RESEARCH PAPERS

# Metabolic patterns of bacterial communities in aerobic compost teas associated with potential biocontrol of soilborne plant diseases

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**Summary.** Aerated compost teas (ACTs) are organic products obtained by forced aeration of composts suspended in liquid phase. These products may be biological control tools alternative to synthetic fungicides, because ACTs contain antagonistic microorganisms. In this study, soilborne disease suppressive ability of seven water ACTs, extracted from five horticultural residue-based composts, from an animal waste anaerobic solid digestate and from a commercial municipal waste compost, was assessed using *in vitro* and *in vivo* systems. All the ACTs inhibited *in vitro* growth of *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, *Sclerotinia minor*, *Sclerotium rolfsii* and *Botrytis cinerea*. Filter or thermal sterilization eliminated *in vitro* suppression, suggesting that microorganisms play key roles in pathogen inhibition. Drenching applications of raw ACTs have potential to reduced disease symptoms caused by *R. solani* on savoy cabbage, *S. minor* on lettuce and *S. rolfsii* on pepper, improved the biomass production and did not show any sign of phytotoxicity. Both *in vitro* and *in vivo* suppressiveness of ACTs may be explained by antagonistic  bacterial communities that provide general suppression activities. The metabolic BIOLOG GN and GP profiles reflected the functional potential of the numerically dominant members of the microbial communities used as inoculum. This study has demonstrated that useful resident microorganisms, including mainly Gram-positive and Gram-negative antagonistic bacteria, are likely to be responsible for biological control activity of ACTs.

**Key words:** metabolic fingerprinting, organic disease management, smart agriculture.

## Introduction

Soilborne plant pathogens can cause severe damage on susceptible vegetable crops, with potentially significant losses in yields and quality production. Infected plants show typical symptoms including, firstly, root, collar and / or crown rots, that can evolve, with advancing disease, to tissue discoloration, vascular wilt and damping-off. Generally, the control of soilborne diseases is very difficult, and chemical soil fumigation, in the past, has been viewed as the only possible remedy because of its effectiveness. However, the transition toward sustainable plant disease management required by the EU, through restrictive policies on the use of synthetic fungicides, has stimulated research into valid alternative methods equally capable of reducing plant disease losses.

Use of aerated compost teas (ACTs) is becoming an attractive disease management option among producers who support sustainable protective methods. ACTs are liquid products generally derived from aerated aqueous extractions of composted biodegradable organic compounds (Ingham *et al.*, 2003). Some variables of this process, including duration, oxygenation rate, compost and extractant type and relative ratio, including the use of additives, may be introduced to improve the specificity

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of the products. These organic products may show positive effects on plants by suppressing plant pathogens with improvement of quantity and quality of the yields (Zaccardelli *et al.*, 2012). Soluble organic molecules, such as humic substances and useful microorganisms, such as antagonistic bacteria, fungi, protozoa and nematodes, play crucial roles in ACT bioactivity. Different mechanisms of action, linked essentially to the main constituents of specific ACTs, have been hypothesized to explain suppressiveness, including direct fungitoxicity of dissolved molecules and biotic antagonistic functions (Martin and Brathwaite, 2013).

Characterization of microbial community structures has been proposed as an advanced approach to understanding the mechanisms governing these biocontrol functions. Previously, two methods, based on fatty-acid metabolism (McKellar *et al.*, 2003) and on T-RFLP molecular technology (Chen *et al.*, 2012), have been used to recognize seed-colonizing compost-derived microbial communities associated with the suppression of *Pythium* damping off on cotton and cucumber. Similarly, the functional method, based on the ability of a microbial community to metabolize a differential set of carbon sources that form the Biolog® panel, can also be very informative, and suitable for characterizing suppressive microbial communities in organic materials (Pane *et al.*, 2013).

The promising development of compost extracts place them among the most innovative organic source products existing in the field of crop disease management (Praveena Deepthi and Narayan Reddy, 2013). However, further insights are still necessary. Although ACTs have been widely demonstrated to suppress a range of foliar pathogens, their use to control soilborne diseases has received only limited attention. Several reviews have indicated the necessity for further studies on mechanisms, to promote the development of quality and efficacy of these tools for the control of soilborne pathogens (Hadar, 2011; Martin and Brathwaite, 2013).

The present study has focussed on the evaluation of the efficacy of seven ACTs for control of soilborne diseases in three pathosystems: pepper/*Sclerotium rolfsii*, savoy cabbage/*Rhizoctonia solani* and lettuce/*Sclerotinia minor*. Furthermore, microbial community physiological profiles were characterized in these ACTs, to elucidate the main underlying mechanism(s) leading to the observed plant disease suppression.

## Materials and methods

### Aerated compost teas

ACTs were produced through 7 d water fermentations of compost (1:5 v/v) in the forced air blower system previously described by Pane *et al.* (2012). The ACTs were identified as follows: ACT1, from a commercial at least 1-y-old biowaste compost, purchased at Gesenu (Perugia, Italy);
ACT2, from on-farm composted sweet corn and other horticultural residues;
ACT3, from on-farm composted artichoke residues;
ACT4, from composted cauliflower residues;
ACT5, from slightly composted solid residues of digestate from anaerobic digestion;
ACT6, from on-farm composted artichoke and fennel residues;
ACT7, from on-farm composted tomato and scarole residues.

At the end of the fermentation cycle, an aliquot of each ACT was stored at 4°C for 1 month, during which all experiments were completed.

### Quantification of bacterial populations

The abundance of culturable total, spore-forming and pseudomonad-like bacteria in ACTs, was estimated using a serial ten-fold dilution ($10^{-1}$ to $10^{-7}$) method. Total bacteria were counted on a selective medium (glucose 1 g $L^{-1}$, proteose peptone 3 g $L^{-1}$, yeast extract 1 g $L^{-1}$, $K_2PO_4$ 1 g $L^{-1}$, agar 15 g $L^{-1}$) amended with 100 mg $L^{-1}$ actidione (cycloheximide). Pseudomonad-like colonies were counted on a selective agar medium without iron, to which actidione was added (Scher and Baker, 1982). Spore-forming bacteria were counted by plating ten-fold dilutions of previously heated suspensions at 90°C for 10 min onto Nutrient Agar (Sadfi *et al.*, 2001).

### Characterization of microbial communities in ACTs

Bacterial community levels of physiological profiles (CLPPs) were assessed using the Biolog® GN2 and GP2 microplates™ system (Biolog Inc.). Aliquots (100 $\mu$L) of ACT diluted at $10^{-3}$ (dilution determined by a preliminary experiment) were inoculated into wells. The plates were incubated at 25°C for 4 d and colour development in each well was recorded as optical density at 590 nm, using the Bio-Rad Microplate Reader 550 (Biorad). Measures were carried

out in triplicate. Average well colour development (AWCD) and Shannon index (H') were determined as described by Gomez *et al.* (2006). Principal component analyses (PCA) were assessed by grouping standardized AWCD data, as described by Pane *et al.* (2013).

**Phytopathogenic fungi**

The fungal plant pathogens used in this study were *Sclerotium rolfsii* (pepper isolate from CRA-ORT collection), *Rhizoctonia solani* (RT10 strain from CRA-CAT collection), *Sclerotinia minor* (lettuce isolate from CRA-ORT collection), *Verticillium dahliae* (melongena isolate from CRA-ORT collection), *Botrytis cinerea* (ISPAVE169 strain from CRA-PAV collection) and *Fusarium oxysporum* f. sp. *lycopersici* (ATTC 16605 strain from CRA-SCS collection). Fungi were maintained on potato dextrose agar (PDA, Oxoid) and each isolate was preliminarily tested for pathogenicity on tested plants.

**Suppressive ACTs assays**

Raw, autoclaved (121°C for 22 min) and filtered (0.22 $\mu$m sterilized millipore membrane, followed by gentle centrifugation to precipitate suspended cells) ACTs, diluted 1:10 (v/v) in water, were used to evaluate whether their microbial components had suppressive effects. Evaluation of suppressiveness was carried out in plate culture assays using the methods of Bernal-Vicente *et al.,* (2008). Twenty mL of sterile PDA were used for each plate; four wells were then punched out using a 0.5 cm sterile cork borer on the edge of the plate at 5 cm from the center. Each of the well bottoms was sealed with two drops of sterile water agar. One hundred $\mu$L of different diluted ACTs were transferred into each well, while sterile water was placed in the wells of the control plates. One disc (0.5 cm) of mycelium of each fungus was inverted and centrally placed between the wells on PDA medium. All plates were incubated at 25°C, until the mycelium had reached the wells in water amended control plates. After incubation, the radius of the clear zone around each well was measured.

The pathogens *S. rolfsii*, *R. solani* and *S. minor* were artificially inoculated, respectively, onto pepper (ecotype Friariello Napoletano), savoy cabbage (cv. Romano) and lettuce (cv. Ballerina), to screen the ACTs for *in vivo* suppressive ability. Fungal inoculums

were prepared, according to a previous report (Pane *et al.,* 2011) as follows: common millet seeds were placed in 0.5 L capacity flasks, and saturated with a potato dextrose broth (PDB) solution (1/10 w/w), and autoclaved twice. Flasks were inoculated with fungi previously cultured on PDA for 15 d, and were then incubated for 21 d at 20°C. The resulting millet colonized by fungal mycelia was air-dried for 3 d, powdered in a mortar and mixed, at a concentration of 0.5% (w/w, dry weight), into a potting substrate of sterilized peat. In the experimental controls, non-inoculated common millet was added, prepared as described above. For each pathogen, the experimental design included seven ACTs applied in six replications, each consisting of a pot (20 cm diam.) where five 1-month-old nursery seedlings were planted and 100 mL of 1:10 water diluted ACT were distributed by drenching. The pots were then placed in a growth chamber (25°C) in a a completely randomized experimental design. Distribution of the pots in the chamber was rearranged randomly every 2 d to avoid effects of environmental heterogeneity. After 15 d, the number of symptomatic plants *per* pot was measured to calculate disease incidence as percentage of diseased plants, using the formula:

$$\text{Disease incidence \%} = \frac{\text{No. of infected plants } per \text{ pot}}{\text{Total No. of plants } per \text{ pot}} \times 100$$

The total fresh weight of plants *per* pot (g pot$^{-1}$) was also recorded to calculate the plant biomass yield, as percentage of fresh weight recovered from uninoculated pots, in accordance with the formula:

$$\text{Plant biomass yeld \%} = \frac{\text{g in infected pots}}{\text{g in healthy pots}} \times 100$$

The experiment was repeated twice.

**Statistical analyses**

Descriptive statistics were performed for all measured parameters. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Duncan's test at *P*<0.05. Relationships among ACT parameters and disease severity were assessed by regression analysis. Principal component analysis (PCA) was performed on OD

data of the 95 carbon sources (GN2 and GP2) at 96 h of incubation. The data were standardized by the average well colour development in each microplate to remove inoculum density effects (Garland 1997).

## Results

### Quantification of bacteria

Population densities of bacterial populations in the ACTs are shown in Table 1. Populations of total bacteria were greatest in ACTs 1, 3 and 7, while least in ACT4. Most culturable thermophilic bacteria were found for ACTs 1, 2, 6 and 7, while ACT5 had the smallest population of these bacteria. Pseudomonad-like populations followed a similar pattern to that of total bacteria, with the greatest populations being in ACT1 and ACT3 and the smallest in ACT4.

### Metabolic CLPPs of ACTs

In order to characterize microbial biodiversity in the ACTs, we used functional CLPPs based on utilization patterns of sole carbon sources from GN2 and GP2 panel set. These two bacterial plates produced dissimilar patterns of Shannon's Diversity Index, as well as AWCD, but they were equally able to distinguish between the different ACTs (Figure 1). The ACT3 and ACT1 communities showed the greatest

functional activity and diversity according to GP2 profiles. However, computed data from GN2 were more variable than those from GP2. Ordination bi-plots for PCA analysis of normalized absorbance values from GP2 and GN2 plates confirmed the observed differences in the relative separation power of both systems (Figure 2). In GP2 plates, data were ordered regarding the first variable (PC1), that accounted for 86%, and the second variable (PC2), that accounted for 13% of their total variance. ACTs 1 and 2 were individually separated from the remaining ACTs, which all closely clustered together. In the case of GP2, instead, three groups of ACT communities, ACTs 1, 4 and 5, ACTs 2 and 3, and ACTs 6 and 7, were each slightly clustered

**Table 1.** Culturable bacterial populations resident in seven different aerated compost teas (ACTs). Different letters within a column indicate significant differences (*P*≤0.05) according to Duncan's test.

| ACTs | Bacterial population (Log CFU mL$^{-1}$) | | |
| | Total | Thermophylic | Pseudomonas-like |
| --- | --- | --- | --- |
| 1 | 8.01 a | 4.86 a | 7.36 a |
| 2 | 6.30 ab | 5.00 a | 5.10 ab |
| 3 | 7.80 a | 4.75 ab | 7.52 a |
| 4 | 5.16 b | 4.40 ab | 4.40 b |
| 5 | 6.79 ab | 3.75 b | 6.42 ab |
| 6 | 6.96 ab | 5.20 a | 6.46 ab |
| 7 | 7.14 a | 5.56 a | 6.06 ab |
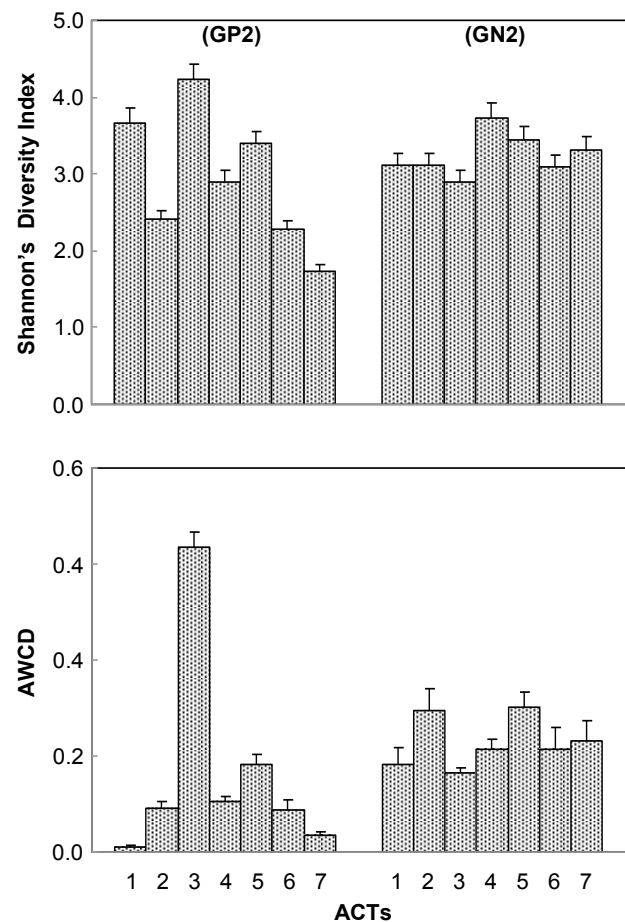


**Figure 1.** Shannon's Diversity Index (H') and Average Well Colour Development (AWCD) calculated for different aerated compost teas (ACTs) from GP2 and GN2 carbon substrate use profiles, measured after 120 h by standardized 590 nm OD.
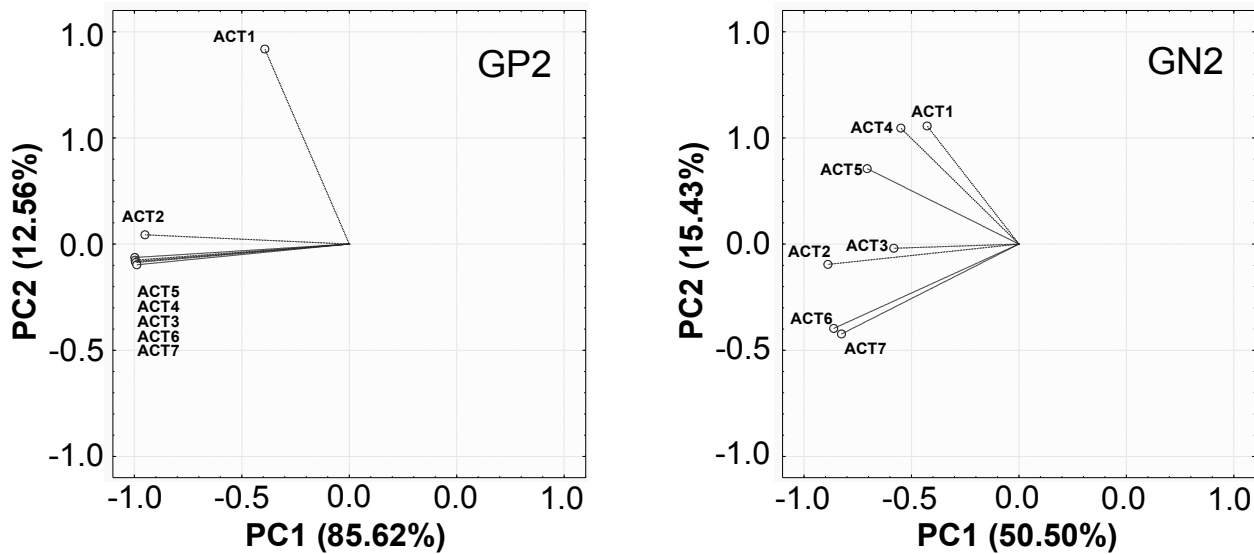
**Figure 2.** Ordination biplots of principal component analysess of GP2 and GN2 substrate utilization patterns, assessed by standardized average well colour development at 120 h, for microbial communities of seven aerated compost teas (ACTs).

both PC1 and PC2 axes, which explained 51% and 15% of the data variance, respectively.

### *In vitro* suppressiveness of ACTs

Well-diffusion Petri dish assays revealed the potential of the ACTs to suppress the mycelial de-velopment of the tested pathogens (Table 2). All ACTs, applied in these challenge experiments, in-hibited *in vitro* growth of *V. dahliae*, *F. oxysporum* f. sp. *lycopersici*, *R. solani*, *S. minor*, *S. rolfsii* and *B. cinerea*. In contrast, no suppressive activity was detected for any of the filter or thermal sterilized ACTs.

**Table 2.** *In vitro* suppression activity of aerated compost teas (ACTs) against *Botrytis cinerea* (BC), *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Rhizoctonia solani* (RS), *Sclerotinia minor* (SM), *Sclerotium rolfsii* (SR) and *Verticillium dahliae* (VD), indicated as mean radii of inhibition zones ± 1 SE, measured in well-cut diffusion plate assays. Different letters within a column indicate significant differences (*P*≤0.05) according to Duncan's test.

| ACTs | Inhibition zone (mm) | | | | | |
|------|------|------|------|------|------|------|
| | **BC** | **FOL** | **RS** | **SM** | **SR** | **VD** |
| 1 | 7.5±0.9  ab | 7.9±0.7  b | 5.5±0.5  bcd | 7.6±0.1  a | 3.9±0.1  b | 7.0±0.4  a |
| 2 | 9.5±0.3  a | 5.5±0.1  c | 7.1±0.4  b | 6.2±1.1  a | 4.4±0.2  ab | 5.5±0.1  bcd |
| 3 | 9.0±0.4  a | 9.7±0.1  a | 6.5±0.6  bc | 8.5±0.4  a | 4.5±0.2  ab | 7.5±0.2  a |
| 4 | 8.0±0.4  ab | 9.2±0.5  ab | 4.9±0.6  cd | 7.2±0.9  a | 5.1±0.5  a | 6.9±0.7  ab |
| 5 | 6.3±0.9  b | 9.3±0.5  ab | 9.2±0.7  a | 7.3±0.9  a | 4.3±0.5  ab | 5.4±0.8  cd |
| 6 | 9.3±0.5  a | 9.2±0.3  ab | 5.8±0.5  bcd | 6.0±0.7  a | 3.8±0.3  b | 6.5±0.3  abc |
| 7 | 6.7±0.5  b | 9.1±0.3  ab | 4.5±0.4  d | 6.5±0.8  a | 5.3±0.1  a | 4.9±0.3  d |

### *In vivo* suppressiveness of ACTs

Suppressive bioassays showed the capability of pot drenching treatments with ACTs to counteract the development of diseases caused by the set of soilborne pathogens assessed. Pot drenching with three of the ACTs significantly decreased *Rhizoctonia* disease incidence on savoy cabbage (Figure 3). In non-treated pots, disease incidence amount was approx. 90%; ACTs 4, 6 and 7 significantly reduced incidence by 39 to 44%. The other four ACTs were ineffective. No difference in plant biomass between treatments and infected control was found (Figure 3). Six of the ACTs, with the exception of ACT3, showed high and significant ability to control let-

tuce drop compared with the non-treated control, in which disease incidence amounted to apprx. 70%. Compared to this, treatments reduced *Sclerotinia*-infected plants by between 43 and 93%; ACT 2 was the most effective (Figure 4). In all treated pots, lettuce biomass has tripled, on average, compared to that of the infected control (Figure 4). All of the ACTs also suppressed *Sclerotium rolfsii* on pepper. In this case, ACT drenching decreased disease incidence from about 80% in the non-treated pots to about 40%, exhibiting a control efficacy of 50%, on average (Figure 5). Protective effects of ACTs allowed, on average, a twofold increase of pepper plant biomass compared to the untreated infected control (Figure 5).
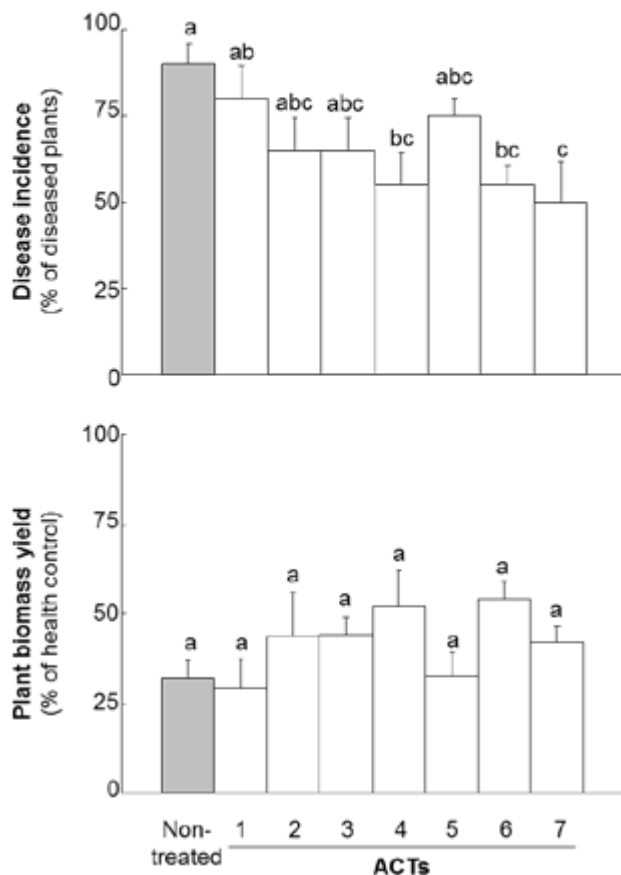


**Figure 3.** Mean disease incidence and mean plant biomass (±1 SE) for savoy cabbage plants drenched with different aerated compost teas (ACTs), growing in media inoculated with *Rhizoctonia solani*. Different letters indicate significant differences (*P*≤0.05) according to Duncan's test.
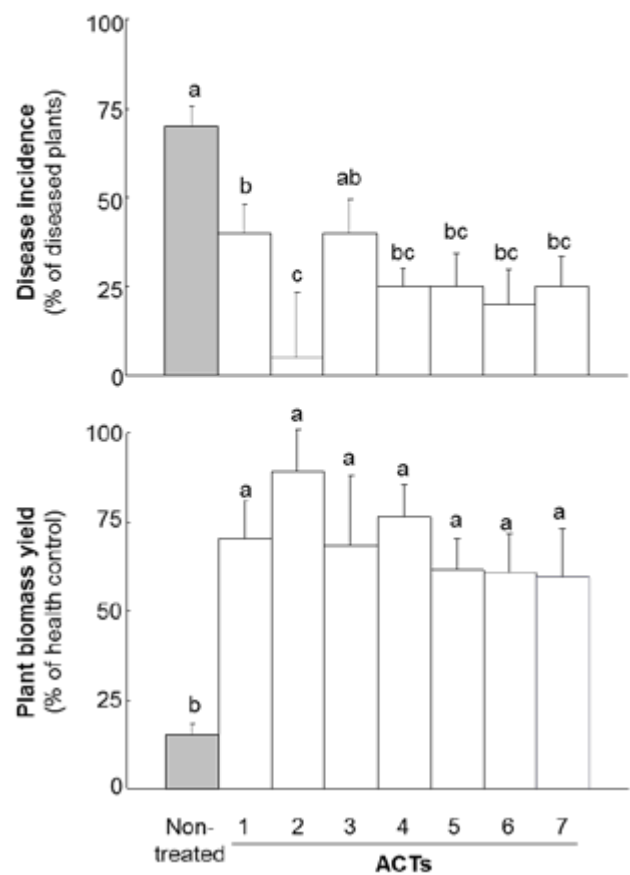


**Figure 4.** Mean lettuce drop incidence and mean plant biomass (±1 SE) for lettuce plants drenched with different aerated compost teas (ACTs), growing in media inoculated with *Sclerotinia minor*. Different letters indicate significant differences (*P*≤0.05) according to Duncan's test.
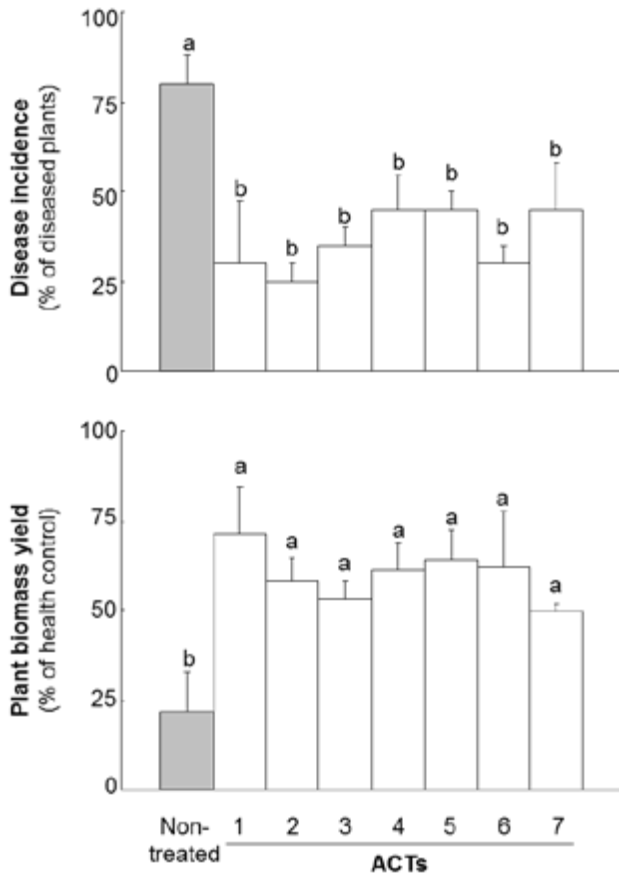
**Figure 5.** Mean disease incidence and mean plant biomass (±1 SE) for pepper plants drenched with different aerated compost teas (ACTs), growing in media inoculated with *Sclerotium rolfsii.* Different letters indicate significant differences (*P*≤0.05) according to Duncan's test.

## Discussion

ACTs can be suitable for beneficial soil drenching applications in order to control the development of the soilborne diseases examined in the research described here. ACTs significantly suppressed pepper southern blight and lettuce drop, and improved plant biomass compared to untreated infected controls. However, only three of the assayed ACTs significantly reduced the incidence of *Rhizoctonia* infected cabbage plants, emphasizing the severe problems that this disease can cause (Tredway and Burpee, 2001). Response to ACTs can be variable among different pathosystems; therefore, the consistency of the results obtained in the present study for a multiple assay of compost-mediated suppres-

siveness is highlighted (Termorshuizen *et al.*, 2007).

Sterilization of ACTs by autoclaving or microfiltration caused complete loss of their *in vitro* inhibitory effects. This indicated that the biotic component of ACTs played crucial roles in determining suppressiveness (Dionne *et al.*, 2012). In agreement with our study, ACT drenching has been previously reported to be more effective against *Pythium ultimum* causing damping-off on cucumber, due to the action of the resident microbial population (Scheuerell and Mahaffee, 2004). Gea *et al.* (2009) made the same observation in compost tea applications against the mushroom pathogen *Verticillium fungicola*. It has recently been reported that the potential uses of compost teas to manage soilborne pathogens, including *R. solani*, were accompanied by increased biomass production in tomato (Xu *et al.*, 2012) and spinach (Cummings *et al.*, 2009).

Because their suppressive properties are generally induced by uncharacterized living microbial communities, ACTs can be considered as biological control tools (Weltzien 1991). Different and complementary strategies could be implemented with microbes present in ACTs to combat pathogens, and reduce disease incidence and severity in many crops. For example, Diánez *et al.* (2013) assessed the presence of siderophores in various grape marc ACTs and their suppressive effects on nine pathogens. Sang *et al.* (2010) reported that induced systemic resistance was triggered in pepper plants by water extracts of compost, and suppressed *Phytophthora capsici* infection. However, antibiosis and hyperparasitism are the most commonly reported phenomena to explain the modes of action of suppressive ACTs (Martin and Brathwaite, 2012). In our study, crude ACTs exhibited *in vitro* antagonistic activity against a set of phytopathogenic fungi, with the formation of clear zones of inhibition between biome challengers in agar plates. This suggests the occurrence of antibiosis mechanisms similar to those reported for compost teas against *Alternaria solani*, *Botrytis cinerea* and *Phytophthora infestans* by Koné *et al.* (2010).

Antibiotic-mediated suppression (antibiosis) involves microbes that produce and secrete one or more compounds with detrimental activity for various plant pathogens (Hoitink and Fahy, 1986). Pane *et al.*, (2011), for example, recorded the presence of chitinolytic enzymes within compost, which were implied to control directly Rhizoctonia disease. Hardy and Sivasithamparam (1991) reported that the main mechanism by which non-sterile compost

extracts suppressed *Phytophthora* spp., was through lysis of sporangia.

In a complex environment, such as that of a compost tea, all microbial ecological services, including suppressive activities, are expressed by the whole community. Techniques based on the assessment of microbial diversity, which use combinations of DNA-based techniques (e.g. analysis of terminal restriction fragment length polymorphisms (T-RLFPs) (Michel *et al.,* 2002) and denaturing gradient gel electrophoresis (DGGE) (Calvo-Bado *et al.,* 2003), may lead to improved understanding of the changes in microbial communities associated with disease control from compost or compost tea applications to various media (Noble and Coventry 2005; Litterick and Wood 2009). Itoh *et al.* (2002), instead, used a functional approach based on the Biolog® method, to successfully discriminate disease suppressive and conducive growing media. The same metabolic profiling was used by Borrero *et al.,* (2006) to characterize functional microbial groups responsible for compost-based suppression of *Fusarium oxysporum* on tomato, and by Pane *et al.,* (2013) for suppression of *Rhizoctonia solani* and *Sclerotinia minor* on cress.

The microflora of ACTs has been described as being dominated by bacteria (Diánez *et al.,* 2007), with microbial diversity favoured by aeration (Ingham and Alms, 2003). The simple measurement of population density of culturable bacteria, however, was found to not discriminate suppressiveness (Palmer *et al.,* 2010; Pane *et al.,* 2012). Here, we used GN2 and GP2 Biolog® plates, referred to Gram-negative and Gram-positive bacteria, respectively (Preston-Mafham *et al.,* 2002), that can be promising for assessing suppressive ACT bacterial CLPPs (Classen *et al.,* 2003). The most representative of these bacterial groups, such as *Pseudomonas-* and *Bacillus*-like microbes, have been widely reported for their antagonistic properties (Tuitert *et al.,* 1998). In our study, the metabolic analyses of ACT microbial communities have shown only slight difference in metabolic patterns, essentially linked to Gram-negative profiles. However, metabolic fingerprints did not produce specific significant correlations with the suppressive potential of the ACTs, but rather revealed the untargeted antagonistic structures activated in the biocontrol interactions. These results are consistent with a general model of suppressiveness (Hadar 2011), since substantially equivalent levels of control efficacy were recorded among ACTs produced from different compost sources. General suppression involves the action of the totality of microbial agent in suppressing pathogens. The characterized microbial diversity could be crucial if it is connected to the antagonistic activity (Palmer *et al.,* 2010). In these cases, the antagonistic role of microbial population structure resulting from the fermentation processes can exceed the specific contribution of each different compost to suppressive properties of ACTs. Pane *et al.* (2012) found that microbial community structures of ACTs produced from different composts were affected, in terms of suppressiveness, by the specific compost extractant that was used in the fermentation phase.

## Acknowledgement

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