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"Gheorghe Asachi" Technical University of Iasi, Romania

EFFECT OF COMPOST AGAINST SOIL-BORNE PLANT PATHOGENS AND ITS IMPACT ON RHIZOSPHERE MICROBIOTA

Massimo Pugliese

University of Torino – Agroinnova. Grugliasco (TO), Italy
Email address: massimo.pugliese@unito.it

Abstract

Compost microbiota and microbial activity play a key role in suppressing soil-borne plant pathogens, starting from rhizosphere, The objective of the present work was to summarize results achieved evaluating compost efficacy against soil-borne pathogens such as *Phytophthora capsici* on courgette and *Fusarium oxysporum* on lettuce and tomato, and explain possible relationships among the targeted host/pathogen and the rhizosphere microbiota due to compost applications. Experimental trials were carried out on potted plants (by mixing compost into the potting substrate) and in two infested fields (by transplanting plants previously grown using potting substrate containing compost). Quantitative Polymerase Chain Reaction - qPCR and the next generation amplicon sequencing technologies were applied on rhizosphere samples. Compost suppressed the diseases by 50-70%, compared to the untreated controls. Moreover, a reduction of the abundance of the soil-borne pathogens up to 100 folds was observed in the soils where compost was applied. The abundance of beneficial microorganisms, such as *Bacillus* and *Trichoderma,* was also influenced and a 10-100 folds increase of it was observed in the rhizosphere of plants treated with compost. However, compost application did not affect the microbial diversity observed applying next generation amplicon sequencing. These findings suggest that compost can be used to reduce plant diseases caused by soil-borne pathogens, most probably improving the abundance of beneficial microorganisms and reducing that of pathogens, but not increasing rhizosphere microbial diversity.

Key words: crop protection, compost suppressiveness, microbiome, plant disease

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1. Introduction

Soil-borne pathogens can affect many plants worldwide, causing root and crown rots, pre- and post-emergence damping-off, vascular wilts and other diseases. Compost can be used to control these pathogens, suppressing plant diseases caused by soil-borne pathogens as well as inducing resistance towards them, as well documented by many authors (De Corato, 2020a; Hadar, 2011; Pugliese et al., 2015; Termorshuizen et al., 2006).

The mechanisms responsible for plant disease suppressiveness of composts are generally very complex, and both microbial activity and microbiota play a major role in the suppression of soil-borne plant pathogens, as Hoitink and Fahy (1986) suggested. Moreover, it has been demonstrated that microbial antagonists are present in suppressive composts and can be isolated from them (Chen et al., 1988; Boulter et al., 2002; De Corato, 2020a; Pugliese et al., 2008).

Many studies have focused on compost suppressiveness of soil-borne diseases caused by *Phytophthora* and *Pythium* species, linking it to the competition for carbon sources and directly to microbial populations and activities (Noble and Coventry, 2005).

In other cases, *Fusarium* wilts are enhanced by the application of composts with higher nitrogen or ammonium content (Hoitink et al., 2001). However, chemical, physical and biological composition can be very different among composts, and, consequently, the ability of compost to suppress soil-borne diseases affecting crops is changeable and often pathogen-specific (Termorshuizen et al., 2006).

Moreover, phytotoxicity problems due to chemical composition, like pH, E.C. and heavy metals, are very well know (Jimenez and Garcia, 1989) and can negatively influence compost applications, in particular for flowers, vegetable and ornamental crops in potting mixes, nurseries and greenhouses. Spermosphere- and rhizosphere-associated microbial communities influence disease development on different plant genotypes (Mazzola and Gu, 2002).

Several advancements based on use of plant disease-suppressive composts have been reached, including novel strategies for the production and application of on-farm compost as well as of compost-based tea (De Corato, 2020a; 2020b). However, a more detailed understanding of the changes occurring for the microbial communities after the application of compost still requires further researches.

The objective of the present work was to summarize results achieved evaluating compost efficacy against the soil-borne pathogens *Phytophthora capsici*, causing Phytophthora blight on courgette, and *Fusarium oxysporum*, causing Fusarium wilt on lettuce and tomato. Another objective was to explain possible relationships among the targeted host/pathogen and the rhizosphere microbiota due to compost application.

2. Material and methods

2.1. Experimental trials on potted plants

In a first set of trials, two different composts were used: (i) a green waste compost (GC), produced starting from green wastes (yard trimming and pruning) composted in a dynamic system for 6 months and sifted with a 10 mm sieve, (ii) the same green compost (GCT), bio-augmented with experimental "*Trichoderma* sp. TW2". The two composts were added at 0, 1, 10 and 20% v/v to a peat substrate (Tecno 2, made with 70% white peat and 30% clay, pH 5.5–6, N 110–190 mg/L, P_2O_5 140–230 mg/L, K_2O 170–280 mg/L, Turco Silvestro terricci, Bastia d'Albenga, SV, Italy) and used for sowing courgette seeds.

One week before sowing, each substrate mixture was infested with 2 g/l of fresh biomass of the soil-borne plant pathogen *Phytophthora capsici* grown in grain-hemp. The fungicide metalaxyl (Ridomil gold, 480 g/l, Syngenta Crop Protection) was applied at the same time as the inoculation and considered as the reference control. Seven days after the infestation with the pathogen, the seedlings were transplanted into pots and placed in a greenhouse on a potting bench.

Each treatment was replicated three times, considering three different pots per treatment and each pot containing 3 plants. A randomized experimental design was applied and the experiment was carried out twice independently. Disease incidence (DI) was evaluated by counting the number of diseased plants in each pot twice during the trials and calculating the percentage of diseased plants.

The fresh biomass of the plants was also weighed. The full description of materials and methods is described in Bellini et al. (2020).

2.2. Experimental trials on field crops

The experiments were carried out under field conditions in Northern Italy on a commercial farm in Moretta (44°45′49.75″N 7°32′29.18″E) as well as on an experimental farm in Carmagnola (44°88′55.188″N 7°68′37.457″E).

The field trials were carried out over two consecutive years, during 2016 and 2017, with the aim to evaluate the efficacy of pre-planting soil treatments against *P. capsici*, *Fusarium oxysporum* f. sp. *lactucae* and *Fusarium oxysporum* f. sp. *lycopersici* starting from the nursery. The two composts previously described were applied at 8 g/seedling at sowing and at 1kg/m^3 of soil before transplanting.

An untreated control was used, while chemical fungicides were applied as a reference chemical control by soil drenching before planting. Disease incidence and severity were scored monthly after transplanting by visually estimation. The full details of materials and methods are described in Cucu et al. (2019), Cucu et al. (2020a) and Cucu et al. (2020b).

2.3. Microbial community analysis

The influence of the treatments with compost on microbial communities was studied at the rhizosphere level by collecting samples from experimental sites at the end of each trials. DNA was extracted and quantitative Polymerase Chain Reaction (qPCR) and the next generation amplicon sequencing (NGS) technologies were applied to study the effects of compost on the rhizosphere microbiota.

The full details of materials and methods are described in Bellini et al. (2020), Cucu et al. (2019), Cucu et al. (2020a), Cucu et al. (2020b).

3. Results and discussion

3.1. Compost suppressiveness

Among the different combinations tested in pots, only the green compost enriched with *Trichoderma* (GCT) applied at 10% was able to significantly reduce the incidence of *Phytophthora capsici* on courgette (Fig. 1).

Regarding the field experimental trials, the results are summarized in Table 1. Composts suppressed the diseases by 50-70% compared to the untreated controls and in a way similar to the application of chemical fungicides.

3.2. Microbial diversity and composition

The results are summarized in Table 2. The abundance of the pathogens (*Phytophthora capsici*, *Fusarium oxysporum* ff. spp. *lactucae* and *lycopersici*) was always reduced (up to 100 folds) in the compost treated soils in comparison to the other soils. For example, *Phytophthora capsici* passed from an average of 7.24 Ypt gene log copy g/dw^{-1} in untreated control to 5.37 in plants treated with compost (Cucu et al., 2020b).

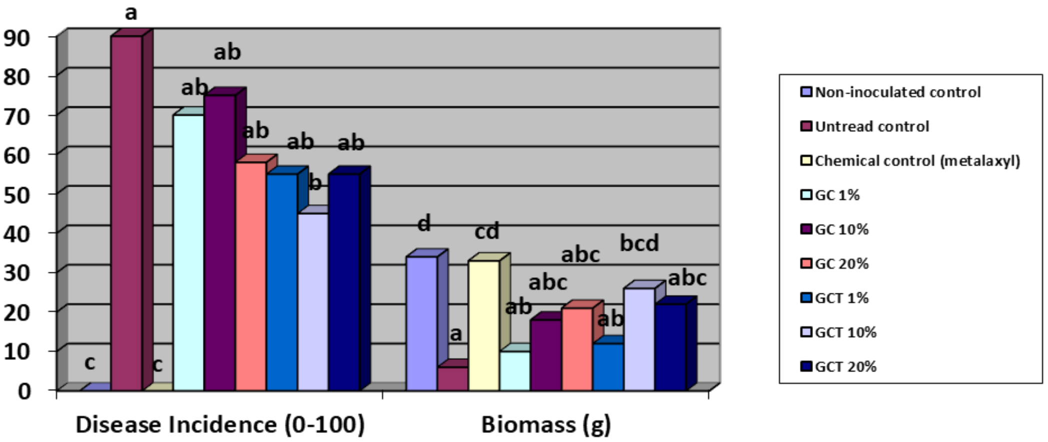

Fig. 1. Efficacy of the compost mixtures to control the soil-borne pathogen *Phytophthora capsici* on courgette plants expressed as disease incidence (%) and fresh biomass (g). The letters refer to the Tukey's post hoc test, which was performed after one-way ANOVA ($P < 0.05$) (Adapted from Bellini et al., 2020)

Table 1. Effect of different compost treatments against *Phytophthora capsici* of courgette, Fusarium wilt of lettuce and tomato in field conditions in 2016 and 2017. Different letters above the number values indicate significant differences between treatments (adapted from Cucu et al., 2019; Cucu et al., 2020a; Cucu et al., 2020b).

Treatment	***Fusarium wilt of tomato***		***Fusarium wilt of lettuce***	***Phytophthora rot on courgette***	
	Disease severity % (2016)	***Disease severity % (2017)***	***Disease severity % (2016-2017)***	***Disease severity % (2016)***	***Disease severity % (2017)***
Green compost (GC)	1.9^{a}	14.5^{a}	30.5^{ab}	17.9^{a}	40.0^{ab}
Green compost Trichoderma (GCT)	10.4^{a}	21.9^{a}	19.2^{a}	16.7^{a}	43.3^{ab}
Untreated control	29.7^{b}	39.9^{b}	61.7^{c}	40.4^{b}	78.3^{c}
Chemical control	Not tested		22.3^{ab} (azoxystrobin)	8.8^{a} (metalaxyl)	34.4^{a} (metalaxyl)

Table 2. Effect of different compost treatments on composition and microbial diversity in field conditions in 2016 and 2017, expressed as significantly improved/reduced in rhizosphere compared to the untreated control (Adapted from Cucu et al., 2019; Cucu et al., 2020a; Cucu et al., 2020b)

Treatment	***Fusarium wilt of tomato***				***Fusarium wilt of lettuce***				***Phytophthora rot on courgette***		
	Pathogen abundance	***Bacterial, archeal and fungal abundance***	***Antagonists abundance (Bacillus, Trichoderma)***	***Functional genes abundance (chiA)***	***Pathogen abundance***	***Bacterial, archeal and fungal abundance***	***Antagonists abundance (Bacillus, Pseudomonas, Trichoderma)***	***Functional genes abundance (chiA, phlD, hcnAB)***	***Pathogen abundance***	***Antagonists abundance (Bacillus, Pseudomonas, Trichoderma)***	***Functional genes abundance (chiA)***
Green compost (GC)	Significantly reduced	Significantly improved (archeal); no variations (bacterial); significantly reduced (fungal)	Significantly improved (*Bacillus*, *Trichoderma*)	Significantly improved	Significantly reduced	Significantly improved (bacterial); no variations (archeal, fungal)	Significantly improved (*Bacillus*, *Pseudomonas*, *Trichoderma*)	Significantly improved (chiA, phlD, hcnAB)	Significantly reduced	Significantly improved (Bacillus and Trichoderma)	Significantly improved
Green compost *Trichoderma* (GCT)	Significantly reduced	Significantly improved (bacterial, archeal); no variations (fungal)	Significantly improved (*Bacillus*, *Trichoderma*)	Significantly improved	Significantly reduced	Significantly improved (bacterial, fungal); significantly reduced (archeal)	Significantly improved (*Bacillus*, *Pseudomonas*, *Trichoderma*)	Significantly improved (chiA, phlD, hcnAB)	Significantly reduced	Significantly improved (Bacillus and Trichoderma)	Significantly improved

Furthermore, the abundance of beneficial microorganisms such as *Trichoderma* and *Bacillus* and of functional genes was also increased (up to 10-100 folds) in the rhizosphere of plants treated with compost compared to the untreated plants. For example, in the case of *Phytophthora capsici/*courgette trial, *Trichoderma* passed from an average of 4.94 ITS log copy g/dw^{-1} in untreated control to 7.05 in plants treated with compost, and *Bacillus* passed from an average of 5.23 16S rRNA gene log copy g/dw^{-1} in untreated plants to 6.07 when compost was applied (Cucu et al., 2020b).

The effects of compost on total bacterial, archaeal and fungal populations varied in different ways for each specific target crop and disease. Regarding next generation amplicon sequencing, differences were observed only when comparing soils from different sites, while compost application did not affect the microbial diversity (Cucu et al., 2020 b).

4. Conclusions

The use of two different composts has been effective in reducing *P. capsici*, *F. oxysporum* f. sp. *lactucae* and f. sp. *lycopersici* abundance and disease severity at two experimental sites in naturally and artificially infested soils.

The results have shown that, in general, the compost-based treatments resulted in an enhancement of the resident *Trichoderma* spp. and *Bacillus* spp. communities from the rhizosphere, which provide beneficial effects on plants and are also responsible in the reduction of the diseases. However, compost application did not affect the microbial diversity according to next generation amplicon sequencing.

The differences in the total microbial community in the rhizosphere highlighted that compost treatments is safe for the rhizosphere non-target microbial communities as well as for the effective control of the soil-borne pathogens.

This summarized overview suggest that compost can be used to reduce plant diseases caused by soil-borne pathogens, most probably modifying the rhizosphere microbiota and microbial activity and in particular by improving the abundance of beneficial microorganisms and reducing that of pathogens, but not by increasing rhizosphere microbial diversity.

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