



International Journal of  
**Agricultural  
Research**

ISSN 1816-4897



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***In vitro* and *in vivo* Suppression of *Fusarium oxysporum*  
f. sp. *radicis-lycopersici* the Causal Agent of Fusarium Crown  
and Root Rot of Tomato by Some Compost Fungi**

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**Abstract:** *Aspergillus* sp., *Trichoderma viride* strain 2 and *T. viride* strain 1 isolated from an animal manure compost are tested for their *in vitro* and *in vivo* antagonistic activity against *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the causal agent of the Fusarium Crown and Root Rot of tomato. Dual culture experiments, observed after incubation at 25°C on PDA during 5 days, showed that all tested fungi significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *radicis-lycopersici* comparatively to the untreated control. Inhibition varied from 25% for *Trichoderma viride* (strain 1) to 100% for *Aspergillus* sp. Competition for media was the predominant mechanism of action noted on PDA. *In vivo*, tomato plants (cv. Riogrande), simultaneously inoculated and treated individually by the compost fungi conidial suspensions ( $10^7$  spores mL<sup>-1</sup>), showed reduced severity of the Fusarium Crown and Root Rot, when observed 30 days after transplantation, comparatively to the untreated control. The compost fungi *T. viride* (strain 1) was the most effective, it decreased severity of the disease by 48%.

**Key words:** Biocontrol, *Aspergillus* sp., *Trichoderma* sp., inhibition, disease severity

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## INTRODUCTION

*Fusarium* Crown and Root Rot of tomato induced by *Fusarium oxysporum* f. sp. *radicis-lycopersici* is one of the most damaging soil-borne diseases of tomato causing heavy economic losses (Rekah *et al.*, 1999). Complete suppression of this fungus from soil is difficult by the use of fungicides (Hibar *et al.*, 2006). The difficulties in controlling this pathogen promoted scientists to search for other alternatives (Sivan and Chet, 1993). Composts prepared from heterogeneous organic wastes are some applicable means for the biological control for several plant diseases especially those caused by soilborne pathogens (Hoitink *et al.*, 1991, 1997). Phytopathogenic fungi such as *Phytophthora* sp. (Aryantha *et al.*, 2000), *Rhizoctonia solani* (Nelson and Hoitink, 1983) and *Fusarium* sp. (Chef *et al.*, 1983; Cotxarrera *et al.*, 2002) were successfully suppressed by composts. Several researches concluded that the microflora of composts plays the major role in suppression of plant pathogens. Indeed, Pasteurization of compost destroys their active microflora and nullifies their efficacy (Hoitink *et al.*, 1991, 1997; Zhang *et al.*, 1998; Bess, 2000; Quarles, 2001; Ingham, 2002; Camozzi, 2003). Beneficial microorganisms present in composts are implicated in various suppressive activities (Hoitink *et al.*, 1991) and strains of *Trichoderma* sp. (Cotxarrera *et al.*, 2002) and *Bacillus subtilis* (Phae *et al.*, 1990), isolated from composts, were shown to be effective antagonists against several pathogens. Strains of *T. hamatum* suppressed *F. oxysporum* (Trialls-Gay *et al.*, 1986). Isolates of *Penicillium* sp. and of



*Aspergillus* sp. were suppressive to *F. solani*, *F. graminearum*, *F. sambucinum* and *F. oxysporum* f. sp. *tuberosi* (Daami-Remadi *et al.*, 2006). Antagonistic interactions with other fungi and mechanisms involved in the biocontrol process are based on antibiosis, parasitism, induced resistance and competition (Hoitink *et al.*, 1997).

Preliminary dual cultures of some compost extracts with *F. oxysporum* f. sp. *radicis-lycopersici*, showed inhibited mycelial growth of this pathogen (Kerkeni *et al.*, 2007a). The aim of this study is to isolate fungi from the most suppressive previously tested compost extracts, to evaluate *in vitro* and *in vivo* their individually effects, on *F. oxysporum* f. sp. *radicis-lycopersici* and to assess their ability to decrease the *Fusarium* Crown and Root Rot of tomato severity.

## MATERIALS AND METHODS

### Pathogen

*F. oxysporum* f. sp. *radicis-lycopersici* used in this study was isolated from tomato plants showing typical symptoms of crown and root rot. It was cultured on PDA at 25°C for one week and stored at 4°C for long preservation.

### Compost Fungi

A mature compost (>12 months), composed of 40% cattle manure, 40% sheep manure and 20% vegetable wastes and produced on 2006 at the composting-unit of the Technical Centre of Organic Agriculture of Chott Mariem-Tunisia, was used for antagonistic fungi isolations.

Potato Dextrose Agar (PDA; Sigma) supplemented with 5 mg L<sup>-1</sup> Penicillium-G was used for fungal isolation. A sample of 10 g of solid compost was suspended in 90 mL of sterilized distilled water in 250 mL bottle. The sample was stirred for 1 h at 200 rpm. A serial dilution up to 10<sup>-3</sup> was carried out and then 100 µL aliquots of this dilution were spread onto PDA medium plates. After incubation at 25°C for 5 days, fungal colonies obtained were individually transferred on PDA. The same procedure was repeated until having a purified fungal culture. Selected compost fungi were identified on the basis of their macroscopic and microscopic characteristics (El-Masry *et al.*, 2002). They were cultured on PDA at 25°C for one week before use.

### *In vitro* Bioassay of the Antagonistic Activity of the Compost Fungi

The study was conducted in the Laboratory of Phytopathology of the Regional Centre of Research in Horticulture and Organic Agriculture of Chott Mariem (Tunisia). The antifungal activity of each tested compost fungi against *Fusarium oxysporum* f. sp. *radicis-lycopersici* was studied via the dual culture technique. The method consists of placing an active mycelial disc (6 mm in diameter) of the pathogen, 1 cm from the edge of a 9 cm Petri plate containing freshly prepared PDA medium. Another disc (6 mm) of the antagonist fungi was deposited in a diametrically opposed position 1 cm away from the other set of the plate. For untreated plates, an agar disc of *F. oxysporum* f. sp. *radicis-lycopersici* was placed at the center of the petri dish. All plates were then incubated at 25°C and evaluated for pathogen growth inhibition after 4 days of incubation. Three replicates were used per elementary treatment.

To determine the inhibition rate of this pathogen by each of the tested compost fungi, the fungal growth of *F. oxysporum* f. sp. *radicis-lycopersici* was recorded by measuring the *F. oxysporum* f. sp. *radicis-lycopersici* colony diameters (average of the two perpendicular diameters). These diameters (control and treated) served for the calculation of the inhibition rate of the fungal growth. This rate is calculated according to the following formula used by Hibar *et al.* (2005):

$$\text{Inhibition rate (\%)} = (1 - (\text{Average diameter of the treated} / \text{Average diameter of the control})) \times 100$$



### **In vivo Bioassay of the Antagonistic Activity of the Compost Fungi**

#### **Plant Material**

Disease suppressiveness of substrates individually amended with the different isolated fungi was tested in a bioassay using tomato (*Lycopersicon esculentum* Mill. Priscas), cv. Riogrande plants. This later was chosen for its sensibility to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Hibar, 2002).

#### **Bioassay**

One-month-old tomato plants, cv. Riogrande, were transferred from alveolar flats into 10 cm diameter plastic pots containing an autoclaved peat (15 min at 120°C).

For plant inoculation, mycelium taken from the edge colony of *F. oxysporum* f. sp. *radicis-lycopersici* was transferred to 150 mL of Potato Dextrose Broth (PDL) and incubated at 25°C for 5 days in a rotary incubator (120 rpm). The liquid culture was filtered and the conidial suspension was adjusted to  $10^7$  spores  $\text{mL}^{-1}$  by means of Malassez cystometer (Hibar *et al.*, 2006). The same procedure was used for the preparation of the inoculum of the tested fungi.

Tomato plants already planted, were inoculated simultaneously by irrigation with 10 mL of conidial suspension ( $10^7$  spores  $\text{mL}^{-1}$ ) of *F. oxysporum* f. sp. *radicis-lycopersici* and 10 mL of conidial suspension ( $10^7$  spores  $\text{mL}^{-1}$ ) of compost fungi (inoculated separately). Plants inoculated with the pathogen and without compost fungi were used as control.

Bioassay was conducted under greenhouse conditions at 25°C and under 12 h photoperiod (Pharand *et al.*, 2002). The plants were watered as needed. Ten replicate pots of each treatment were randomly placed. No fertilizer was added to plants. The experiment was conducted twice. Disease severity was determined 30 days after plantating (Woo *et al.*, 1996), based on a symptom severity scale, where: 0 = asymptomatic plants; 1 = weakly infected plants (<50% of leaves chlorotic or wilted); 2 = high infected plants (>50% of leaves wilted but plants not dead) and 3 = dead plants. At the end of the bioassay, the height, the mean shoot and root fresh and dry weights of plants per elementary treatment were determined.

#### **Experimental Design and Statistical Analysis**

Data were arranged as a completely randomized design. Ten replicate pots of each elementary were used and the whole bioassay was repeated twice. Data were analyzed using SPSS statistical program version 11.0. and subjected to Analysis of Variance (ANOVA). Means were compared according to the Duncan test.

## **RESULTS**

### **In vitro Inhibition of the *F. oxysporum* f. sp. *radicis-lycopersici* Growth by the Tested Compost Fungi**

The results shown in Table 1 showed that compost fungi, significantly reduced the mycelial growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, after incubation at 25°C for 5 days. All fungi tested were effective in reducing the mycelial growth more than 20% compared to the untreated

Table 1: Inhibition rate of *Fusarium oxysporum* f. sp. *radicis-lycopersici* colonies in presence of the compost fungi (PDA, after five days of incubation at 25°C)

Parameters	Inhibition ratio of <i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i> (%)
<i>Aspergillus</i> sp.	100a
<i>T. viride</i> (strain 2)	72b
<i>T. viride</i> (strain 1)	25c

Different letter(s) within columns represent values that are significantly different at  $p = 0.05$  based on ANOVA and Duncan test. Each value represents the mean of 3 values



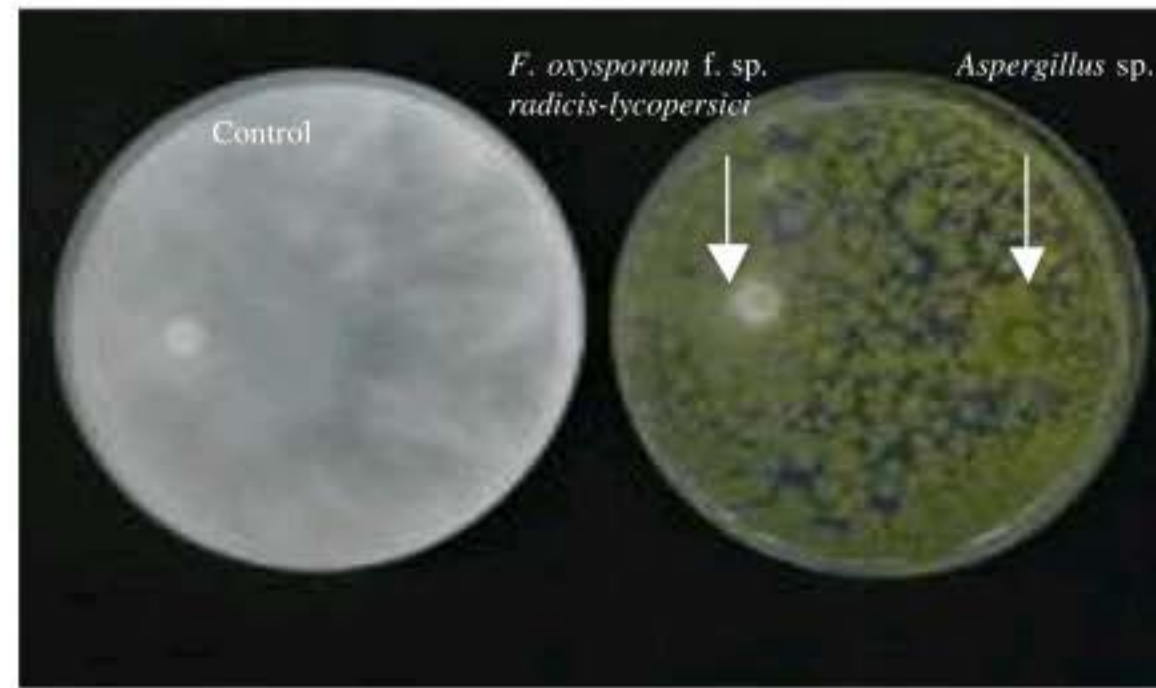


Fig. 1: Total overlapping and inhibition of *Fusarium oxysporum* f. sp. *radicis-lycopersici* by the compost fungus *Aspergillus* sp.

Table 2: *Fusarium* crown and root rot severity on tomato plants observed after 30 days, in sterilized peat treated with compost fungi in comparison to the untreated control (means of ten plants). Disease severity ranked from 0 (asymptomatic plants) to 3 (dead plants)

Treatments	<i>Trichoderma viride</i> (strain 2)	<i>Aspergillus</i> sp.	<i>Trichoderma viride</i> (strain 1)	Control
Disease severity	1.9ab	2.2a	1.3b	2.5a

Different letter(s) represent values that are significantly different at  $p = 0.05$  based on ANOVA and Duncan test

control. Inhibition varied from 25 to 100%. The most effective fungi were *Aspergillus* sp. and *Trichoderma viride* (strain 2), where pathogen growth was limited by 100 (Fig. 1) and 72%, respectively. The fungus *T. viride* (strain 1) showed lesser efficiency (25%).

### ***In vivo* Inhibition of the *F. oxysporum* f. sp. *radicis-lycopersici* Growth by the Tested Compost Fungi**

#### **Disease Severity**

The ability of the tested compost fungi to suppress *F. oxysporum* f. sp. *radicis-lycopersici* on tomato plants, in sterilized peat, was assessed one month post inoculation. Symptoms developed by *F. oxysporum* f. sp. *radicis-lycopersici* were lesser in plants grown in substrates treated with *T. viride* (strain 1) (Table 2). This later showed a remarkable efficiency in reducing *Fusarium* crown and root rot disease severity in comparison to the *in vitro* essay. *In vivo*, it decreased disease severity by nearly 50%, compared to the untreated control. Results showed also that *Aspergillus* sp., which was the most effective *in vitro*, was comparable to the control. *T. viride* (strain 2) reduced the disease development but its efficiency was lesser than *T. viride* (strain 1).

#### **Plant Growth Parameters**

##### **Plant Height**

Results in the Table 3 showed that the amendment of substrates with compost fungi had a significant effect on the tomato plant height. The presence of *Trichoderma viride* (strain 2) in the substrate with *F. oxysporum* f. sp. *radicis-lycopersici* enhanced significantly the plant height comparatively to the control. Tomato plants growing in this substrate had 23.75 cm. Whereas, those growing in the control substrates had only 13.4 cm. Statistically, plants inoculated with *Aspergillus* sp. and *T. viride* (strain 1) were not different to the control.





Fig. 2: Effect of the treatment of substrates with compost fungi on the plant growth parameters of one month old tomato; Control: substrates with *Fusarium oxysporum* f. sp. *radicis-lycopersici*. (25°C, under 12 h photoperiod)

Table 3: Effect of the treatment of substrates with compost fungi on plant height, shoot and root fresh weights and shoot and root dry weights, of one month old tomato. Control: substrates with *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Parameters	Control a	Treatments		
		<i>T. viride</i> (strain 2)	<i>Aspergillus</i> sp.	<i>T. viride</i> (strain 1)
Plant height (cm)	13.40b	23.75a	13.95b	17.60b
Shoot fresh weight (g)	2.00c	6.54a	3.18b	3.30b
Root fresh weight (g)	1.15b	2.82a	1.47ab	2.14a
Shoot dry weight (g)	1.25b	1.62a	1.31b	1.26b
Root dry weight (g)	0.21a	0.10a	0.18a	0.16a

Different letter(s) represent values that are significantly different at p = 0.05 based on ANOVA and Duncan test; Each value represents the mean of 10 values

#### Plant Shoot and Root Weights

Table 3 showed that only the presence of *T. viride* strain 2 in the substrates resulted in a pronounced increase in the tomato shoot fresh weight, compared to the control and to substrates amended with the other compost fungi. The improvement in shoot fresh weight of plants growing in the presence of *T. viride* (strain 2) amounted to more than 50%, in comparison to the untreated control (Table 3 and Fig. 2). Root fresh weight increase for this treatment, was not different to the substrates inoculated with *T. viride* (strain 1).

Table 3 showed also that the same substrate, inoculated with *T. viride* (strain 2) improved significantly tomato shoot dry weight, in comparison to all treatments. Whereas, no difference was obtained between all plants concerning root dry weights.

### DISCUSSION

These results showed for the first time in Tunisia that fungi isolated from an animal manure compost are suppressive against the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici*.



The current study showed that fungi isolated from compost were able to inhibit the growth of *F. oxysporum* f. sp. *radicis-lycopersici*, the causal agent of *Fusarium* crown and root rot of tomato. This results joined that obtained by Phae *et al.* (1990), Zhang *et al.* (1998) and Bess (2000), showing that compost contain microorganisms suppressive to plant pathogens. *in vitro*, *Aspergillus* sp., *Trichoderma viride* (strain 2) and *T. viride* (strain 1) showed an inhibitory effect towards *F. oxysporum* f. sp. *radicis-lycopersici*. *Aspergillus* sp. was the best in reducing the mycelial growth of *F. oxysporum* f. sp. *radicis-lycopersici* by 100%.

Earlier study conducted *in vitro* by Kerkeni *et al.* (2007a) showed that the whole compost extract used for these fungi isolation, inhibited the growth of this same isolate of *F. oxysporum* f. sp. *radicis-lycopersici* by only 42.6%. This suggests that composts and compost extracts contain biocontrol agents that are more efficient when used alone, probably this is the case with *Aspergillus* sp. and *T. viride* strain 2.

In the contrast, bioassay conducted *in vivo*, showed that this fungus was the least in reducing the disease severity on tomato plants. *T. viride* (strain 1) was the most effective *in vivo* but the least suppressive antagonist *in vitro* (25%). The fungus *T. viride* (strain 2) was always intermediate in suppression but the best in enhancing plant growth comparatively to the control and the other compost fungi. The variable efficiency of the tested fungi may be attributed to a variable mode of action and/or a variable type of antifungal metabolites produced by the antagonists (Williams and Asher, 1996). Tests based on *in vitro* mycelial inhibition do not always correlate with biocontrol efficacy under natural conditions. This finding proved that *in vitro* and *in vivo* results may be divergent as may be due to the variable physical and chemical properties within niches occupied by the biocontrol agents which may affect both root colonization and expression of biocontrol mechanisms.

The effectiveness of compost fungi against plant diseases as biocontrol agents was previously reported by Daami-Remadi *et al.* (2006), where an antagonistic effect of some filamentous fungi, isolated from compost, was noted against the *Fusarium* sp. complex, causing dry rot of potato. El Masry *et al.* (2002) and Muhammad and Amusa (2003) also isolated from compost, several fungal microorganisms such as *Aspergillus niger*, *Rhizopus* sp., *Drechslera* sp. and *Trichoderma harzianum*, which had an inhibitory effect against pathogens such as *Pythium aphanidermatum*, *Fusarium oxysporum* and *Rhizoctonia solani*. Cotxarrera *et al.* (2002), found that *Trichoderma asperellum* isolated from compost decrease disease severity of the *Fusarium wilt* of tomato. In a previous work, we also showed that compost fungi had an antagonistic effect against *Pythium ultimum* (Kerkeni *et al.*, 2007b).

Competition for nutrients present in the media, traduced by an overlapping of the pathogen colonies by that of the tested fungi, was the main mechanism, employed by compost fungi in antagonism of *F. oxysporum* f. sp. *radicis-lycopersici* in the dual culture. The *F. oxysporum* f. sp. *radicis-lycopersici* colonies overlapping by antagonists is probably due to a physical contact between pathogen and the compost fungi leading to the pathogen parasitism and its mycelium destruction. The same mechanism was observed in the dual culture of some compost fungi and *Pythium ultimum* (Kerkeni *et al.*, 2007b). In fact, multiple mechanisms of action including mycoparasitism, lysis, induction of mycelial cords and early chlamydospores formation were observed in dual cultures of some compost fungi with four *Fusarium* species (Ayed *et al.*, 2006; Daami-Remadi *et al.*, 2006).

Howell (2003), reported that biocontrol agents produce enzymes such as chitinase, protease and cellulase. These enzymes have been proved to be involved in the antagonistic activity. They act by breaking down and dissolving the polysaccharides, responsible for the rigidity of fungal cells walls. Chérif and Benhamou (1990) and Hibar *et al.* (2005) found that *Trichoderma* strains could produce enzymes that can diffuse in culture media. These substances induced reduction in *F. oxysporum* f. sp. *radicis-lycopersici* growth. However, in *in vivo* bioassay, Kerkeni *et al.* (2007b) did not show any correlation between the suppression of *Pythium ultimum* and production of cellulase in the growing substrate.



The compost fungus *T. viride* (strain 1) was the most effective in inhibiting the growth of *F. oxysporum in vivo*. Protective effect of this least fungus may be attributed to the induction of systemic resistance on tomato plants. This mechanism by which biocontrol agents acted, is not frequently detected *in vitro* (Schisler *et al.*, 1997).

These results showed that isolated compost fungi are suitable products to suppress plant pathogenic fungi. All tested fungi showed an antagonistic activity *in vitro* and *in vivo* against *Fusarium oxysporum* f. sp. *radicis-lycopersici*, causal agent of *Fusarium crown* and root rot of tomato. This finding is interesting and showed the importance of antagonist potential employed and also the ability of compost isolated fungi to support environmental conditions. They could be a promising way for the biological control of plant diseases and so could reduce the need of fungicides use.

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