

#### Research Article

# Antifungal activity and growth promotion of three types of compost extracts against *Fusarium oxysporum* and *Fusarium solani* associated with peach seedling decline in nurseries

Sabrine Mannai, Najwa Benfradj, Najet Horrigue-Raouani and Naima Boughalleb-M'Hamdi

High Institute of Agronomy of Chott Mariem, Department of Biological Sciences and Plant Protection, 4042 Sousse, UR13AGR03, University of Sousse, Tunisia.

**Abstract:** Three types of composts (T1, T2, and T3) composed of variable percents of bovine, ovine, fowl manures, green waste and olive pomace were used in this study. Composts were effective in controlling Fusarium oxysporum and Fusarium solani associated with peach decline. In vitro evaluation of four doses of the three composts extracts against mycelium growth of F. solani and F. oxysporum showed that the compost extracts have a significant effect on mycelial growth. The highest inhibition percent was obtained with 10% dose for the three compost extracts, with values more than 48.8% and 50% of F. solani and F. oxysporum, respectively. However, the filtration of the tea composts revealed to be ineffective against the hyphal reduction for both pathogens. The in-vivo experiments exhibited the efficacy of these composts in reducing the seedlings root rot. In fact, T1, T2 and T3 reduced the root rot and browning of plants inoculated with F. solani by 50.19% and plants inoculated with F. oxysporum by 41.86%, 46.45% and 48.3%, respectively. Furthermore, these composts seemed to improve the sanitary state of peach seedlings inoculated with F. oxysporum. However, the improvement of sanitary state of peach inoculated with F. solani was just noted in case of the composts T1 and T2. The treatment of peach seedlings with these improved plant growth by increasing height and root weight of seedlings inoculated with F. oxysporum. However, none of these composts was able to stimulate growth of plants inoculated with F. solani. Root weight of plants inoculated with F. solani was negatively correlated with bovine manure in the compost, positively with fowl (r = 0.69) and sheep manure content (r = 0.69)0.69). Besides, a significant negative correlation among sanitary state index of plants inoculated with F. oxysporum in the case of olive pomace (r = -0.92) and the polyphenols content (r = -0.74) of compost was found.

**Keywords:** Compost extracts, peach decline, *Fusarium* spp., *in vitro* and *in vivo* inhibition

#### Introduction

Peach decline, as a result of seedlings root and collar rot in nurseries, is one of the most

Handling Editor: Vahe Minassian

\*Corresponding author, e-mail: n.boughalleb@laposte.net Received: 1 March 2018 Accepted: 23 July 2018

Published online: 1 August 2018

destructive diseases causing a dramatic reduction in plant growth (Stylianides *et al.* 1985; Haygood *et al.* 1986; Yang *et al.* 2012). Several investigations have shown that *Fusarium* spp., such as *F. equiseti*, *F. moniliforme*, *F. oxysporum* and *F. solani* are frequently isolated from peach orchards with replant symptoms (Wensley 1956; Hine 1961; Nyczepir and Lewis 1984; Nyczepir and

Pusey 1986). However, *F. solani* and *F. oxysporum* were the most predominant species in the stem and root lesions of declining young peach seedlings (Rodriguez and Hernández 2004). Furthermore, Nyczepir and Pusey (1986) reported that these species were responsible for necrosis of peach feeder roots in greenhouse tests and decreased shoot growth and plant height (Nyczepir and Pusey 1986).

agrochemicals Currently, the method remains the most used in the management of peach tree diseases. However, extensive use of fungicides has generated a number of negative effects including environmental pollution and development of pathogen resistance due to the repeated applications of chemicals (Oruc, 2010; Zhang, 2004). Therefore, economic and environmental pressures to reduce reliance on chemical management practices have created a renewed interest in the use of organic soil amendments for suppressing disease and improving general plant health. The application of compost to soil was shown to alter the balance of the soil microflora and suppress soil-borne diseases in field crops (Hoitink et al., 1997).

Compost is often reported as a substrate that is able to suppress soil-borne plant pathogens and the efficiency depends on the type of compost and pathosystem (Termorshuizen *et al.*, 2006). Composts have been shown to suppress several soil pathogens in woody ornamentals and forest tree nurseries (Hoitink and Kuter, 1984; Hoitink *et al.*, 1991; Blok *et al.*, 2002), turfgrass (Craft and Nelson, 1996) and many different vegetable crops (De Ceuster and Hoitink, 1999; Termorshuizen *et al.*, 2006). However, there are few reports documenting the successful use of compost in managing the fruit trees decline.

Composts has two actions (indirect and direct) on plant health. Indirect action is due to its influence on soil structure and balanced nutrient intake (Epstein *et al.*, 1976). The direct action of the compost is more important than the indirect one and it is mainly due to its beneficial microflora and could result in a

reduction of both soil-borne and foliar diseases (Hoitink and Grebus, 1994).

The efficacy of compost to suppress diseases can include one or a combination of competitions for nutrients. antibiosis, production of extracellular enzymes and compounds (El-Masry et al., 2002; Steyaert et al., 2003), parasitism and predation (Hoitink and Fahy, 1986) and host-mediated induction of resistance (Alfano et al., 2007; Yogev et al., 2010). As a result, it is often difficult to determine the exact suppression mechanisms, especially in compost due to the complex structure of the microbial community (Boulter et al., 2000; 2002).

The aim of the present investigation was to (i) evaluate the *in vitro* effectiveness of three composts and their extracts to reduce the development of *F. oxysporum* and *F. solani* associated with peach seedling decline; (ii) in vivo study of disease-suppressive effects of composts on *F. oxysporum* and *F. solani* on peach seedlings.

#### **Materials and Methods**

#### Pathogen isolates used

Two pathogenic isolates of *Fusarium* oxysporum (MF993097) and *Fusarium solani* (MF993094) isolated from peach seedling from Tunisian nurseries in 2013 were used in this study.

#### Composts used

Three types of composts (T1, T2, and T3) were used in this study. They were composed of variable fractions of bovine, ovine, fowl manures, green waste and olive pomace (Table 1), in a specific experimental composting process in the Technical Center of Organic Agriculture (CTAB) at Chott-Mariem, Sousse, Tunisia. The extraction was carried out according to the aerobic method developed by Weltzien (1992). Water was added to different composts (1: 1, volume/volume). The mixture was incubated at 20 to 22 °C with a daily agitation for 5 to 10min. After seven days, the mixtures were filtered.

**Table 1** Composition of used composts.

	Bovine	Fowl	Ovine	Green	Olive
Compos	t manure	manure	manure	waste	pomace
	(%)	(%)	(%)	(%)	(%)
T1	30	30	30	5	5
T2	70	25	0	0	5
T3	70	25	0	5	0

#### **Determination of composts composition**

The Walkley and Black (1934) protocol as modified and described by Naanaa and Susini (1988) was followed. In brief, 0.5g of each fine dry compost sample was put in a beaker. Then, 5 ml of potassium dichromate (8%) and 10ml of concentrated sulfuric acid H<sub>2</sub>SO<sub>4</sub> were added. After cooling, the solution for 30min, 25ml of H<sub>2</sub>O was added and the suspension was whiskered with a glass rod. After standing overnight in the dark, the samples were run in colorimetric analysis and from the calibration curve and the given colorimeter values the carbon values in mg for each sample were deduced. The carbon values for each sample were deduced according to this formula (Naanaa and Susini, 1988):

$$C(\%) = \frac{C(mg) \times 100}{P \times 1000}$$

With:

P: compost weight in g (0.5 g)

C: carbon content in mg.

The determination of the organic matter (OM) is based on the determination of the C% carbon content, using the formula of Naanaa and Susini (1988):

$$OM = (C\% \times 1.724)$$

The methodology of Liang et al., (2003) was used to determine the polyphenols content. Thus, the preparation of the compost extract was made by adding 3 g of the compost to 150 ml of freshly boiled distilled water for 10 min. After filtration of this solution on a 0.2 mm mesh, 1ml of the filtrate was put in a sterilized beaker (25 ml) and mixed with 5 ml of a dye solution  $(3.6 \ 10-3 \ M \ FeSO_4 + 3.5 \ 10-3 \ M$  $KNaC_4H_4O_6$ ), 15 ml of the buffer (0.067 M Na<sub>2</sub>HPO<sub>4</sub> and 0.067M KH<sub>2</sub>PO<sub>4</sub>) and 4ml H<sub>2</sub>O. The absorbance readings were done at 540 nm by a spectrophotometer using a calibration solution prepared with distilled water instead of the compost extract. Polyphenols contents were calculated in mg/g for each sample using the following formula:

Polyphenols (mg.g<sup>-1</sup>) =  $((E1-E2) \times 3.9133 \times 150)/3$ E1: absorbance at 540 nm of the reaction solution.

E2: absorbance at 540 nm of the control reaction solution (5 ml H<sub>2</sub>O + 5 ml dyeing solution + 15 ml of the buffer).

#### In vitro effect of compost extracts on Fusarium spp. hyphal growth

The methodology of Marin et al. (2014) with some modifications was used to study the effect of compost extracts on hyphal growth of Fusarium spp. This technique involves the centrifugation of the compost juice at 10000 rpm for 10 min to remove excess of organic matter. Subsequently, the supernatants were divided into two equal aliquots, one of which was filtered through sterile syringe filters (0.22 ul). The crude supernatant was added to the PDA (Potato Dextrose Agar) medium (45 °C) at different concentrations (0.5, 1, 5 and 10% v: v). Concerning the filtered fractions, six concentrations of each compost juice were used (0.5, 1, 5, 10, 15 and 20% v: v). For the control, the volume of the compost juice was replaced by the same volume of sterile distilled water. Then, plug of 0.6 cm in diameter of pathogen was put in the center of each petri dish (90 mm in diameter). The petri dishes were incubated at 25 °C, in darkness for six days. Three replicates were performed for each concentration and each isolate. The percent of growth inhibition (PI) with respect to the control was calculated according to the following formula (Hmouni et al., 1996): PI =  $((1 - T) / C) \times 100$  Where, T = mean diameter of the colonies in the presence of the compost extract,

C = average diameter of the control colonies.

#### In vivo effect of composts extracts on reducing Fusariums pp. severity

Four-week-old peach seedlings 'Garnem' were used in this study. These seedlings were grown

in pots (23 cm diameter x 23 cm deep) in a greenhouse. The determination of composts and their extracts effect on the aggressiveness of *Fusarium* species was made according to the method of Van Schoor *et al.*, (2009). For this, 12.5% (v/v) of the compost was used with the potting mix. Pots were arranged in a completely randomized design and watered when needed.

To prepare the inoculums of pathogen, ten discs of each pathogen of 7-days-old cultures grown on PDA medium, were incubated for 7 days in an Erlenmeyer flask containing 150ml of PDB (Potato-Dextrose-Browth) stirring (120 rpm). The resulting conidial suspension was adjusted to 10<sup>7</sup> spores/ml hemocytometer. Molasses using planting peach seedlings, 50ml of 10<sup>7</sup> suspension were added Fusarium spp. immediately to each seedling.

On the other hand, the liquid compost extract was prepared in an open plastic bucket, by mixing 1 L of compost with 10 L of water (v/v; 1: 10) and allowed to stand for 24 h. The liquid was then separated from the solid compost by filtering it through cheesecloth. Plants were watered every week during three months with freshly made compost extracts (500 ml / plant). The experiment was conducted as a completely randomized design, and each isolate was tested using three peach seedlings. The inoculated and treated seedlings were grown in a greenhouse, and were harvested after three months.

At the harvest, the disease severity was noted according to the sanitary state (health) of the vegetative part of plants. This parameter is rated onto 0-5 scale, where:  $\mathbf{0} = \text{no}$  obvious symptoms;  $\mathbf{1} = \text{moderate}$  discoloration of plant leaves ( $\leq 25\%$ );  $\mathbf{2} = \text{moderate}$  discoloration and falling leaves ( $\leq 50\%$ );  $\mathbf{3} = \text{moderate}$  discoloration of plant collar, stem and leaves ( $\leq 75\%$ );  $\mathbf{4} = \text{extensive}$  discoloration of plant collar and stem with falling leaves (> 75%); and  $\mathbf{5} = \text{dead}$  plant. Then, peach seedlings were removed from the potting bags and washed under running water to remove excess potting mix adhering to roots. For each seedling the height, root weight and root rot were noted.

Root rot was rated onto a 0-5 scale (0 = no obvious symptoms; 1 = moderate discoloration of root tissue; 2 = moderate discoloration of tissue with some lesion; 3 = extensive discoloration of tissue; 4 = extensive discoloration of tissue with girdling lesions; and 5 = dead plant) (Tewoldemedhin *et al.*, 2011).

#### Statistical analysis

Data were subjected to a one-way analysis of (ANOVA) by using Statistical variance Package for the Social Sciences software (SPSS), version 20.0. The *in vitro* assays were according completely analyzed to randomized factorial model with two factors (compost extract tested and doses used). The in vivo assays were analyzed in a completely randomized model. For all the tests conducted in vitro and in vivo, means were separated using Student-Newman-Keul's (SNK) test (P  $\leq 0.05$ ).

Correlation analyses between the disease severity and plant growth parameters with the composition and some characteristics of composts tested were carried out using Pearson's correlation analysis at  $P \leq 0.05$ .

#### Results

#### **Composts composition**

Results showed that the percent of organic carbon and matter, of the different composts were close. Indeed, the organic carbon percent ranged from 4.63 for T2 to 5.08 for T1. The organic matter percent also ranged from 7.98 for T2 to 8.74 for T1. For the polyphenol content, the highest value (8.61 mg/g) was found in T2, whereas, the lowest (1.37 mg/g) was found in T3 (Table 2).

**Table 2** Some chemical parameters of analyzed composts.

Composts <sup>1</sup>	Organic carbon (%)	Organic matter (%)	Polyphenol content (mg/g)
T1	5.08	8.74	4.30
T2	4.63	7.98	8.61
T3	4.87	8.39	1.37

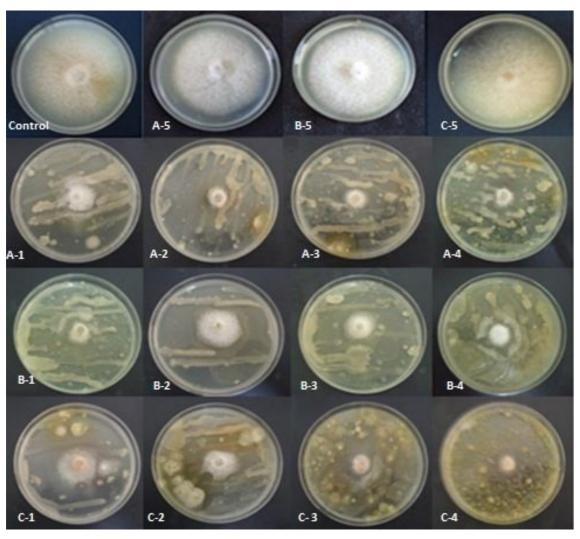
For composts refer to table 1.

### In vitro effect of compost extracts on Fusarium spp. hyphal growth

The variance analysis revealed a significant effect ( $P \le 5\%$ ) of the dose on the hyphal growth inhibition of F. solani. Indeed, the highest percent of growth inhibition was obtained with the dose D4 (10%) for the three compost extracts (Fig. 1 and table 3). These growth inhibition percents were 48.84%, 54.26% and 56.59% for T1, T2 and T3 respectively. While, the efficacy of the tested

compost extracts against *F. solani* was statistically comparable.

The *in vitro* evaluation of the efficiency of different doses of the compost extracts on F. oxysporum hyphal growth showed a significant inhibition (P  $\leq$  5%). The highest percent of growth inhibition was obtained with D4 (10%) for the three compost extracts used. Indeed, the inhibition percents recorded were 54.95%, 57.83% and 69.01% for T1, T2 and T3 respectively (Table 3).



**Figure 1** *In-vitro* evaluation of different doses (1, 2, 3, 4) of three composts (A, B, C) on radial growth of *Fusarium solani* recorded after six days of incubation on PDA at 25 °C in comparison to the untreated control (above, on the left corner). **A:** Compost T1, **B:** Compost T2, **C:** Compost T3; (1 to 4): doses of unfiltered composts at the rates of 0.5%; 1%; 5%; and 10%. A-5, B-5, C-5 (above to the right) dose 10% of filtered composts. For composts refer to table 1.

**Table 3** Percent hyphal growth inhibition of *Fusarium solani* and *Fusarium oxysporum* at different doses of three unfiltered compost extracts recorded six days after incubation at 25 °C.

Concentrations	Fusarium solani	į		Fusarium oxysporum			
	T1	T2	Т3	T1	T2	Т3	
Dose1	$42.64 \pm 12.53^{a^*A}$	$42.64 \pm 8.86$ abA	$28.68 \pm 2.53$ aA	$40.26 \pm 8.94^{a^*A}$	$46.65 \pm 8.50$ abA	$46.65 \pm 15.35$ Aa	
Dose2	44.96 ± 9.35aB**	32.95 ± 1.48aA	$27.52 \pm 2.33$ aA	47.28 ± 11.59aA**	$42.81 \pm 15.24abA$	$46.33 \pm 15.65$ Aa	
Dose3	$44.19 \pm 1.27$ aA	$47.29 \pm 15.24abA$	$47.29 \pm 5.94$ bA	$47.28 \pm 11.01$ aA	$33.87 \pm 11.96$ aA	$63.90 \pm 2.18$ Ab	
Dose4	$48.84 \pm 1.27$ aA	$54.26 \pm 3.69$ bA	$56.59 \pm 10.52$ bA	$54.95 \pm 5.04$ aA	$57.83 \pm 3.61$ bA	$69.01 \pm 1.92$ Ab	

<sup>(\*)</sup> Means  $\pm$  standard error in the column followed by the same small letter are not significantly different according to SNK test at  $P \le 0.05$ .

For T1, T2 and T3: composts refer to table 1.

The compost extract T3 was the most effective compared to T1 and T2. In fact, 63.9% mycelial growth inhibition was obtained at D3 (5%). However, the inhibition percent generated by T1 and T2 was between 47.28% and 33.87% for the same dose (Table 3 and Fig. 2).

The sterilization of compost extracts using micro-filters  $(0.22\mu\text{m})$  completely nullified their inhibitory effect on hyphal growth of *Fusarium oxysporum* and *F. solani* even at highest concentration (Fig. 1 and 2 A-5 to C-5).

### In vivo effect of compost extracts in reducing Fusarium spp. severity

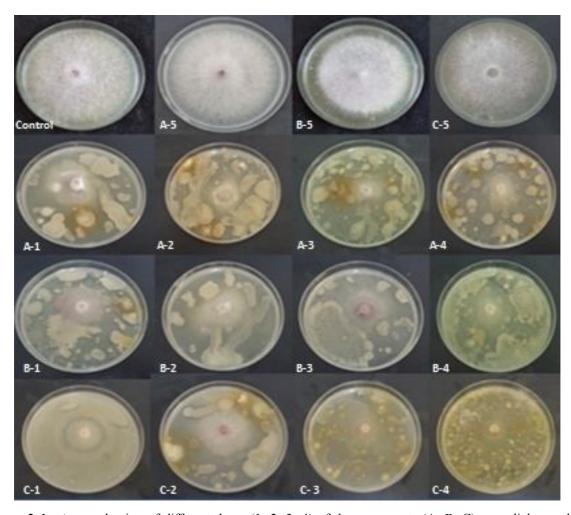
The variance analysis of disease severity parameters recorded three months after inoculation showed that the three composts tested reduced root rot induced by F. solani by 50.19%. Whereas, only T1 and T2 improved in a non-significant way the sanitary state of the plants inoculated by F. solani by 22% and 16.5%, respectively (Table 4 and fig. 3). On the other hand, these three composts did not stimulate the plant growth parameters (height and root weight) (table 4). For F. oxysporum, T1, T2 and T3 reduced significantly browning of the root system by 41.86%, 46.45% and 48.3%, respectively. In addition, T1 and T2

improved the sanitary state of the plants in a non-significant way by 50%. Also, the treatment with the composts T1 and T2 improved plant growth by increasing the height by 26% and 11.27%, respectively and the root weight by 35.11% and 10.67%, respectively (Fig. 4 and table 4).

## Correlations between the efficacy of composts on the severity index of *Fusarium* spp. and plant growth parameters with the composts composition

Pearson's correlation analysis demonstrated that root weight of plants inoculated with F. solani was significantly and negatively related to bovine manure percent of compost (r = -0.69), positively and significantly correlated with fowl (r = 0.69) and sheep manure percent (r = 0.69). Thus, the peach plant growth was decreased with a high concentration of bovine manure and low concentration of sheep and fowl manure in compost. Besides, there was a significant negative correlation of sanitary state index of plants inoculated with F. oxysporum and olive pomace percentage in compost (r = -0.92) and the polyphenols content (r = -0.74)of compost (Table 5).

<sup>(\*\*)</sup> Means  $\pm$  standard error in a row for each pathogen, followed by the same capital letter are not significantly different according to SNK test at  $P \le 0.05$ .



**Figure 2** *In-vitro* evaluation of different doses (1, 2, 3, 4) of three composts (A, B, C) on radial growth of *Fusarium oxysporum* recorded after six days of incubation on PDA at 25 °C in comparison to the untreated control (Plate above on the extreme left). **A:** Compost T1, **B:** Compost T2, **C:** Compost T3; (1 to 4): doses of unfiltered composts at the rates of 0.5%; 1%; 5%; and 10%. A-5, B-5, C-5 (above to the right) dose 10% of filtered composts. For composts refer to table 1.

**Table 4** Effect of composts and their extracts on root rot, sanitary state, growth and root weight of 'Garnem' peach seedlings grown in greenhouse and artificially infested with *Fusarium solani* MF993094 and *Fusarium oxysporum* MF993097 isolates, as recorded after three months.

					Fusarium oxysporum			
Compost	Root rot	Sanitary state	Height (cm)	Root weight (g)	Root rot	Sanitary state	Height (cm)	Root weight (g)
NIC	$1.33 \pm 0.58^{a^*}$	$1.67 \pm 0.58^{a}$	$62.40 \pm 2.26^{a}$	$4.79 \pm 0.37^{a}$	$1.33 \pm 0.58^{a}$	$1.67 \pm 0.58^{a}$	$62.40 \pm 2.26^{a}$	$4.79 \pm 0.37^{a}$
IC	$2.67\pm0.58^a$	$2.00\pm1.00^a$	$55.00 \pm 10.11^{a}$	$4.50 \pm 0.55^{a}$	$2.67\pm0.58^b$	$2.00 \pm 1.00^{a}$	$55.00 \pm 10.11^{a}$	$4.50 \pm 0.55^a$
T1	$1.33\pm0.58^a$	$1.56 \pm 0.58^a$	$48.96 \pm 9.53^a$	$4.37\pm1.52^a$	$1.00\pm0.00^a$	$1.00\pm0.00^a$	$69.30 \pm 18.25^{a}$	$6.08\pm1.21^a$
T2	$1.33\pm0.58^a$	$1.67\pm0.58^a$	$46.60 \pm 4.52^a$	$4.22\pm1.29^a$	$1.33\pm0.58^a$	$1.00\pm0.00^a$	$61.20 \pm 6.54^a$	$4.98\pm1.02^a$
T3	$1.33\pm0.58^a$	$2.00 \pm 1.00^{a}$	$52.97 \pm 9.58^{a}$	$4.16 \pm 0.81^{a}$	$1.33\pm0.58^a$	$2.33\pm0.58^a$	$52.93 \pm 7.87^{a}$	$4.41 \pm 0.61^{a}$

<sup>(\*)</sup> Means  $\pm$  standard error in the column followed by the same letter are not significantly different according to SNK test at  $P \le 0.05$ . NIC:uninoculated control; IC: inoculated control; for T1, T2 and T3 composts refer to table 1.



**Figure 3** Effect of three composts on peach seedlings growth and sanitary state, 3 months after inoculation with *F. solani*. **A:** Inoculated control; **B:** plant inoculated and treated with T1; **C:** plant inoculated and treated with T2; **D:** plant inoculated and treated with T3. For composts T1, T2 and T3 refer to table 1.



**Figure 4** Effect of three composts on peach seedling growth, 3 months after inoculation with *F. oxysporum*. **A:** Inoculated control; **B:** plant inoculated and treated with T1; **C:** plant inoculated and treated with T2; **D:** plant inoculated and treated with T3. For composts refer to table 1.

**Table 5** Correlation coefficients among the efficacy of composts on the severity index of *Fusarium* spp., plant growth parameters, and the composition of tested composts.

Entries	Inoculated with F. oxysporum				Inoculated with F. solani			
	Н	RR	RW	SS	Н	RR	RW	SS
СО	0.26	-0.32	0.40	0.03	0.29	0.00	0.57	-0.19
MO	0.25	-0.32	0.40	0.04	0.29	0.00	0.57	-0.19
Polyphenol	0.23	0.04	0.15	-0.74*	-0.37	0.00	-0.06	-0.16
Bovine	-0.48	0.38	-0.62	0.46	-0.11	0.00	-0.69*	0.35
Fowl	0.48	-0.38	0.62	-0.46	0.11	0.00	$0.69^{*}$	-0.35
Sheep	0.48	-0.38	0.62	-0.46	0.11	0.00	$0.69^{*}$	-0.35
Green waste	-0.00	-0.19	0.12	0.46	0.37	0.00	0.33	0.00
Olive pomace	0.49	-0.19	0.499	-0.92**	-0.25	0.00	0.36	-0.35

<sup>(\*\*)</sup> significant correlation at  $P \le 0.01$ .

#### **Discussion**

The test of different doses of composts extracts *in vitro* against *F. solani* and *F. oxysporum* showed their efficacy to inhibit the hyphal growth. Several previous studies have shown that water extracts of composts have a strong inhibitory effect on mycelium growth of *Fusarium* spp. isolated from different other plants (El kinany *et al.*, 2017; Suárez-Estrella *et al.*, 2012). There are no reports documenting the successful use of compost *in vitro* to inhibit hyphal growth of pathogenic *Fusarium* spp. associated with peach seedling decline.

However, in our study, the sterilized compost extract, at different concentrations (0.5; 1; 5; 10; 15 and 20%), didn't show any inhibitory effect on the mycelial growth of *F. solani* and *F. oxysporum*. These findings are in agreement with previous studies reporting that sterilization largely negates the disease-suppressive capacity of composts (Larkin *et al.*, 1993; Mandelbaum *et al.*, 1988; Reuveni *et al.*, 2002), which suggests that compost efficiency is associated with microbial activity (Hoitink and Fahy, 1986).

Effect of compost extracts appears to be associated with live microorganisms through: induced resistance, antibiosis or competition for nutrients or space (Scheuerell and Mahaffee, 2002).

For in vivo tests, the analysis of the variance of disease severity parameters recorded three months after inoculation showed that the three composts tested reduced root rot and root browning caused by F. solani and F. oxysporum on peach seedlings. These findings are in agreement with previous studies reporting the capacity of composts to reduce the incidence of crown and root-rot diseases in tomato as well as population of the causal pathogen, Fusarium oxysporum f. sp. radicis-lycopersici. In another study, different Fusarium pathogens were suppressed by exposure to the same indicating a relatively composts, spectrum of effectiveness for each of the tested composts (Yogev et al., 2006). There are no reports documenting the successful use of compost in managing pathogenic Fusarium spp. associated with peach seedling decline.

Compost amendments can modify the microbial community composition, enhance the antagonism among microbes and decrease the plant pathogens activity (Hoitink and Boehm, 1999). Also, composts are important source of nutrients usable by the microorganisms (Bailey and Lazarovits, 2003). As a consequence, compost amendments generally enhance the development of the microflora,, increase the soil microbial activity and so increase the competition effects in the soil (Bailey and Lazarovits, 2003). In the present study, treatment

<sup>(\*)</sup> Significant correlation at  $P \le 0.05$ .

H: Height, RR: root rot index, RW: root weight, SS: sanitary state

with the composts T1 and T2 improved plants growth by increasing their height and root weight in the presence of F. oxysporum. Similar results have been reported by Van Schoor et al. (2009) who showed that the compost and compost extracts significantly increased seedling growth parameters for several cases of the Apple Replant Disease (ARD) soils tested, suggesting that they can reduce the effects of ARD by supplying nutrients. In addition, Pharand et al. (2002) suggested that the beneficial effect of compost in reducing disease symptoms is associated with induced plant resistance to fungal colonization whereby, various Fusarium wilts, e.g. that of chrysanthemum, flax, cucumber and sweet basil were also suppressed by composts (Chef et al., 1983; Kannangara et al., 2000; Reuveni et al., 2002).

On the contrary, in the present study, the compost T3 did not stimulate the growth parameters of peach seedlings; this may be due to the compost source (absence of sheep manure and olive pomace in T3) and/or some other characteristics of compost like the polyphenol content, the organic carbon and organic matter.

The general biological activity of the soil is also stimulated by the addition of an available carbon source (Magarey, 1999) and soils with a diversity of beneficial microorganisms are more likely to be suppressive to disease development (Lazarovits, 2001). In our study, a non-significant positive correlation was observed between the percent of carbon matter of composts and the root weights of peach plants inoculated with *F. oxysporum* and *F. solani*.

In addition, results of this study showed a negative significant correlation between the root weights of plants inoculated with *F. solani*, and the percentage of bovine manure in the compost. Also, the same plant growth parameter is positively and significantly related with percent of fowl and sheep manure used in the composition. In the same way, previous researchers showed that the compost sources may influence the efficacy of compost extracts. Indeed, the composts derived from animal manures and undigested plant materials

are considered more suitable for producing disease suppressive extracts (Elad Shteinberg, 1994; Scheuerell and Mahaffee, 2004; 2006). Scheuerell and Mahaffee (2004) observed that the extracts prepared from composted chicken manure significantly suppressed damping-off of cucumber caused by Pythium ultimum compared to extracts derived from other classes like vermicompost. In addition, the cattle manure has been reported effective for the control of Fusarium root and stem rot in cucumber plants (Kannangara et al., 2004) and Fusarium crown and root rot in tomato plants (Raviv et al., 2005). There are also reports of the suppression of Fusarium spp. diseases that have focused on different composts, like coffee-waste composts for the control of Fusarium wilt in melon plants (Ros et al., 2005), pulp and paper mill (Pharand et al., 2002) or tomato residues (Cheuk et al., 2005) for the control of Fusarium crown and root rot in tomato plants.

Moreover, in our study there was a negative correlation of the sanitary state index with the olive pomace percentage and the polyphenols content of composts. These findings are in agreement with previous studies reporting the capacity of olive mill waste (OMW) and polyphenols, for high level of antifungal activity against F. solani and Rhizoctonia solani (Yangui et al., 2008). Several researchers have demonstrated that only a few micro-organisms are able to survive in OMW, because it contains various simple and complex phenolic compounds characterized by high antimicrobial activity (Capasso et al., 1995; Kistner et al., 2004). Furthermore, species of *Bacillus*, Burkholderia and Pseudomonas were isolated from OMW which exhibited antimicrobial activity against two soil-borne plant pathogenic fungi F. solani and R. solani (Yangui et al., 2008). In addition, some phytopathogenic like Pseudomonas syringaepv. savastanoi, Corynebacterium michiganense and Xanthomonas campestris are inhibited by polyphenols present in OMW in their original concentration (18.86 mg.ml<sup>-1</sup>) (Ciafardini and Zullo, 2003).

Thus, the olive wastes are of great interest for agronomic use and for potential contribution to the biological control of peach decline disease. The specific composition of this product such as the presence of phytotoxic compounds including polyphenols, organic acids, lipids, salts, the lignin and cellulose content (Komilis *et al.*, 2005) and their relative concentrations are very important.

#### **Conflict of Interests**

We have no conflict of interest

#### References

- Alfano, G., Lewis Ivey, M. L., Cakir, C., Bos, J. I. B., Miller, S. A., Madden, L. V., Kamoun, S. and Hoitink, H. A. J. 2007. Systemic modulation of gene expression in tomato by *Trichoderma hamatum*. Phytopathology, 97: 429-437.
- Bailey, K. L., Lazarovits, G., 2003. Suppressing soilborne diseases with residue management and organic amendments. Soil Tillage Research, 72: 169-180.
- Blok, W. J., Coenen, T., Puji, A. S. and Termorshuizen, A. J., 2002. The Netherlands-Suppressing disease in potting mixes with composted biowastes. Biocycle, 43, 58.
- Boulter, J. I., Boland, G. J. and Trevors, J. T. 2000 Compost: a study of the development process and end-product potential for suppression of turfgrass disease. World Journal of Microbiology and Biotechnology, 16: 115-134.
- Boulter, J. I., Boland, G. J. and Trevors, J. T. 2002. Evaluation of composts for suppression of dollar spot (*Sclerotinia homoeocarpa*) of turfgrass. Plant Disease, 86: 405-410.
- Capasso, R., Evidenti, A., Schivo, L., Marcialis, M. A. and Cristinzio, G. 1995. Antibacterial polyphenols from olive mill waste waters. Journal of Applied Bacteriology, 79: 393-398.
- Chef, D. G., Hoitink, H. A. J. and Madden, L. V. 1983. Effects of organic components in container media on suppression of *Fusarium*

- wilt of chrysanthemum and flax. Phytopathology, 73: 279-281.
- Cheuk, W., Kwang, V. L., Copeman, R., Joliffe, P. and Fraser, S. 2005. Disease suppression on greenhouse tomatoes using plant waste compost. Journal of Environmental Science and Health Part B, 40: 449-461.
- Ciafardini, G. and Zullo, B. A. 2003. Antibacterial activity of oil mill water polyphenols on the phytopathogen *Xanthomonas campestris* spp. Annals of Microbiology, 53: 283-290.
- Craft, C. M. and Nelson, E. B. 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. Applied and Environmental Microbiology, 62: 1550-1557.
- De Ceuster, T. J. J. and Hoitink, H. A. J. 1999. Prospects for composts and biocontrol agents as substitutes for methyl bromide in biological control of plant diseases. Compost Science and Utilization, 7: 6-15.
- Elad, Y. and Shteinberg, D. 1994. Effect of compost water extracts on grey mold (*Botrytis cinerea*). Crop Protection, 13: 109-114.
- El kinany, S., Achbani, E., Haggoud, A., Ibijbijen, J., Belmalha, S., Rachidi, F., Echchgadda, G. and Bouamri, R. 2017. *In vitro* evaluation of compost extracts efficiency as biocontrol agent of date palm *Fusarium* wilt. African Journal of Microbiology Research, 11: 1155-1161.
- El-Masry, M., Khalil, A., Hassouna, M. and Ibrahim, H. 2002. *In situ* and *in vitro* suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World Journal of Microbiology and Biotechnology, 18: 551-558.
- Epstein, E., Taylor, J. M. and Chaney, R. L. 1976. Effects of sewage sludge and sludge compost applied to soil on some soil physical and chemical properties. Journal of Environmental Quality, 5: 422-426.
- Haygood, R. A., Graves, C. H., and Ridings, W. H. 1986. *Phytophthora* root rot and stem

- canker of peach trees in Mississippi. Plant Disease, 70: 866-868.
- Hine, R. B. 1961. Role of fungi in peach replant problem. Plant Disease, 45: 462-465.
- Hmouni, A., Hajlaoui, M. R. and Mlaiki, A. 1996. Résistance de *Botrytis cinerea*aux benzimidazoles et aux dicarboximides dans les cultures abritées de tomate en Tunisie. *OEPP/EPPO Bull.* 26: 697-705.
- Hoitink, H. A. J. and Boehm, M. J. 1999. Biocontrol within the context of soil microbial communities: a substratedependent phenomenon. Annual Review of Phytopathology, 37: 427-446.
- Hoitink, H. A. J. and Grebus, M. E. 1994. Status of biological control of plant diseases with composts. Compost Science and Utilization, 2: 6-12.
- Hoitink, H. A. J. and Fahy, P. C. 1986. Basis for the control of soilborne plant pathogens with composts. Annual Review of Phytopathology, 24: 93-114
- Hoitink, H. A. J., Inbar, Y., Boehm, M. J. 1991. Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. Plant Disease, 75: 869-873.
- Hoitink, H. A. J. andKuter, G. A. 1984. Role of composts in suppression of soilborne plant-pathogens of ornamental plants. Biocycle, 25: 40-42.
- Hoitink, H. A. J., Stone, A. G. and Han, D. Y. 1997. Suppression of plant diseases by composts. HortScience, 32: 184-187.
- Kannangara, T., Utkhede, R. S. and Bactawar, B. 2004. Compost effect on greenhouse cucumbers and suppression of plant pathogen *Fusarium oxysporum*. Compost Science and Utilization, 12: 308-313.
- Kannangara, T., Utkhede, R., Paul, J. W. and Punja, Z. K. 2000.Effects of mesophilic and thermophilic composts on suppression of *Fusarium* root and stem rot of greenhouse cucumber. Canadian Journal of Microbiology, 46: 1021-1028.
- Kistner, T., Nitz, G. and Schnitzler, W. H. 2004. Adding olive mill waste water to hydroponic nutrient solutions: a potential

- agent against microbial diseases. Acta Horticulturae, 659: 315-321.
- Komilis, D. P., Karatzas, E. and Halvadakis, C. P. 2005. The effect of olive mill waste water on seed germination after various pretreatment techniques. Journal of Environmental Management, 74: 339-348.
- Larkin, R. P., Hopkins, R. P. and Martin, F. N. 1993. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to *Fusarium* wilt of watermelon. Phytopathology, 83: 1097-1104.
- Lazarovits, G. 2001. Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. Canadian Journal of Plant Pathology, 23: 1-7.
- Liang, Y., Lu, J., Zhang, L., Wu, S. and Wu, Y. 2003. Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. Food Chemistry, 80: 283-290.
- Magarey, R. C. 1999. Reduced productivity in long term monoculture: where are we placed? Australasian Plant Pathology, 28: 11-20.
- Mandelbaum, R., Hadar, Y. and Chen, Y. 1988. Composting of agricultural wastes for their use as container media: effect of heat treatments on suppression of *Pythium aphanidermatum* and microbial activities in substrates containing compost. Biological Wastes, 26: 261-274.
- Marin, F., Dianez, F., Santos, M., Carretero, F., Gea, F. J., Castaneda, C. Navarro. M. J. and Yau, J. A. 2014. Control of *Phytophthora capsici* and *Phytophthora parasitica* on pepper (*Capsicum annuum*L.) with compost teas from different sources, and their effects on plant growth promotion. Phytopathologia Mediterranea, 53: 216-228.
- Naanaa, M. and Susini, J. 1988. Méthodes d'analyse physique et chimique des sols. Directions des sols. Ministère de l'agriculture, 118: 61-63.
- Nyczepir, A. P. and Lewis, S. A. 1984. Incidence of *Fusarium* and *Pythium* spp. in

- peach feeder roots as related to dibromochloropropane application for control of *Criconemella xenoplax*. Plant Disease, 68: 497-499.
- Nyczepir, A. P. and Pusey, P. L. 1986. Association of *Criconemella xenoplax* and *Fusarium* spp. with root necrosis and growth of peach. Journal of Nematology, 18: 217-220.
- Oruc, H. H. 2010. Fungicides and their effects on animals. In: Fungicides, Carisse, O. (Ed.) In-Tech Publishers. pp. 349-362. doi: 10.5772/555.
- Pharand, B., Carisse, O. and Benhamou, N. 2002. Cytological espects of compost-mediated induced resistance against *Fusarium* crown and root rot in tomato. Phytopathology, 92: 424-438.
- Raviv, M., Oka, Y., Katan, J., Hadar, Y., Yogev, A., Medina, S., Krasnovsky, A. and Ziadna, H. 2005. High-nitrogen compost as a medium for organic container-grown crops. Bioresource Technology, 96: 419-427.
- Reuveni, R., Raviv, M., Krasnovsky, A., Freiman, L., Medina, Sh., Bar, A. and Orion, D. 2002. Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop Protection, 21: 583-587.
- Rodriguez, R. P. and Hernández, E. 2004. Mucor foliar spot and mycoflora in stem and root lesions of peach. Journal of Agriculture of the University of Puerto Rico, 88: 155-160.
- Ros, M., Hernandez, M. T., Garcia, C., Bernal, A. and Pascual, J. A. 2005. Biopesticide effect of green compost against *Fusarium* wilt on melon plants. Journal of Applied Microbiology, 98: 845-854.
- Scheuerell, S. and Mahaffee, W. 2002. Compost tea: principles and prospects for plant disease control. Compost Science & Utilization, 10: 313-338.
- Scheuerell, S. J. and Mahaffee, W. F. 2006. Variability associated with suppression of gray mold (*Botrytis cinerea*) on Geranium by foliar applications of nonaerated and aerated compost teas. Plant Disease, 90: 1201-1208.
- Scheuerell, S. J. and Mahaffee, W. F. 2004. Compost tea as a container medium drench

- for suppressing seedling damping-off caused by *Pythium ultimum*. Phytopathology, 94: 1156-1163.
- Stylianidis, D. C., Chitzanidis, A. and Theochari-Athanasiou, I. 1985. Evaluation of resistance to *Phytophthora* spp. and *Rhizoctonia solani* in stone fruit rootstocks. Options Méditerranéennes, 85: 73-78.
- Steyaert, J. M., Ridgway, H. J., Elad, Y. and Stewart, A. 2003. Genetic basis of mycoparasitism: a mechanisms of biological control by species of *Trichoderma*. New Zealand Journal of Crop and Horticultural Science: 31: 281-291.
- Suárez-Estrella, F., Bustamante, M. A., Moral, R., Vargas-García1, M. C., López, M. J. and Moreno, J. 2012. *In vitro* control of *Fusarium* wilt using agroindustrial subproduct-based composts. Journal of Plant Pathology, 94: 59-70.
- Termorshuizen, A. J., Van Rijn, E., Van der Gaag, D. J., Alabouvette, C., Chend, Y., Lagerlof, J., Malandrakis, A. A. Paplomatas, E. J. Ramert, B., Ryckeboer, J., Steinberg, C. and Zmora-Nahum, S. 2006. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. Soil Biology & Biochemistry, 38: 2461-2477.
- Tewoldemedhin, Y. T., Mazzola, M., Spies, C. F. J. and McLeod, A., 2011. Characterization of fungi (*Fusarium* and *Rhizoctonia*) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. European Journal of Plant Pathology, 130: 215-229.
- Van Schoor, L., Denman. S. and Cook, N. C. 2009. Characterization of apple replant disease under South African conditions and potential biological management strategies. Scientia Horticulturae, pp. 153-162.
- Wensley, R. N. 1956. The peach replant problem in Ontario: IV. Fungi associated with replant failure and their importance in fumigated and nonfumigated soils. Canadian Journal of Botany, 34: 967-981.
- Weltzien, H. C. 1992. Biocontrol of foliar fungal disease with compost extracts. In: Microbial Ecology of Leaves, Andrews, J.

- H. and Hirano, S. S. (Eds.). New York: Springer-Verlag, pp: 430-450.
- Yangui, T., Rhouma, A., Triki, M. A., Gargouri, K. and Bouzid, J. 2008. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Protection, 27: 189-197.
- Yang, J., Ruegger, P. M., McKenry, M. V., Becker, J. O., and Borneman, J. 2012. Correlations between Root-Associated Microorganisms and Peach Replant Disease Symptoms in a California Soil. PLoSONE7 (10): e46420. doi:10.1371/journal.pone.0046420.
- Yogev, A., Raviv, M., Cohen, R., Ganayim, N., Aba-Tuama, M., Yunis, H., Hadar, Y. and Katan, J. 2006. Prevention and control of *Fusarium* crown rot in cucumber in greenhouses. Phytoparasitica, 34: 313 (Abstr.).
- Yogev, A., Raviv, M., Hadar, Y., Cohen, R., Wolf, S., Gil, L. and Katan, J. 2010. Induced resistance as a putative component of compost suppressiveness. Biological Control, 54: 46-51.
- Zhang, J. Y. 2004. The hazards and countermeasures of chemical pesticides. Yunnan Environmental Science, 23: 23-26.

## فعالیت ضدقارچی و تحریک رشدی سه نوع عصاره کمپوست علیه Fusarium oxysporum و عامل مرگ گیاهچههای هلو در نهالستانها

سابرین منی، نجوا بنفرد، نجت هریگ-راوانی و نایما بفلبه ماحمدی\*

مؤسسه تحقیقات زراعی چوت ماریم، گروه علوم زیستی و گیاهپزشکی، دانشگاه سوس، سوس، تونس. \* پست الکترونیکی نویسنده مسئول مکاتبه: n.boughalleb@laposte.net دریافت: ۱۰ اسفند ۱۳۹۶؛ پذیرش: ۱ مرداد ۱۳۹۷

چکیده: در این پژوهش سه نوع کمپوست T1، T1 و T3 شامل درصد متفاوتی از کود گاوی، کود گوسفندی، کود مرغی، زباله سبز و تفاله زیتون مورد بررسی قرار گرفت. کمپوستها در کنترل قارچهای Fusarium oxysporum و Fusarium solani عامل بیماری مرگ گیاهچه هلو مؤثر بودند. ارزیابیها نشان داد که چهار غلظت از عصارههای حاصل از کمپوستها توانستند رشد میسلیوم قارچهای F. solani و F. oxysporum را مهار نمایند. در غلظت ۱۰ درصد بالاترین مهار به میزان چای روی کاهش رشد هر دو گونه قارچ بی تأثیر بود. همچنین آزمایشها نشان داد که کمپوستها روی پوسیدگی ریشه گیاهچهها مؤثر بودند. در واقع تیمارهای یک تا سه پوسیدگی ریشه و قهوهای شدن گیاه توسط F. solani را به میزان ۵۱/۱۹ درصد کنترل نمودند. اما پوسیدگی ریشه در گیاهان تلقیح شده با F. oxysporum بهترتیب به میزان ۴۱/۸۶ ۴۶/۴۵ و۴۸/۳۰ درصد کنترل گردید. بهعلاوه، بهنظر میرسد که این کمپوستها باعث بهبود وضعیت درمان گیاهچههای تلقیح شده با قارچ F. oxysporum شدند. اما در مورد قارچ F. solani تیمارهای اول و دوم تأثیر بیشتری داشتند. گیاهچههای هلو که با قارچ F. oxysporum تلقیح شده بودند در حضور کمپوست موجب تحریک افزایش ارتفاع بوته و وزن ریشه شدند. هیچیک از کمپوستها نتوانستند گیاهچههای تلقیح شده با F. solani را تحریک به رشد نمایند. وزن ریشه در بوتههای تلقیح شده با F. solani در حضور کود گاوی رابطه منفی داشت اما با کود مرغی (r = ٠/۶۹) و کود گوسفندی (۴۹ - ۰/۶۹) رابطه مثبت نشان داد. به علاوه یک رابطه منفی میان بهبود شاخص بهداشتی در گیاهان تلقیح شده با F. oxysporum و تفاله زیتون ( $r = -\frac{1}{2}$ ) و میزان پلیفنلها ( $r = -\frac{1}{2}$ ) مشاهده شد.

واژگان کلیدی: عصاره کمپوست، مرگ گیاهچه هلو، فوزاریوم، تلقیح در محیط زنده و غیرزنده