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Suppressive effect of non-aerated compost teas on foliar fungal pathogens of tomato

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ABSTRACT

Compost teas are fermented watery extracts of composted materials that are used for their ability to decrease plant disease. Non-aerated compost teas (NCT) prepared from five types of compost were tested for their ability to inhibit the growth of *Alternaria solani*, *Botrytis cinerea*, and *Phytophthora infestans in vitro*. Weekly applications of NCT were also used in greenhouse trials to assess their suppressive effect on powdery mildew (*Oidium neolycopersici*) and gray mold (*B. cinerea*) on tomato plants. All NCT significantly inhibited the mycelial growth of *A. solani* (37–66%), *B. cinerea* (57–75%), and *P. infestans* (100%), whereas sterilized teas did not inhibit growth of the tested pathogens. Although NCT failed to efficiently control powdery mildew, they were able to control tomato gray mold for up to 9 weeks in greenhouse experiments. Among the tested compost teas, NCT prepared from sheep manure compost consistently provided the highest inhibition of mycelial growth and the highest disease suppression, in particular of gray mold (>95% disease reduction). The overall relative efficacy of the various NCT did not correlate well with microbial communities or physico-chemical composition of the prepared NCT. Results also suggest that the presence of the microorganisms in the NCT is a prerequisite for inhibition.

1. Introduction

Compost teas are fermented watery extracts of composted materials that are used for their beneficial effect on plants (Litterick et al., 2004). The most widely described benefit of compost teas is their ability to decrease plant disease when used as soil drenches or foliar sprays (Scheuerell and Mahaffee, 2002, 2004). In this respect, compost teas are viewed as potential alternatives to the use of synthetic chemical fungicides as they provide a means of controlling plant pathogens that are deemed safer for health and the environment (Siddiqui et al., 2009). Although disease suppressive effects of compost teas have been reported in numerous agricultural systems, their efficacy remains variable (Scheuerell and Mahaffee, 2006).

The potential parameters that affect the efficacy of compost teas are twofold: the target pathosystem (pathogen and host plant) and the preparation methodologies of the teas (aeration, compost type, nutrient additives, duration of fermentation, etc.) (Scheuerell and Mahaffee, 2006). Among compost tea preparation factors, the compost type has been studied to determine its effect on the efficacy of the prepared tea. In previous reports, the type of compost is deemed a crucial factor in the efficacy of the produced tea. Indeed, many authors reported that teas prepared from manure-based composts are generally more effective against plant pathogens than teas from plant-based composts (Weltzien, 1991; Al-Dahmani et al., 2003; Haggag and Saber, 2007). Conversely, others have concluded that the type of the compost does not directly affect the efficacy of the tea and that both manure-based composts and other compost types can provide an efficient tea to control plant pathogens (Elad and Shtienberg, 1994; Scheuerell and Mahaffee, 2006).

Compost teas are reported to control plant pathogens through different mechanisms. The most reported factor influencing the efficacy of compost teas in inhibiting the development of plant disease is their microbial content. The microorganisms present in the tea may act as pathogen antagonists through their ability to compete for space and nutrients (Al-Mughrabi et al., 2008), to destroy pathogens by parasitism (El-Masry et al., 2002), to produce antimicrobial compounds, or to induce systemic resistance in plants (Zhang et al., 1998). Other work hypothesized that the physico-chemical properties of the compost teas, namely nutrients and organic molecules such as humic or phenolic compounds (Hoitink et al., 1997; Siddiqui et al., 2008), may protect the plant against disease through improved nutritional status, direct toxicity toward the pathogen or induced systemic resistance.

In this study, non-aerated compost teas (NCT) prepared from various compost types were tested for their effect on the growth of different tomato (*Solanum lycopersicum* L.) foliar pathogens and for their effect on the development of two tomato foliar

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diseases namely powdery mildew (*Oidium neolycopersici*) and gray mold (*Botrytis cinerea*) in an effort to gain insight into their suppressive nature. NCT was favored over aerated compost teas (ACT) since they are regarded as the simplest technology for the preparation of compost teas (no specialized aeration equipment necessary) while generally considered to be as effective as ACT in controlling disease (Welke, 2005; Scheuerell and Mahaffee, 2006).

2. Materials and methods

2.1. Microbial material

Four tomato pathogens were used to assess the suppressiveness of the prepared NCT. *Alternaria solani* Sorauer and *B. cinerea* Pers. were maintained on potato dextrose agar (PDA; Becton Dickinson, Sparks, MD) at 24 °C. *Phytophthora infestans* (Mont.) de Bary was maintained on rye agar (Klimczak and Prell, 1984) containing 200 g/l boiled (90 min) rye grain filtrate, 5 g/l dextrose, and 12 g/l agar at 24 °C. These three isolates were obtained from the Laboratoire de diagnostic en phytoprotection (MAPAQ, Québec, Canada). The biotroph *O. neolycopersici* L. Kiss was maintained on tomato plants under greenhouse conditions.

2.2. Preparation of compost teas

Commercially-available composts produced from chicken manure, sheep manure (four sources; SM1–SM4), bovine manure, shrimp powder, or seaweed were used for the production of NCT. Each NCT was prepared by mixing 1 L of homogenized compost with 5 L of water (from an agricultural well) in a 10-L container according to a modified method of Elad and Shtienberg (1994). The mixture was loosely covered and stored in the dark at 18 °C for 14 days. The NCT were stirred on the 7th and 14th day of incubation. Following the incubation period, each mixture was filtered through eight layers of cheesecloth and conserved at 4 °C until use.

2.3. Physico-chemical and microbial analysis of NCT

The mineral contents of the produced NCT were analyzed using standard methods. NH_4 and PO_4 were determined by colorimetry according to the methods of Nkonge and Ballance (1982) and Tran and Simard (1993), respectively. NO_3, SO_4, and Cl were quantified by ionic chromatography (Dionex, Sunnyvale, CA). K and Na were determined by atomic emission and Ca, Mg, Fe, Cu, Mn, and Zn were quantified by atomic absorption spectroscopy (Perkin-Elmer Atomic Absorption Spectrometer 3300, Ueberlingen, Germany). The electrical conductivity (EC) and pH were also determined for each NCT.

The presence of coliforms and *Escherichia coli* in the prepared NCT were analyzed in duplicate on *E. coli*/Coliform Petrifilm count plates (3M, London, Canada) according to the manufacturer's directions. The populations of fungi, actinomycetes, and bacteria (total, Gram negative, and fluorescent pseudomonads) were analyzed in each NCT on different selective culture media. Fungi were grown on Malt agar (Becton Dickinson) amended with 0.3 g/l streptomycin (Sigma–Aldrich, St. Louis, MO). Actinomycetes were cultured on Actinomycete Isolation Agar (Becton Dickinson) amended with glycerol (10 g/l; Sigma–Aldrich). Total bacteria were grown on Tryptic Soy Agar (Sigma–Aldrich) and Gram negative bacteria were cultured on Tryptic Soy Agar amended with crystal violet (0.002 g/l; Sigma–Aldrich). Finally, fluorescent pseudomonads were grown on Pseudomonas Agar F (Becton Dickinson) amended with glycerol (10 g/l; Sigma–Aldrich), ampicillin (0.5 g/l; Sigma–Aldrich), chloramphenicol (0.125 g/l; Sigma–Aldrich), and chlorothalonil (1.1 g/l; Syngenta, Guelph, Canada). Each NCT was 10-fold serial diluted (10^0–10^{-7}) and 50 μl of each dilution were plated on the selective media. After an incubation period of 2–4 days at 25 °C, the most probable number technique (MPN) (Woomer et al., 1988; Min, 1999) was used to estimate the different microbial populations on each selective medium. Four replicates for each NCT were performed to determine MPN.

2.4. Effect of NCT on mycelial growth

To determine their effect on mycelial growth of *A. solani*, *B. cinerea*, and *P. infestans*, the produced NCT or agricultural well water (control) were incorporated into PDA (15% vol:vol) (Diánez et al., 2006). To prepare the NCT containing media, PDA was sterilized and cooled to 45 °C. The NCT were then mixed with the cooled PDA and immediately poured into the Petri dishes. In addition, the effect of sterilized NCT on mycelial growth was evaluated by incorporating autoclaved (121 °C for 20 min) or microfiltered (0.2 μm) NCT into PDA (15% vol:vol), prepared as described above. Agar plugs (0.5 cm diameter) covered with actively growing mycelia of the three pathogens were individually inoculated on the media and incubated 6–7 days in the dark at 25 °C. After the incubation period, mycelial growth of each fungus was measured as the average of two perpendicular diameters of the colony. All experiments were conducted according to a completely randomized design with four repetitions.

2.5. Effect of NCT on tomato disease severity

Tomato seeds (cultivar Bush Beefsteak) were sown in rock wool. After 2 weeks, the tomato seedlings were individually transplanted into 30-cm pots containing Promix substrate (Premier Tech, Rivière-du-Loup, Canada). Seedlings were grown under greenhouse conditions at 19 °C (night) and 23 °C (day) with a 16-h photoperiod and 85% relative humidity. Plants were fertilized daily with a nutrient solution containing 200–80–240 μg/ml of N–P–K.

When tomato plants reached the four leaf stage, leaves were artificially inoculated with either *B. cinerea* or *O. neolycopersici* spores 3 days after (preventive) or before (curative) the initial treatment with NCT. *Botrytis cinerea* inoculum was prepared as described in Scheuerell and Mahaffee (2006), adjusted to 1×10^5 conidia/ml suspension, and uniformly sprayed onto each leaf of each plant (20 ml per plant). Tomato leaves heavily infected with *O. neolycopersici* were shaken 1 day prior to inoculation to ensure that freshly formed conidia were available. Leaf segments bearing conidia were harvested and used to inoculate the experimental plants by manually shaking the infected leaf segments over the plants to dislodge the conidia. Non-inoculated plants were also included to evaluate potential phytotoxicity of the tested NCT. Each of the five tested NCT (bovine manure, sheep manure SM1, chicken manure, shrimp, and seaweed compost NCT) or agricultural well water (control) was applied weekly throughout the experiment by spraying of the entire foliar surface until runoff.

Disease severity was evaluated weekly as the percentage (%) of visible symptom area of gray mold (*B. cinerea*) or powdery mildew (*O. neolycopersici*) on the fourth true leaf of each plant. Symptoms of phytotoxicity (scorching, burning, or spotting of tomato plant leaves) were noted and plant height was measured. The experiments were conducted according to completely randomized block designs with five repetitions. The experiments were repeated twice.

2.6. Statistical analysis

Analysis of variance (ANOVA) was carried out with the GLM procedure of SAS (SAS Institute, 1999) and, when significant ($P < 0.05$), treatment means were compared using Fisher's protected least significant difference (LSD) test. In the tomato disease severity tests, ANOVA of the experimental data showed no significant difference between the two experiments. The data were therefore combined and analyze as a single experiment.

3. Results

3.1. Physico-chemical and microbial analysis of NCT

Physico-chemical properties of the prepared NCT are shown in Table 1. pH of NCT ranged from 5.92 (sheep manure SM1) to 6.57 (chicken manure). The agricultural well water used to produce the NCT had a pH of 7.13. The highest EC was found in NCT from chicken manure compost (1.05 mS) followed by NCT from seaweed compost (0.48 mS). NCT from bovine manure, sheep manure (SM1), and shrimp composts showed lower EC (0.08–0.10 mS). Water had an EC of 0.02 mS. Overall, NCT produced from chicken manure compost had the highest quantities of macro- and microelements (Table 1). NCT from seaweed compost had the highest concentrations of Fe, Mn and the second most elevated quantities of NH_4, PO_4, Cl, SO_4, K, Ca, Mg, Na, Fe, Cu, and Zn. NCT from bovine manure, sheep manure (SM1), and shrimp composts all had comparable macro- and micronutrient concentrations, which were generally equivalent to what was found in the agricultural well water control.

Escherichia coli was not present in any of the NCT or in the agricultural well water control (data not shown). NCT from chicken manure and seaweed composts showed an average of 28 and 5 coliforms/ml, respectively, whereas no coliforms were detected in the other prepared NCT or in the agricultural well water.

Microbial populations in the produced NCT are shown in Table 2. NCT from sheep manure (SM1) compost provided the highest concentration of total bacteria, Gram negative bacteria, actinomycetes, and fluorescent pseudomonads. NCT from shrimp and seaweed compost had the lowest concentration of total bacteria. Chicken manure compost NCT had the lowest Gram negative bacteria. Finally, the NCT from bovine manure compost also had the highest concentration of Gram negative bacteria as well as the lowest fluorescent pseudomonads concentration.

When NCT from different sources of sheep manure compost were evaluated, there was no significant difference in microbial populations (total bacteria, Gram negative bacteria, actinomycetes, fluorescent pseudomonads, and fungi) between any of the four tested NCT (SM1–SM4) (data not shown).

3.2. Effect of NCT on mycelial growth

All NCT inhibited the *in vitro* growth of the three tested pathogens when compared to the water control. NCT from sheep manure (SM1), bovine manure, and shrimp compost provided the highest inhibitory effect on *A. solani* and *B. cinerea*, reducing mycelial growth by approximately 66% and 75%, respectively (Fig. 1). NCT from chicken manure compost had a lower inhibitory effect than those from sheep manure (SM1) and shrimp compost. NCT from seaweed compost showed the lowest inhibitory effect among the tested NCT. All tested NCT demonstrated 100% inhibition of *P. infestans* (data not shown).

When sterilized by autoclaving or by microfiltration, there was no significant inhibition of any of the sterilized NCT on the mycelial growth of the three tested pathogens (data not shown).

3.3. Effect of NCT on tomato disease severity

When NCT were sprayed on non-inoculated tomato plants, there was no appearance of phytotoxic symptoms (scorching, burning, or spotting of tomato plant leaves) in any of the treatments (data not shown). Moreover, the application of NCT to the non-inoculated tomato plants showed no significant effect on plant height for any of the treatments (data not shown).

A preventive application of the five NCT (3 days before artificial inoculation with *O. neolycopersici*) provided a significant decrease in powdery mildew severity over the first 4 weeks of the trials (Fig. 2). NCT from sheep (SM1) and chicken manure composts demonstrated the lowest disease severity after 3 and 4 weeks, reducing powdery mildew by 45–62% when compared to the water control. NCT from bovine manure, shrimp, and seaweed composts also significantly reduced powdery mildew severity after 3 and 4 weeks but to a lesser extent (23–35% disease reduction) than sheep (SM1) and chicken manure compost NCT.

A curative application of the NCT (3 days after artificial inoculation) also demonstrated a reduction in powdery mildew severity (Fig. 3), albeit to a lesser extent than the preventive treatments. Throughout the curative treatment trial, NCT from sheep manure (SM1) compost provided the lowest powdery mildew severity among the NCT (48% and 21% disease reduction for weeks 2 and 3, respectively). There was no significant difference in powdery mildew severity between the water control and shrimp and seaweed compost NCT after 3 weeks and between the control and NCT from chicken manure compost after 4 weeks.

A preventive application of the five NCT (3 days before artificial inoculation with *B. cinerea*) provided a significant decrease in gray mold severity over a 9-week period when compared to the water control (Fig. 4). NCT produced from sheep manure (SM1) gave the lowest gray mold severity, reducing disease by 99% and 97% after 8 and 9 weeks, respectively. NCT from chicken manure compost gave the second lowest disease severity (88–92% reduction) after 8–9 weeks. NCT from bovine manure and shrimp composts significantly reduced gray mold severity between 72% and 85% after 8–9 weeks compared to the control. Seaweed compost NCT generally showed the highest disease severity among tested NCT.

A curative application NCT from chicken manure, sheep manure (SM1), and bovine manure (3 days after artificial inoculation with *B. cinerea*) provided a significant decrease in gray mold severity

Table 1

Physico-chemical properties of non-aerated compost teas (NCT) from chicken manure, bovine manure, sheep manure, seaweed, and shrimp composts.

NCT	pH	EC	Macro- and micronutrient concentration (mg/l)												
			NO_3	NH_4	PO_4	Cl	SO_4	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Water	7.13	0.02	–	–	–	15 c	2 c	1 c	20 b	4 c	5 d	–	0.04 c	0.13 b	0.04 c
Chicken manure	6.57	1.05	13	10.9 a	72 a	155 a	31 a	421 a	39 a	16 a	65 a	1.9 a	0.68 a	0.21 ab	0.60 a
Bovine manure	6.35	0.08	–	–	1 c	16 c	2 c	8 c	12 b	2 d	8 c	–	0.05 c	0.04 b	–
Sheep manure (SM1)	5.92	0.09	–	–	–	17 c	3 c	3 c	13 b	3 cd	7 c	0.1 b	0.07 c	–	–
Seaweed	6.49	0.48	–	0.37 b	40 b	83 b	13 b	158 b	16 b	7 b	46 b	1.3 a	0.34 b	0.43 a	0.26 b
Shrimp	6.39	0.10	–	–	2 c	18 c	5 c	10 c	13 b	3 cd	9 c	0.1 b	0.06 c	–	–

Within columns, macro- and micronutrient means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

EC, electrical conductivity in milliSimens (mS).

–, below detection limits.

Table 2
Microbial populations of non-aerated compost teas (NCT) from chicken manure, bovine manure, sheep manure, seaweed, and shrimp composts.

NCT	Total bacteria ($\times 10^6$)	Gram negative bacteria ($\times 10^4$)	Actinomycetes ($\times 10^4$)	Fluorescent pseudomonads ($\times 10^1$)	Fungi ($\times 10^3$)
Chicken manure	3.2 (0.8–12.2)	1.0 (0.3–3.8)	1.8 (0.5–6.8)	70.0 (18.4–266)	3.2 (0.8–12.2)
Bovine manure	3.2 (0.8–12.2)	280 (73.7–1060)	7.0 (1.8–26.6)	6.0 (1.6–22.8)	2.8 (0.7–10.6)
Sheep manure (SM1)	12.0 (3.1–45.6)	280 (73.7–1060)	23.0 (6.0–87.4)	100 (26.3–380)	1.4 (0.4–5.3)
Shrimp	1.2 (0.3–4.6)	7.0 (1.8–26.6)	2.2 (0.6–8.4)	70.0 (18.4–266)	2.8 (0.7–10.6)
Seaweed	1.0 (0.3–3.8)	5.0 (1.3–19.0)	3.2 (0.8–12.2)	90.0 (23.7–342)	3.2 (0.8–12.2)

Means were calculated by the most probable number method in MPN/ml.
Values in brackets represent the low and high confidence limits (95%).

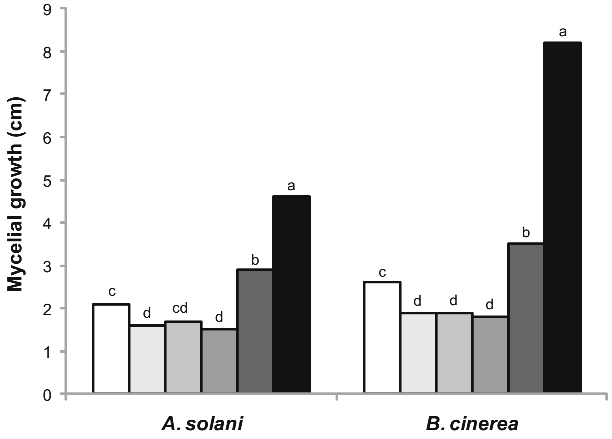

Fig. 1. Effect of non-aerated compost teas on mycelial growth of *Alternaria solani* and *Botrytis cinerea in vitro*. Compost teas were incorporated into PDA (15% vol:vol). For each fungal pathogen, means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$). Chicken manure compost ☐; sheep manure (SM1) compost ☐; bovine manure compost ▨; shrimp compost ▨; seaweed compost ▨; water control ■.

over a 5- to 9-week period when compared to the water control (Fig. 5). Throughout the curative treatment trial, NCT from sheep manure (SM1) compost provided the lowest disease severity followed by either chicken manure compost NCT or bovine manure compost NCT. While initially able to reduce gray mold severity, seaweed compost NCT and shrimp compost NCT did not significantly decrease severity as compared to the control after 8 and 9 weeks of culture, respectively (Fig. 5).

3.4. Effect of different sheep compost NCT on mycelial growth

In addition to NCT prepared from sheep manure compost SM1, three other NCT were prepared from different sources of commercially-available sheep manure composts (SM2, SM3, SM4) and tested against *A. solani*, *B. cinerea*, and *P. infestans in vitro*. NCT from SM1, SM2, SM3, and SM4 inhibited the mycelial growth of *A. solani* and *P. infestans* to almost equivalent levels (Fig. 6). NCT from SM3 and SM4 provided a significant reduction in *B. cinerea* mycelial growth, although these were significantly less inhibitory than SM1 and SM2. When sterilized by autoclaving or by microfiltration, there was no significant inhibition of any of the sterilized sheep manure NCT on the mycelial growth of the three tested pathogens (data not shown).

4. Discussion

Compost teas are perceived as potential alternatives to synthetic chemical fungicides (Siddiqui et al., 2009). Although most accounts describe compost teas produced from various compost types demonstrating an inhibitory effect on plant pathogens in numerous plant pathosystems, these effects remain variable (Scheuerell and Mahaffee, 2006) and therefore difficult to use reliably. In recent work, the mechanisms of action underlying the efficacy of compost teas to control plant pathogens have been reported as single or multiple mechanisms involving microbial antagonism (through antibiosis, parasitism, competition for nutrients/space or induced plant resistance; Zhang et al., 1998; El-Masry et al., 2002; Al-Mughrabi et al., 2008) or suppressive physicochemical properties (improved nutrient status of the plant, toxic

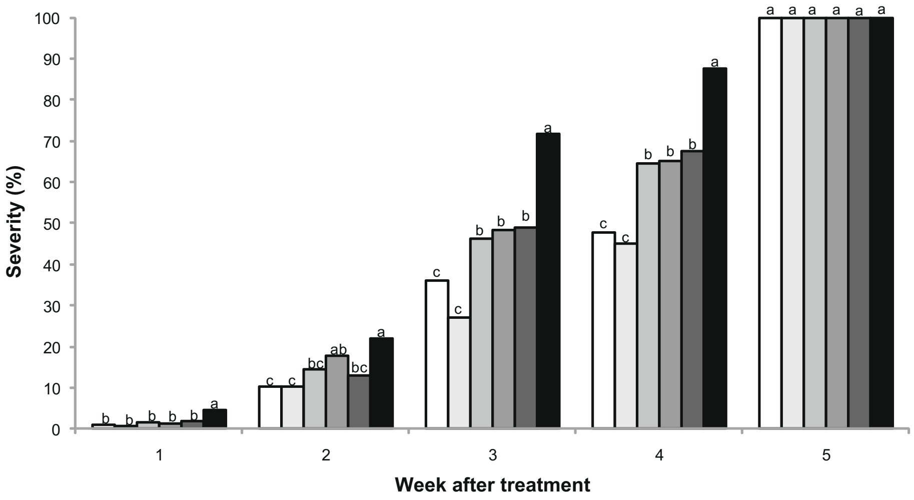

Fig. 2. Effect of a preventive application of non-aerated compost teas on the severity of tomato powdery mildew (*Oidium neolycopersici*) in greenhouse assays. Compost teas were applied until runoff on artificially-inoculated tomato plants. For each week, means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$). Chicken manure compost ☐; sheep manure (SM1) compost ☐; bovine manure compost ▨; shrimp compost ▨; seaweed compost ▨; water control ■.

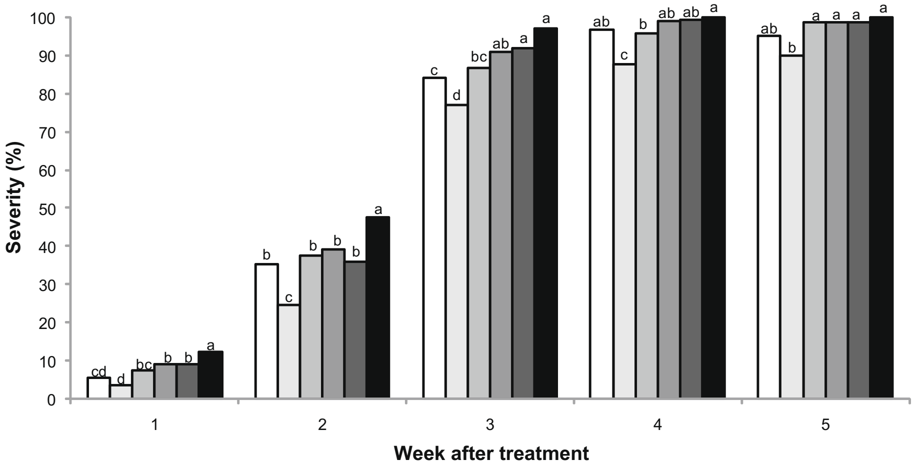

Fig. 3. Effect of a curative application of non-aerated compost teas on the severity of tomato powdery mildew (*Oidium neolycopersici*) in greenhouse assays. Compost teas were applied until runoff on artificially-inoculated tomato plants. For each week, means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$). Chicken manure compost □; sheep manure (SM1) compost ▢; bovine manure compost ▨; shrimp compost ▨; seaweed compost ▩; water control ■.

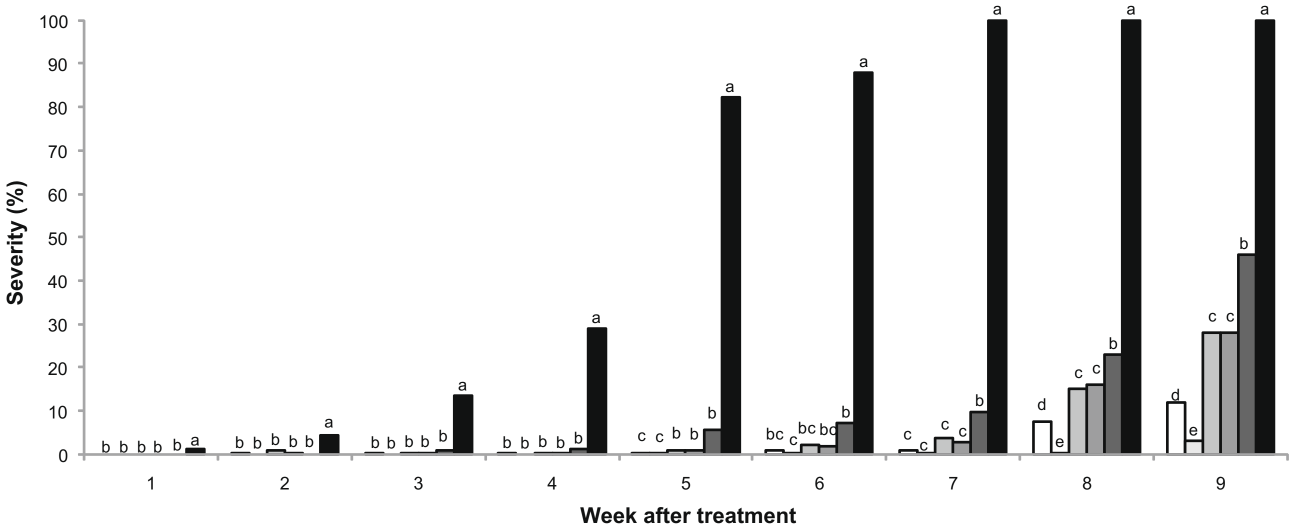

Fig. 4. Effect of a preventive application of non-aerated compost teas on the severity of gray mold of tomato (*Botrytis cinerea*) in greenhouse assays. Compost teas were applied until runoff on artificially-inoculated tomato plants. For each week, means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$). Chicken manure compost □; sheep manure (SM1) compost ▢; bovine manure compost ▨; shrimp compost ▨; seaweed compost ▩; water control ■.

compounds, or induced resistance; Hoitink et al., 1997; Siddiqui et al., 2008). In this study, NCT prepared from various compost types were evaluated for their ability to control tomato pathogens to gain insight into properties involved in their suppressive effects.

In vitro assays showed that the five tested NCT provided significant inhibition of mycelial growth of *A. solani*, *B. cinerea*, and *P. infestans*. Among the tested NCT, those prepared from sheep manure (SM1), bovine manure, and shrimp composts gave the highest inhibition whereas NCT prepared from chicken manure and, in particular, seaweed compost always had a lesser effect. This suggests that the NCT (and their intrinsic components) have a direct inhibitory effect on the tested pathogens. When NCT were sterilized by microfiltration or autoclaving, they had completely lost their direct inhibitory effect on the mycelial growth of the tested pathogens. This indicates that toxic compounds (extracted from the organic and inorganic compost materials or produced by the microbial populations within the NCT) were not initially present in the NCT or at least not at levels that impede the growth of the tested pathogens. This also implies that the presence of microbial antagonists in the NCT is necessary for direct inhibition of mycelial growth. These antagonists could potentially use competition or parasitism to exert their direct effect on the pathogens and may also produce antimicrobial compounds (antibiosis) when in direct confrontation with the pathogens. Along these lines, previous reports have shown that biosynthesis of toxic metabolites in bacteria and fungi could be enhanced or newly induced in the presence of a competing microorganism (Sonnenbichler et al., 1994; Martinez et al., 2006).

In *in vivo* trials, all tested NCT demonstrated inhibition of the development of both diseases, in particular gray mold (*B. cinerea*). Although powdery mildew (*O. neolycopersici*) severity was significantly lower than the control, application of the tested NCT failed to efficiently control this disease.

The preventive application of NCT 3 days before inoculation by the pathogens was more effective in controlling development of the diseases than a curative application (3 days after inoculation with the pathogens). This would indicate that the NCT suppressive effect is partly because of inhibition of fungal spore germination

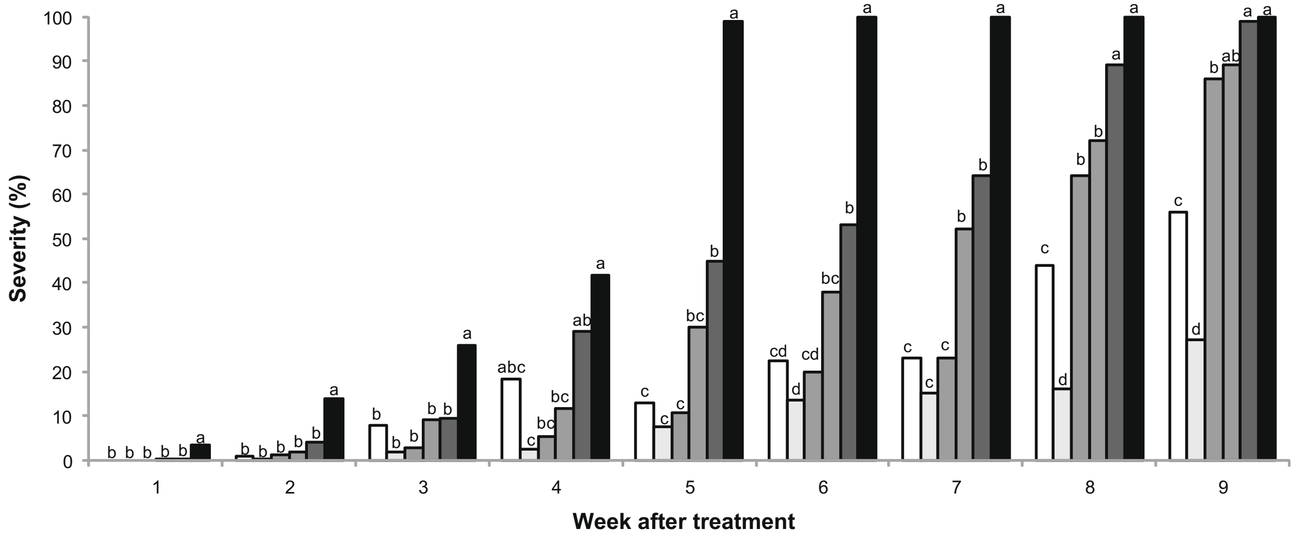

Fig. 5. Effect of a curative application of non-aerated compost teas on the severity of gray mold of tomato (*Botrytis cinerea*) in greenhouse assays. Compost teas were applied until runoff on artificially-inoculated tomato plants. For each week, means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$). Chicken manure compost ☐; sheep manure (SM1) compost ☐; bovine manure compost ▨; shrimp compost ▨; seaweed compost ▨; water control ■.

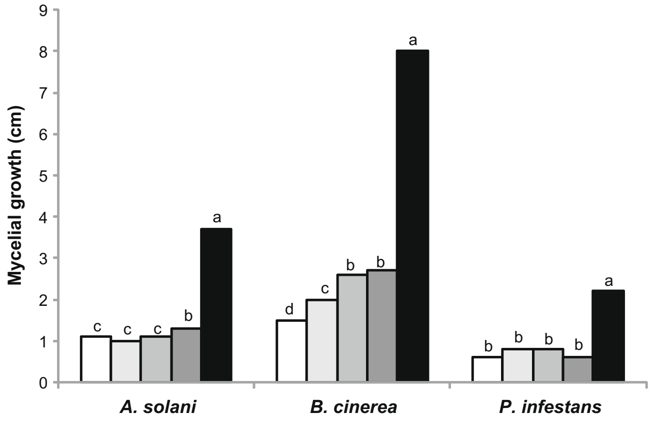

Fig. 6. Effect of non-aerated sheep compost teas prepared from different commercial sheep manure composts on mycelial growth of *Alternaria solani*, *Botrytis cinerea*, and *Phytophthora infestans in vitro*. Compost teas were incorporated into PDA (15% vol:vol). For each fungal pathogen, means with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$). Sheep manure composts: SM1 ☐; SM2 ☐; SM3 ▨; SM4 ▨; water control ■.

and/or penetration inside the plant cells as have been shown previously (Diánez et al., 2007; Siddiqui et al., 2009). NCT from sheep manure (SM1) compost always provided the highest inhibition of disease development in all trials. In particular, a preventive application of NCT from sheep manure (SM1) compost was able to reduce gray mold severity by more than 95% over a 9-week period. NCT from chicken manure compost consistently provided the second highest disease inhibition whereas NCT from seaweed compost was always the least inhibitory. NCT from bovine manure, chicken:bovine (1:9 vol:vol) manure, and grape marc composts using a similar production methodology (1:5 compost/water ratio; 14-day incubation) had been previously shown to reduce gray mold on tomato by about 74–80% in growth chambers and NCT from bovine manure reduced gray mold in greenhouse tomatoes by 30% and 37% after 6 and 8 weeks, respectively (Elad and Shtienberg, 1994). This same work showed a significant inhibition (40–52%) of the development of another powdery mildew fungus, *Leveillula taurica*, on tomato plants with weekly applications of bovine manure composts.

When taken together, *in vitro* and *in vivo* results appeared to initially correlate well with total bacteria concentrations in individual NCT. The most efficient NCT from sheep manure (SM1) compost showed the highest total bacteria concentration, which was approximately fourfold higher than the next most efficient NCT (chicken and bovine manure composts). NCT from sheep manure (SM1) compost also had a 10- and 12-fold higher total bacteria concentration than NCT from shrimp and seaweed composts, respectively, which were the least efficient NCT in this study. Although, NCT from sheep manure (SM1) compost also had the highest concentration of Gram negative bacteria, actinomycetes and fluorescent pseudomonads, these particular factors did not correlate well with the relative efficacy of the other NCT. Previous studies had also postulated that high total bacteria content was linked to the efficacy of various NCT (Weltzien, 1991; Diánez et al., 2007; Al-Mughrabi et al., 2008). However, results from assays with NCT prepared from four sources of sheep manure compost had variable efficacy against the tested pathogens *in vitro* although they had similar microbial population counts including total bacteria. Contrary to the previous assays, this indicated that total bacterial counts in the NCT did not correlate with their relative efficacy. Other work described that particular bacterial isolates from NCT could effectively control gray mold when applied alone (Elad and Shtienberg, 1994) and that increasing the bacterial counts in NCT through the use of fermentation additives did not necessarily correlate with the reduction of gray mold (Scheuerell and Mahaffee, 2006). This may indicate that specific microorganisms (potential biological control agents) would be more important in the suppressive effect than the high total counts of bacteria.

In some cases, it was shown that NCT were more efficient in reducing the severity of gray mold on tomato plants than it was in inhibiting the mycelial growth of *B. cinerea in vitro*. The additional indirect effect of these NCT against *B. cinerea in vivo* might have been a result of their higher concentration in nutrients (Table 1). Although improved nutritional status may protect the plant against disease (Siddiqui et al., 2008), there was no increase in plant height in plants NCT treatment and absolute concentrations of macro- and micronutrients would make the prepared NCT weak fertilizers at best. It is more probable that this indirect effect was provided by particular microorganisms or compounds within the NCT that, while not directly inhibitory to *B. cinerea* mycelial growth *in vitro*, had a possible effect on the induction of intrinsic plant defenses that help stave off gray mold as has been reported in other systems (Zhang et al., 1998; Haggag and Saber, 2007; Siddiqui et al., 2009). The higher nutrient concentration in some NCT may have also supported the growth of the antagonistic microorganisms on the leaf surface.

With respect to potential microbial safety issues of NCT, although *E. coli* was not present in any of the NCT, it is noteworthy that NCT prepared from chicken manure and seaweed composts consistently showed the presence of coliforms. Both of these NCT (chicken manure NCT in particular) showed coliform counts that are above many USA state or Canadian guidelines for irrigation water. Although coliforms in NCT have been reported to fall below detectable levels when applied to plants (Welke, 2005), care should be taken to limit or eliminate potential human pathogens from compost teas, in particular if preparation includes additives destined to enhance microbial growth (Ingram and Millner, 2007).

Overall, results from this study showed that NCT from various compost types provided a significant inhibition of the mycelial growth of foliar pathogens of tomato and significantly reduced the development of powdery mildew (*O. neolycopersici*) and, in particular, gray mold (*B. cinerea*) on tomato plants. NCT prepared from sheep manure compost (SM1) consistently provided the highest inhibition throughout the study. The relative efficacy of all the prepared NCT appeared to initially correlate well with the concentration of total bacteria in the respective NCT, but did not correlate well with other microbial communities (Gram negative bacteria, fluorescent pseudomonads, actinomycetes, fungi) or with macro- and micronutrient concentrations. However, results from the various sheep manure compost NCT would indicate that specific microorganisms (potential biological control agents) would be more important in the suppressive effect than the high total count of unspecified bacteria. Although the loss of *in vitro* efficacy upon sterilization of the NCT indicates that microbial communities must be present to exert their antagonistic effect (competition, parasitism, antibiosis) against the pathogens and suggests that microbial communities or particular microorganisms are involved directly in disease suppression, the induction of plant defense reaction by particular microorganisms or by organic and inorganic compounds in the NCT cannot be ruled out. Future work will attempt to identify microbial antagonists in highly suppressive NCT as well as identify compost tea preparation parameters that enhance populations of these antagonists.

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References

Al-Dahmani, J.H., Abassi, P.A., Miller, S.A., Hoitink, H.A.J., 2003. Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. Plant Disease 87, 913–919.

Al-Mughrabi, K.I., Berthélémé, C., Livingston, T., Burgoyne, A., Poirier, R., Vikram, A., 2008. Aerobic compost tea, compost and a combination of both reduce the severity of common scab (*Streptomyces scabiei*) on potato tubers. Journal of Plant Sciences 3, 168–175.

Diánez, F., Santos, M., Boix, A., de Cara, M., Trillas, I., Avilés, M., Tello, J.C., 2006. Grape marc compost tea suppressiveness to plant pathogenic fungi: role of siderophores. Compost Science and Utilization 14, 48–53.

Diánez, F., Santos, M., Tello, J.C., 2007. Suppressive effects of grape marc compost on phytopathogenic oomycetes. Archives of Phytopathology and Plant Protection 40, 1–18.

Elad, Y., Shtienberg, D., 1994. Effect of compost water extracts on grey mould (*Botrytis cinerea*). Crop Protection 13, 109–114.

El-Masry, M.H., Khalil, A.I., Hassouna, M.S., Ibrahim, H.A.H., 2002. *In situ* and *in vitro* suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World Journal of Microbiology and Biotechnology 18, 551–558.

Haggag, W.M., Saber, M.S.M., 2007. Suppression of early blight on tomato and purple blight on onion by foliar sprays of aerated and non-aerated compost teas. Journal of Food Agriculture and Environment 5, 302–309.

Hoitink, H.A.J., Stone, A.G., Han, D.Y., 1997. Suppression of plant diseases by composts. HortScience 32, 184–187.

Ingram, D.T., Millner, P.D., 2007. Factors affecting compost tea as a potential source of *Escherichia coli* and *Salmonella* on fresh produce. Journal of Food Protection 70, 828–834.

Klimczak, L.J., Prell, H.H., 1984. Isolation and characterization of mitochondrial DNA of the oomycetous fungus *Phytophthora infestans*. Current Genetics 8, 323–326.

Litterick, A.M., Harrier, L., Wallace, P., Watson, C.A., Wood, M., 2004. The role of uncomposted materials, composts, manures and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production – a review. Critical Reviews in Plant Sciences 23, 453–479.

Martinez, C., Avis, T.J., Simard, J.-N., Labonté, J., Bélanger, R.R., Tweddell, R.J., 2006. The role of antibiosis in the antagonism of different bacteria towards *Helminthosporium solani*, the causal agent of potato silver scurf. Phytoprotection 87, 69–75.

Min, H., 1999. Microbial Research Techniques. Sciences Press, Beijing, China.

Nkonge, C., Ballance, G.M., 1982. A sensitive colorimetric procedure for nitrogen determining in micro-Kjeldahl digests. Journal of Agricultural and Food Chemistry 30, 416–420.

SAS Institute, 1999. SAS/STAT User's Guide, Version 8. SAS Institute, Cary, NC.

Scheuerell, S.J., Mahaffee, W.F., 2002. Compost tea: principles and prospects for plant disease control. Compost Science and Utilization 10, 313–338.

Scheuerell, S.J., Mahaffee, W.F., 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. Phytopathology 94, 1156–1163.

Scheuerell, S.J., Mahaffee, W.F., 2006. Variability associated with suppression of gray mold (*Botrytis cinerea*) on geranium by foliar applications of nonaerated compost teas. Plant Disease 90, 1201–1208.

Siddiqui, Y., Meon, S., Ismail, M.R., Ali, A., 2008. *Trichoderma*-fortified compost extracts for the control of choanephora wet rot in okra production. Crop Protection 27, 385–390.

Siddiqui, Y., Meon, S., Ismail, R., Rahmani, M., 2009. Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. Biological Control 49, 38–44.

Sonnenbichler, J., Dietrich, J., Peipp, H., 1994. Secondary fungal metabolites and their biological activities. V. Investigations concerning the induction of the biosynthesis of toxic secondary metabolites in basidiomycetes. Biological Chemistry Hoppe-Seyler 375, 71–79.

Tran, T.S., Simard, R.R., 1993. Mehlich III-extractable elements. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, FL, pp. 43–49.

Welke, S.E., 2005. The effect of compost extract on the yield of strawberries and the severity of *Botrytis cinerea*. Journal of Sustainable Agriculture 25, 57–68.

Weltzien, H.C., 1991. Biocontrol of foliar fungal diseases with compost extracts. In: Andrews, J.H., Hirano, S.S. (Eds.), Microbial Ecology of Leaves. Springer-Verlag, New York, NY, pp. 430–450.

Woomer, P.L., Singleton, P.W., Bohlool, B.B., 1988. Reliability of the most probable number technique for enumerating rhizobia in tropical soils. Applied and Environmental Microbiology 54, 1494–1497.

Zhang, W.D., Han, Y., Dick, W.A., Davis, K.R., Hoitink, H.A.J., 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology 88, 450–455.