Journal of Plant Diseases and Protection, **115** (5), 220–228, 2008, ISSN 1861-3829.

Selection of antagonists from compost to control soil-borne pathogens

Selektion von Antagonisten aus Kompost zur Kontrolle bodenbürtiger Pathogene

M. Pugliese*, B.P. Liu, M.L. Gullino & A. Garibaldi
Centre of Competence for the Innovation in the Agro-Environmental field (AGROINNOVA), University of Torino, via Leonardo da Vinci, 44, IT-10095 Grugliasco (TO), Italy
* Corresponding author, e-mail massimo.pugliese@unito.it

Received 5 February 2008; accepted 29 May 2008

Summary

One-hundred and one microorganisms have been selected and tested for their antagonistic activity towards soil-borne plant pathogens from a compost originated from urban organic and yard wastes. Among them, twenty eight microorganisms, tested under laboratory conditions on tomato seedlings growing on perlite medium in Petri plates, controlled tomato wilt caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. In a second round of trials, they were assessed under greenhouse condition on three pathosystems: *Fusarium oxysporum* f.sp. *basilici* on basil, *Phytophthora nicotianae* on tomato and *Rhizoctonia solani* on bean. The *Fusarium* strain K5 showed a disease control of 69% and an increase in biomass production of basil of 32% compared to inoculated control. In the case of tomato/*P. nicotianae*, the bacteria strain B17 showed a disease control of 82% and an increase of 216% of biomass production of tomato. Two microorganisms, E19 and P11 controlled root and stem rot caused by *R. solani* on bean and increased the biomass of bean up to 163%. None of the microorganisms was able to control all the soil-borne pathogens. Three *Fusarium* (K7, K9 and K11) and two *Trichoderma* (E28 and E36) isolates showed the best results and were tested in a third round of trials mixed together and at different dosages. Two *Fusarium* isolates, K7 and K9, were able to control *F. oxysporum* f. sp. *basilici* and confirmed they could be used in the future as commercial antagonists.

Key words: biological control, *Fusarium oxysporum* f. sp. *basilici*, *Fusarium oxysporum* f. sp. *racidis-lycopersici*, *Rhizoctonia solani*, *Phytophthora nicotianae*, *Trichoderma* sp.

Zusammenfassung

Einhundertundeins verschiedene Mikroorganismen wurden aus einem Kompost isoliert, der aus Haushalts- und Gartenabfällen stammte. Die Isolate wurden hinsichtlich ihrer antagonistischen Wirkung gegenüber bodenbürtigen Pflanzenpathogenen untersucht. Einundzwanzig dieser Isolate wirkten gegenüber der durch *Fusarium oxysporum* f. sp. *radicis-lycopersici* verursachten Tomatenwelke an Tomatensämlingen, die im Labor in Petrischalen auf Perlit-Medium angezogen wurden. Diese Isolate wurden dann in einer zweiten Versuchsrunde im Gewächshaus an den Pathosystemen *Fusarium oxysporum* f. sp. *basilici* an Basilikum, *Phytophthora nicotianae* an Tomaten und *Rhizoctonia solani* an Bohnen getestet. Der *Fusarium*-Stamm K5 reduzierte die Anzahl abgetöteter Basilikumpflanzen um 69% und erhöhte die Biomasse um 32% im Vergleich zur nicht mit diesem Stamm behandelten, mit *F. oxysporum* f. sp. *basilici* inokulierten Kontrolle. Der Bakterienstamm B17 verminderte die Zahl durch *P. nicotianae* abgetöteten Tomatenpflanzen um 82% und erhöhte die Biomasse der Pflanzen um 216%. Die Mikroorganismen E19 und P11 verminderten die Anzahl der durch *R. solani*, den Verursacher der Wurzel- und Stängelfäule der Bohne abgetöteten Pflanzen um 49 bzw. 42%; gleichzeitig wurde die Biomasse der Bohnenpflanzen um bis zu 163% erhöht. Keiner dieser Mikroorganismen wirkte gegenüber allen untersuchten bodenbürtigen Pathogenen antagonistisch. Drei *Fusarium*-(K7, K9 und K11) und zwei *Trichoderma*-Isolate (E28 und E36) zeigten die beste Wirkung und wurden in einer dritten Versuchsrunde kombiniert in verschiedenen Dosierungen getestet. Die beiden *Fusarium*-Isolate K7 und K9 waren in der Lage, *F. oxysporum* f. sp. *basilici* zu kontrollieren und besitzen daher das Potenzial, zukünftig kommerziell im Basilikumanbau eingesetzt zu werden.

Stichwörter: biologische Kontrolle, *Fusarium oxysporum* f. sp. *basilici*, *Fusarium oxysporum* f. sp. *racidis-lycopersici*, *Rhizoctonia solani*, *Phytophthora nicotianae*, *Trichoderma* sp.

1 Introduction

Soil-borne diseases are the cause of severe losses of economically important crops. Chemical pesticides have been widely used for several decades to control soil-borne pathogens, but in recent years, prohibitions or severe restrictions to many commonly used pesticides and fumigants, methyl bromide among others, were taken for health and environmental concerns (Gullino et al. 2005). The development of effective methods to control soil-borne pathogens is a slow and mid-long term goal that needs many research activities. These approaches include solarization, biofumigation, biological soil disinfestation, grafting, use of organic amendments, application of biocontrol agents, crop rotation and, last but not least, use of composts (Katan 2005).

Composts attract research interests for their contribution both to recycling waste and reducing usage of non-renewable resources such as peat (Termorshuizen et al. 2006). Composts are usually free of plant and human pathogens and weed seeds, and without odor (Ryckeboer 2002). Suppression of soil-borne plant diseases with composts has been widely studied and was recently summarized by Noble and Coventry (2005). Heat treatment of composts generally results in a loss in disease suppressiveness, indicating that the mechanism is often or predominantly due to biological causes. Fortifying composts with beneficial microorganisms is one possible factor that can help increasing the efficacy and reliability of disease control (De Clercq et al. 2004).

In Piedmont (northern Italy) different composts were tested for their suppressiveness towards soil-borne pathogens. Among them, one compost originating from urban organic and yard wastes showed a good suppressive activity (Pugliese et al. 2007).

The objective of the present work was to isolate microorganisms from that compost and to test them for their activity against soil-borne pathogens: *Fusarium oxysporum* f. sp. *racidis-lycopersici*, *Fusarium oxysporum* f. sp. *basilici*, *Rhizoctonia solani* and *Phytophthora nicotianae*.

2 Materials and methods

2.1 Compost description

A compost prepared from green wastes, organic domestic wastes and urban sludges by ACEA Pinerolese SpA (Pinerolo, Torino, Italy), that showed a good suppressive activity in previous trials (Pugliese et al. 2007) was used as source of microorganisms. Chemical-physical analysis of the compost was performed at the soil analysis laboratory of the composting plant. Physical and chemical properties of the compost and peat amendment are summarized in Table 1.

2.2 Microbial density

Bacteria and fungi were estimated using a standard dilution-plating procedure. Five samples of about 200 g were collected from three "big bags" (500 l) of compost, coded as "A" "B" "C". From each sample, 0.5 g of compost were diluted into bottles containing 50 ml of sterile deionized water. One drop of polisorbate 20 (Tween 20, Croda International Plc, Snaith, Goole, United Kingdom) was added to each bottle and, after shaking for 20 min at room temperature, serial dilutions were prepared into tubes. Five different media were used for plating 1000 µl aliquots of serial diluted suspensions on Petri plates (9 cm diameter). A suspension in the range of 10^{-3} to 10^{-5} was plated on media selective for *Fusarium* (Komada 1975) and for *Trichoderma* (Elad et al. 1981). A suspension in the range of 10^{-2} to 10^{-4} was plated on a medium selective for oomycetes (Masago et al. 1977). A suspension in the range of 10^{-4} to 10^{-6} was plated on potato dextrose agar (PDA; 39 g PDA in 1 l of deionized water, autoclaved at 120°C for 15 min) for isolation of fungi, and a suspension in the range of 10^{-6} to 10^{-8} was plated on lysogeny broth LB (Bertani 1951) for isolation of bacteria. Five plates of each medium were inoculated for each dilution. Plates were incubated at room temperature (20–25°C) for 4-5 days before counting the colony forming unit (CFU).

2.3 Isolation of microorganisms

On the basis of their morphology, different single colonies were isolated from the plates after 4–5 d of incubation and divided in two groups according to their capacity to grow at temperatures higher than 38°C (being potentially harmful to human beings) or not. All microorganisms were given a code by letters and numbers. Letters represent a group and numbers represent the progressive order. Microorganisms were divided in five groups: "K" for isolates from Komada, "E" for isolates from Elad, "OO" for oomycetes from Masago, "P" for fungi from PDA, "B" for bacteria from LB. Among all isolates, those not growing at 38°C were selected to be tested on the first round of trials against *F. oxysporum* f.sp. *radicis-lycopersici* on tomato.

Table 1: Physical and chemical properties of the compost and the peat amendment before introduction of antagonists

Type of substrate	C/N	C-total (g kg^{-1})	N-total (g kg^{-1})	Organic matter (%)	EC (mS cm^{-1})	pH
Compost	16.1	281	19	54.6	1.4	8.5
Peat	23.8	190	8	33.0	0.3	6.2

2.4 Test of microorganisms under laboratory conditions

Plates (15 cm diameter) were filled with 28–30 g of perlite (previously sterilized at 120°C for 15 min) and prepared for the trials with the pathosystem *F. oxysporum* f. sp. *radicis-lycopersici*/tomato. Ten ml of sterilized water were sprayed on the surface of the perlite. Three ml conidia suspension of each microorganism (1×10^7 CFU ml^{-1}) and of *F. oxysporum* f. sp. *radicis-lycopersici* (3×10^5 CFU ml^{-1}) were sprayed on the surface of the perlite. Three control treatments were also prepared: water only, pathogen only, and a commercial formulation of *Streptomyces griseoviridis* strain K61 (MYCOSTOP, Isagro Ricerca srl, Novara, Italy). Three plates for each treatment were prepared and the test was repeated once. The plates were stored in a dark chamber at 20 ± 2°C, covered with a black plastic bag. Seven days later, 20 tomato (cv Cuor di bue) seeds were sown on the surface of the perlite in each plate, and 5 ml of sterilized deionized water were sprayed on the surface of the seeds. Plates were mantained in a growth chamber at 18 ± 2°C, 90 ± 5% RH and light 12 h/d. After 10 d, the germinated seeds were counted and 20 d later also the healthy tomato seedings were counted.

2.5 Evaluation of antagonistic activity of selected microorganisms against soil-borne pathogens under greenhouse conditions

Twenty-eight microorganisms that were effective with the pathosystem *F. oxysporum* f. sp. *radicis-lycopersici*/tomato under laboratory conditions were selected to be tested on three pathosystems under greenhouse conditions: *F. oxysporum* f.sp. *basilici*/basil, *P. nicotianae*/tomato and *R. solani*/bean.

One oomycete (OO5) was propagated in flasks (1000 ml) on wheat plus hemp (200 g of wheat kernels and 100 g of hemp kernels in 320 ml deionized water, autoclaved at 120°C for 30 min). Nineteen fungi were propagated on wheat (300 g of wheat kernels in 320 ml deionized water, autoclaved at 120°C for 30 min). All fungi and oomycete strains were incubated for at least 14 days at room temperature (20–25°C). The growth curves at 0, 6, 24, 30, 48, 54, 72 h of the 8 bacteria were compared with the optical density at 592 nm in LB liquid medium in order to assess the CFU concentrations. Bacteria were grown on a rotary shaker for 48 h in flask (250 ml) containing 70 ml of sterilized LB medium. A final bacterial suspension of 5×10^8 CFU ml^{-1} was prepared by diluting with deionized water.

P. nicotianae was propagated in flasks on wheat plus hemp (200 g of wheat kernels and 100 g of hemp kernels in 320 ml of deionized water, autoclaved at 120°C for 30 min), while *R. solani* was propagated in flasks on wheat (300 g of wheat kernels in 320 ml of deionized water, autoclaved at 120°C for 30 min). *F. oxysporum* was incubated on casein liquid medium for 10–15 days on a rotary shaker, then centrifuged and mixed with talc to produce chlamydospores (Locke and Colhoun 1974).

Fungi and oomycetes were added to peat after steam-disinfection at 120°C for 20 minutes at 10 g l^{-1} of inoculum, while bacteria were added at 5×10^9 CFU l^{-1}. Inoculated peat was stored for 7 d at room temperature.

In the case of basil, after 7 d, a conidial suspension of *Fusarium oxysporum* f. sp. *basilici* at 5×10^4 CFU g^{-1} concentration was added to the substrates, five pots of 1 l volume (10 × 10 × 12 mm) were filled with the mix and 50 basil seeds were sown in each pot. The pots were layed on a bench in the greenhouse with a randomized experimental block design. Germinated plants were counted 10 days after sowing, and diseased plants were counted every 7 days. Thirty-forty days from sowing, healthy plants were counted and above-ground biomass was weighed.

In the tomato trials, *P. nicotianae* was added to peat at a concentration of 2 g l^{-1} after 7 d, the five pots of 1 l volume

each (10 × 10 × 12 mm) were prepared and 10 tomato seeds were sown in each pot. The pots were put on a bench in the greenhouse with randomized experimental block design. Fourteen days after sowing, the healthy and dead plants were counted and the above-ground biomass of the healthy ones was weighed.

In the bean trials, *R. solani* was mixed to peat at a concentration of 1 g l^{-1} of inoculum 7 days after microorganisms inoculation, then five pots of 3 l volume each (14 × 14 × 15 mm) were prepared and eight bean seeds/pot were sown. The pots were layed on a bench in the greenhouse with randomized experimental block design. Fourteen days after sowing, disease severity and above-ground biomass were assessed.

Temperatures were mantained at 25–28°C and no fertilizers were used. All trials were repeated once.

2.6 Evaluation of antagonistic activity of the most efficient microorganisms mixed together under greenhouse conditions

Five microorganisms (K7, K9, K11, E28 and E36) that showed to control soil-borne pathogens in one or more pathosystem were selected for a third round of trials against *R. solani* on bean and *F. oxysporum* f.sp. *basilici* on basil. The microorganisms were added to peat substrate alone at 5 g l^{-1} dosage of fungi biomass, mixed all together at 1 g l^{-1} dosage each and then stored at room temperature. A mix of K9 and E36 at 2.5 g l^{-1} dosage each of fungi biomass was also added to peat. Seven days later, in the case of basil, a conidial suspension of *F. oxysporum* f. sp. *basilici* at 1 × 10^4 CFU g^{-1} concentration was added to peat, five pots of 1 l volume (10 × 10 × 12 mm) were filled with the substrate and 50 basil seeds were sown in each pot. Total germination was counted 10 d after sowing, then diseased plants were counted every 7 d. The pots were disposed on a bench in an iron-glass greenhouse with a randomized experimental block desing. Temperatures were maintained at 25–28°C and no fertilizers were added to the substrate. Thirty-forty days from sowing, healthy plants were counted and weighed. In the bean trials, inoculum of *R. solani* was added to peat at a concentration of 1 g l^{-1}, then five pots of 3 l volume each (14 × 14 × 15 mm) were prepared and eight seeds were sown. Fourteen days after sowing, disease severity and biomass were assessed. All trials were repeated once.

2.7 Statistical analysis

Analysis of variance was carried out with the statistical programme SPSS 12.1. After ANOVA, Tukey's "Honestly Significantly Different" was used as post-hoc analysis, with a significance defined at the $P < 0.05$ level unless stated otherwise.

3 Results

3.1 Isolation and selection of microorganisms

Microbial densities in the compost, as expected, showed a higher concentration of bacteria (5.6 × 10^8 CFU g^{-1}), while total fungi concentration was 1.3 × 10^6 CFU g^{-1}, *Fusarium* concentration was 3.5 × 10^4 CFU g^{-1}, *Trichoderma* concentration was 1.1 × 10^5 CFU g^{-1} and Oomycetes concentration was 6.7 × 10^3 CFU g^{-1}.

In total, 168 colonies were isolated. They are from K1 to K46, from E1 to E40, from OO1 to OO6, from P1 to P27, from B1 to B29, from BF1 to BF20. Among them, 101 microorganisms (60%) did not grow at 38°C. Bacteria were the biggest group among those able to grow at T > 38°C (Table 2).

3.2 Test of microorganisms under laboratory conditions

Among the 101 microorganisms tested for their efficacy to control *F. oxysporum* f. sp. *radicis-lycopersici*, 28 showed a significant disease reduction, in several cases also higher than that provided by commercial formulation of *S. griseoviridis* (Table 3). K5, K7 and E7 showed the best results, with a disease reduction of, respectively, 87, 67 and 66% compared to inoculated control, while *S. griseoviridis* provided 24% efficacy (Table 3).

3.3 Test of microorganisms against three pathosystems under greenhouse condition

Among the 28 microorganisms tested, K5, K7, K34 and K44 showed the best results both in terms of disease control and biomass produced in the case of the pathosystem *F. oxysporum* f. sp. *basilici*/basil. K5 showed a disease control of 69%, and an increase in biomass of 32% compared to peat inoculated control. Nine microorganisms, in particular E28, E36, K11, P11 and BF10, showed an increase in disease severity and, consequently, a decrease in biomass production compared to the inoculated control (Table 4).

Among the 28 microorganisms tested, E12, E15, E 36, B3, B13, B15, B17, B23, B24 and B29 controlled *P. nicotianae* on tomato. Among them B3, B13, B15, B17, B23, B24 and B29 were also able to increase the above-ground biomass of tomato (Table 5).

The microorganisms E19 and P11 showed the best results against *R. solani* on bean. They were able to control the pathogen at, respectively, 49 and 42%, and they increased the biomass of bean up to 163% compared to inoculated control. The microorganism E18 showed a slight increase in disease incidence with consequently a decrease in biomass, but not statistically significant (Table 6).

Table 2: Microorganisms isolated from compost on different media

Culture media	LB (bacteria)	Komada (*Fusarium*)	Elad (*Trichoderma*)	Masago (oomycetes)	PDA (fungi)
Termophilic (T > 38°C)	B1, 2, 7, 8, 10, 11, 20, 21, 22, 25, 27; BF1, 2, 3, 4, 6, 7, 9, 12, 13, 14, 15, 16, 17, 18, 19	K2, 4, 13, 17, 18, 20, 21, 23, 24, 26, 31, 33, 37, 38, 40, 41, 42, 43, 46	E1, 3, 6, 8, 9, 16, 17, 22, 31, 37	(none)	P2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 19, 21, 22, 25
Mesophilic (T < 38°C)	B3, 4, 5, 6, 9, 12, 13, 14, 15, 16, 17, 18, 19, 23, 24, 26, 28, 29; BF5, 8, 10, 11, 20	K1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 19, 22, 25, 27, 28, 29, 30, 32, 34, 35, 36, 39, 44, 45	E2, 4, 5, 7, 10, 11, 12, 13, 14, 15, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 38, 39, 40	OO1, 2, 3, 4, 5, 6	P1, 8, 11, 14, 15, 16, 17, 18, 20, 23, 24, 26, 27

Table 3: Evaluation of the control of *Fusarium oxysporum* f. sp. *radicis-lycopersici* by microorganisms selected from compost under laboratory condition

Microorganism		N° of alive tomato seedlings	% disease control
Fusarium	K5	16.7 ab*	87
	K6	11.8 abcde	62
	K7	12.8 abc	67
	K9	12.2 abcd	64
	K11	6.5 cdefghij	34
	K34	6.2 cdefghij	32
	K44	11.2 bcdef	58
	K46	8.0 cdefghi	42
Trichoderma	E7	12.7 abc	66
	E12	7.7 cdefghij	40
	E14	7.5 cdefghij	39
	E15	8.3 cdefghi	44
	E18	6.5 cdefghij	34
	E19	3.5 fghij	18
	E23	11.0 bcdefg	57
	E28	3.7 fghij	19
	E36	5.7 cdefghij	30
Fungi	P11	6.3 cdefghij	33
Oomycetes	OO5	10.2 bcdefgh	53
Bacteria	BF10	7.5 cdefghij	39
	B3	3.3 ghij	17
	B5	3.2 hij	17
	B13	4.3 efghij	23
	B15	1.7 ij	9
	B17	3.0 hij	16
	B23	3.5 fghij	18
	B24	5.3 cdefghij	28
	B29	2.3 ij	12
Water control		19.2 a	100
Inoculated control		0.0 j	0
S. griseoviridis		4.7 defghij	24

* Tukey's HSD test ($P < 0.05$).

3.4 *Evaluation of the efficacy of the best five microorganisms in two pathosystems:* Rhizoctonia solani*/bean and* Fusarium oxysporum *f. sp.* basilici*/basil.*

The tested microorganisms did not reduce the biomass of basil when peat was not inoculated with *F. oxysporum* f.sp. *basilici*, and the biomass was higher than control when a mix of five microorganisms was added to peat. When the pathogen was added, only E36 showed an increase in disease incidence but there were no differences on biomass. K9 and K7 showed the best results both in terms of disease control and in biomass production, with a percentage of biomass, respectively, of 98 and 83% compared to inoculated control (Table 7). In the pathosystem *R. solani/*bean, results showed no statistical differences when the microorganisms were added to peat with or without pathogen inoculation, compared to control (Table 8).

4 Discussion

According to Hoitink and Fahy (1986), most microorganisms that lead to compost suppressive activity generally recolonize the compost pile from the outer low-temperature layer after the second composting phase, known as peak heating, in which compost temperature can reach 60–75°C and most pathogens and beneficial microorganisms are killed. For this reason, most of the microbial communities in compost may come from the original composting substrate environment.

Results showed that 28 microorganims selected from compost made from green wastes, organic domestic wastes and urban sludges were able to control *F. oxysporum* f. sp. *radicis-lycopersici* under laboratory condition and that 23 of them were able to reduce disease incidence of at least one pathogen in greenhouse trials (Table 9).

Table 4: Activity of microorganisms isolated from compost against *F. oxysporum* f. sp. *basilici* on basil, showed as percentage of dead plants and biomass

Treatment	Microorganism	Substrate	No. of dead plants	% disease control	Biomass (g)	% biomass relative to peat inoculated control
1	K5	Peat	5.4 abc*	69	32.0 abc*	132
2	K6	Peat	7.7 abcdef	56	29.5 abcd	121
3	K7	Peat	6.2 abcd	64	28.1 abcde	116
4	K9	Peat	9.0 abcdefg	48	26.3 abcdef	108
5	K11	Peat	27.6 lm	−58	5.8 ij	24
6	K34	Peat	6.8 abcde	61	22.8 bcdefg	94
7	K44	Peat	7.0 abcde	60	30.4 abcd	125
8	K46	Peat	12.7 cdefgh	27	16.1 efghij	66
9	E7	Peat	12.6 cdefgh	28	22.4 bcdefgh	92
10	E12	Peat	19.6 hijkl	−12	16.6 efghij	68
11	E14	Peat	19.3 hijkl	−11	15.7 efghij	65
12	E15	Peat	19.3 hijkl	−11	18.0 defghi	74
13	E18	Peat	14.0 cdefgh	20	20.1 cdefgh	83
14	E19	Peat	15.8 defghij	10	9.7 hij	40
15	E23	Peat	18.4 ghijkl	−6	14.7 fghij	60
16	E28	Peat	29.4 m	−69	6.0 ij	25
17	E36	Peat	25.0 jklm	−43	10.8 ghij	44
18	P11	Peat	23.9 ijklm	−37	5.5 ij	23
19	OO5	Peat	8.2 abcdef	53	33.8 ab	139
20	BF10	Peat	26.9 klm	−54	4.9 j	20
21	B3	Peat	14.7 cdefghi	16	22.5 bcdefg	93
22	B5	Peat	16.1 efghij	8	27.1 abcdef	112
23	B13	Peat	13.9 cdefgh	20	25.8 abcdef	106
24	B15	Peat	17.4 fghijk	0	23.3 bcdefg	96
25	B17	Peat	15.8 defghij	10	25.8 abcdef	106
26	B23	Peat	14.9 cdefghi	15	22.8 bcdefg	94
27	B24	Peat	11.0 bcdefgh	37	27.0 abcdef	111
28	B29	Peat	15.9 defghij	9	23.4 bcdefg	96
29	–	Peat inoculated control	17.4 fghijk	0	24.3 bcdef	100
30	–	Peat control	0.0 a	100	37.9 a	156
31	–	Compost inoculated control	1.8 ab	90	16	66
32	–	Compost control	0.0 a	100	31.4	129

* See Table 3.

None of the tested microorganisms was able to control each of the different pathogens investigated. In several cases, microorganisms showed an increase in disease incidence compared to peat inoculated control, that could be explained by the high amount of inoculum added to the substrate (10 g l^{-1}) that influenced the microflora and consequently plants growth. A host plant may face infection by multiple pathogens, furthermore, compost is known as a product that varies considerably in chemical, physical and biotic composition, and, consequently, also in its ability to suppress soil-borne diseases (Termorshuizen et al. 2006).

The effects of the 28 microorganisms against basil wilt caused by *F. oxysporum* f. sp. *basilici* showed different results. The microorganisms K5, K7, OO5 and K44 showed a reduction in disease incidence and improved basil biomass, while K11 and E28 were conducive to the disease compared with the inoculated control and statistically increased disease incidence. Compost confirmed its suppressive activity against *F. oxysporum* f.sp. *basilici* (Reuveni et al. 2002) and microorganisms isolated from it could be used in biological control of the disease (Minuto et al. 1994).

Trichoderma strains E12, E15 and E36 controlled tomato root rot caused by *P. nicotianae*, confirming that *Trichoderma* spp. generally are good antagonists of *Phytophthora* spp. (Ambadkar and Jadhav 2007). Similar results were reported by Grasso et al. (2003), when several microorganisms isolated from the rhizosphere of gerbera plants were antagonists to *Phytophthora cryptogea* on gerbera.

The 28 tested microorganisms provided a different response in disease control. *Fusarium* strain K7 controlled *F. oxysporum* f. sp. *basilici* and *R. solani* but showed no significant effect against *P. nicotianae*. At the same time, *Fusarium* strain K9 controlled *F. oxysporum* f. sp. *basilici* and *P. nicotianae* but not *R. solani*. The microorganism P11 reduced *R. solani* incidence on bean, but showed to increase basil wilt caused by *F. oxysporum* f. sp. *basilici* (Table 9). The microorganisms K5, K7 and K44 controlled *F. oxysporum* f. sp. *radicis lycopersici* and *F. oxysporum* f. sp. *basilici* but were not able to control *P. nicotianae* and *R. solani*. The microrganisms K6, K9, E7, E23 and OO5 showed to control *F. oxysporum* f. sp. *radicis lycopersici* in Petri dishes but were not effective in greenhouse trials.

Table 5: Activity of microorganisms isolated from compost against *Phytophthora nicotianae* on tomato, showed as percentage of disease suppression and biomass

Treatment	Microorganism	Substrate	No. of alive plants	% disease control	Biomass (mg)	% biomass relative to peat inoculated control
1	K5	Peat	3.1 bc*	28	364 d	121
2	K6	Peat	0.0 d	−59	0 e	0
3	K7	Peat	0.8 d	−37	259 de	86
4	K9	Peat	2.2 c	3	651 bc	216
5	K11	Peat	1.1 cd	−28	394 d	130
6	K34	Peat	0.7 d	−39	170 e	56
7	K44	Peat	0.1 d	−56	54 e	18
8	K46	Peat	1.0 cd	−31	316 d	105
9	E7	Peat	0.1 d	−56	11 e	4
10	E12	Peat	3.7 b	45	300 d	99
11	E14	Peat	2.4 bc	8	222 de	74
12	E15	Peat	3.9 b	51	466 cd	154
13	E18	Peat	3.4 bc	37	340 d	113
14	E19	Peat	0.7 d	−39	166 e	55
15	E23	Peat	1.1 cd	−28	352 d	117
16	E28	Peat	1.7 cd	−11	511 c	169
17	E36	Peat	5.6 a	99	289 de	96
18	P11	Peat	1.0 cd	−31	273 de	90
19	OO5	Peat	1.3 cd	−23	275 de	91
20	BF10	Peat	3.0 bc	25	307 d	102
21	B3	Peat	4.7 a	73	728 bc	241
22	B5	Peat	3.2 bc	31	480 cd	159
23	B13	Peat	4.4 ab	65	656 bc	217
24	B15	Peat	3.7 b	45	547 c	181
25	B17	Peat	5.0 a	82	954 b	316
26	B23	Peat	4.1 b	56	734 bc	243
27	B24	Peat	3.8 b	48	628 bc	208
28	B29	Peat	3.9 b	51	831 b	275
29	–	Peat inoculated control	2.1 c	0	302 d	100
30	–	Peat control	5.7 a	100	1624 a	538
31	–	Compost inoculated control	4.0 abc	54	334 d	111
32	–	Compost control	5.3 ab	89	628 bc	208

* See Table 3.

The results of the third round of trials showed that two *Fusaria*, K7 and K9, have a good antagonistic activity also at lower dosages (5 g l^{-1}), and are probably the most important responsible for compost suppressiveness against *F. oxysporum* f. sp. *basilici* on basil. According to Pugliese et al. (2007), the compost was not suppressive against *R. solani*, and the results confirm that none of the microorganisms isolated from that compost was able to control the pathogen.

Carisse et al. (2003) isolated microorganisms from composts and tested them for control of damping-off of cucumber caused by *Pythium ultimum*. Microorganisms showed different levels of disease control when assessed one by one. Postma et al. (2003) were able to increase disease suppressiveness of compost against *R. solani* on sugar beet and tomato after enrichment with *Verticillium biguttatum*. Trillas et al. (2006) also reported that compost enriched with the biological control agent *Trichoderma asperellum* (strain T34) at 10^3 cfu ml^{-1} dosage reduced the incidence of *R. solani*. On the contrary, Dianez et al. (2007) reported the suppressive activity of compost against *Pythium* damping-off on cucumber and *Phytophthora* root rot was not improved on tomato using selected microorganisms isolated from grape marc compost.

The antagonistic activity of the genus *Trichoderma* and of non-pathogenic strains of *F. oxysporum* has been repeatedly shown and compost can be a natural resource for these antagonists (Garibaldi et al. 1987; Garibaldi 1988; Harman et al. 2004). The possibility to isolate the microorganisms responsible of the suppressive activity of compost and to use them for controlling soil-borne pathogens seems a good strategy not only in organic farming but also in traditional agriculture, particularly in soilless systems for many economically important crops.

Future researches may focus on study how to increase the suppressiveness of compost enriched with the most effective microrganisms and their mixtures, and to investigate on the mode of action of these selected microorganisms in order to produce high suppressive substrates.

Table 6: Activity of microorganisms isolated from compost against *Rhizoctonia solani* on bean, showed as percentage of disease suppression and biomass

Treatment	Microorganism	Substrate	DI (0–100)	% disease suppression	Biomass (g)	% biomass relative to peat inoculated control
1	K5	Peat	76 def*	13	15.3 bcd	143
2	K6	Peat	75 cdef	15	11.9 de	111
3	K7	Peat	69 cde	22	18.4 bcd	172
4	K9	Peat	82.5 ef	5	14.8 bcd	138
5	K11	Peat	69 cde	22	20.5 abcd	192
6	K34	Peat	75.5 cdef	14	13.8 de	129
7	K44	Peat	68 cde	23	18.1 bcd	169
8	K46	Peat	80.5 def	8	13.2 de	123
9	E7	Peat	79 def	10	15.7 bcd	147
10	E12	Peat	75.5 cdef	14	17.5 bcd	164
11	E14	Peat	85.5 ef	1	10.2 de	95
12	E15	Peat	86 ef	1	8.9 de	83
13	E18	Peat	90 ef	−4	8.4 de	79
14	E19	Peat	48 bc	49	28.1 ab	263
15	E23	Peat	81.5 ef	6	11.9 de	111
16	E28	Peat	70.5 cde	20	17.7 bcd	165
17	E36	Peat	81 ef	7	11.1 de	104
18	P11	Peat	53 bcd	42	28.1 abc	263
19	OO5	Peat	84.5 ef	3	12.0 de	112
20	BF10	Peat	63 bcde	30	20.3 abcd	190
21	B3	Peat	77.5 def	11	16.7 bcd	156
22	B5	Peat	77.5 def	11	13.3 de	124
23	B13	Peat	74 cdef	16	18.3 bcd	171
24	B15	Peat	81.5 ef	6	12.6 de	118
25	B17	Peat	63.5 bcde	29	21.6 abcd	202
26	B23	Peat	81 ef	7	14.3 cde	134
27	B24	Peat	73.5 cdef	17	19.3 bcd	180
28	B29	Peat	74 cdef	16	13.1 de	122
29	–	Peat inoculated control	86.5 ef	0	10.7 de	100
30	–	Peat control	7.5 a	100	33.7 a	315
31	–	Compost inoculated control	99 f	−16	0.9 e	8
32	–	Compost control	40 b	59	21.4 abcd	200

* See Table 3.

Acknowledgements

The activities of Liu Bao-Ping, from North East Agricultural University of Harbin in China, have been carried out with the financial support of the European Union, within the Asia-link project "Organic farming: ethical, economical, scientific and technical aspects in a global perspective" CN/ASIALINK/028 (108-962) under the Asia-Link programme supported by the European Commission.

Literature

Ambadkar, C.V., V.T. Jadhav, 2007: Evaluation of *Trichoderma spp*. against *Phytophthora spp*. Int. J. Plant Sci. **2**, 52-54.

Bertani, G., 1951: Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. **62**, 293-300.

Carisse, O., J. Bernier, N. Benhamou, 2003: Selection of biological agents from composts for control of damping-off of cucumber caused by *Pythium ultimum*. Plant Pathol. **25**, 258-267.

De Clercq, D., L. Vandesteene, J. Coosemans, J. Ryckeboer, 2004: Use of compost as suppressor of plant diseases. In: P. Lens, B. Hamelers, H. Hoitink, W. Bidlingmaier (eds.): Resource Recovery and Reuse in Organic Solid Waste Management, pp. 317-337. IWA Publishing, London.

Dianez, F., M. Santos, J.C. Tello, 2007: Suppressive effects of grape marc compost on phytopathogenic oomycetes. Arch. Phytopathol. Plant Protect. **40**, 1-18.

Elad, Y., I. Chet, Y. Henis, 1981: A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica **9**, 59-67.

Garibaldi, A., 1988: Research on substrates suppressive to *Fusarium oxysporum* and *Rhizoctonia solani*. Acta Hort. **221**, 271-277.

Garibaldi, A., F. Brunatti, M.L. Gullino, 1987: Evaluation of several antagonists and different methods of application against fusarium wilt of carnation. EPPO Bull. **17**, 625-629.

Table 7: Activity of the most efficient microorganisms isolated from compost against *Fusarium oxysporum* f. sp. *basilici* on basil

Treatment	Microorganism	Dosage (g l^{-1})	Inoculated with pathogen	Substrate	No. of of dead plants	% disease control	Biomass (g)	% biomass relative to peat inoculated control
1	K9	5	Yes	Peat	2.7 ab*	72	36.6 bcd*	99
2	E36	5	Yes	Peat	13.1 e	−38	22.7 cde	61
3	K11	5	Yes	Peat	8.7 cd	8	20.9 de	56
4	E28	5	Yes	Peat	8.2 cd	14	21.9 de	59
5	K7	5	Yes	Peat	3.9 ab	59	33.8 bcde	91
6	K9+E36	2.5+2.5	Yes	Peat	5.7 bcd	40	26.9 bcde	73
7	K9+E36+K11+E28+K7	1+1+1+1+1	Yes	Peat	6.2 bcd	35	30.0 bcde	81
8	K9	5	No	Peat	0 a	–	30.8 bcde	83
9	E36	5	No	Peat	0 a	–	31.8 bcde	86
10	K11	5	No	Peat	0 a	–	27.8 bcde	75
11	E28	5	No	Peat	0 a	–	33.6 bcde	91
12	K7	5	No	Peat	0 a	–	39.8 bc	108
13	K9+E36	2.5+2.5	No	Peat	0 a	–	33.2 bcde	90
14	K9+E36+K11+E28+K7	1+1+1+1+1	No	Peat	0 a	–	40.6 b	110
15	–	–	Yes	Peat	9.5 de	0	18.6 e	50
16	–	–	No	Peat	0 a	100	37.0 bcd	100
17	–	–	Yes	Compost	5 bc	47	33.3 bcde	90
18	–	–	No	Compost	0 a	100	59.2 a	160

* See Table 3.

Table 8: Activity of the most efficient microorganisms isolated from compost against *Rhizoctonia solani* on bean

Treatment	Microorganism	Dosage (g l^{-1})	Inoculated with pathogen	Substrate	DI (0–100)	% disease control	Biomass (g)	% biomass relative to peat inoculated control
1	K9	5	Yes	Peat	92.3 cd*	1	6.6 cde*	17
2	E36	5	Yes	Peat	91.3 cd	2	8.7 cde	22
3	K11	5	Yes	Peat	94.5 cd	−2	4.7 de	12
4	E28	5	Yes	Peat	93 cd	0	5.3 de	13
5	K7	5	Yes	Peat	88.8 c	5	9.7 cd	25
6	K9+E36	2.5+2.5	Yes	Peat	92.3 cd	1	6.8 cde	17
7	K9+E36+K11+E28+K7	1+1+1+1+1	Yes	Peat	85.8 c	9	13.6 c	34
8	K9	5	No	Peat	5 a	–	40.9 a	104
9	E36	5	No	Peat	7.5 a	–	40.1 a	102
10	K11	5	No	Peat	6.3 a	–	39.8 a	101
11	E28	5	No	Peat	5 a	–	39.8 a	101
12	K7	5	No	Peat	3.8 a	–	41.1 a	104
13	K9+ E36	2.5+2.5	No	Peat	3.8 a	–	40.7 a	103
14	K9+E36+K11+E28+K7	1+1+1+1+1	No	Peat	5 a	–	39.4 a	100
15	–	–	Yes	Peat	93.3 cd	0	5.3 de	13
16	–	–	No	Peat	7.5 a	100	39.5 a	100
17	–	–	Yes	Compost	98.8 d	−6	1.1 e	3
18	–	–	No	Compost	28.8 b	75	25.1 b	64

* See Table 3.

GRASSO, V., A. MINUTO, A. GARIBALDI, 2003: Selected microbial strains suppress *Phytophthora cryptogea* in gerbera crops produced in open and closed soilless systems. Phytopathol. Medit. **42**, 55-64.

GULLINO, M.L., C. CLINI, A. GARIBALDI, 2005: Life without methyl bromide:the Italian experience in replacing the fumigation. Commun. Agric. Appl. Biol. Sci. **70**, 13-25.

HARMAN, G.E., C.R. HOWELL, A. VITERBO, I. CHET, M. LORITO, 2004: *Trichoderma* species – opportunistic, avirulent plant symbionts. Nature Rev. Microbiol. **2**, 43-56.

HOITINK, H.A.J., P.C. FAHY, 1986: Basis for the control of soilborne plant pathogens with composts. Annu. Rev. Phytopathol. **24**, 93-114.

KATAN, J., 2005: Soil disinfestation: one minute before methyl

Table 9: An overview of the activity of 28 microorganisms isolated from compost against soil-borne pathogens

Microorganism		Tomato	Basil	Tomato	Bean
		Fusarium oxysporum f. sp. *radicis-lycopersici*	*Fusarium oxysporum* f. sp. *basilici*	*Phytophthora nicotianae*	*Rhizoctonia solani*
Fusarium	K5	++++*	+++	+	x
	K6	+++	++	–	x
	K7	+++	+++	–	+
	K9	+++	++	x	x
	K11	+	–	–	+
	K34	+	+++	–	x
	K44	++	+++	–	+
	K46	++	+	–	x
Trichoderma	E7	+++	+	–	x
	E12	++	–	++	x
	E14	+	–	x	x
	E15	++	–	++	x
	E18	+	+	+	–
	E19	x	x	–	++
	E23	++	–	–	x
	E28	x	–	–	+
	E36	+	–	++++	x
Fungi	P11	+	–	–	++
Oomycetes	OO5	++	++	–	x
Bacteria	BF10	+	–	+	+
	B3	x	x	+++	x
	B5	x	x	+	x
	B13	+	+	+++	x
	B15	x	x	++	x
	B17	x	x	++++	+
	B23	x	x	++	x
	B24	+	+	++	x
	B29	x	x	++	x

* ++++: high control; +++: good control; ++: moderate control; +: low control; –: disease induction; x: no disease control.

bromide phase out. Acta Hort. **698**, 19-26.

Komada, H., 1975: Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protect. Res. **8**, 114-125.

Masago, H., M. Yoshikawa, M. Fuhada, N. Nakanishi, 1977: Selective inhibition of *Pythium spp*. on a medium for direct isolation of *Pytophthora spp*. from soil and plants. Phytopathology **67**, 425-428.

Minuto, G., A. Garibaldi, M.L. Gullino, 1994: Biological control of Fusarium wilt of basil (*Ocimum basilicum* L.). Brighton Crop Protect. Conf., Pests. Dis. **2**, 811-816.

Noble, R., E. Coventry, 2005: Suppression of soil-borne plant diseases with composts: a review. Biocontrol Sci. Technol. **15**, 3-20.

Postma, J., M. Montanari, P.H.J.F. Van Den Boogert, 2003: Microbial enrichment to enhance the disease suppressive activity of compost. Eur. J. Soil Biol. **39**, 157-163.

Pugliese, M., A. Garibaldi, M.L. Gullino, 2007: The use of compost in horticulture for controlling soil-borne pathogens. Phytopathology **97**, s95.

Ryckeboer, J., 2002: The Fate of Plant Pathogens during Anaerobic Digestion and Composting. Biocycle **43**, 50-53.

Reuveni, R., M. Raviv, A. Krasnovsky, L. Freiman, S. Medina, A. Bar, D. Orion, 2002: Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop Prot. **21**, 583-587.

Termorshuizen, A.J., E. Van Rijn, D.J. Van Der Gaag, C. Alabouvette, Y. Chen, J. Lagerlöf, A.A. Malandrakis, E.J. Paplomatas, B. Rämert, J. Ryckeboer, C. Steinberg, S. Zmora-Nahum, 2006: Suppressiveness of 18 composts against 7 pathosystems: variability in pathogen response. Soil Biol. Biochem. **38**, 2461-2477.

Trillas, I.M., E. Casanova, L. Cotxarrera, J. Ordovàs, C. Borrero, M. Avilés, 2006: Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biol. Control **39**, 32-38.