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In Vivo Evaluation of Compost Extracts for the Control of the Potato Fusarium Wilt Caused by *Fusarium oxysporum* f. sp. *tuberosi*

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ABSTRACT

Nine compost extracts (C1, C2, C3, C4, C5, C6, C7, C8 and C9) based on animal manures were evaluated for their inhibitory effect against *Fusarium oxysporum* f. sp. *tuberosi* the causal agent of the potato (*Solanum tuberosum* L.) Fusarium vascular wilt. They were compared to one healthy control (non-treated and non-inoculated plants) and to an inoculated control (plants inoculated and non-treated). Fusarium wilt severity was assessed based on the Leaf Damage Index (LDI) and on plant growth parameters 90 days post-planting. Potato plants grown in a mixture of perlite and peat treated with different compost extracts and inoculated with *Fusarium oxysporum* f. sp. *tuberosi* showed a highly significant decrease in the LDI as compared to those inoculated and non-treated. Moreover, the development of the pathogen was completely suppressed by C2, C3 and C9 extracts. The presence of compost extracts in the growing medium allowed for a significant improvement in plant-growth parameters as compared to inoculated control and even to those of healthy control (non-treated and non-inoculated plants). Despite the presence of inoculum in the substrate, increase of plant growth in comparison to the inoculated control, exceeded 43% for the tuber fresh weight, 75% for the shoot fresh weight and 78% for the root dry weight. The C8 extract was usually the most efficient in increasing the plant growth and yield parameters as compared to the other extracts. This study demonstrated the usefulness of compost extracts as an effective organic fertilizers and as an efficient biological tool for plant protection.

Keywords: control, disease severity, growth parameters, inoculation, leaf damage index, *Solanum tuberosum L.*

INTRODUCTION

In these last years, *Fusarium oxysporum* f. sp. *tuberosi* was frequently isolated in Tunisia from potato tubers showing dry rot and from wilting. This fungus infected plants through roots and colonized xylem vessels of stems (Daami-Remadi and El Mahjoub 2004; Ayed *et al.* 2006). Several strategies used to control this pathogen were mainly based on cultural practices (mainly rotation), physical control (soil solarization), and use of disease free seed tubers. However, due to its soil-borne origin, complete suppression of this disease from soil was difficult. Moreover, fungicides lost their utility due to security regulations, non-target effects and development of fungicide-resistant strains (Ozbay and Newman 2004; Daami-Remadi *et al.* 2006). Thus, more efficient control alternatives are required (Sivan and Chet 1993). The efficiency of composts and compost extracts, in the control of several pathogens, has been widely studied (Weltzien 1992; Elad and Shtienberg 1994; Al-Dahmani *et al.* 2003; Hibar *et al.* 2006; Khanfir Ben Jenana *et al.* 2009). For the biological control of *Fusarium* spp., compost extracts have potential value (Weltzein 1992; McQuilken *et al.* 1994; Hoitink *et al.* 1997; Cotxarrera *et al.* 2002; Pharand *et al.* 2002). In fact, Tratch and Bettiol (1997) showed that spraying compost extract diluted to 10% inhibited the mycelial growth of *Fusarium oxysporum* on several plants. *In vitro* and *in vivo*, Znaidi (2002) and Hibar *et al.* (2006) also found that compost extracts can reduce the development of *Fusarium* spp. infecting potato and tomato.

Few studies have evaluated the biocontrol of soil-borne pathogens by mixing compost extract with growing media and culture substrates. In fact, researches were mainly focused on application of compost extracts as foliar sprays or by using the solid form of the compost in the growing substrate. The compost extracts used in the present study were found to be effective when used as fertilizers (Kerkeni *et al.* 2010) and as biological treatment against plant pathogens in some vegetables. However, changes in their initial composition and quality were reported to affect their antimicrobial potential (Trillas *et al.* 2006; Lozano *et al.* 2009). In fact, only compost extracts with quality standards should be considered for use in the field and for plant protection.

The aim of the present work is to evaluate the effectiveness of some compost extracts (having already shown promising antifungal activity *in vitro* (Kerkeni *et al.* 2007a)), in reducing Fusarium wilt severity on potato plants and to elucidate their effects on plant growth and production.

MATERIALS AND METHODS

Biological material

Compost extracts. Nine extracts prepared from different composts (C1, C2, C3, C4, C5, C6, C7, C8 and C9) and primarily composed of different animal manures (poultry, sheep, cattle and horse manures) were used (**Table 1**). Original composts were produced in the composting unit of the Technical Center of Organic Agriculture of Chott-Mariem (Tunisia), according to an aerobic process (Znaidi 2002). Extract-production consists on suspending composts in tap water (1:5, v/v) in 20-liter plastic container and stirring the mixture daily for about 10 min during an extraction period of 5 days (Weltzein 1992). After the incubation period, the mixtures were filtered through cheesecloth (250 µm) and the obtained extracts were stored at 4°C. They were taken out 30 min

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Table 1 Initial composition of composts used for preparation of extracts.

Composts	Compositions
C1	50% CM + 25% SM + 25% PM
C2	60% CM + 30% SM + 10% ground straw
C3	50% CM + 25% SM + 25% HM
C4	50% CM + 20% SM + 20% PM + 10% ground straw
C5	25% CM + 25% SM + 25% PM + 25% HM
C6	30% CM + 30% SM + 30% PM +10% ground straw
C7	40% CM + 40% SM + 20% vegetable wastes
C8	25% CM + 25% SM + 25% PM + 15% HM +10% ground straw
C9	25% CM + 25% SM + 25% PM + 23.5% HM + 1.5 natural phosphate

C1-C9: compost 1-compost 9; CM: cattle manure; SM: sheep manure; PM: poultry manure; HM: horse manure

before use.

To increase the effectiveness of microorganisms contained in these extracts and decrease the amount necessary for the realization of the test, 5 ml of each extract were suspended in 200 ml of Potato Dextrose Broth (PDB) and incubated at 25°C for one week in a rotary incubator (150 rpm). After incubation, the liquid culture was adjusted to 500 ml with sterile distilled water (required volume to moisten the culture substrate of 5 plastic pots of 25 cm diameter).

Potato cultivar

Potato tubers cv. 'Spunta', the most cultivated in Tunisia, were used in this test. They were superficially disinfected by soaking for 5 min in a solution of 10% sodium hypochlorite (Aiglol Production, Zaouiet Sousse, Tunisia), rinsed abundantly with running water, dried in the open air and then placed under favourable environmental conditions to sprout (15-20°C, 60-80% relative humidity and natural room light) for approximately 14 days prior to use.

Inoculum preparation

The isolate of *Fusarium oxysporum* f. sp. *tuberosi*, used in this study was isolated from potato plants showing wilt symptoms (Ayed *et al.* 2006). It was cultured on Potato Dextrose Agar (PDA) for 10 days at 25°C before use (Daami-Remadi *et al.* 2006). Inoculum was prepared by transferring mycelium to 200 ml of sterile liquid culture of PDB and incubating the cultures at 25°C for 5 days in a rotary incubator (120 rpm). The liquid culture was filtered and the conidial suspension was adjusted to 10^7 conidia/ml (Wang and Jeffers 2000) by using a Malassez cytometer (Fa. Laboroptik. Friedrichsdorf, Tiefe 0.2 mm, 0.0025 mm^2, HBG, Germany).

In vivo experiments

After their germination, tubers were planted in plastic pots (25 cm diameter) containing a mixture of peat and perlite (1: 1, v/v) previously sterilized at 110°C for 1 h and humidified by compost extracts (100 ml/pot). Pathogen inoculation was performed six weeks after plant emergence by watering pots with 100 ml of a conidial suspension of *Fusarium oxysporum* f. sp. *tuberosi* (10^7 conidia/ml).

A healthy control (non-inoculated and non-treated plants) and an inoculated control (plants only inoculated with pathogen) were used to compare the activity of compost extracts. Plants were maintained under cover at 25°C and watered regularly to promote normal growth.

Effect of compost extracts on Fusarium wilt severity was assessed 90 days post-planting via the Leaf Damage Index and according to 0-4 scale depending on symptom severity on leaves as described by Béye and Lafay (1985). It was determined as: 0 = asymptomatic leaf, 1 = leaf wilted, 2 = leaf with hemiplegic yellowing, 3 = leaf with necrosis, 4 = dead leaf and calculated per potato plant by the following formula:

$$LDI = \sum notes / max$$

LDI: Index of Leaf Damage
∑ notes: Total notes.
Max: 4 times of developed-leaves number.

Potato plant height, tuber fresh weight, fresh and dry weights of shoots and roots were also measured 90 days post-planting for assessing the effect of the tested composts extracts on potato growth and production.

Statistical analyses

Data were arranged as a completely randomized design. Five replicate pots per elementary treatment were used and the whole bioassay was repeated twice. Data were analyzed using SPSS statistical program version 11.0 and subjected to analysis of variance (ANOVA). Means were compared using the Duncan's Multiple Range Test and the differences between means were deemed to be significant at $P < 0.05$.

RESULTS

Incidence of compost extracts on disease severity assessed by the LDI

The Leaf Damage Index (LDI) noted on potato plants cv. 'Spunta' inoculated with *F. oxysporum* f. sp. *tuberosi* was assessed 90 days post-planting. As shown in **Figs. 1** and **2**, the application of compost extracts into the growing substrate has resulted in a significant ($P < 0.05$) decrease of wilt severity as compared to inoculated and non-treated control. The LDI recorded on plants treated with C5 and C6 extracts (the least effective as their LDI are statistically comparable to the inoculated control) did not exceed 1.15 as compared to 2 noted on the inoculated control plants. Therefore, a large variability within the same treatment was noted (IC, C1, C5 and C6). For example, the LDI of the inoculated control ranged between 0.75 and 3.43 whereas for C1 extract, Fusarium wilt severity varied from 0 to 3.

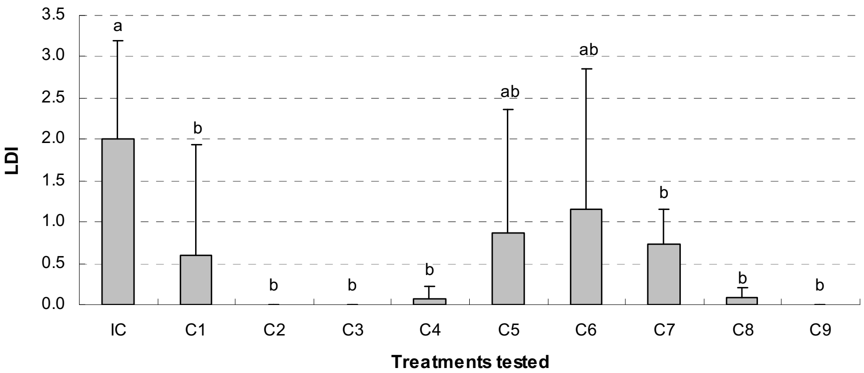

Fig. 1 Leaf Damage Index (LDI) noted, 90 days post-planting, on potato plants inoculated with *Fusarium oxysporum* f. sp. *tuberosi* and treated with compost extracts as compared to the inoculated and non-treated control plants. Bars with the same letter are not significantly different based on the Duncan's multiple range test (at $P \leq 0.05$); Bars are the means of 5 plants; IC: Inoculated and non-treated control.

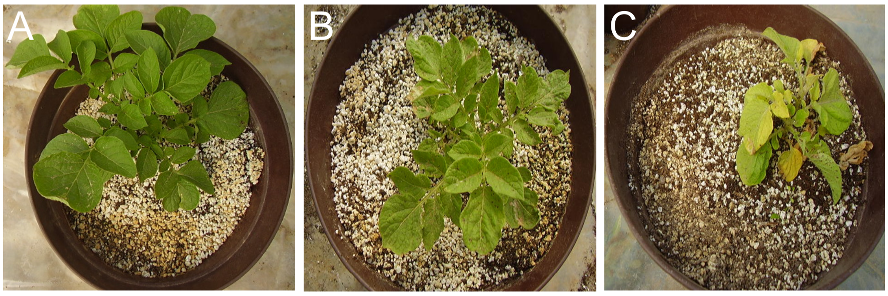

Fig. 2 (**A**) Potato plants inoculated with *Fusarium oxysporum* f. sp. *tuberosi* and treated with C2 extract; (**B**) Potato plants inoculated and treated with C5 extract; (**C**) Inoculated and non-treated plants (inoculated control, IC).

The extracts C2, C3 and C9 exhibited a remarkable potency in suppressing disease development.

Effect of compost extracts on potato plant growth

1. Plant height

Results presented in **Fig. 3A**, showed that height of plants inoculated with *Fusarium oxysporum* f. sp. *tuberosi* decreased significantly as compared to the non-inoculated plants. However, application compost extracts tested has resulted in a significant ($P \leq 0.05$) increase in plant height as compared to the inoculated control and some of them yielded significantly similar values for heights as healthy control (non-treated and non-inoculated plants). Compost extracts C1, C7 and C9 were the most effective extracts in increasing this parameter by more than 30% as compared to the inoculated control.

2. Shoot fresh weight

For non-treated plants, the presence of the pathogen had significantly decreased the fresh weight of shoots as compared to the healthy control ones. All compost extracts tested had enhanced this parameter except C1, C2, C4 and C7. Increase of shoot weight was more pronounced (about 75%) in plants treated with C8 extract (**Fig. 3B**).

3. Root fresh weight

Addition of compost extracts to the growing media had enhanced the root growth as compared to the inoculated control plants (**Fig. 3C**). The extracts C4 and C8 were the most effective in improving the roots weight which increased by 48.5 and 50%, respectively, as compared to the inoculated and non-treated control.

4. Tuber fresh weight

Fig. 3D reveals a significant increase of the tuber fresh weight of plants treated with C5, C6, C8 and C9, as compared to the inoculated control. Moreover, results showed no significant difference between the extracts C1, C2, C3, C4, C7 and the inoculated control. The C8 extract enhanced the tuber fresh weight of plants by 43%, in comparison to the inoculated control.

5. Shoot and root dry weights

The shoot and root dry weights of potato plants are illustrated in **Fig. 4**. For the shoot dry weight, **Fig. 4A** reveals significant differences between extracts. Plants growing in media supplemented with C3 or C8 extracts exhibited the higher shoot dry weights as compared to inoculated and non-inoculated plants (healthy control). The higher increase in the shoot dry weight (of about 50%) was obtained with the application of C8 extract.

According to **Fig. 4B**, the dry weight of roots was highly improved by C8 extract (78%) comparatively to the inoculated control. Moreover, extracts C8 and C6 were found to be more effective than the non inoculated control (healthy control). Means comparisons showed that compost extracts C1, C2, C3, C4, C7 and the healthy control had no significant difference with the inoculated control.

DISCUSSION

The efficacy of compost extracts, prepared from heterogeneous organic wastes, against several pathogenic fungi was previously demonstrated (Weltzien 1992; Elad and Shtienberg 1994; Zhang *et al.* 1998; Scheuerell and Mahaffe 2004; Kerkeni *et al.* 2007a). Pathogens inhibition induced by compost extracts resulted from a combination of chemical and biological mechanisms including microbial competition for nutriments with pathogen (Chen *et al.* 1988), production of antibiotics and extra cellular lytic enzymes, induction of plant defence (Segarra *et al.* 2009), parasitism and predation (Zhang *et al.* 1998). Elad and Shtienberg (1994) showed that compost extracts prepared from animal manures reduced the incidence of leaf grey mould caused by *Botrytis cinerea* by more than 56%. Nelson and Boehm (2002) reported that different extracts prepared from 100% chicken manure, food waste, yard waste or brewery sludge were efficient in reducing root rot caused by *Pythium* sp. Hibar *et al.* (2006) also found that compost extract, composed of 50% cattle manure, 30% sheep manure, 20% poultry manure and 10% ground straw, had successfully controlled *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato. Results founded in this current study confirmed these previous findings. In fact, the use of compost extracts obtained from various animal manures and tested against *Fusarium oxysporum* f. sp. *tuberosi* showed a significant decrease in the LDI and consequently symptom severity on potato plants. However, the compost extracts tested were found to affect the development of this pathogen by various degrees. In fact, C2 (60% CM + 30% SM + 10% ground straw), C3 (50% CM + 25% SM + 25% HM) and C9 (25% CM + 25% SM + 25% PM + 23.5% HM + 1.5% natural phosphate) were highly efficient in suppressing this disease, while C5 (25% CM + 25% SM + 25% PM + 25% HM) and C6 (30% CM + 30% SM + 30% PM + 10% ground straw) extracts had no significant effects suggesting the role of their initial composition on their effectiveness. A large variability was noted within the same treatment (as is the case of IC, C1, C5 and C6 treatments), that could be attributed to a delay in the infection process.

In the same context, Hibar *et al.* (2006) found that addition of compost extracts to the growing media decreased wilt symptoms caused by *F. oxysporum* f.sp. *rasicis-lycopercisi* on tomato plants. Segarra *et al.* (2009) also noted decrease in disease severity of *Erysiphe polygoni* on tomato leaves by 19% by using compost extracts. When applied as foliar sprays, compost extracts had also limited by 29% the late blight (*Phytophthora infestans*) severity on potato (Al-Mughrabi 2007).

Reduction of the development of *F. oxysporum* f. sp. *tuberosi* in response to compost extract application is pre-

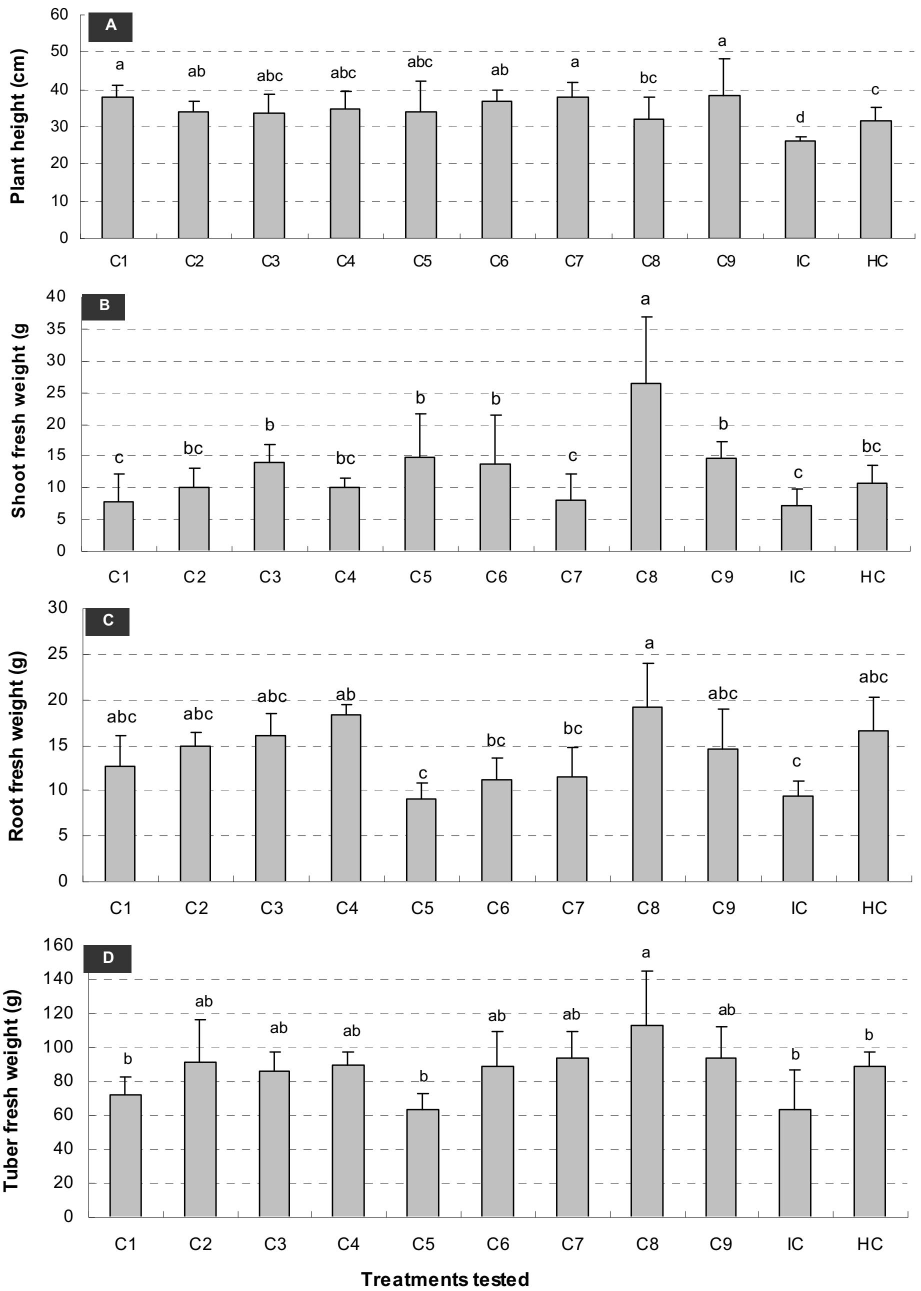

Fig. 3 Effects of the compost extracts tested on growth and yield parameters noted, 90 days post-planting, on potato plants inoculated with *Fusarium oxysporum* f. sp. *tuberosi* as compared to the controls. C1-C9: Compost extract 1-compost extract 9; IC: Inoculated and non-treated control; HC: Healthy control i.e. non-inoculated and non-treated; Bars with the same letters are not significantly different based on the Duncan's multiple range test (at $P \leq 0.05$); Bars are the means of 5 plants.

sumably due to the presence of antagonistic microorganisms such as bacteria and fungi (Segarra *et al.* 2009). Support to this assumption was given by Hoitink *et al.* (1997) who reported suppression of symptoms caused by various phytopathogenic fungi by antagonistic microorganisms associated to or involved in organic amendments like compost.

In this context, we previously analysed the origin of tested extracts (composts) for their microbial content and we have reported the occurrence of many antagonistic bacteria and fungi of the genera *Aeromonas*, *Serratia*, *Pseudomonas* and *Trichoderma* which were able to inhibit the development of many plant pathogens (Kerkeni *et al.* 2007b, 2008). We also founded that these extracts were rich in actinomycetes especially the C3 extract. However, microbial analyses of C2 showed that this extract is highly colonized by the antagonistic fungi (*Trichoderma* spp.). The higher antifungal activity of these extracts is presumably due to their microbial populations and their interactions.

As previously reported at the beginning of this paper, these extracts were preliminarily tested *in vitro* for their antifungal activity (Kerkeni *et al.* 2007a). Comparison with *in vitro* results revealed an improvement of the activity of these extracts. Indeed, the highest inhibition ratio obtained *in vitro* was of about 46% with the C4 while in the current study we noted a total suppression of the pathogen by using the C2, C3 and C9 compost extracts. The enhancement of extract activity is presumably due to the amplification of the suppressive potential of antagonistic microflora contained in these extracts (after incubation and agitation). According to Segarra *et al.* (2009), the effects of compost extracts could also be due to their richness in inorganic salts, organic carbon and phenols, which can affect directly pathogen growth and phyllosphere microorganisms.

The *in vitro* results of chemical and biological control, conducted by Ayed (2005) against *F. oxysporum* f. sp. *tuberosi*, showed that the efficacy of our compost extracts was less important as compared to synthetic products such as Tachigaren 360 (hymexazol) and organic products trade as Funga stop, which have inhibited the mycelial growth of

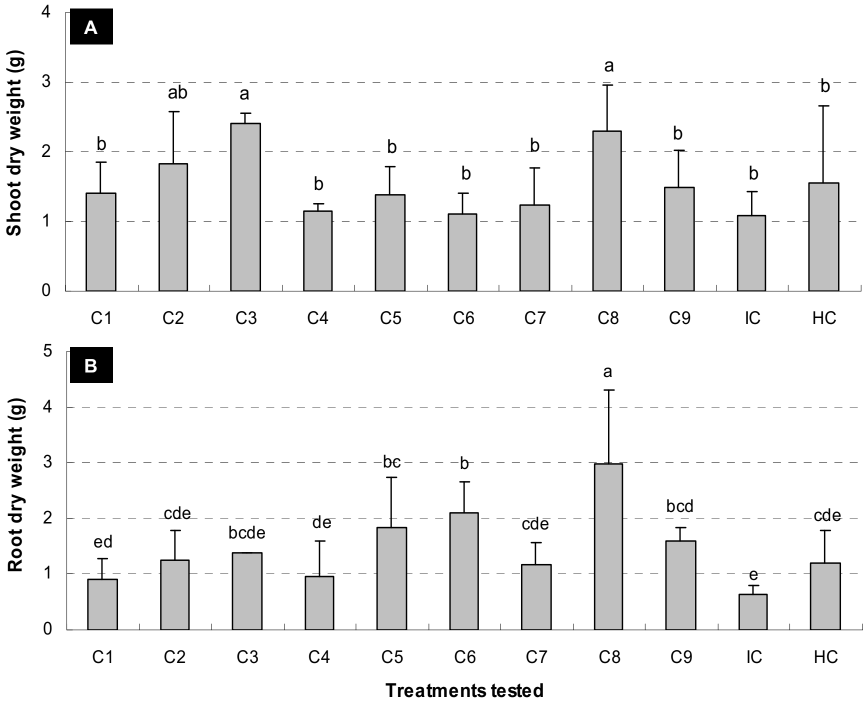

Fig. 4 Effects of the compost extracts tested on the dry weight of shoots (A) and roots (B) of potato plants inoculated with *F. usarium oxysporum* f. sp. *tuberosi* noted 90 days post-planting as compared to the controls. C1-C9: Compost extract 1-compost extract 9; IC: Inoculated and non-treated control; HC: Healthy control i.e. non-inoculated and non-treated. Bars with the same letters are not significantly different based on the Duncan's multiple range test (at $P \leq 0.05$); Bars are the means of 5 plants.

this fungus up to 77% and 76%, respectively against 46% obtained with compost extracts. However, comparison with the Polyversum, which is an organic product based on *Pythium oligandrum*, revealed that tested extracts were more effective, since the highest inhibition ratio achieved by this product had not exceed 12.6%. In addition, this study (Ayed 2005) showed that *T. harzianum*, an antagonistic fungus isolated from a Tunisian soil, had limited this pathogen by 43% as compared 46% for our compost extract C4 (Kerkeni *et al.* 2007a).

Results showed also that addition of compost extracts to growing media had improved potato plant growth despite the presence of the pathogen. Moreover, this improvement was better than the non-inoculated plants. Similarly, Hibar *et al.* (2006) reported that tomato plants treated by compost extracts and inoculated by *F. oxysporum* f. sp. *radicis-lycopersici* had vigorous root system and a better vegetative growth than non-treated plants. With the same pathogen, Haruna *et al.* (2012) found that application of compost extracts as soil drench, induced better plant height, heavier fresh root and shoot weight and higher yield of tomato than plants treated with fungicide.

The compost extract C8 was usually the most efficient in increasing growth parameters as compared to the other extracts. This is presumably due to it's equilibrate initial composition (**Table 1**). Positive effect of compost extracts on growth may be also due to the presence of nutrients and beneficial microorganisms (mostly bacteria) that were known to also promote root growth (plant growth-promoting bacteria). These bacteria are located in the "phyllosphere" and are able to stimulate plant growth. According to Brinton *et al.* (1996), extracts influence the phyllosphere thanks to its microbial population. Moreover, correlations were found between the microbial activity in the phyllosphere and disease control (Krause *et al.* 2003; Idris *et al.* 2007; Segarra *et al.* 2009). This shows the usefulness of extracts of composts as organic fertilizer solutions in addition to their role in plant protection.

To summarize, the present study showed that compost extracts prepared from various animal manures had negative effects on *F. oxysporum* f. sp. *tuberosi* and they demonstrated their usefulness as fertilizers. They could constitute a promising alternative for a biological control of some plant diseases and reduce the abusive use of synthetic fungicides and fertilizers. However, a more field comprehensive studies are needed to confirm our findings in the field under natural conditions.

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