

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

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**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 ( $r = 0.69$ ). 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

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

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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).

Currently, the agrochemicals 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.

| Compost | Bovine manure (%) | Fowl manure (%) | Ovine manure (%) | Green waste (%) | Olive 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):

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

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):

$$\text{OM} = (C\% \times 1.724)$$

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<sup>-3</sup> M FeSO<sub>4</sub> + 3.5 10<sup>-3</sup> M KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>), 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:

$$\text{Polyphenols (mg.g}^{-1}\text{)} = ((E1-E2) \times 3.9133 \times 150)/3$$

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 μl). 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) × 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) with stirring (120 rpm). The resulting conidial suspension was adjusted to  $10^7$  spores/ml using Molasses hemocytometer. After planting peach seedlings, 50ml of  $10^7$  *Fusarium* spp. suspension were added 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: **0** = no obvious symptoms; **1** = moderate discoloration of plant leaves ( $\leq 25\%$ ); **2** = moderate discoloration and falling leaves ( $\leq 50\%$ ); **3** = moderate discoloration of plant collar, stem and leaves ( $\leq 75\%$ ); **4** = extensive discoloration of plant collar and stem with falling leaves ( $> 75\%$ ); and **5** = 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 variance (ANOVA) by using Statistical Package for the Social Sciences software (SPSS), version 20.0. The *in vitro* assays were analyzed according to a completely 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                      |

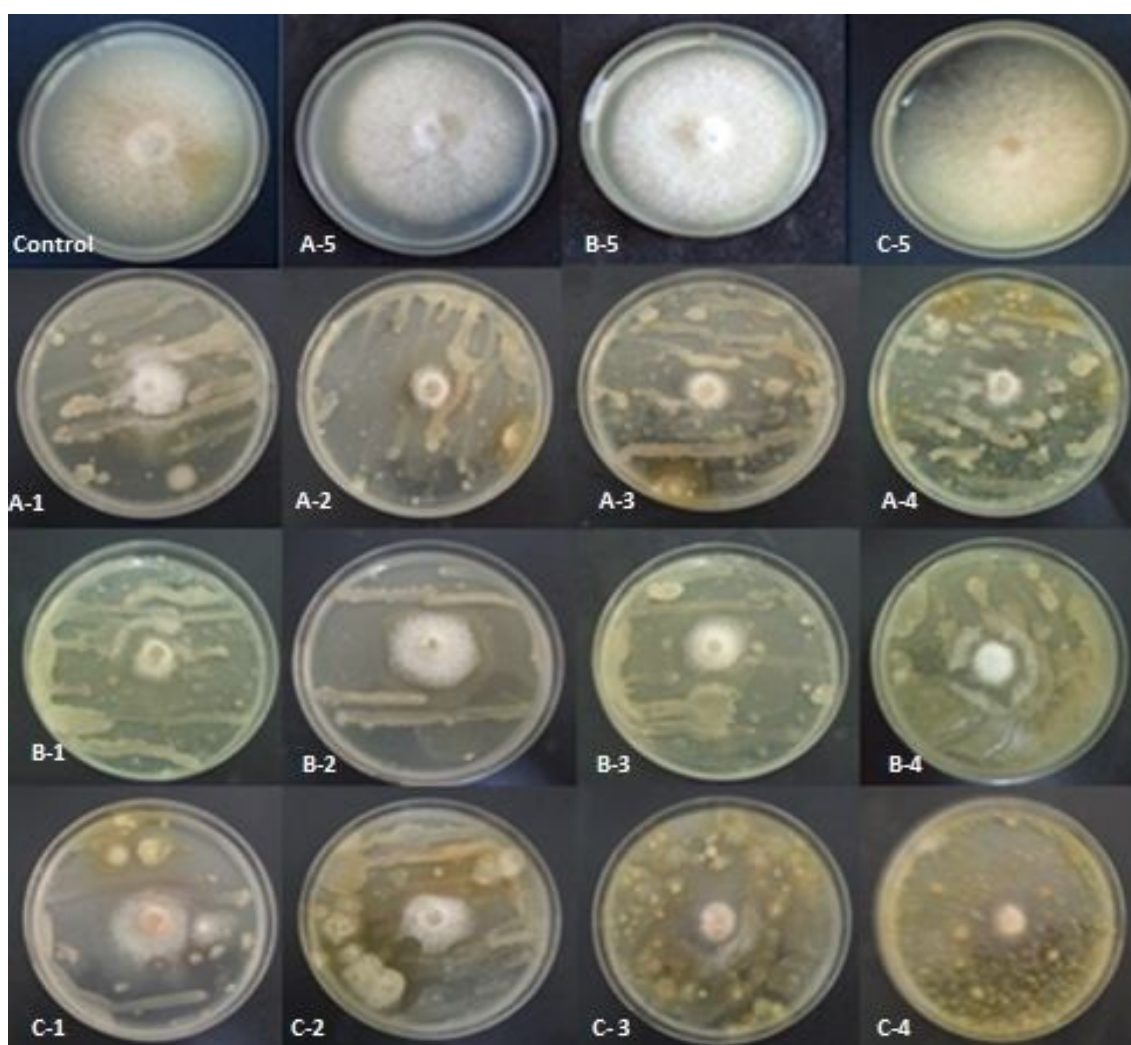
For composts refer to table 1.

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

The variance analysis revealed a significant effect ( $P \leq 5\%$ ) 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 | <i>Fusarium solani</i>       |                  |                 | <i>Fusarium oxysporum</i>     |                  |                 |
|----------------|------------------------------|------------------|-----------------|-------------------------------|------------------|-----------------|
|                | T1                           | T2               | T3              | T1                            | T2               | T3              |
| Dose1          | 42.64 ± 12.53 <sup>aA</sup>  | 42.64 ± 8.86abA  | 28.68 ± 2.53aA  | 40.26 ± 8.94 <sup>aA</sup>    | 46.65 ± 8.50abA  | 46.65 ± 15.35Aa |
| Dose2          | 44.96 ± 9.35aB <sup>**</sup> | 32.95 ± 1.48aA   | 27.52 ± 2.33aA  | 47.28 ± 11.59aA <sup>**</sup> | 42.81 ± 15.24abA | 46.33 ± 15.65Aa |
| Dose3          | 44.19 ± 1.27aA               | 47.29 ± 15.24abA | 47.29 ± 5.94bA  | 47.28 ± 11.01aA               | 33.87 ± 11.96aA  | 63.90 ± 2.18Ab  |
| Dose4          | 48.84 ± 1.27aA               | 54.26 ± 3.69bA   | 56.59 ± 10.52bA | 54.95 ± 5.04aA                | 57.83 ± 3.61bA   | 69.01 ± 1.92Ab  |

(\*) Means ± standard error in the column followed by the same small letter are not significantly different according to SNK test at  $P \leq 0.05$ .

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

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 (0.22µm) 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).

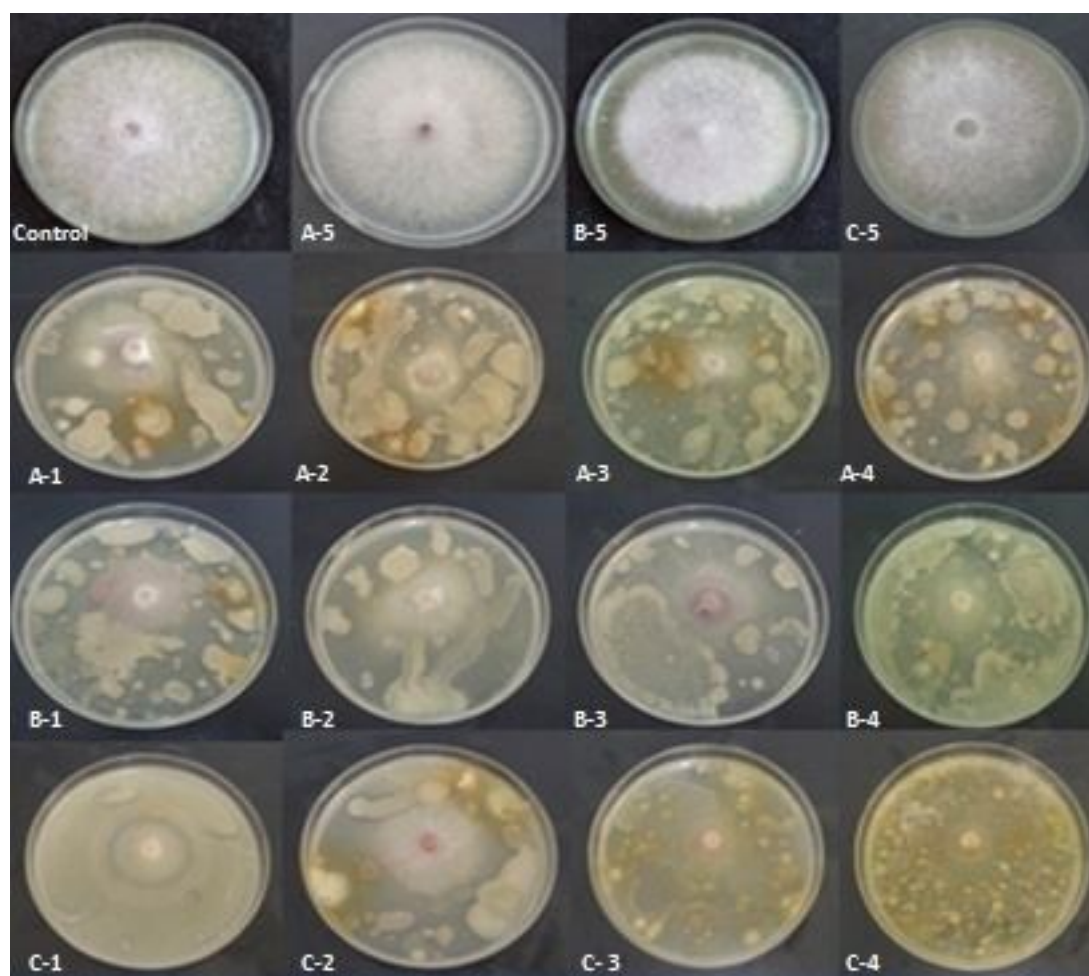
#### **In vivo effect of compost extracts in reducing *Fusarium* spp. severity**

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).



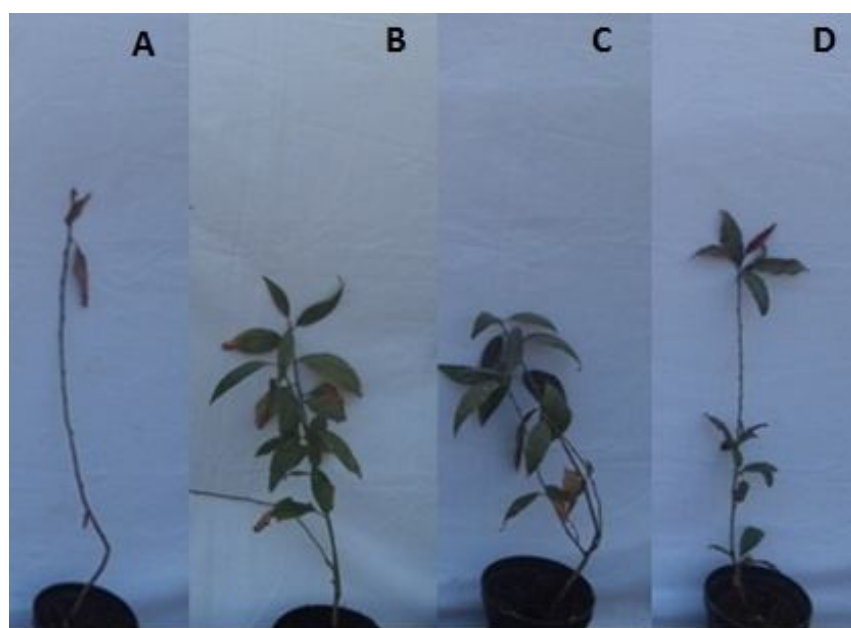
**Figure 2** *In-vitro* evaluation of different doses (1, 2, 3, 4) of three composts (A, B, C) on radial growth of *Fusarium oxysporum* recorded after six days of incubation on PDA at 25 °C in comparison to the untreated control (Plate above on the extreme left). **A:** Compost T1, **B:** Compost T2, **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.

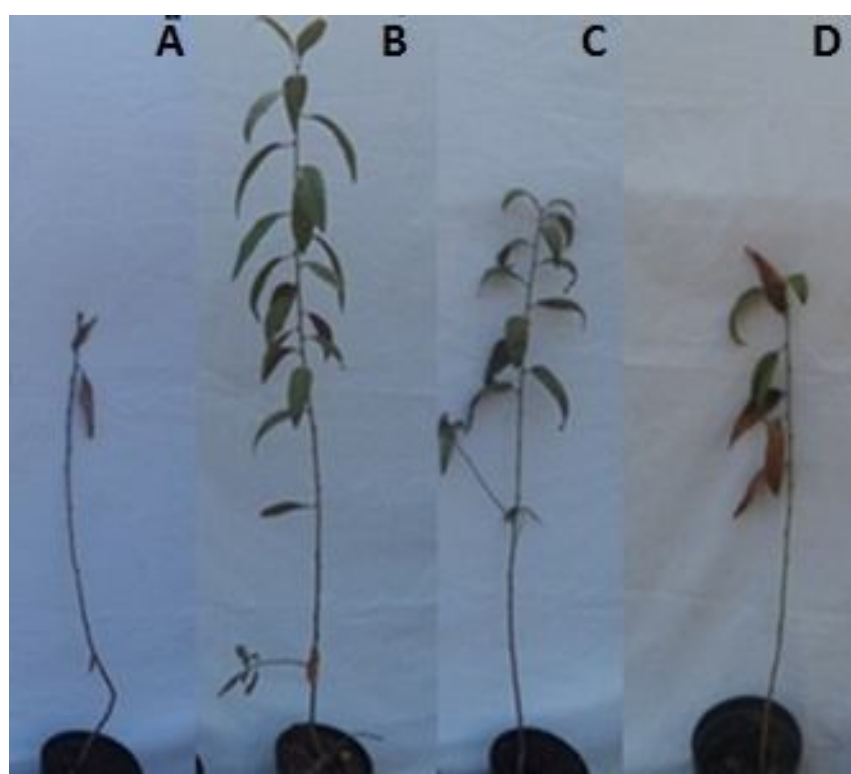
| Compost | <i>Fusarium solani</i>    |                          |                            |                          | <i>Fusarium oxysporum</i> |                          |                            |                          |
|---------|---------------------------|--------------------------|----------------------------|--------------------------|---------------------------|--------------------------|----------------------------|--------------------------|
|         | Root rot                  | Sanitary state           | Height (cm)                | Root weight (g)          | Root rot                  | Sanitary state           | Height (cm)                | Root weight (g)          |
| NIC     | 1.33 ± 0.58 <sup>a*</sup> | 1.67 ± 0.58 <sup>a</sup> | 62.40 ± 2.26 <sup>a</sup>  | 4.79 ± 0.37 <sup>a</sup> | 1.33 ± 0.58 <sup>a</sup>  | 1.67 ± 0.58 <sup>a</sup> | 62.40 ± 2.26 <sup>a</sup>  | 4.79 ± 0.37 <sup>a</sup> |
| IC      | 2.67 ± 0.58 <sup>a</sup>  | 2.00 ± 1.00 <sup>a</sup> | 55.00 ± 10.11 <sup>a</sup> | 4.50 ± 0.55 <sup>a</sup> | 2.67 ± 0.58 <sup>b</sup>  | 2.00 ± 1.00 <sup>a</sup> | 55.00 ± 10.11 <sup>a</sup> | 4.50 ± 0.55 <sup>a</sup> |
| T1      | 1.33 ± 0.58 <sup>a</sup>  | 1.56 ± 0.58 <sup>a</sup> | 48.96 ± 9.53 <sup>a</sup>  | 4.37 ± 1.52 <sup>a</sup> | 1.00 ± 0.00 <sup>a</sup>  | 1.00 ± 0.00 <sup>a</sup> | 69.30 ± 18.25 <sup>a</sup> | 6.08 ± 1.21 <sup>a</sup> |
| T2      | 1.33 ± 0.58 <sup>a</sup>  | 1.67 ± 0.58 <sup>a</sup> | 46.60 ± 4.52 <sup>a</sup>  | 4.22 ± 1.29 <sup>a</sup> | 1.33 ± 0.58 <sup>a</sup>  | 1.00 ± 0.00 <sup>a</sup> | 61.20 ± 6.54 <sup>a</sup>  | 4.98 ± 1.02 <sup>a</sup> |
| T3      | 1.33 ± 0.58 <sup>a</sup>  | 2.00 ± 1.00 <sup>a</sup> | 52.97 ± 9.58 <sup>a</sup>  | 4.16 ± 0.81 <sup>a</sup> | 1.33 ± 0.58 <sup>a</sup>  | 2.33 ± 0.58 <sup>a</sup> | 52.93 ± 7.87 <sup>a</sup>  | 4.41 ± 0.61 <sup>a</sup> |

(\*) Means ± standard error in the column followed by the same letter are not significantly different according to SNK test at P ≤ 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 <i>F. oxysporum</i> |       |       |         | Inoculated with <i>F. solani</i> |      |        |       |
|--------------|-------------------------------------|-------|-------|---------|----------------------------------|------|--------|-------|
|              | H                                   | RR    | RW    | SS      | H                                | RR   | RW     | SS    |
| CO           | 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 |

(\*\*) significant correlation at  $P \leq 0.01$ .

(\*) Significant correlation at  $P \leq 0.05$ .

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

## 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 the population of the causal pathogen, *Fusarium oxysporum* f. sp. *radicis-lycopersici*. In another study, different *Fusarium* pathogens were suppressed by exposure to the same composts, indicating a relatively broad 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

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 and 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 bacteria like *Pseudomonas syringa* sp. *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

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

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**چکیده:** در این پژوهش سه نوع کمپوست T1، T2 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 میسلیم قارچ‌های *F. solani* و *F. oxysporum* را مهار نمایند. در غلظت ۱۰ درصد بالاترین مهار به میزان ۴۸/۸ و ۵۰ درصد به ترتیب در قارچ‌های *F. solani* و *F. oxysporum* مشاهده شد. اما عصاره کمپوست جای روی کاهش رشد هر دو گونه قارچ بی‌تأثیر 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* به ترتیب به میزان ۴۱/۸۶، ۴۶/۴۵ و ۴۸/۳۰ 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 ( $r = 0/69$ ) و کود گوسفندی ( $r = 0/69$ ) رابطه مثبت نشان داد. به‌علاوه یک رابطه منفی میان بهبود شاخص بهداشتی در گیاهان تلقیح شده با *F. oxysporum* و تفاله زیتون ( $r = -0/92$ ) و میزان پلی‌فنل‌ها ( $r = -0/74$ ) مشاهده شد.

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