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RESEARCH PAPERS

Effectiveness of composts and *Trichoderma* strains for control of Fusarium wilt of tomato

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Summary. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a major limiting disease in tomato production in Morocco. Commercial and locally produced Moroccan composts and peat were found to reduce Fusarium wilt in tomato plants. We explored the presence of *Trichoderma* strains in these materials, and in six soils sampled in the North West of Morocco, where a low incidence of Fusarium wilt had been previously observed. The most abundant *Trichoderma*-like fungus was selected from each soil, compost or peat sample. Twelve *Trichoderma* strains were isolated and identified molecularly. *Trichoderma asperellum* CT9 and *Trichoderma virens* ST11 showed the greatest overall antagonistic activity against FOL, *Rhizoctonia solani*, *Botrytis cinerea* and *Pythium ultimum*. The three strains evaluated in *in planta* tests, CT9, ST11 and *T. virens* ST10, reduced tomato Fusarium wilt, and strain ST11 also promoted growth of tomato plants.

Key words: biological control, *Solanum lycopersicum*, antagonism, growth promotion.

Introduction

Tomato is one of the most important economic crops in Morocco, this country being, after Mexico, world's second exporter of tomatoes in 2012, mainly to the EU. *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a major pathogen affecting tomato production in Morocco. Although the use of resistant cultivars provides some of control of tomato Fusarium wilt, soil fumigation with methyl bromide (MB) is also used to control the disease in this country. However, production and consumption of this ozone-depleting fumigant is being phased out for developing countries, such as Morocco.

The use of composts is a common agricultural practice to improve soil quality and also to dispose of organic wastes (Pérez-Piqueres *et al.*, 2006). In addition, compost has proved to be effective in controlling soil-borne diseases, including Fusarium wilt (Garbeva *et al.*, 2004; Suárez-Estrella *et al.*, 2007). Mechanisms such as microbial competition for nutrients, and antibiotic production, or the activation of disease-resistance pathways in plants, have been proposed for disease control through compost application (Hoitink and Fahy, 1986).

Numerous bacteria and fungi, including *Trichoderma* isolates, or combinations of microorganisms, collected from field tomato plants have proved to be effective in controlling Fusarium wilt in tomato (Larkin and Fravel, 1998; Srivastava *et al.*, 2010). *Trichoderma* is a genus of cosmopolitan filamentous fungi, and its members are considered to be opportunistic, avirulent plant symbionts (Harman *et al.*, 2004). These fungi are efficient biological control agents against plant pathogenic fungi, oomycetes, and nem-

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atodes (Monte, 2001; Druzhinina *et al.,* 2011). Selected strains have also shown direct beneficial effects for plants by promoting growth and development, stimulating defenses against pathogens or increasing tolerance to abiotic stress (Shoresh *et al.,* 2010; Hermosa *et al.,* 2012). The capacity of *Trichoderma* strains to suppress Fusarium wilt in tomato has been reported (Cotxarrera *et al.,* 2002). The enrichment of composts with *Trichoderma asperellum* or *T. harzianum* has been also proposed to enhance their suppressive capacity of Fusarium wilt and other soil-borne pathogens (Trillas *et al.,* 2006; Sant *et al.,* 2010; Blaya *et al.,* 2013).

FOL control is a priority need in Morocco due to the important economic losses caused by this pathogen in tomato production. In Mediterranean European countries, such as Spain, France, Italy and Greece, the use of biocontrol agents as well as composting have been reported as appropriate strategies to control this disease. A similar strategy could be deployed in Morocco, but using native biocontrol strains. We have therefore investigated the potential of different composts, including commercial products, to suppress Fusarium wilt. In addition, to seek new potential antagonists, several *Trichoderma* spp. strains were isolated from composts and horticultural soils, characterized molecularly, and screened in *in vitro* and *in vivo* assays for their biocontrol capabilities. This research was conducted as a first step in the development of an alternative biological control strategy for Fusarium wilt management in Morocco.

Materials and methods

Composts and soils

Five composts, six soils and a homogeneous mixture of blonde and black German peat (Floraska, Spain) were used in this study. Of the five composts, Ferticompost and Planta-mix are commercial (Ecofertil Maroc, Casablanca, Morocco), and the other three were farm composts from different animal manures (sheep, cow or poultry), as commonly used for the fertilization of tomato crops in the Tangier-Tetouan region of Morocco. Soil samples were collected from the top layer (0–20 cm) of horticultural soils previously cultivated with tomato in four different geographical locations of the Larache, Tangier and Fahs-Anjra provinces in Morocco. The physicochemical parameters of soils and peat are shown in Table 1.

Effects of composts on tomato seedling emergence

Tomato seeds (*Solanum lycopersicum* var. Marmande), sterilized in 2% sodium hypochlorite for 20 min and washed thoroughly in sterile distilled water, were placed in 20 cm diam. pots containing 100% compost, a mix of compost and peat (20/80%, v/v) or 100% peat, this latter used as an experimental control for comparison. The pots were kept for 10 d at 25ºC in a growth chamber, at 40% humidity and in darkness, after which the proportions (%) of tomato seedling emergence were calculated. Ten replicate pots (four seeds per pot) were used for each of the three plant growth media.

Effects of composts on Fusarium wilt of tomato

Composts were assayed to determine whether they had the ability to reduce Fusarium wilt on tomato plants (Table 2). A race 2 isolate of FOL (strain 42-87 from Prof. K. Lairini, University Abdelmalek Essaadi, Tangier, Morocco), was used throughout this study. Tomato seeds var. Marmande, sterilized as described above, were planted in pots containing the substrate under assay and grown for 21 d. The substrates assayed were sand (used as a negative experimental control), 100% peat, and five different composts, each as a 20:80% (v:v) compost:peat mixture. Ten pots (pot volume 216 cm^3) per substrate were used, and one plant was grown per pot. Each 21-d-old tomato plant was drenched with 3 mL of a conidium suspension of 10^7 conidia mL^{-1} of FOL. The inoculum obtained from a FOL culture grown in potato dextrose broth (PDB, Difco) for 7 d on a rotary shaker at 150 rpm at 25ºC and blended. Pots were kept in a growth chamber under conditions of 25°C and a 16 h light/8 h dark photoperiod, and were watered as needed. The plants were examined daily and a FOL disease index was determined for 10 d using a scale from 0 to 4 to reflect the degree of wilt symptoms due to the pathogen (0 = asymptomatic, 1 = yellowing, 2 = vascular discoloration, 3 = wilting, 4 = plant dead).

Isolation and identification of *Trichoderma* strains

Trichoderma strains were isolated from compost, peat and six soils. The soils were selected because of their low incidence of Fusarium wilt, and were from horticultural fields from the Moroccan Tangier-Tetouan region, where compost application is a normal

Table 1. Physicochemical parameters of soils and peat used in this work.

Sample	pH	Electrical conductivity (ms cm^{-1})	Organic matter (%)	Water content (%)
Soil 1	6.7 ± 0.03	1.99 ± 0.64	83.71 ± 0.61	81.1 ± 0.12
Soil 2	6.6 ± 0.03	1.77 ± 0.03	89.13 ± 0.05	79.7 ± 0.20
Soil 3	6.8 ± 0.04	2.34 ± 0.05	74.03 ± 1.60	86.9 ± 0.09
Soil 4	6.56 ± 0.18	0.82 ± 0.37	73.76 ± 0.38	86.8 ± 0.28
Soil 5	6.5 ± 0.31	0.52 ± 0.54	46.89 ± 1.93	87.7 ± 0.35
Soil 6	6.7 ± 0.21	0.36 ± 0.13	48.65 ± 0.75	75.43 ± 0.56
Peat	3.79 ± 0.16	0.77 ± 0.02	94.37 ± 2.98	68.25 ± 0.78

Table 2. Mean Fusarium wilt severity indices on tomato plants grown for 21 d in five different compost-peat mixes (20:80), peat or sand, and 10 d after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*.

Treatments	Fusarium wilt disease index (0–4 scale)
Planta-mix	1.6^{ab}
Ferticompost	1.2^{a}
Composted sheep manure	1.6^{ab}
Composted cow manure	1.3^{ab}
Composted poultry manure	1.3^{ab}
Peat	2.3^{bc}
Sand	3.5^{c}

Ten plants were used for each treatment. Values with different superscript letters are significantly different according to Friedman test ($P < 0.05$).

practice used to suppress soil-borne plant pathogens.

Aliquots of composts and soils were sown on *Trichoderma*-selective medium (Elad *et al.*, 1981) plates and incubated at 30ºC for 5 d. The most abundant *Trichoderma*-like colony of each soil, compost or peat was then selected for isolation, and monoconidium cultures were obtained. *Trichoderma* spp. isolates were grown on potato dextrose agar (PDA, Difco) and kept at 4ºC for further studies. Twelve *Trichoderma* spp. strains isolated from soils, composts or peat were subjected to molecular identification. Mycelia for DNA extractions were obtained from PDB cultures incubated at 28°C and 200 rpm for 2 d. They were collected by filtration, washed with distilled water, frozen, and lyophilized. Fungal genomic DNA was isolated as previously described (Hermosa *et al.*, 2000).

Amplification and sequencing of the ITS1-ITS4 region of the nuclear rDNA gene cluster and of a fragment of the translation elongation factor 1-E (*tef1*) gene were carried out, respectively, with the ITS1/ITS4 and EF1-728F/tef1-rev primer pairs, as previously described (Sadfi-Zouaoui *et al.*, 2009).

In vitro antagonistic activity of *Trichoderma* strains against plant pathogens

In vitro confrontation assays were conducted with 12 isolated *Trichoderma* strains (Table 3), against the pathogens FOL 42-87, *Rhizoctonia solani* CECT 2815 (Spanish Type Culture Collection, Burjassot, Spain) and *Pythium ultimum* 8 (University of Naples, Italy) on PDA plates, and *Botrytis cinerea* 98 (isolated from diseased strawberry plants) on malt extract agar (MEA, Difco). These tests were carried out as previously described (Rubio *et al.*, 2009). Pathogens grown alone were used as experimental controls. Plates were photographed after 5 d and images were subjected to analysis using ImageJ software (imagej.nih.gov/ij) to calculate colony size. Dual cultures were carried out in triplicate and results were expressed as pathogen colony size (cm^2).

In addition, for comparative purposes, growth assays on cellophane sheets and 14 kDa-cut-off dialysis cellulose membranes were carried out on PDA (for FOL and *R. solani*) or MEA (for *B. cinerea*) plates

Table 3. Sources and accession numbers of *Trichoderma* strains isolated in this research.

Ref.	Identified as	Source	Origin or reference	GenBank accession ITS1-ITS4/*tef1*
ST1	*T. asperellum*	Soil 1	Douar Laghdira	KJ652493 / KJ677260
ST2	*T. virens*	Soil 2	Douar Laghdira	KJ652494 / KJ677261
ST3	*T. virens*	Soil 3	Douar Laghdira	KJ652494 / KJ677262
ST4	*T. asperellum*	Soil 4	Douar Mjahdine	KJ652495 / KJ677263
CT5	*T. virens*	Planta-mix	Ecofertil Maroc (Casablanca)	KJ652494 / KJ677264
CT6	*T. virens*	Ferticompost	Ecofertil Maroc (Casablanca)	KJ652497 / KJ677265
CT7	*T. hamatum*	Composted sheep manure	Tangier	KJ652496 / KJ677266
CT8	*T. virens*	Composted cow manure	Tangier	KJ652497 / KJ677267
CT9	*T. asperellum*	Composted poultry manure	Tangier	KJ652495 / KJ677268
ST10	*T. virens*	Soil 5	Khmis Bni Arouss	KJ652494 / KJ677269
ST11	*T. virens*	Soil 6	Fedan Chapeau	KJ652494 / KJ677270
PT12	*T. virens*	German peat	Floraska (Almería, Spain)	KJ652494 / KJ677271

as previously described (Rubio *et al.*, 2009). The antagonistic activity of *T. asperellum* CT9 and three *Trichoderma* strains previously described as biocontrol agents (Hermosa *et al.*, 2004; Rubio *et al.*, 2009), was evaluated in this assay. The three reference strains were *T. asperellum* IMI 296237 (CABI Bioscience, Egham, United Kingdom), referred to as T25; *T. harzianum* CECT 2413, referred to as T34; and *T. virens* NBT 59 (Newbiotechnic S.A., Seville, Spain), referred to as T59. Each pathogen was tested in triplicate. Growth diameters were measured after 2 d for *R. solani*, 4 d for *B. cinerea* and 5 d for FOL. Results were expressed as the percentage of growth inhibition of each pathogen by each *Trichoderma* strain, with respect to the mean colony diameters of each pathogen grown alone.

Control of Fusarium wilt of tomato by the *Trichoderma* spp. strains CT9, ST10 and ST11

Tomato seeds var. Marmande, sterilized as described above, were planted in trays containing sand, previously autoclaved 120°C for 1 h on two successive days, and were grown for 18 d. Each tomato plant was irrigated with a 1 mL conidium suspension of 10^7 conidia mL^{-1} of a *Trichoderma* isolate and, after 24 h, transplanted into a 6 cm diam. pot containing sand infested with FOL, which was added to the sand at 10^4 conidia g^{-1}. Ten pots per condition, and one plant per pot, were used. *Trichoderma* conidia were obtained by growing the isolates for 7 d on PDA. FOL inoculum was obtained as described above. Pots were kept for 30 d in a growth chamber under the conditions described above, watered with the Long-Ashton nutrient solution (Hewitt and Smith, 1974) as needed, and plant dry weight was measured. The tests considered in this assay included: a control without fungi; only FOL-infested; treatment with *Trichoderma* isolate plus FOL-infested, and only treatment with *Trichoderma* isolate. *Trichoderma asperellum* CT9, *T. virens* ST10 and *T. virens* ST11 were evaluated. Results were expressed as plant dry weight and as FOL disease index, using the 0-4 severity scale described above.

Statistical analyses

Analysis of variance (ANOVA) was conducted on the data of compost effect on tomato emergence and the evaluation of the antagonistic activity of *Trichoderma* strains, using a General Linear Model implemented in SPSS v. 10 (SPSS Inc., USA). Pairwise

comparisons were made using Tukey's test at the 95 % significance level. In the case of Fusarium wilt disease index, the Friedman non-parametric method was performed at the 95% significance level.

Results

Effects of composts on tomato emergence

Ten days after planting the seeds, the emergence rate of tomato seedlings was greatest (mean = 75%) on 100% peat, and this was considered as the control. Seedling emergence rates lower than 20% were obtained for all the 100%-compost applications, except for composted sheep manure, which gave 63% emergence. When composts were used in mixtures with peat, compost:peat (20:80, v:v), tomato seedling emergence ranged from 55 to 72%.

Effects of composts on Fusarium wilt of tomato

Mixed 20:80 compost:peat substrates elicited significant reductions in FOL wilt in comparison with sand, used as a non FOL-suppressive substrate (Table 2). While an average FOL disease index of 3.5 was observed for tomato plants grown in sand, compost:peat substrates showed lower mean FOL disease indices, ranging from 1.2 to 1.6.

Isolation and identification of *Trichoderma* strains

Twelve *Trichoderma* spp. strains were isolated from the samples of five composts, one peat and six soil samples (Table 3), and their identities were confirmed at species level by analysis of the nucleotide sequences of the ITS1-ITS4 region and a fragment of the *tef1* gene. The nuclear rDNA regions, containing the ITS1, ITS2 and 5.8S gene sequences, ranged from 564 to 584 bp in length. An initial alignment of the 12 *Trichoderma* strains identified five different sequence types, which were compared with information available in databases. Eight strains were identified as *T. virens*, three as *T. asperellum* and one as *T. hamatum*. The *tef1* gene fragment was about 600 bp in length and seven different sequence types were identified. The alignment and sequence analysis of this gene confirmed the identities of the 12 strains. The sequences were deposited in the GenBank database and their accession numbers are shown in Table 3.

In vitro antagonistic activity of *Trichoderma* strains against plant pathogens

Plate confrontation experiments between the 12 *Trichoderma* strains and the pathogens FOL, *B. cinerea*, *R. solani* and *P. ultimum* were performed (Table 4). For the four pathogens, the largest colonies were recorded from control plates. In the confrontation plates, all *Trichoderma* strains surrounded the colony of FOL and overgrew the colonies of *R. solani* and *B. cinerea*. Only *T. asperellum* CT9 was able to overgrow the colony of *P. ultimum*. This pathogen was not inhibited by the other *Trichoderma* strains, and, except *T. hamatum* CT7, the other strains were overgrown by *P. ultimum*. Strain-dependent inhibitory behaviour was observed against the target pathogens. *Trichoderma asperellum* strains displayed wide antagonistic potential against the four target pathogens. In general terms, *T. asperellum* CT9 and *T. virens* ST11 were the best antagonistic strains, and *T. virens* ST10 was the least antagonistic. These three strains were included in a subsequent *in vivo* assay.

Since strain CT9 showed the best biocontrol capacity in dual culture assays, its antagonistic activity was compared with those of three *Trichoderma* strains selected in previous studies for their biocontrol potential (Table 5). The greatest inhibition values were observed for strains T59 and CT9 against *B. cinerea*, and strain T34 against FOL. No differences were observed among the four strains against *R. solani*. The two *T. asperellum* strains, CT9 and T25, showed different antagonistic activity against FOL on cellophane, and against *B. cinerea* on both cellophane and dialysis membrane, indicating strain-dependent inhibitory behaviour.

Control of Fusarium wilt of tomato by the *Trichoderma* spp. strains CT9, ST10 and ST11

The three *Trichoderma* strains selected for their different antagonistic behaviour in dual confrontation assays were evaluated *in vivo* in FOL-infested and non-FOL-infested tomato plants (Table 6). All three *Trichoderma* strains reduced Fusarium wilt. In addition, no significant differences in plant dry weight were observed between un-inoculated control plants and tomato plants inoculated with *Fusarium* and treated with CT9 or ST11. Moreover, when tomato plants were not inoculated with FOL, *T. virens* ST11 exerted a significant plant growth promotion effect as compared to the controls.

Table 4. Mean colony size (cm^2) of *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Rhizoctonia solani* (Rs) *Botrytis cinerea* (Bc) and *Pythium ultimum* (Pu) after 5 d growing alone (control) or in dual culture with the 12 different *Trichoderma* spp. strains isolated in this work.

Fungus	FOL	Rs	Bc	Pu
Pathogen (control)	18.25 ± 0.19^{a}	57.85 ± 0.99^{a}	38.49 ± 0.22^{a}	48.60 ± 0.24^{a}
T. asperellum ST1	8.13 ± 0.25^{f}	15.57 ± 1.0^{de}	4.21 ± 0.60^{i}	17.56 ± 0.32^{i}
T. virens ST2	10.45 ± 0.28^{e}	10.46 ± 2.42^{fg}	12.65 ± 0.08^{c}	28.10 ± 0.63^{f}
T. virens ST3	14.60 ± 0.35^{b}	19.06 ± 1.36^{c}	12.83 ± 0.11^{c}	33.22 ± 0.52^{cd}
T. asperellum ST4	8.51 ± 0.27^{f}	12.97 ± 0.39^{ef}	8.77 ± 0.86^{d}	24.58 ± 0.22^{gh}
T. virens CT5	11.96 ± 0.72^{de}	18.72 ± 1.50^{cd}	7.12 ± 0.08^{efg}	31.74 ± 0.79^{de}
T. virens CT6	10.75 ± 0.71^{e}	11.31 ± 0.65^{fg}	8.50 ± 0.04^{de}	30.99 ± 0.27^{e}
T. hamatum CT7	4.55 ± 0.34^{g}	30.31 ± 0.84^{b}	5.86 ± 0.09^{gh}	25.84 ± 0.69^{gh}
T. virens CT8	13.54 ± 0.85^{bcd}	17.30 ± 0.99^{cd}	11.69 ± 0.89^{c}	33.60 ± 0.16^{cd}
T. asperellum CT9	6.06 ± 0.09^{g}	2.97 ± 0.21^{h}	7.79 ± 0.38^{def}	5.35 ± 0.21^{j}
T. virens ST10	13.78 ± 0.60^{bcd}	31.84 ± 0.89^{b}	20.62 ± 0.15^{b}	35.91 ± 0.85^{b}
T. virens ST11	12.00 ± 0.51^{cde}	3.41 ± 0.27^{h}	4.70 ± 0.76^{hi}	23.87 ± 0.93^{h}
T. virens PT12	13.14 ± 0.75^{bcd}	8.48 ± 0.16^{g}	6.53 ± 0.28^{fg}	27.55 ± 0.25^{f}

Values are means of three replicates with the corresponding standard deviation. For each column, values with different superscript letters are significantly different according to Tukey's test ($P < 0.05$).

Table 5. Mean colony growth inhibition (percent) for *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Rhizoctonia solani* (Rs) and *Botrytis cinerea* (Bc) by hydrolytic enzymes / metabolites from *Trichoderma asperellum* CT9, *T. asperellum* T25, *T. harzianum* T34 or *T. virens* T59 grown on cellophane or dialysis membrane (dialysis) for 5 d (FOL), 4 d (Rs) or 2 d (Bc).

Fungal strain	FOL		Rs		Bc	
	Cellophane	Dialysis	Cellophane	Dialysis	Cellophane	Dialysis
CT9	49.2 ± 3.8^{a}	41.1 ± 4.5^{ab}	49.3 ± 1.9^{a}	62.5 ± 2.9^{a}	76.0 ± 8.2^{a}	70.0 ± 6.0^{a}
T25	31.4 ± 11.7^{b}	37.8 ± 8.2^{a}	53.1 ± 8.6^{ab}	49.1 ± 14.3^{b}	29.4 ± 4.6^{b}	26.7 ± 2.9^{b}
T34	74.4 ± 1.4^{c}	68.6 ± 2.8^{c}	59.6 ± 3.9^{b}	65.8 ± 5.1^{a}	45.7 ± 1.7^{c}	52.2 ± 10.0^{c}
T59	42.2 ± 3.1^{a}	44.8 ± 3.1^{b}	56.6 ± 7.8^{ab}	59.2 ± 3.8^{ab}	100.0 ± 0.0^{d}	92.6 ± 5.8^{d}

Values are means of three replicates with the corresponding standard deviation. For each column, values with different superscript letters are significantly different according to Tukey's test ($P < 0.05$).

Discussion

The beneficial effects of compost amendments for the control of Fusarium wilt in tomato have been demonstrated before (Cotxarrera *et al.*, 2002; Kouki *et al.*, 2012). However, the application of large amounts of compost has been traditionally associated with phytotoxycity (Giordano *et al.* 1975), suggesting that a dilution of compost with peat is necessary before soil application. Using a 20:80 compost peat mixture in the present study, no negative effects on seedling emergence were observed, accordingly with previous reports (Zucconi *et al*., 1981). Negative effects on plant establishment due to the application of large

Table 6. Mean plant dry weights and Fusarium wilt severity indices for tomato plants treated with the *Trichoderma* spp. strains CT9, ST10 and ST11, in a 30 d pot assay.

Treatment	Plant dry weight (g)	Fusarium wilt disease index (0-4 scale)
Control	0.43 ± 0.14^{b}	0^{a}
FOL	0.13 ± 0.02^{c}	3.5^{b}
CT9	0.48 ± 0.17^{b}	0^{a}
ST10	0.33 ± 0.03^{bc}	0^{a}
ST11	0.84 ± 0.14^{a}	0^{a}
CT9 + FOL	0.49 ± 0.16^{b}	0^{a}
ST10 + FOL	0.21 ± 0.08^{c}	1^{a}
ST11 + FOL	0.54 ± 0.04^{b}	0^{a}

Values are means of ten plants with the corresponding standard deviation. Values of plant dry weights accompanied by different letters are significantly different according to Tukey's test ($P < 0.05$). Values of Fusarium wilt indices with different superscript letters are significantly different according to Friedman test ($P < 0.05$). Control: untreated and uninoculated tomato plants.

amounts of compost have also been related to an excessive supply of soluble salts (Iglesias-Jiménez and Pérez-García, 1989). We did not observe any correlation between the salinity/conductivity parameters (data not shown) and percentages of tomato seedling emergence for any of the five composts tested.

In our study, the tomato plants grown in the five composts after FOL application showed less Fusarium wilt than those grown in sand. The microbial nature of Fusarium wilt suppressiveness in compost-amended substrates has been demonstrated previously (Cotxarrera *et al.*, 2002; Borrero *et al.*, 2006; Suárez-Estrella *et al.*, 2007; Kouki *et al.*, 2012).

We explored the presence of *Trichoderma* strains in these plant growth substrates, and also in Moroccan soils in which tomatoes are regularly produced with a very low incidence of Fusarium wilt. Despite the unknown background of these soils, compost addition for horticultural production is a common management strategy in the North-West of Morocco. Thus, one of the main objectives is this research was the isolation of native *Trichoderma* strains with biocontrol abilities, taking into account the basic principle according to which microorganisms isolated from a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species (Cook, 1993). Furthermore, it has also been demonstrated that eco-composts are good sources of bio-protective microbial agents (Suárez-Estrella *et al.*, 2013). It is also well established that *Trichoderma* is an efficient biocontrol agent of soil-borne oomycetes and fungi (Monte, 2001) and that some isolates reduce Fusarium wilt diseases in different crops, including tomato (Larkin and Fravel, 1998; Cotxarrera *et al.*, 2002; Suárez-Estrella *et al.*, 2007). In the present study, *Trichoderma* fungi were the most abundant in all samples of composts, soils and peat plated on a selective *Trichoderma* medium, and the ITS1-ITS4 region and a fragment of *tef1* gene sequences identified the 12 strains isolated (Table 3). No correlation was observed between species and their origin. For example, *T. virens* was isolated from plant and animal composts, and from the peat and soils collected in different locations. *Trichoderma hamatum* and *T. virens* have also been isolated previously from compost:peat mixtures (Thornton *et al.*, 2002). *Trichoderma hamatum*, which has been reported to be the most widely distributed *Trichoderma* species and to be present in forest soils in North America, Russia and Nepal and in North Africa agricultural and oasis ecosystems (Danielson and Davey, 1973; Sadfi-Zouaoui *et al.*, 2009), was only isolated from composted sheep manure. In addition, no correlation was observed between physicochemical characteristics of the soils, such as electrical conductivity and organic matter content, and the predominant *Trichoderma* sp.

The antagonistic potential of the 12 *Trichoderma* strains tested in dual cultures demonstrate that their aggressiveness is dependent on the target pathogen, as previously described (Hermosa *et al.*, 2000) (Table 4). Here, it is important to note that the three *T. asperellum* strains were the best antagonists for three of the four phytopathogens assayed, and that only *T. asperellum* CT9 was able to overgrow the colonies of *P. ultimum*. It would be risky to extrapolate the biocontrol activity of a given strain under laboratory conditions to natural environments, but the use of *in vitro* antagonism assays can serve to select potential biocontrol agents against particular pathogens (Hermosa *et al.*, 2000). We have previously observed that *F. oxysporum* f. sp. *dianthi* is more sensitive to attack by *T. asperellum* strains than pathogens such as *B. ci-*

nerea, *Phoma betae* and *Rosellinia necatrix* (Hermosa *et al.*, 2000).

The biocontrol potential of strain CT9 was confirmed in a comparative study with the model strain *T. harzianum* T34 described by Rubio *et al.* (2009) and the previously described biocontrol strains *T. asperellum* T25 and *T. virens* T59 (Hermosa *et al.*, 2004). Results obtained for CT9 from cellophane and dialysis membrane assays (Table 5) indicate that low molecular weight compounds are the major contributors to the antifungal activity of this strain against the three plant pathogens tested. This ability confers to CT9 overall biocontrol potential at a similar level to those displayed by the reference biocontrol agents.

Another *T. asperellum* strain has previously been shown to control tomato Fusarium wilt (Cotxarrera *et al.*, 2002). In the present study, three strains of *T. asperellum* and *T. virens* since the three strains belong to different species (CT9, ST10 and ST11) were selected for their different antagonistic properties against four plant pathogens in dual cultures and evaluated for their ability to reduce Fusarium wilt in tomato plants. Although strain ST10 reduced the negative effects of FOL in tomato, complete protection (100% healthy plants) was observed only for the CT9 and ST11 treatments. This result is in agreement with the lesser antagonistic behaviour of ST10 against FOL in dual cultures. Moreover, in the absence of FOL the significant increase observed in tomato dry weight for the ST11 treatment with respect to the control suggests that *T. virens* ST11 exerted growth promotion effects in tomato plants. Previous research has shown that *Trichoderma* promotes growth responses in plants (Chang *et al.*, 1986), and this positive effect was related to its ability to increase plant root development, to solubilize nutrients and to produce the phytohormone indol-3-acetic acid (Altomare *et al.*, 1999; Contreras-Cornejo *et al.*, 2009). After *Trichoderma* applications, positive and negative growth responses in tomato are dependent on the plant's genetic background and the *Trichoderma* strain employed (Tucci *et al.*, 2011; Rubio *et al.*, 2012). When plants are challenged with an attacker, growth is suppressed and defenses are activated in order to balance energy costs (Hermosa *et al.*, 2012; Kazan and Manners, 2012). The fact that no significant difference in dry weight was observed between *T. asperellum* CT9- or *T. virens* ST10-treated plants and their respective conditions under FOL pressure suggests that these strains antagonize the pathogen directly and that the plant's energy continues to be invested in growth instead of in increasing defense responses. However, according to *in vitro* dual culture tests, plants treated with CT9 showed significant increases in dry weight with respect to ST10 under FOL pressure, indicating that CT9 would be a better biocontrol agent against this pathogen. Future research will include the validation of biocontrol efficacy of the selected strains under crop production situations before their commercial development for Moroccan horticulture.

In conclusion, the results presented in this study have demonstrated that selection of *Trichoderma* strains from Moroccan agrosystems, in this case from composts or soils amended with composts, was suitable for the biocontrol of tomato Fusarium wilt because these strains are adapted to environmental conditions where they will be applied.

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