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In vitro evaluation of compost extracts efficiency as biocontrol agent of date palm *Fusarium* wilt

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Bayoud, vascular wilt of date palm caused by *Fusarium oxysporum* f. sp. *albedinis* (*Foa*), is widely distributed in all date palm growing regions of Morocco. It is the most serious disease of the date palm. Compost is recognized for their ability to improve soil characteristics and to protect the crops against biotic and abiotic stress. In this experiment, *in vitro* effects of different concentrations of sterilized and unsterilized compost extract on the growth of *F. oxysporum* f. sp. *albedinis* were evaluated. All concentration of unsterilized compost extract decreased radial growth of *Foa*. In fact, fungal radial growth inhibition ranged from 20 to 97%. Higher antifungal activity was noted in 30 and 40% concentration (more than 93%). Nevertheless, sterile compost extract inhibited mycelia growth only for the 40% concentration with 18% fungal growth inhibition, while lower concentrations were not effective.

Key words: Date palm, *Fusarium oxysporum* f. sp. *albedinis*, compost extract, mycelium growth, inhibition rate.

INTRODUCTION

The date palm, *Phoenix dactylifera* L. is one of the most important species in the palm family (*Palmaceae*) which includes 200 genera and more than 2500 species (El Hadrami and Al-Khayri, 2012; Hadrami and Hadrami, 2009). The genus *Phoenix* consists of fourteen species distributed in the tropical and sub-tropical regions (Al-balqa, 2016). *P. dactylifera* L. is claimed to encompass over 5000 cultivars, some of which have been more or less characterized in detail (El-Hadrami and Al-Khayri, 2012). Date palm is of great economic importance to oasis agriculture where abiotic factors are extreme. Otherwise it creates favorable conditions for improving growth of secondary crops like olive tree, wheat and others leguminous plants. In the world, 100 million trees were estimated with an average of production of 7.62 million tons (FAO, 2010). Moroccan palm groves alone cover 50 000 ha corresponding to 5 million tree and 100.000 tons/year (Sedra, 2012). In Morocco, 223 cultivars have been absolutely characterized since 1992 (Saaidi, 1992; http://www.agriculture.gov.ma).

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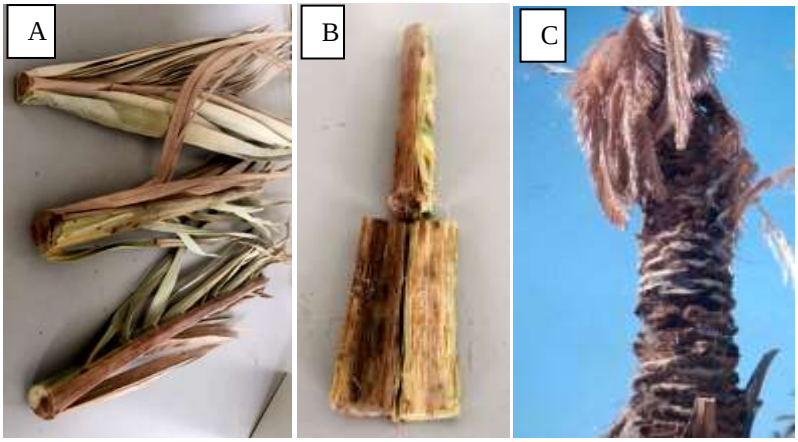

Figure 1. Bayoud symptoms: **A,** unilateral wilting of rachis; **B,** Fusarium wilt within rachis; **C,** late stage of infection (plant death).

Economically, the date palm fruit and by-product are precious for their nutritional and dietetic properties and income generating for oasis' populations (Al-shahib and Marshall, 2003; Chao and Krueger, 2007; Saafi et al., 2008). "Bayoud" disease (Figure 1) is the most destructive fungal disease of date palm. Its causal agent is *Fusarium oxysporum* f. sp. *albedinis* (*Foa*). The impact of the disease is very serious in North Africa, particularly in Morocco where 2/3 of palm tree were destroyed so far (Chakroune et al., 2008; Pereau-Leroy, 1958). In addition, *Fusarium* wilt has killed more than 10 million palm trees during the last 100 years (Dihazi et al., 2012; Saaidi, 1992). The control of the disease using chemicals products is not effective and implies negative effects on environmental and human health (Bernal-Vicente et al., 2008; Boulter et al., 2000; Brimner and Boland, 2003). Prophylactic methods are not of interest due to the contamination of several date palm groves and to their non-durable impact (Jaiti et al., 2007; Saaidi, 1992). Planting resistant cultivars constitutes the only efficient and economic method to control *Fusarium* wilt despite the fact the available cultivars have produced poor date (Saaidi, 1992). In Morocco, cultivars that are sensitive are economically important (Mejhoul and Boufegous).

Another alternative method to control phytopathogenic fungi consists of applications of compost and/or its extract (Alberto et al., 2016; El-Masry et al., 2002; Markakis et al., 2016; Pane et al., 2013, 2012; Sghir et al., 2015). The compost extract is an organic product obtained after fermentation of compost in liquid phase for a few days to up two weeks or just for few hours of mixing with or without aeration (short preparation) (Ingham, 2003; Lanthier, 2007). A number of factors which are involved in the compost extraction process, such as temperature, aeration, organic matter and microbiological properties, are responsible for their efficiency in plant disease suppression (Pane et al., 2012). The use of compost extract as a biological control agent (BCA) against soil borne diseases has increased in the last years. Several researchers showed that the compost extract can control several pathogenic fungi like *Botrytis cinerea, Alternaria alternata, Pyrenochaeta lycopersici* (Palou et al., 2013; Pane et al., 2012), *Pythium debaryanum, Sclerotium bataticola* and *Fusarium oxysporum* f. sp. *lycopersici* (El-Masry et al., 2002). Nine compost extracts based on animal manures (cattle manure, sheep manure, chicken manure and horse manure) were used *in vitro* against numerous pathogenic fungi causing different plant diseases (*Fusarium oxysporum* f. sp. *radici-lycopercisi*, *Fusarium solani*, *Fusarium graminerum, Fusicoccum amugdalis, Alternaria sp*., *Colletotrichum coccodes, Botrytis cinerea, Sclerotinia sclerotiorumn Aspergilus niger, Rhizoctonia solani, Rhizoctonia bataticola, Phytium* sp. and *Verticilium dahliae*) and revealed important results (Kerkeni and Khedher, 2007). The suppressiveness of compost extract is mostly due to its microbial community (Koné et al., 2010; Lin et al., 2014; Pane et al., 2012; Powell and Barry, 2017; Suárez-Estrella et al., 2013; Ventorino et al., 2016). These microbes exert their antagonism by microbiostasis, antibiosis and hyperparasitism and/or stimulate systemic resistance in host plants (Le Page and Bousquet, 2007). However, other researchers reported that compost actions are due to physical, chemical, biochemical and microbiological dynamic interactions with plant-pathogen system (Le Page and Bousquet, 2007). Indeed, several organic chemicals present in compost or released by compost-inhabiting microorganisms have been identified as providing disease suppressive effects, including phenolic compounds, volatile fatty acids and salicylic acid (Le Page and Bousquet, 2007). It is also demonstrated that compost or its extract induce systemic resistance against pathogenic fungi in numerous plants (Kavroulakis et al., 2005; Sang et al., 2010).

Therefore, the main objective of this study was to investigate the suppressive capacity of unsterilized (UCE) and sterilized (SCE) compost extract against *Foa* growth,

Table 1. Rates of raw materials used in composting process.

Raw matter	CM (%)	OMWW (%)	DOMW (%)	OMW (%)	VS (%)
Rate (%)	47	5	12	17	19

CM: Chicken manure, OMWW: olive mill waste water, DOMW: dry olive mill waste, OMW: two phases olive mill waste, VS: vine shoot.

Table 2. Physical and chemical properties of different composting raw materials.

Parameter	CM	OMWW	DOMW	OMW	VS
Moisture (%)	63.5	77.7	19.8	54.2	39.2
pH	7.9	4.9	8.5	6.3	8.7
CE (mS. Cm^{-1})	12.0	29.9	6.6	2.4	3.3
C/N	7.4	27.0	44.8	29	41
Organic carbon (%)	13.2	6.7	76.1	20.3	29.2
Total nitrogen (%)	1.8	0.3	1.7	0.7	0.7
P2O5 (%)	1.3	0.3	0.3	1.0	0.2
K2O (%)	1.1	3.0	1.0	0.4	0.5

CM: Chicken manure, OMWW: olive mill waste water, DOMW: dry olive mill waste, OMW: two phases olive mill waste, VS: vine shoot.

focusing on the abiotic and biotic factors involved in this suppressive mechanism.

MATERIALS AND METHODS

Fungal isolate

In the Tafilalet date palm grove, the leaf samples were collected from symptomatic and non-symptomatic Mejhoul cultivar trees and used for *Fusarium* species isolation and identification. Twenty fragments of date palm leaf of each sample were surface-sterilized for 5 min with a 4% sodium hypochlorite solution, rinsed twice in sterile distilled water and dried in a laminar flow cabinet. The growth medium potato dextrose agar (PDA) was used for fungal isolation. The plates were incubated at 28°C in the dark for 7 days. All *Fusarium* isolates were subcultured on PDA medium and incubated for purification and spores production for 7 days in the dark and two weeks of light. After that, cultural characters were assessed by microscopic examination. The morphology of macroconidia, microconidia and the chlamydospores was assessed and the identification was made using the criteria of Sedra (2012).

Compost source

The compost used in this experiment was produced in a private composting unit in Meknes, Morocco using a mixture of agricultural waste (chicken manure and vine shoot) and agro-industrial waste (olive mill waste and olive mill waste water) (Table 1). The windrow composting system was used in which mixtures were subjected to interval turning over every two weeks. Different raw material and compost were analyzed in a private laboratory to determine their physical and chemical characteristics (Table 2).

Compost extract preparation

Compost extract were prepared following the method of El Masry et al. (2002). The mature compost was suspended in phosphate buffer containing K_2HPO_4 (8 g/l) and NaH_2PO_4 (0.34 g/l) at a ratio of 1:2 (w/v). Then, it was well shacked (150 rpm) for 72 h under room temperature in natural photoperiod (24/11°C day/night). The mixture was splitted in sterile centrifuge tubes (50 ml) and centrifuged at 500 g (gravity) for 10 min, to remove large particles, then, at 1000 *g* for 10 min to obtain the active supernatant (compost extract). A portion of compost extract was autoclaved (120°C for 20 min) to obtain a sterile compost extract (SCE).

In vitro suppressive effect of compost extract

The suppressive effect of the compost extract against *F. oxysporum* f. sp. *albedinis* (*Foa*) was examined using well-cut diffusion technique (Pane et al., 2013). Both sterilized and unsterilized composts extracts were used in five different concentrations: 10, 15, 20, 30 and 40% (v/v). PDA medium (before cooling step) was used for preparation of compost extract concentrations, and mixtures were cooled into Petri dishes (90 mm of diameter). For control plates, the PDA medium was supplemented with phosphate buffer PBS sterile. An active mycelia disk (5 mm in diameter) of pathogen was placed at the center of the Petri plates. All Petri plates were then incubated at 28°C and evaluated for pathogen growth monitoring during 8 days of incubation. Five replicate were used per elementary concentration and experiment was repeated twice.

To determine the inhibition rate (IR) of the pathogen by applying each of the tested compost extract, the radial fungal growth of *Foa* was monitored by measuring the colony diameters for the control and treated plates at 0, 2, 4, 6 and 8 days. The inhibition rate was calculated according to the formula used by Hibar et al. (2006):

IR (%) = (1–(Average diameter of the treated / Average diameter of the control)) x 100

Five repetitions were carried out for each UCE or SCE concentrations and controls. All plates were incubated at 26°C until

Table 3. Physical and chemical properties of mature compost.

Parameter	Value
Moisture (%)	44.83
pH	6.69
Organic matter (%)	32.48
Total Kjeldhal nitrogen (NTK) (%)	1.29
Phosphorus (P_2O_5) (%)	1.74
Potassium (K_2O) (%)	1.22
Organic carbon (%)	16.24
C/N	12.59
Electric conductivity ($ms.cm^{-1}$)	24.69

control plates were fully covered by mycelium (8 days). After incubation, linear reduction of the radius-growth of *Foa* was measured.

Experimental design and statistical analysis

Petri plates were distributed in a completely randomized design with five Petri plates per elementary treatment and the whole experiment was repeated two times. Data were analyzed using SPSS statistical program version 12.0 and subjected to the analysis of regression relation between unsterilized extract concentration and rate inhibition of *Foa*.

RESULTS AND DISCUSSION

Organic matter, C/N ratio, total nitrogen, phosphorus, potassium and pH of raw material and final compost are presented in Table 3. The compost produced and used showed pH with suitable value of 6.69 and the electrical conductivity of about 24 $ms.cm^{-1}$. Compost had more than 32% organic matter content. The total nitrogen, the phosphorus and the potassium were greater than 1%. The C/N ration is suitable for sustainable agriculture, because ratio of 27 in OMSW (Table 1) in raw materials was previously confirmed for increasing pore space and allowing bulk oxygenation (Barje et al., 2016). In addition, the same compost composition was used to promote date palm (cv. Mejhoul) at 1:3 ratio (v/v). It has increased significantly all growth parameters as well as nutrient and chlorophyll content without any toxicity (data not shown).

After 8 days of incubation at 28°C, results shown in Figures 2, 3 and 4 revealed that unsterilized compost extract caused an inhibiting effect on mycelium development of *Foa* when compared with untreated control (*Foa* alone). However, the mycelia growth of *Foa* measured in the different Petri plates during incubation varied with the compost extract concentration (Figure 4). The results of the regression analysis showed a significant relationship, at 5% levels, between suppressive effect and concentration of unsterilized compost extract (Table 4). The regression coefficient was 0.966 (Table 5).

The most effective concentrations were 30 and 40% where the pathogen development was completely inhibited (more than 97%). For the remaining concentrations (10, 15 and 20%), mycelium growth decrease ranged from 20 to 44% as compared to the control (Figures 1, 2 and Table 6). After the period of incubation, some compost-inhabiting microorganisms (bacteria and fungi species) were observed to develop in the Petri dishes and had antagonistic effect against *Foa* (Figure 2). On the other hand, no suppressive effect towards *Foa* was observed in the sterilized compost extract except for high concentration (40%) which showed an inhibitory effect of about 18% (Figure 3). This reduction of mycelium growth in the 40% concentration of sterilized compost extract may be due to some chemical compounds elaborated during composting process and remained after the sterilization step or some thermostable extracellular metabolites. These results confirm the findings of Kerkeni and Khedher (2007) and El-Massry et al. (2002) who showed that compost extract prepared from various animal manures had high inhibitory effects of *F. oxysporum* f. sp. *radices-lycopersici* which can be attributed to active microorganisms inhabiting the unsterilized compost extract. Indeed, as confirmed by other research, compost extract might have contained antagonistic mycoparasites, such as *Trichoderma* sp., *Penicillium* sp. and *Petriella* sp. (Danon et al., 2007; Zmora-Nahum et al., 2008) or plant growth promoting bacteria (PGPB) like genera of *Azotobacter, Pseudomonas, Stenotrophomonas, Bacillus, Flavobacterium, Streptomyces* or Actinomyces group found in the chestnut compost which has colonized mycelia and inhibited sclerotia germination of *Sclerotium rolfsii* (Danon et al., 2007; Ventorino et al., 2016; Zmora-nahum et al., 2008). In addition, it could be other antagonistic microorganisms inhabiting compost such as *Nocardiopsis* sp., *Streptomyces violaceorubidus* and *Streptomyces* sp. which were identified and screened for their antifungal activities by bio-active substance production (peptides) (Su et al., 2014). These peptides were characterized for their antibiosis mechanism (surfactins) and tested for growth inhibition of tomato pathogens such as *Alternaria solani* and *Botrytis cinerea* (On et al., 2015). It was also reported that the sterilization of compost destroyed its active microorganisms and consequently nullified their antagonistic effect (El Khaldi et al., 2016; Hoitink et al., 1997; Raviv, 2014; Zhang et al., 1998) and the growth inhibition was related to pH value and ammonium (NH_4^+) concentration in the culture medium (Danon et al., 2007; Zmora-Nahum et al., 2008). Fungal colonies grown in plates containing weak concentration of UCE showed that fungal species inhabiting compost were inhibited by high compost extract (Figure 2). Hence, microbial activities have particularly reduced growth radius of *Foa* pathogen. For sterilized compost extract, no limited growth was detected in weak percentage because only chemical

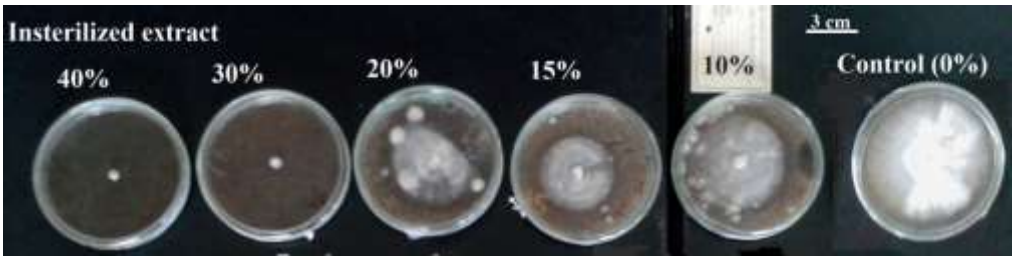

Figure 2. Mycelium growth of *F. oxysporum* f.sp albedinis in response to different unsterilized compost extract concentrations after 8 days of incubation in PDA medium. Control plate contains Foa pathogen alone.

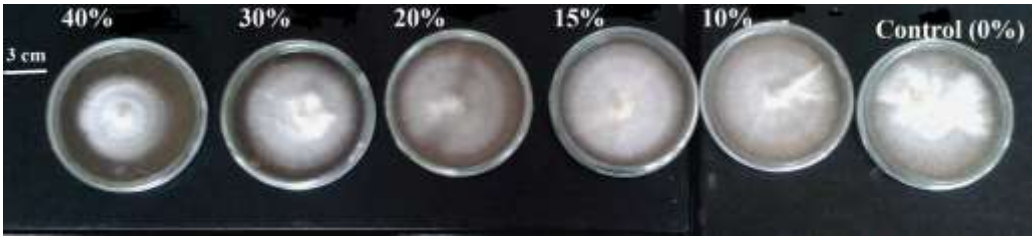

Figure 3. Mycelium growth of *F. oxysporum* f.sp albedinis in response to different sterilized compost extract concentrations after 8 days of incubation in PDA. Control plate contains *Foa* pathogen alone.

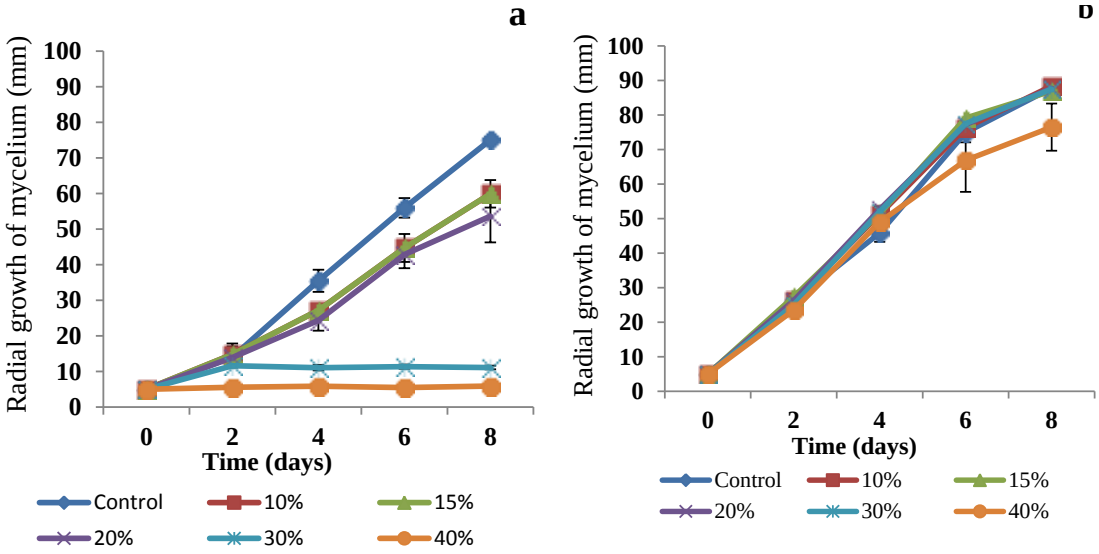

Figure 4. Evolution of mycelia growth of *F. oxysporum* f.sp *albedinis* with unsterilized compost extract (a) and sterilized compost extract (b).

Table 4. Significant regressions of compost extract and rate inhibition of radius growth of *Foa*

Model		Sum of squares	ddl	Average of squares	D	Sig.
1	**Regression**	**4532.931**	**1**	**4532.931**	**41.623**	**0.008**
	Residue	326.717	3	108.906		
	Total	4859.648	4			

Table 5. Model of linear regression.

Model	R	R^2	R^2 ajusted	standard error of the estimation
1	0.966	0.933	0.910	10.43578

Table 6. Rate inhibition of *F. oxysporum* f.sp *albedinis* at different concentration of sterilized and unsterilized compost extracts.

Concentration	10%	15%	20%	30%	40%
Unsterilized compost extract	20.1±0.5	37.3±2.7	44.5 ± 3.2	93.0 ± 5.1	97.9±1.7
Sterilized compost extract	4.8±0.5	6.6±0.7	5.6±0.1	5.9±0.53	18.4±2.6

factors have influenced mycelium growth in elevated SCE concentration.

Conclusion and perspective

This study revealed that unsterilized compost extracts were efficient for *in vitro* suppression of *Foa*. They could constitute an alternative for biological control of “Bayoud”. Further studies are required to isolate and select effective microorganisms inhabiting this compost. Also, it is necessary to evaluate the antifungal activity of this compost *in vivo* and in naturally infected soils in date palm grove.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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