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Effect of Substrate Culture on the Development of Fusarium Crown and Root Rot of Tomato

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ABSTRACT

Fusarium crown and root rot of tomato is a new damaging disease of tomato plants in Tunisia. A study of the effect of culture substrate on the development of this disease revealed that *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) is highly virulent when tomato plants are transplanted to sand, topsoil or perlite. Adding compost to these substrates significantly reduced the expression of this pathogen. Indeed, disease incidence measured in a mixture of perlite and compost was only about 6.6%; however this value exceeded 43% when tomato plants were transplanted only to perlite. These results suggest the suppressive effect of compost on the development of Forl and on soil-borne pathogens in general.

Keywords: disease incidence, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, soilless culture, substrate

INTRODUCTION

In Tunisia, tomato culture is an important crop vegetable; indeed, the surface currently reserved for this culture is about 19.1 thousand ha, representing 13.7% of the total surface reserved for vegetable cultures (Mahroug 2007).

Tomatoes can be cultivated quite well in a soilless system as can vegetables or ornamental species (Savvas *et al.* 2008). The main diseases in tomato aerial parts are grey mold (*Botrytis cinerea*) and Cercospora leaf mold (*Cercospora fuliginea*). These diseases can be controlled by spraying fungicides as well as using biocontrol agents such as *Trichoderma harzianum* (Moyano *et al.* 2003). The main soil-borne systemic diseases are Fusarium crown and root rot (*Fusarium oxysporum* f. sp. *radicis-lycopersici*) (Forl), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), late blight (*Phytophthora infestans*) and Pythium damping-off (*Pythium aphanidermatum*) (Schwarz and Grosch 2003). Of the soil-borne diseases, Fusarium crown and root rot (FCRR) is the most serious, especially in a soilless cultivation system. This disease, newly recorded in Tunisia during the 2000-2001 crop season (Hajlaoui *et al.* 2001; Hibar 2002), caused heavy losses reaching 90% of plants in some geothermal greenhouses.

The use of recycled rockwool substrates has stopped the development of Forl (Minuto *et al.* 2007). It was suggested that this suppressive effect against Forl was induced by indigenous microflora still resident (Minuto *et al.* 2007). These results are similar to those obtained with *Pythium aphanidermatum* in cucumber (Postma *et al.* 2000; Postma 2004) grown on rockwool in a closed soilless system and contribute to demonstrate the relevant role played by the resident microflora (Calvo-Bado *et al.* 2006).

In Tunisia, Forl is considered as a new emergent pathogen, so no control strategy is available to remedy this problem. Recourse to soilless systems seems to be one of the proposed solutions.

Perlite, sand and peat-based substrates are excellent media for tomato (Hanna 2005, 2006). Moreover, the use of compost has considerably reduced the incidence of soil-borne pathogens in tomato culture (Elad and Shteinberg 1994; Yohalem *et al.* 1996; Reuveni *et al.* 2002; Al-Dahmani *et al.* 2003; Daami-Remadi *et al.* 2006). In an attempt to know more about the development of Forl in a soilless cultivation system, and to distinguish the beneficial effect of compost, different culture substrates were tested, *in vivo*, on disease incidence.

MATERIALS AND METHODS

Fungal isolates

Forl isolates used in this study were obtained from tomato plants showing typical crown and root rot symptoms at the "5ème saison" exploitation in Hammet Gabès in South Tunisia where tomato culture heated with geothermal water is practised.

Fungal pathogen was isolated by planting plant tissues (surface-disinfected with 1% sodium hypochlorite for 2 min) on PDA (Potato Dextrose Agar) and incubating cultures at 25°C for 5 days (Katan *et al.* 1991). Isolates were identified as *F. oxysporum* morphologically based on characteristics of the macroconidia, phialids, microconidia, chlamydospores, and colony growth traits (Leslie and Summerell 2006). The *forma specialis* of this pathogen was identified using pathogenicity tests (Hibar 2002), based on which the more virulent isolates were selected for this study. The four isolates used in this study are presented in **Table 1**.

Table 1 Forl isolates used for study.

Isolates	Host plant (Cultivar)	Date of isolation
Fo1-05	Durintha	2005
Fo2-05	Romana	2005
Fo3-05	Romana	2005
Fo4-05	Bochra	2005

Tomato cultivars

Tomato seeds (*Lycopersicon esculentum* Mill. cv. 'Riogrande', susceptible to Forl) were sterilised by immersion in absolute ethanol for 7 min, followed by extensive rinsing in sterile distilled

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water (Benhamou *et al.* 1997). Seeds were sown in alveolus plates filled with previously sterilised peat. Seedlings were grown in a growth chamber at 24 to 26°C with a 12-h photoperiod, provided by incandescent lamps (delivering ≈3 $\mu mol.m^{-2}.s^{-1}$), and 70% humidity. They were watered daily and fertilized twice a week with a standard nutrient solution according to Pharand *et al.* (2002).

Experiments were performed with 5-week-old tomato plants carrying five or six fully expanded leaves (Benhamou and Bélanger 1998).

Substrate culture

In this study, six culture substrates were tested: peat, perlite, sand, topsoil, a mixture of compost and perlite (50: 50) and a mixture of compost and sand (50: 50).

Peat and perlite were sterilized by autoclaving at 120°C and at 1 bar for 1½ h. Topsoil was drawn from a plot were Forl had never been detected. Sand was provided from a sandpit. Compost was prepared in the Technical Centre of Organic Agriculture of Tunisia from basic material (Znaidi 2002), mentioned in **Table 2**.

Table 2 Proportions in basic materials for the preparation of the used compost.

Basic materials	Percentage of basic materials
Dairy cow (*Bos taurus*,,Holstein-Friesian) manure	50%
Barbarine sheep (*Ovis aries*) manure	20%
Chicken (*Gallus Gallus*) droppings	20%
Crushed wheat (*Triticum aestivum*)straw	10%

Effect of culture substrate on disease incidence

The effect of the six culture substrates on FCRR disease incidence was evaluated in a growth chamber. Experiments were performed with 5-week-old tomato plants carrying five or six fully expanded leaves (Benhamou and Bélanger 1998). Plants were carefully removed from alveolus plates and transplanted into pots (500 cm^3 volume) filled with one of the substrates.

After the pathogen was cultured in the potato sucrose (PS) medium at 28°C (Yamamoto *et al.* 1990), a spore suspension was obtained. The cultured liquid medium was filtered, and the concentration (10^7 spores/ml) was determined using a Malassez Blade (2*2.5 mm, 0.2 mm depth, quality standard, ref. MAS). After one week, plants with 6-7 leaves were inoculated with 10 ml of the spore suspension of the pathogen applied as a drench. The control plants were similarly treated with sterile distilled water.

Disease severity was recorded on a 0 to 3 visual scale, in which 0 = no symptoms; 1 = light yellowing of leaves, light or moderate rot on taproot and secondary roots and crown rot; 2 = moderate or severe yellowing of leaves with or without wilting, stunting, severe rot on taproot and secondary roots, crown rot with or without hypocotyls rot, and vascular discoloration in the stem; and 3 = dead seedlings (Vakalounakis and Fragkiadakis 1999).

Disease incidence percentage was determined using the following formula (Song *et al.* 2004):

$$\text{Disease incidence}\,(\%) = \left(\frac{\sum \text{scale} \times \text{number of infected plants}}{\text{highest scale} \times \text{total number of plants}} \right) \times 100$$

In this assay we evaluated the effect of culture substrate on plant growth by measuring the weight of the fresh and dry part of shoots and roots.

Ten plants per elementary treatment were used and variance analysis of the treatment effect on measured data was performed by using the general linear model procedure of SPSS (SPSS 10.0). Experiments were analyzed using standard analysis of variance (ANOVA) with factorial treatment structure and interactions. When F values were significant at p>0.05, differences among the treatments were determined by the SNK (Student Newman Keul's) test.

RESULTS AND DISCUSSION

Effect of culture substrate on the disease incidence of Forl

The evaluation of disease incidence under growth chamber conditions showed that culture substrate has a significant effect on the development of FCRR of tomato. Important disease incidence (i.e. when disease incidence exceeded 50%) occurred when tomato plants were transplanted on sand, topsoil, perlite or peat. However, the lowest disease incidence (6.6%) was obtained when tomato culture were transplanted on the mixture of perlite and compost (**Fig. 1**).

By observing the aggressiveness of Forl when tomato plants were transplanted only on perlite (43.3%), we concluded that compost has a suppressive effect on the development of Forl. This beneficial effect of compost was also clear when tomato plants were transplanted on sand. Indeed, disease incidence was reduced from 53.3%, when tomato plants were transplanted only on sand, to 30% when compost was added to sand.

The effect of culture substrate on the development of Forl was studied by Yu and Komada (1998). These authors demonstrated that disease incidence of FCRR of tomato was more important on tomato plants transplanted on rockwool than on those transplanted on bark fiber of hinoki (*Chamaecyparis obtusa*). These same authors demonstrate that these two culture substrates had no effect on leaf number and plant height.

Culture substrate has not only an effect on disease incidence but also on the virulence of pathogens. Indeed, a *Fusarium avenaceum* inoculum produced on puffed wheat was twice as virulent as that produced on marsh reed grass straw amended with malt extract (Winder 1999).

In this study, we noted that addition of compost significantly reduced the disease incidence of Forl. The beneficial effect of compost has been noted by many researchers. Szczech (1999) demonstrated that addition of vermicompost, produced from cattle manure, to different culture substrates (sphagnum peat, pine sawdust and brown coal) has reduced the incidence of Fusarium wilt of tomato cv. 'Remiz F1'. The same author reported that disease incidence values, measured on tomato plants transplanted on the substrate composed of 30% vermicompost and 70% peat, was more important than hose obtained when tomato plants were transplanted on 100% vermicompost.

Similarly, Pharand *et al.* (2002) demonstrated that transplanting tomato plants cv. 'Bonny Best' inoculated with Forl in a mixture of sphagnum peat moss with composted pulp and paper mill residues (3:1 v/v) has significantly reduced disease incidence compared to plants transplanted only on peat.

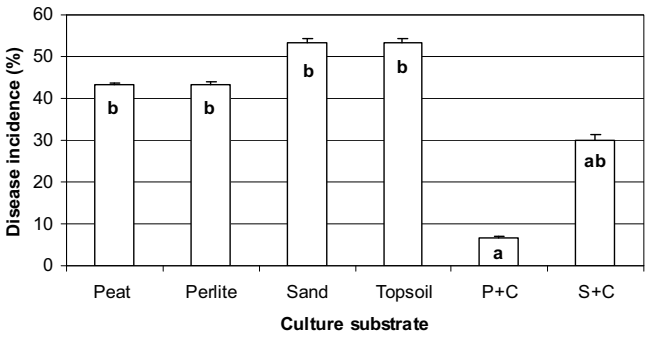

Fig. 1 Disease incidence of Fusarium crown and root rot of tomato (cv. 'Riogrande') measured in the different culture substrates, 60 days after inoculation with Forl. P+C: perlite + compost; S+C: sand+ compost. Values represent the average of 10 plants per elementary treatment. Within columns, means followed by the same letters are not significantly different (*P*=0.05) according to S.N.K. test.

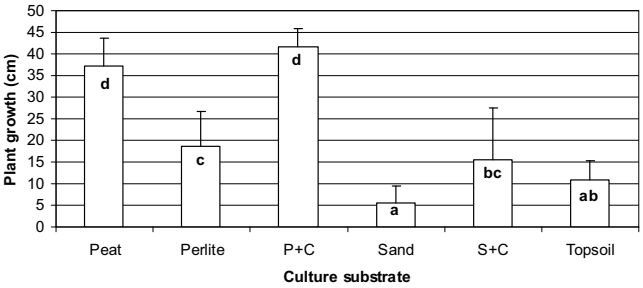

Fig. 2 Plant growth (cm) of tomato plants (cv. 'Riogrande') measured in the different culture substrates, 60 days after inoculation with Forl. P+C: perlite + compost; S+C: sand+ compost. Values represent the average of 10 plants per elementary treatment. Within columns, means followed by the same letters are not significantly different (*P*=0.05) according to S.N.K. test.

Effect of culture substrate on the plant growth

Tomato plants transplanted onto a mixture of perlite and compost were tallest (41 cm), i.e. greatest growth compared to those transplanted only on perlite, which were shorter (18.6 cm) (**Fig. 2**). In this assay, shortest plants occurred when tomato plants were transplanted onto sand or topsoil (5 and 10 cm, respectively). We also noted that peat provided sufficient plant growth with an average of 35 cm.

As shown in **Fig. 3** the beneficial effect of compost on plant height was obviously clear.

Effect of culture substrate on the fresh and dry weight of the shoot part

The highest fresh shoot weight (25 g) was observed on plants transplanted into a mixture of perlite and compost, significantly more than those obtained with other substrates (**Table 3**).

Similarly with dry weight, the best results were obtained with plants transplanted onto a mixture of perlite and compost or onto peat.

Effect of culture substrate on the fresh and dry weight of the root part

Tomato plants transplanted onto a mixture of perlite and compost showed the best root development with an average weight exceeding 13 g. With the other substrates, the fresh weight of the root part never exceeded 4 g (**Table 4**). Considering dry weight of roots, best values were found on a mixture of perlite and compost, significantly higher than all other substrates.

In this study the lowest values of plant growth was obtained when tomato plants were transplanted on sand, unlike the findings of Haddad (2007) who found, in a study done in the South of Tunisia, that tomato plants cv. 'Amel' transplanted onto sand produced more fruits than those transplanted onto perlite or gravel.

This contradiction may be explained by the fact that in the South of Tunisia a traditional method based on a suitable draining system is used.

We also note in this study that addition of compost to the culture substrate significantly improved plant growth and the fresh and the dry weights of the shoot and root parts. Similar results demonstrated the beneficial effect of com-

Table 3 The fresh and the dry weight (g) of the shoot part of tomato plants transplanted into different substrates.

Culture substrate	Fresh weight of the shoot part	Dry weight of the shoot part
Peat	24.98 c	3.12 c
Perlite	8.52 ab	0.61 ab
Perlite+compost	24.14 c	2.98 c
Sand	4.23 a	0.11 a
Sand+compost	10.06 b	0.70 b
Topsoil	5.86 ab	0.19 a

Values represent the average of 10 plants per treatment. Within columns, means followed by the same letters are not significantly different (P=0.05) according to SNK test

Table 4 The fresh and the dry weight (g) of the root part of tomato plants transplanted into different substrates.

Culture substrate	Fresh weight of the root part	Dry weight of the root part
Peat	3.52 b	0.57 b
Perlite	2.33 ab	0.14 a
Perlite+compost	13.24 c	2.45 c
Sand	1.02 ab	0.05 a
Sand+compost	3.3 b	0.65 b
Topsoil	0.84 a	0.04 a

Values represent the average of 10 plants per treatment. Within columns, means followed by the same letters are not significantly different (P=0.05) according to S.N.K. test

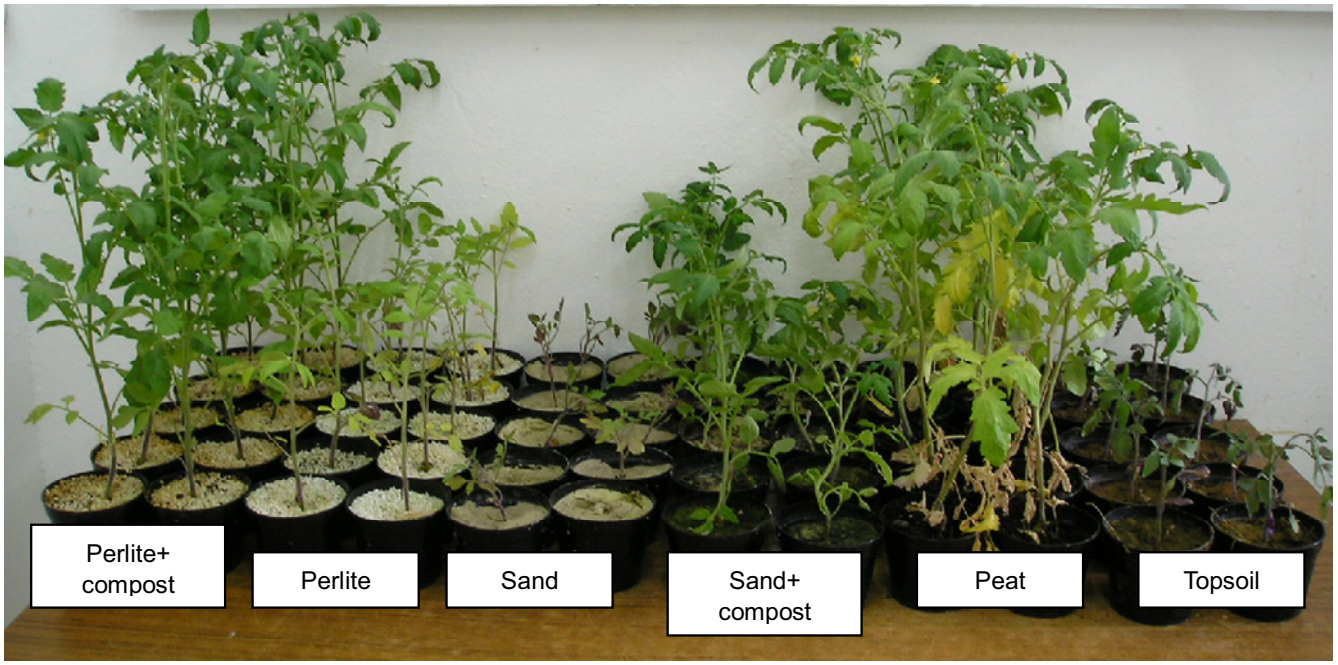

Fig. 3 Plant growth of tomato plants (cv. 'Riogrande') in the different culture substrates, 60 days after inoculation with Forl.

post on plant growth. Indeed, Hibar *et al.* (2006) found that tomato plants transplanted onto peat treated with compost extracts had more vegetative growth than those transplanted only on peat or on a mixture of peat and perlite.

Finally and to be more informative, this study should be repeated under greenhouse conditions to select the best culture substrate.

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