fruit number than plants treated with VCT at the low concentration (Table 1.8). Interestingly by 75 DAS, the fruit number in VCT-treated plants was significantly more than plants receiving commercial cytokinin treatments except for compound X. Plants treated with compound X had significantly more fruits per plant (Table 1.9).

The effect of treatment on fruit size followed similar trend as fruit number. At 55 DAS, plants receiving the low concentration of VCT had the smallest fruit compared to all other commercial cytokinins (Table 1.8). However at 75 DAS, the fruit size for plants

treated with the low concentration VCT was greater than or equal to the fruit size obtained with the high concentration of VCT and all other cytokinins, except compound X (Table 1.9).

DISCUSSION

To our knowledge this research is the first to quantify the concentration of phyto-hormones (IPA, IAA, ABA) in vermicompost tea or to compare the effect of VCT and comparable amounts of hormone on plant growth and yield. The low concentration of VCT at 40 DAS outperformed the low concentration of IPA in its effect on plant fresh weight (Table 1.5), fruit number per plant (Table1.9) and in the size of individual fruit at 75 DAS (Table 1.9). In contrast, the low concentration of IPA resulted in longer shoots (Table 1.2), more leaves per plant (Table 1.4) than the low concentration of VCT.

The effects of the higher concentration of VCT and the high concentration of IPA on plant growth were comparable (Tables 1.2, 1.3, 1.4, 1.5, 1.6, 1.7), but the high concentration VCT resulted in significantly more fruit per branch (Table 1.9). In addition, the plants treated with the high concentration of VCT had more vegetative branches per plant. These results suggest that IPA tended to support vegetative growth, whereas the VCT supported reproductive growth and thus had a greater effect on final yield, including fruit size, than the IPA. Only compound X at the high concentration produced an equal number of fruit per plant and per branch as the high concentration of VCT. Moreover compound X resulted in larger fruits than the high concentration of VCT.

It is possible that the high concentration of IAA in the VCT contributed to the growth enhancing effect of the VCT, which would not be obtained with the application of IPA or the other commercial cytokinins. Since plants not receiving VCT were treated

with Shive's nutrient solution weekly, the better performance between VCT and commercial hormones was not likely due to low fertility, although this possibility cannot be ruled out given that significantly greater total amounts of N, P and K were provided to the plants treated with even the low concentration of VCT compared to plants receiving Shive's nutrient solution. The results obtained in the research are in consistent with those of Edwards et al. (2006) demonstrating increased plant growth, flowering and yields of tomato treated with VCT.

In summary, the phytohormones play an important role in enhancing plant growth, but clearly there are other factors within VCT that contribute in improving the plant growth. Incorporation of vermicompost tea may have improved the properties of soil in the pots, such as moisture retention and aeration, compared to the ones that received only hormones and Shive's nutrient solution. As observed by Edwards et al. (2006), it is probably an interaction of several factors (mineral nutrients, micro-organisms, plant growth regulators) that that increase plant growth and yield with the application of VCT. Our experiments did not include analysis of the plant nutrient content or physical and chemical properties of the soil. This first experiment provides evidences that VCTs contain phytohormones, but that VCT, due to having additional components, including IAA, additional cytokinins not quantified in the RIA analysis, and other essential metabolites, had a greater beneficial effect on tomato plant growth and yield than equal amounts of isopentenyladenonine. Thus, it would be of great practical

value to determine the full range of plant hormones and other growth promoting substances present in a wide range of vermicompost teas.

Table 1.2 Shoot length (cm) of the tomato plants at 14 to 57 days after sowing (DAS).

Treatments	**14 DAS**	**28 DAS**	**40 DAS**	**57 DAS**
T_1 - Control	5.75	[z]26.00bc	48.83 b	78.5
T_2 - VCT_low conc.	9.5	10.88 d	27.50 c	46.5
T_3 -VCT_high conc.	5.78	11.43 d	49.33 b	70
T_4 - IPA_low conc.	10.45	35.00 ab	57.50 ab	73.75
T_5 - IPA_high conc.	9.83	38.50 a	61.75 ab	81
T_6 - BA_high conc.	9.53	33.13 ab	52.50 ab	74.5
T_7 - Compd X high conc.	6.5	18.88 cd	65.50 a	74
P-*value*	0.2287	0.0001	0.0024	0.1282

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.3. Root Length (cm) of the tomato plants at 14 to 57 days after sowing (DAS).

Treatments	**14 DAS**	**28 DAS**	**40 DAS**	**57 DAS**
T_1 - Control	[z] 2.75 b	21.00 b	5.63 c	26.5
T_2 - VCT_low conc.	3.50 b	8.38 c	11.25 bc	44.5
T_3 -VCT_high conc.	3.45 b	4.63 c	14.25 b	24.75
T_4 - IPA_low conc.	5.35 a	14.00 bc	14.75 b	25.5
T_5 - IPA_high conc.	2.68 b	14.50 bc	17.98 b	20.25
T_6 - BA_high conc.	3.40 b	13.75 bc	17.40 b	21
T_7 - Compd X high conc.	4.25 ab	32.25 a	31.25 a	28
P-*value*	0.0415	0.0006	0.0004	0.1081

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.4. Number of leaves per tomato plant at 14 to 57 days after sowing (DAS).

Treatments	**14 DAS**	**28 DAS**	**40 DAS**	**57 DAS**
T_1 - Control	[z] 7 b	30 b	68 b	39 e
T_2 - VCT_low conc.	9 b	14 c	23 c	67 d
T_3 -VCT_high conc.	5 b	15 c	96 ab	107 ab
T_4 - IPA_low conc.	16 a	30 b	83 ab	99 abc
T_5 - IPA_high conc.	11 ab	39 ab	70 b	87 c
T_6 - BA_high conc.	9 b	38 ab	81 ab	92 bc
T_7 - Compd X high conc.	9 b	40 a	101 a	111 a
P-*value*	0.0384	0.0001	0.0004	0.0001

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.5. Fresh Weight (g/plant) of the tomato plants at 14 to 57 days after sowing (DAS).

Treatments	**14 DAS**	**28 DAS**	**40 DAS**	**57 DAS**
T_1 - Control	[z] 0.19 b	0.77 b	3.21 c	39.63
T_2 - VCT_low conc.	0.36 b	5.14 b	25.38 a	53.09
T_3 -VCT_high conc.	0.05 b	4.16 b	21.11 b	77.65
T_4 - IPA_low conc.	1.19 a	13.89 a	21.81 b	42.36
T_5 - IPA_high conc.	0.53 b	18.47 a	22.07 b	50.14
T_6 - BA_high conc.	0.49 b	14.74 a	22.40 b	48.87
T_7 - Compd X high conc.	0.54 b	19.48 a	37.80 a	66.63
P-*value*	0.011	0.0001	0.0019	0.267

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.6. Dry weight (g/plant) of the tomato plants at 14 to 57 days after sowing (DAS).

Treatments	14 DAS	28 DAS	40 DAS	57 DAS
T_1 - Control	[z] 0.02 c	0.13 d	0.42 c	44.5
T_2 - VCT_low conc.	0.04 bc	0.92 cd	1.63 bc	26.5
T_3 -VCT_high conc.	0.02 c	1.26 bcd	4.03 a	24.75
T_4 - IPA_low conc.	0.07 a	2.01 abc	2.99 ab	25.5
T_5 - IPA_high conc.	0.06 ab	2.61 a	3.38 ab	20.25
T_6 - BA_high conc.	0.04 abc	2.14 ab	3.28 ab	21
T_7 - Compd X high conc.	0.03 bc	2.79 a	4.42 a	28
P-*value*	0.0354	0.0011	0.0039	0.1081

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.7 Flowering (number/plant) of the tomato plants at 14 to 57 days after sowing (DAS).

Treatments	14 DAS	28 DAS	40 DAS	57 DAS
T_1 - Control	-	[z] 1.25 bc	1.00 c	5.25 d
T_2 - VCT_low conc.	-	0.00 c	3.00 b	6.00 d
T_3 -VCT_high conc.	-	2.00 ab	3.50 ab	12.75 a
T_4 - IPA_low conc.	-	2.75 ab	4.25 ab	9.50 c
T_5 - IPA_high conc.	-	2.00 ab	3.75 ab	10.25 bc
T_6 - BA_high conc.	-	1.75 abc	4.00 ab	10.25 bc
T_7 - Compd X high conc.	-	3.50 a	5.00 a	12.25 ab
P-*value*	-	0.0241	0.0166	0.0001

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.8: Yield parameters of tomato plants at 55 days after sowing.

Treatments	# of fruit	Fruit Dia(mm)	# of Veg Br	# of Fruit Br
T_1 - Control	[z] 5 bc	14.98 a	11 c	3
T_2 - VCT_low conc.	2 c	4.38 b	13 bc	3
T_3 -VCT_high conc.	10 ab	15.59 a	26 a	4
T_4 - IPA_low conc.	11 a	15.58 a	18 b	6
T_5 - IPA_high conc.	10 ab	15.88 a	16 bc	5
T_6 - BA_high conc.	6 abc	16.25 a	12 bc	4
T_7 - Compd X high conc.	10 ab	15.18 a	17 bc	9
P-*value*	0.0073	0.0045	0.0009	0.3749

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$.

Table 1.9: Yield parameters of tomato plants at 75 days after sowing.

Treatments	# of fruit	Fruit Diameter (mm)	# of Veg Br	# of Fruit Br
Control	[z] 8 c	14.98 d	12.20 e	3.80 e
VCT_low concentration	13 b	21.81 b	23.40 d	10.20 d
VCT_high concentration	17 a	21.87 b	34.20 b	17.60 a
IPA_low concentration	9 c	19.36 c	30.20 c	12.40 bc
IPA_high concentration	10 c	19.92 c	30.80 c	11.60 cd
BA_high concentration	9 c	21.51 b	31.00 bc	14.40 b
Compound X high conc.	15 a	24.13 a	38.00 a	17.60 a
P-*value*	0.0008	<.0001	<.0001	<.0001

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P \leq 0.05$

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CHAPTER 2

Effect of vermicompost tea on suppression of root knot nematode in tomato.

Introduction

Vermicompost teas are watery extracts of vermicomposted materials made by steeping and brewing and used for their beneficial effects on plants (Litterick et al. 2004). Most current research on organic amendments such as compost teas (CT) and vermicompost teas (VCT) is on improving plant nutrition and crop yield. However, there is evidence (Siddiqui and Alum, 1987; Jaffee, 2002; Ploeg and Stapleton, 2001) that compost and vermicompost teas have a role in disease and nematode suppression. VCT application as a foliar spray has been shown to suppress a range of foliar diseases (Al-Dahmani et al, 2003) but less work has been done with the use of compost tea as a soil drench for seed or root disease suppression.

Soil drench directly supplies soluble plant nutrients, beneficial metabolites and bio-stimulants released by microorganisms to the soil. Microrganisms supplied to the soil in VCT enhances decomposition, mineralization and disease suppression (Granastein, 1999). Vermicompost teas have been shown to control or prevent various types of diseases, pythium, phytopthora, rhizoctonia, plectospora, verticillium, powdry and downy mildews, bacterial blights, leaf spots, apple scab and grey mold (Aroncon et al., 2007). There are some commendable studies on the bio-efficiency of VCT on plant disease suppression. Scheuerell and Mahaffee (2004) observed that VCT suppressed the growth of *Pythium ultimum* under greenhouse conditions in a soilless media. Edwards et al.

(2007) documented the suppression of aphids, arthropods and spider mites by VCTs in a greenhouse experiment. Siddiqui et al. (2008) investigated the effect of rice straw compost tea (RST) and *Trichoderma* rich rice straw compost (T-RST) tea on the wet rot incidence of okra caused by *Chaenophora.* They found that T-RST reduced the severity to a greater extent than RST. However, plants receiving RST alone had significantly better growth. This shows that biofertilizers or microbial enriched compost teas can provide another biological approach for plant disease management. The use of VCT as a soil drench for nematode suppression has received little attention (Scheurell and Mahaffee, 2004). There are a few studies documenting the effect of VCT on nematode populations. Edward et al. (2007) found that VCT suppressed parasitic nematode attacks under greenhouse conditions. The beneficial microbes present in VCT are believed to out-compete pathogens for nutrients by taking over colonization sites or prevent plant disease by directly parasitizing pathogens. Pieterse et al. (2003) suggested that VCT application can stimulate the plant to make physiological changes that decrease its vulnerability to infection through a process known as induced systemic resistance. It has been proposed that increasing the population of total and active bacteria in aerobic compost tea will generally increase plant disease suppression (Ingham, 2000).

For VCT production, the most important factors that influence the potential for effectively managing plant diseases are compost quality, fermentation time, additives that are added during fermentation and also the species of earthworm being used. Other important factors include fermentation, temperature, pH and application methods

(Ingham, 2005; Weltzein, 1991; Aroncon, 2007). It is commonly believed that the effect of CT/VCT is partly due to the presence of microorganisms present in them. There are several modes of action involved in disease suppression depending on the preparation, application method, and raw materials used to make the VCT. However, there are few published results on the disease suppression mechanism of VCTs.

Root Knot Nematode

Nematodes are microscopic organisms found in many habitats. Although nematodes represent a relatively small amount of biomass within soil, their presence across many trophic levels in soils is vitally important within the soil environment and ecosystem processes (http://orgprints.org/6694/3/Annex_Effects_on_microorganisms.pdf). *Meloidogyne incognita*, commonly known as root knot nematode, was used in this research. Plants infected by root knot nematode are chlorotic, stunted, have reduced yield, and frequently die. However, the extent of damage caused by root knot nematode infestation depends upon the host, cultural conditions, and more importantly on the time of infection. Root knot nematode infestation causes swellings, or “knots” in the root system of the host plant.

Research Objectives

The objectives of this study were to 1) determine if vermicompost tea when applied as a soil drench suppresses root knot nematode (*Meloidogyne incognita)* in tomato plants, and 2) examine the microbial population (bacterial and fungal) in the VCT using ribosomal intergenic spacer analysis (RISA).

MATERIALS AND METHODS

Plant Material

'Roma' tomato (*Lycopersicum esculentum*) cultivar (Gurney's Seed and Nursery Co.) was used in this research. Prior to the start of the experiment, 10-cm plastic pots were filled with University of California Soil Mix I (Baker, 1957). The soil was cleaned by removing stones and other unwanted materials and then homogenized properly. Plants were grown in a glasshouse for 30 days. Glasshouse temperature set points were 30 °C maximum day and 18 °C minimum nights.

Vermicompost Tea

The nematode suppressing vermicompost tea (nsVCT) was obtained from Salton Sea Farms in Thermal, CA. It was made from garden wastes, leaf litter, vegetable residues from packing houses and horse manure acted upon by earthworms (*Eisenia fetida*), placed in 5-m x 5-m piles that were turned over weekly to ensure even aeration. At 90 days the fully matured vermicompost was extracted in distilled water and then supplemented with fish emulsion and sugar solution. The solution was then placed in a standard commercial compost tea brewer (GEOTEA 250) and actively aerated for 24 hrs. The tea was immediately frozen and stored at –80° C for subsequent analysis and use in the experiment.

Determination of hormone concentration of VCT

The hormones present in the VCT were identified and quantified by radioimmunoassy (RIA) using the method of Cuttings (1983), which separates acidic and neutral hormones, as modified by Bertling and Bangerth (1995). Three samples of the nematode-suppressing VCT used in the experiment were analyzed.

Aliquots of VCT were defrosted in the refrigerator and filtered through cheese cloth. ^{14}C-Indole Acetic acid (IAA) (~ 4000 dpm; specific activity of 57 mCi/mmole, Sigma) was added to the filtrate as an internal standard to quantify losses during purification. Samples (25 ml) (adjusted to pH > 8.5 with 0.1 M ammonium acetate) were applied to a 5.5-ml polyvinylpolypyrrolidone (PVPP) column, attached above a 5-ml [2-(diethylamino) ethyl-Sephadex] (DEAE) anion exchange column. The tandem columns were eluted with 30 ml of 0.01 M ammonium acetate (pH 8.0). Cytokinins were collected in a Sep-Pak C18 cartridge (Waters Corp., Milford, MA) attached to the end of the DEAE column. The PVPP column and Sep-Pak C18 cartridge containing the cytokinins were removed. The acidic hormones were eluted from the DEAE column with 25 ml of 1.5 M acetic acid and collected in a second Sep-Pak C18 cartridge attached to the DEAE column. All Sep-Paks were washed with 5 ml distilled water and the hormones eluted from the cartridges with 4 ml (cytokinins) or 5 ml (acidic hormones) 50% HPLC-grade methanol and dried under vacuum. The acidic fraction, containing IAA and ABA, was methylated with diazomethane (Arndt, 1935) prior to radioimmunoassay (RIA) analysis.

Polyclonal antibody production, for use in RIA, includes the process of coupling each hormone to bovine serum albumin and the subsequent inoculation of rabbits with the coupled conjugate to produce antisera (Cutting et al., 1983). Antiserum was produced by Robert Sargent, a commercial company. Tracer compounds consisted of ^{3}H-labeled IAA, ABA and isopentenyladenine (IPA) and were obtained commercially (Amersham Life Science, Inc., Arlington Heights, IL and Sigma, St. Louis, MO). All radioimmunoassays were performed in triplicate. Hormone concentration was calculated from the measured radioactivity of the bound phase, which inversely related to the hormone concentration of the samples, based on a standard curve using the spline approximation method. Assays were reliable from 0.01 ng/100 µl to 50 ng/100 µl for each of the three hormones analyzed. Samples were diluted to obtain concentrations within this range. Recovery of ^{14}C-IAA internal standard was used to correct for losses during purification.

Greenhouse Experiment

The ability of VCT to suppress nematodes was evaluated in a greenhouse pot experiment. There were ten treatments, each replicated six times in a completely randomized design. The treatments included were: T_1 – Control, T_2 – 50% VCT + 5000 eggs, T_3 - 50% VCT + 10000 eggs, T_4 – 100% VCT + 5000 eggs, T_5 – 100% VCT + 10000 eggs, T_6 – IPA + 5000 eggs, T_7 – IAA + 5000 eggs, T_8 - 5000 eggs, T_9 - 10000 eggs, and T_{10}- VCT. One 4-week-old tomato plant was transplanted into each pot. A week later treatments T2, T4, T6 and T8 received 5,000 nematode eggs and treatments

T3, T5 and T9 received 10,000 nematodes eggs per pot. Each pot of one tomato plant was a replicate. Nematode suppressing VCT and hormone treatments were given to the plants once every two weeks. The amount of nsVCT (100% concentration) applied per plant was equivalent to the amount of nsVCT applied in a commercial tomato field per plant. This was calculated based upon the fact that the amount of nsVCT added in the field to control nematode infestation is 189 L/hectare applied every two weeks. From this, the approximate amount of nsVCT supplied to one tomato plant in an hectare of 10,000 plants was calculated per application and for the entire growing season. . Thus, the amount of nsVCT applied in this experiment was 10 ml/plant for plants with 50% VCT and 20 ml/plant with 100% VCT during 1-month of the experiment.

Thirty days later, roots were assessed for the extent of damage by counting the number of root galls. A seedling was classified as healthy if it grew normally with no symptoms or signs of infection. Infection symptoms of damaged seedlings included stunting, wilting, yellowing, and reduced seedling viability. Fresh weight of the plant shoot was measured and root gall index calculated for the extracted root. At the end of 30 days after planting, plants were removed from the pots and washed to remove the soil. Treatment effects on eggs count [log(n + 1) transformed], root galling (0-10 scale, Bridge and Page, 1980), root fresh weight of root, and shoot fresh and dry weight were analyzed in a GLM procedure using SAS statistical software (SAS Institute, Cary, NC).

Examining bacteria and fungi in VCT samples

Biodiversity assessment of microbes not only looks at the number and distribution of species, but also the functional parameters of the organisms. The following techniques were used to analyze the diverse microbial community present in the VCT: (1) bacterial analysis was by ibosomal intergenic spacer analysis (RISA), cloning and sequencing of selected bands; and (2) fungal analysis was by cloning and sequencing of fungal SSU sequences via USER cloning.

Sample Collection

Four replicate (different batches) samples of VCT and nsVCT were collected. For each of the 8 sample-type-batch-combinations, several 0.5-ml aliquots were collected and frozen at -80° C within an hour of collection.

Fungal ITS analyses.

DNA was extracted from vermicompost tea samples (500 µl) using FastDNA SPIN Kit for soil (QBiogene, Carlsbad, CA) as described by the manufacturer, using a 30-second bead-beading step with a FastPrep instrument setting of 5.5. DNA was further purified and size-fractionated by electrophoresis in 1% agarose gels. DNA larger than 3 kb was excised without exposure to UV or ethidium bromide, and recovered using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) following the manufacturer's instructions, except that the gel pieces were not exposed to heat.

Fungal rRNA intergenic fragments were amplified in a 50 µl PCR preparation having the following final concentrations or amounts: 5 µl of compost tea DNA, 50 mM Tris (pH 8.3), 500 µg/ml BSA, 2.5 mM $MgCl_2$, 250 µM of each dNTP, 400 nM of each primer, and 0.5 U *Taq* DNA polymerase. The primers were ITS1F-user (GGGAAAGUCTTGGTCATTTAGAGGAAGTAA) and ITS4-user (GGAGACAUTCCTCCGCTTATTGATATGC). All reagents were combined and heated at 94 ^{o}C for 5 min. forty cycles of PCR were then performed at 94^{o} C for 20s, 56^{o} C for 30s, and 72^{o} C for 60s, followed by 72^{o} C for 5 min. PCRs was performed in 50-µl glass capillary tubes in a 1002 Rapid Cycler (Idaho Technologies, Idaho Falls, Idaho). The PCR products were resolved by electrophoresis on 2% agarose gels, stained with ethidium bromide, excised under UV light and cloned using the User Friendly Cloning Kit (New England Biolabs, Ipswich, MA).

Nucleotide sequences of selected rRNA intergenic fragments were determined using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Sequence identities were determined using BLAST (NCBI) (Altschul et al. 1997).

Phylogenetic tree.

Fungal ITS rRNA gene sequences obtained from the vermicompost teas and their closest relatives determined by BLAST (Altschul et al. 1997) were aligned using the Clustal W

algorithm (Thompson et al., 1994) in Vector NTI v.10 (Invitrogen, Carlsbad, CA). A phylogenetic tree was constructed from these aligned sequences using the Phylip v. 3.66 program Dnapars (Felsenstein, 2005), which produces unrooted parsimony trees (Eck and Dayhoff, 1966; Kluge and Farris, 1969).

RESULTS

Analysis of hormones concentration

The concentration of isopentenyladenine (IPA) in the VCT was 1.06 µg per 100 ml. At the start of this experiment, only IPA had been identified in the nsVCT. The amount of IPA found in nsVCT was similar to that the growth promoting VCT used in chapter 1. Therefore, commercially available IAA was supplied in this experiment at the amount detected previously in the growth promoting VCT.

Nematode analysis

Roots of the tomato plants were significantly affected by inoculation with nematode eggs. The efficiency of nsVCT in the management of root knot nematode was assessed from the reduction in root galling expressed in terms of the Root Knot Index (RKI). The only roots with no galls were from the control plants and the VCT amended pots (T_{10}), which were not inoculated. Plants treated with IAA and IPA showed high galling compared to plants in other treatments. Galling index was lowest for treatment T_5 – 100% VCT + 10,000 eggs (Table 2.1). Galling was significantly reduced by adding 100% VCT when the pot was inoculated with 5,000 eggs (treatment T_4 – 100% VCT + 5000 eggs; average gall index 1.2, $P<0.0001$). It was also less in pots that were amended with nsVCT than the pots amended with hormones T_6 – IPA + 5,000 eggs (average gall index 6.1) or T_7 – IAA + 5,000 eggs (average gall index – 5.3).

Most of the treatments affected shoot fresh and dry weights (Table 2.1, p = 0.05). Plants treated with nsVCT had the highest fresh weight per plant shoot followed by treatment T1 – control. The plants treated with nsVCT but no nematode eggs weighed more than plants in treatment T_4 – 100% VCT + 5,000 eggs and T_5 – 100% VCT + 10,000 eggs. Treatment T_5 – 100% VCT + 10,000 eggs performed better than T_2 – 50% VCT + 5,000 eggs and T_3 - 50% VCT + 1,0000 eggs. Treatments T_6 – IPA + 5,000 eggs and T_7 – IAA + 5,000 eggs had reduced shoot fresh and dry weights. The fresh weight of plants treated with VCT alone (T_{10}- VCT) was the highest among all r treatments (Table 2.1). Plants in treatments 4 and 5 (100% VCT + 5000 eggs and 100% VCT + 10,000 eggs, respectively) higher fresh and dry weights than plants in the nematode-free control treatment, suggesting that nsVCT has a plant growth enhancement affect in addition to reducing nematode damage to roots.

The root weights of the plants were significantly affected by galling (Table 2.1). Only the roots of the tomato plants in the control or the VCT amended pots (T1 and T10 respectively) had no galls. Plants with higher root weight were observed when the soil was amended with the vermicompost teas. Interestingly, root weight was found to be the least in treatments 4 and 5, which gave the highest shoot weight (100% VCT + 5000 eggs and 100% VCT + 10,000 eggs, respectively) but the root weight was still signifcnatly greater than that of the untreated control (Table 2.1).) Highest root weight was observed in hormone treated plants; IAA average root weight was 445.2 g per plant. Root weight was comparatively less in pots that were amended with nsVCT than the pots amended with

hormones T_6 – IPA + 5000 eggs (average root weight 437.6) and T_7 – IAA + 5000 eggs (average root weight 445.21; Table 2.1).

Number of Nematodes

Very high numbers of *M. incognita* J2 were extracted from tomato roots for the T_3 - 50% VCT + 10000 eggs treatment. This was followed by treatment T_6 – IPA + 5000 eggs and T_7 – IAA + 5000 eggs. (Table 2.1). Nematode root infestation was not very different between the plants treated with commercial hormones and plants inoculated with 10,000 eggs. Treatments T_2 – 50% VCT + 5,000 eggs, T_4 – 100% VCT + 5,000 eggs and T_5 – 100% VCT + 10,000 eggs, however, had relatively less infestation.

Microbial analysis

The analysis of VCT and nsVCT showed that the bacterial composition of VCT (Fig 2.1) was not as consistent or as clear as the fungal composition (Fig 2.2).

Analysis of bacterial, fungal and stramenopile rRNA genes

PCR was used to examine the amounts of bacterial, fungal and stramenopile rRNA genes in the VCT samples (Fig. 2.1). In Figure 2.1, the gel lanes are arranged by the type of VCTs, with the growth promoting VCT on the left side and the nsVCT on the right. Strong fungal PCR bands were produced from both of the VCT samples. In the bacterial analysis, only a few of the samples produced a PCR product. In the stramenopile analysis, PCR products were generally more abundant in the nsVCT root samples from

the plants with smaller shoot weights. A phylogenetic tree of stramenopile phylotypes was constructed using at least one representative small-subunit rRNA gene sequence from each phylotype. Reference sequences are designated by their accession number and taxon. The scale bar length is 0.05, and represents the number of nucleotide changes per position. Vertical lines designate the four most abundant phylotypes.

Stramenopile primers were used to amplify rRNA genes from VCTs. A nucleotide sequence analysis from VCT samples showed stramenopile genes belonging to Ascomycota phylum, saccharomycetaceae and Mycosphaerellaceae. (Fig. 2.3). The most abundant phylotypes had high sequence identity to: *Candida ethanolica, Candida humilis and Candida tropicalis* (97–99% identity), *S.cerevisae* (98% identity), *Torulospora sp.* (98–99% identity), *Davidiella tassiana*, *Davidiella macrospora*(97–99% identity) and *Cladosporium macrosporum/uredinicola* (97- 99% identity)

DISCUSSION

Vermicompost extract is being viewed as a potential alternative to the use of fertilizers and pesticides by many organic growers. VCT is generally prepared from agro-industrial wastes, such as animal and green wastes, by steeping them in water followed by active aeration and occasionally with additives. It is either applied as a foliar spray or soil drench. Another merit of using VCT is a reduction in disease incidence. Generally, a foliar spray is used in the control of foliar diseases and soil drench application is used to increase plant growth and to control root diseases. Although disease suppressive effects of different types of vermicompost extracts have been observed in many studies, their efficacy remains variable (Schuerelle and Mahaffee, 2006). There are no publications on the mechanism of action of nsVCTs.

Presence of phyto-hormones in VCT

We identified the hormones IPA in the VCT, and speculated that plant growth hormones present in VCT promote plant growth and yield. However, the effect of VCTs on the suppression of plant diseases is unknown. This study also aimed at determining the effect of growth hormones in suppressing the root knot nematode infestation. From the results obtained by the application of commercial growth hormones, as seen in galling index and nematode number, it appears that the plant growth regulators IAA and IPA exacerbate the nematode infestation rather than suppress it.

Exogenous application of kinetin alone or in combination with auxin encouraged the formation of giant cell and galls in plant root infected with *Meloidogyne* species (Sandstedt and Schuster, 1977). Additionally, the presence of auxins has also been confirmed by Balasubramanian and Rangaswami (1966) in tomato root galls induced by *Meloidogyne javanica, M. hapla and M. incognita*. They suggested that the hormones may play a prominent role in the completion of the life cycle of root knot nematodes.

The function of accumulating cytokinins and auxins by the female nematodes most likely is in establishing and maintaining an active sink so parasites can feed on essential nutrients supplied to the sink (Dimalla and Staden, 1977). Their results suggest that cytokinins occurring in egg masses and larvae are not synthesized in their tissues, but are obtained and accumulated from the host tissue. This means that any additional plant growth regulators applied exogenously to the plant will likely increase nematode damage. This is consistent with our results. When we applied commercial IPA and IAA the number of root galls increased. The amount of IAA applied was similar to the amount of IPA identified in the nsVCT, but the plants treated with nsVCT were significantly healthier than the plants treated with just the IPA or IAA alone. This suggests that apart from phyto-hormones present in the VCT, there are other secondary metabolites or active compounds present in the vermicompost extract which have a fundamental role in plant metabolism and might help in fight plant nematode infestation.

Presence of microorganisms and nematode suppression by nsVCT

There are many reports in the literature of the ability of compost or vermicompost to suppress diseases but very little documentation of the effect of vermicompost teas on plant and soil borne pathogens. The efficiency of nsVCT in suppressing nematode infestations was studied by observing galling index and counting nematode eggs. Most of the treatments with nsVCT had fewer nematode eggs (P = 0.05, Table 2.1). Our results show that when VCT was applied at 100% concentration, nematodes were suppressed more effectively than the 50% concentration of VCT, especially when the pots were infested with a greater number of eggs. Edward et al. (2007) showed that VCT when applied as soil drench reduced root galling by *M. hapla* from 60-90% in tomato plants as compared to other treatments.

Analysis of nsVCT showed the presence of both bacterial and fungal species but fungal species were more prominent than bacterial. Some of the fungal genera such as *Trichoderma, Penicillium* and *Aspergillus*, and bacterial genera, like *Bacillus, Pseudomonas* and *Pantoea,* have been reported to be antagonistic to some soil-borne disease-causing fungi and are also known to be suppressive to nematodes (Sharon et al., 2001,)The fungal species found in the nsVCT in our analysis mostly turned out to be yeast. However, there were also parasitic fungi present, including *Cladosporium* sp. and *Davidiella* sp. These fungi have shown some ability in suppressing other fungal diseases. For example *Cladosporium oxysporum, Cladosporium macrocarpum* and *Cladosporium uredinicola,* both found in our nsVCT, are just few of the fungi that have capacity to

parasitize powdery mildew pathogens (Potera, 1994). It was reported by Orozco et al. (1996) that the presence of *Cladosporium macrocarpum* in asparagus at harvest results in a decrease in *Idriella bolleyi*, as well as F*usarium* and *Penicillium* spp. Bansal and Mukerji (1994) established that the application of mycorrhiza as a soil amendment resulted in alteration in the mycorrhizosphere mycoflora by suppressing pathogenic fungi (e.g. *Fusarium* species) and the stimulation of saprophytes like (e.g. *Cladosporium* species) and thus increasing the resistance to many soil borne pathogens. Since there are no research studies describing the direct effect of *Cladosporium* on the nematodes, a logical follow-up study is to culture the *Cladosporium* found in the VCT to determine their effect on nematodes.

Mechanisms of action by nsVCT

There are three mechanisms by which beneficial microbes inhibit plant pathogens: they out compete the pathogen for a space and nutrient source, directly parasitize the pathogen, or stimulate the plant to make the physiological changes that will decrease its susceptibility to infection (induced systemic resistance) Pieterse et al. 2003). It is clear from our experiment that the hormones tested in this research do not have any effect on suppressing the nematode population. Rather, they stimulate the growth of root knot nematodes in the root of the tomato plant. Contrasting results have been observed in nematode suppression with the application of compost. In a study by Thoden et al. (2011), both a reduction in root knot nematodes and an increase in crop yields were reported after the application of composts. In contrast, Kimpinski et al. (2003) observed

no reduction of *Meloidogyne hapla* populations but found increased yields in field in which the soil was treated with compost or cattle manure. Similarly, some green manure crops such as oil radish also failed to reduce *M. chitwoodi* under field conditions (Thoden et al, 2011)

It was shown by Edward et al. (2006) that the pathogen suppressing property of any vermicompost tea is microbial, since the vermicompost tea lost the suppression property when the vermicompost was sterilized. There are a multitude of factors that affect the disease suppressing quality of vermicompost extract. The potential parameters include vermicompost tea preparation factors such as aeration/non-aeration, type of compost, nutrient additives, duration of fermentation etc (Schuerell and Mahaffee, 2006). Additionally, improved nutritional status of the plant by improving the nutrient properties of the vermicompost tea by adding some additives (Siddiqui et al., 2008 and Hoitnik et al., 1997) may also protect the plant against the disease.

For nematodes, there are numerous papers showing that fungal endophytes help to increase plant resistance to nematodes (Sikora et al., 2008). A non-pathogenic *Fusarium* sp. was reported to play a role in resistance to root knot nematodes in tomato (Hallmann and Sikora, 1994). *Trichoderma* spp. have been developed as a biocontrol agent for the control of soilborne fungi, such as *Pythium* spp., *Rhizoctonia solani*, and *Fusarium* spp; they are also known to control nematodes (Sharon et al., 2001).

In summary, assuming that there are broad ranges of microorganisms that pass from vermicompost to VCT,their application could help in suppressing plant disease although the mechanism underlying nematode suppression is not yet clearly understood. The most common soil amendment that goes into compost is wastes or by-products of agricultural industries, such as animal manures and plant residues (Oka, 2010). One of the problems with using this control method for nematodes is inconsistent nematode suppression, which is highly influenced by the type of amendment and the soil type.

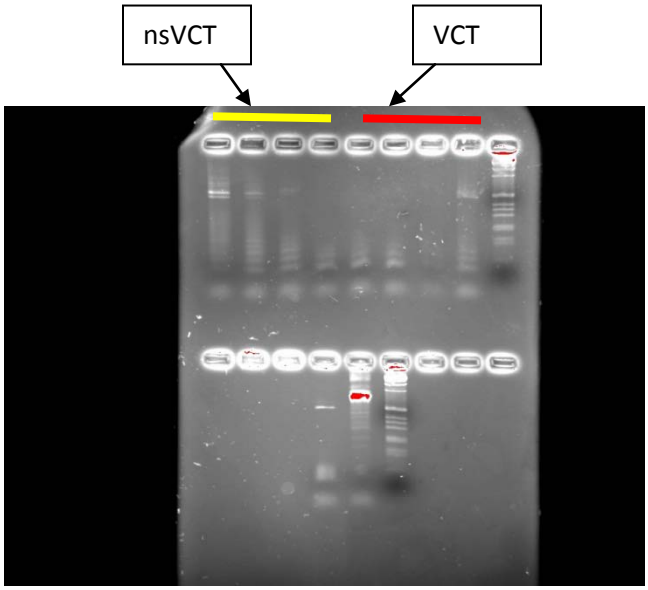

Fig 2.1 Bacterial Analysis: Intergenic 16S to 23S rDNA patterns of dominant bacterial populations in the nsVCT.and growth-promoting VCT used in Chapter 1.

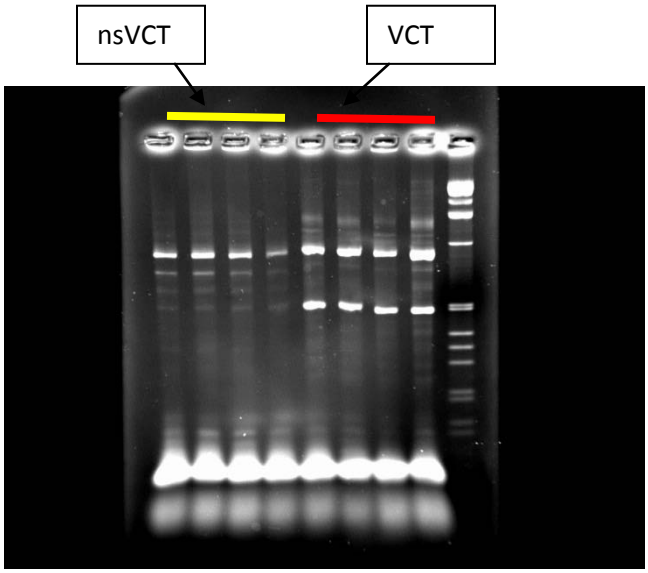

Fig 2.2 Fungal Analysis: Intergenic 16S to 23S rDNA patterns of dominant fungal populations in the nsVCT and growth-promoting VCT used in the Chapter 1.

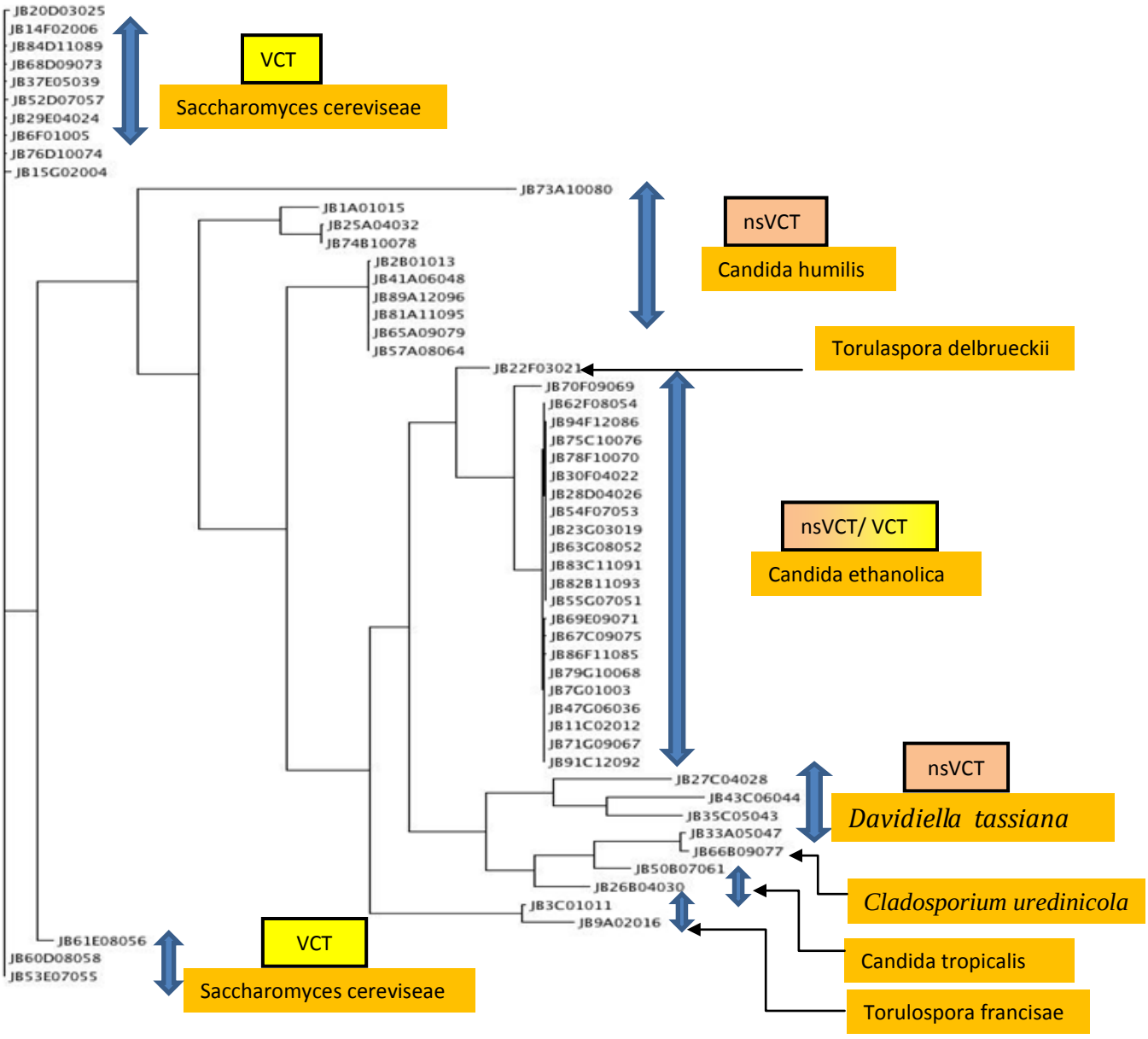

Fig 2.3. Phylogenetic tree of stramenopile phylotypes from growth-promoting VCT and nsVCT. The unrooted parsimony tree was constructed using at least one representative small-subunit rRNA gene sequence from each phylotype taxon. The scale bar length is 0.05, and represents the number of nucleotide changes per position. Vertical lines designate the four most abundant phylotypes

Table 2.1 Various parameters for nematode analysis in roots and tomato plant growth.

Treatment	Gall Index	Eggs count	Root wt (g)	Shoot fresh wt (g)	Shoot dry wt (g)
T_1 – Control	0	0	175.60 e	68.44 d	12.46 e
T_2 – 50% VCT + 5000 eggs	[z] 2.67 e	5.60 d	330.71 c	72.83 bcd	17.20 d
T_3 - 50% VCT + 10000 eggs	4.67 d	6.05 b	418.68 b	71.23 cd	18.83 d
T_4 – 100% VCT + 5000 eggs	1.83 f	5.29 e	245.56 d	75.60 abc	22.20 c
T_5 – 100% VCT + 10000 eggs	2.17 ef	5.52 d	226.56 d	77.71 ab	27.21 b
T_6 – IPA + 5000 eggs	6.17 c	5.83 c	437.63 b	42.61 f	7.54 f
T_7 – IAA + 5000 eggs	6.40 c	5.84 c	501.25 a	46.32 ef	7.02 f
T_8 - 5000 eggs	7.83 b	5.82 c	491.17 a	51.48 e	6.12 f
T_9 - 10000 eggs	8.67 a	6.21 a	525.40 a	41.94 f	6.06 f
T_{10}- VCT	0	0	156.32 e	79.37 a	30.00 a
	<.0001	<.0001	<.0001	<.0001	<.0001

[z] Means followed by different letters are significantly different by Fisher's Protected LSD Test at $P < 0.05$.

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