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Suppressive Effect of Mature Compost of Date Palm By-Products on *Fusarium oxysporum* f. sp. *albedinis*

[1]K. Chakroune, [1]M. Bouakka, [2]R. Lahlali and [1]A. Hakkou
[1]Laboratory of Biochemistry, Faculty of Sciences, University Mohammed I, 60000 Oujda, Morocco
[2]Institut de Recherche en Biologie Végétale (IRBV), Département de Sciences Biologiques, Université de Montréal, 4101 Sherbrooke Est Montréal, Qc, H1X 2B2, Canada

Abstract: The aim of this study was to evaluate the possibility of exploiting mature compost of date palm by products in order to control *Fusarium oxysporium* f. sp. *albedinis* disease (Foa) commonly known as Bayoud. The obtained results showed that applying compost in mixture with peat and vermiculite S2 reduced significantly the losses of date palm seedlings of susceptible cultivar Bouffaggousse Gharasse due to the presence of Foa. However, the use of mixture substrate containing only peat and vermiculite S1 resulted in the death of 90% of the palm seedlings, as compared to non-infested S1. Also, the Foa population density and seedlings date palm mortality dropped in substrate mixtures S4 and S5 as the mature compost increased in soil substrate independently of date palm cultivar. The suppressive effect of compost seems reinforced by its richness of microorganisms having an antagonistic activity against Foa such as *Aspergillus*, *Penicillium* and *Bacillus*.

Key words: Compost, date palm, *Fusarium oxysporum* f. sp. *albedinis*, antagonistic microorganisms, suppressive effect

INTRODUCTION

Bayoud disease is the most destructive fungal diseases of date palm (*Phoenix dactylifera* L.). This disease was caused by *Fusarium oxysporium* f. sp. *albedinis* (Foa) which penetrates through the roots, induces withering of leaves and ultimately leads to the death of infected trees. The impact of this disease is very serious in North Africa, particularly in Morocco where 2/3 of palm trees were destroyed so far (Fernandez *et al*., 1998). Indeed, biological characteristics of *Fusarium oxysporum* f. sp. *albedinis* (Foa) and its date palm host (*Phoenix dactylifera* L.), complicates attempts to control the disease caused by Foa. The chemical control is spread due the fragility of the oasis ecosystem and to its non guaranteed efficiency. The prophylactic and quarantined measures cannot stop the disease. As far most vascular disease caused by soilborne pathogens, the use of the resistant cultivars remain the only effective method, but in the case of the date palm, the selection is not easy, besides, most of the resistant selected cultivars produced dates of poor quality of fruits and the date palm breeding system is laborious and offered only as a long-term plan (Pereau-Leroy, 1958; Toutain, 1968; El Hadrami *et al*., 2005).

During last decades, great emphasis has been placed on environmental protection. This has resulted in considerable pressure to reduce use of synthetic chemical substances, especially in agriculture. This has necessitated the scientific community to investigate alternative possibilities such as biological control, suppressive soils and other techniques. So, several researches are dedicated to the evaluation of compost as a mean of biological control (Hoitink *et al*., 1993; Veeken *et al*., 2005). Compost issued from diverse waste (agricultural, industrial or domestic) showed their capacities to protect the cultures against numerous enemies such as adventitious, insects, mollusks, nematodes, fungi, bacteria and viruses (Veeken *et al*., 2005).

Generally suppressive soils is a term used to describe a soil in which plants do not suffer from certain diseases or where disease severity is reduced, although a pathogen might be present and the host plant is susceptible to the disease; the opposite of a conducive soil. This phenomenon was first described in relation to date palms and Foa in Algeria and Morocco (Amir and Sabaou, 1983; Sedra *et al*., 1990; El Hassni *et al*., 2007). Consequently, some bacterial, yeasts and fungal isolates was reported as potential antagonistic candidates

Corresponding Author: A. Hakkou, Laboratory of Biochemistry, Faculty of Sciences, University Mohammed I, 60000 Oujda, Morocco

against Foa. This biocontrol strategy is very interesting but, it remains without application in orchard.

In a previous study (Chakroune *et al*., 2005), we demonstrated that composting of the by-products of date palm infected by *Fusarium oxysporum* f. sp. *albedinis* engenders stable and homogenous compost. Following on this research, our main objective in this study was to evaluate the ability of mature compost to protect date palm seedlings against *Fusarium oxysporum* f. sp. *albedinis*. We also wanted to determine the effect of this compost on the behaviour of this pathogen in different substrates. Finally, the inhibition activity of some selected micro-organisms issued from mature compost against Foa in *in vitro* dual culture assays has been evaluated.

MATERIALS AND METHODS

Pathogen isolate and compost: *Fusarium oxysporum* f. sp. *albedinis* isolate used in this study was isolated from Bayoud diseased rachis of susceptible date palm cultivar Bouffaggousse Gharasse in highly infested grove in the region of the Figuig, Morocco. This strain was stored in the oxide of ammonium at 4°C for no more than 6 months according to the method described by Locke and Colhoun (1974) and then transferred to Potato Dextrose Agar (PDA) medium at 25±1°C before use. This study was conducted in laboratory of Biochemistry (Faculty of Sciences, University Mohammed I, Oujda) during year 2005.

Mature compost used in this research was produced from by-products of date palm trees according to the method described previously by Chakroune *et al*. (2005). Different types of compost mixtures were tested as substrate culture in this study. All compost was obtained from trees dying from grove in the region of Figuig.

Compost effect on date palm seedling protection against Foa: To test disease development on a Foa sensitive date palm cultivar, seeds were obtained from cultivar Boufaggousse Gharasse and grown on sterile peat during 2 weeks at 38°C until germination. Two types of substrates were tested as growth media for these seedlings. Germinated seeds were then transferred in both pots culture containing a mixture of peat and vermiculite S1 (9v:1v) and peat, compost and vermiculite S2 (4.5v:4.5v:1v). Before use, both types of substrate mixtures were moistened with water and aerated by mixing every second day for two weeks.

The prepared substrates were inoculated with a spore suspension of *F. oxysporum* f. sp. *albedinis* at 10^6 cfu mL^{-1} of substrate. Conidia were collected from fungal cultures grown on PDA plates using sterile distilled water. Non-inoculated substrates were used as controls. Ten repetitions were carried out for each culture substrate and control. This experiment was conducted in a growth chamber maintained at 25±2°C with 16 h of photoperiod during nine months.

Substrate mixtures effect on date palm seedlings cultivars protection against Foa: In this experiment, date palm seedlings belonging to Bouskri, Aguellide and Ennajda cultivars were tested for their resistance to *F. oxysporium* f. sp. *albidenis* (Foa). The date palm seedlings were obtained from seeds of each cultivar as previously described. Three substrate mixtures were evaluated for each date palm cultivar. They were S3 (100% soil), S4 [mixture of soil and the compost at ratio of (2v:1v)] and S5 [mixture of soil and the compost at ratio of (1v:1v)]. For each date palm seedling cultivar, ten plants were used for each substrate infested with Foa at 10^5 spores g^{-1} of dry substrate. This experiment was conducted in growth chamber at 25±2°C with 16 h of photoperiod during ten months.

At monthly intervals a soil sample of 30 g was removed from rhizosphere of 3 to 4 date palm seedlings for each tested cultivar and each substrate culture using core drilling methods. Sampling was performed between a depth of 8 and 18 cm. Samples were suspended in 100 mL of sterile distilled water and shaken for 30 min. An aliquot of 0.1 mL of each sample was spread onto Petri dishes containing 20 mL of gelosed medium (Komada, 1975) and then incubated at 27°C for 6 days in the darkness and for 5 days under natural lighting at ambient temperature.

Screening of bacteria and fungi from mature compost for antagonism against Foa: The population of bacteria and fungi in mature compost was determined using suspension dilution technique. Isolations were performed on PDA medium for fungi and gelosed medium based on peptone (5 g L^{-1}) and yeast extract (3 g L^{-1}) for bacterial isolates. An amount of 20 g of mature compost was mixed in 100 mL sterile distilled water and shaken for 30 min. After serial dilutions, 0.1 mL were plated on Petri dishes containing 20 mL of PDA or gelosed medium. Petri dishes were sealed and kept at 27°C until development of microorganisms at least 3 days. Colonies were sub-cultured separately on Petri dishes containing the gelosed medium favourable for their development. Fungal isolates were characterized based on morphological characteristics using various identification keys. In contrast, all bacterial isolates were identified based on analyses of fatty acid methyl-esters (FAMEs) of total cellular fatty acids by gas chromatography using the MDI system INC, Novark, USA (Berg *et al*., 2005).

Bacterial isolates were screened for their ability to produce antifungal substances against *Fusarium oxysporium* f.sp *albedinus* by *in vitro* dual culture assays on Potato dextrose agar (PDA) (Landa *et al.*, 1997). Each bacterial isolate was spotted at 4 equidistant points along the perimeter of the plate (3 plates per isolates). After 48 h of incubation at 28°C in the dark, a 5 mm plug from the leading edge of a 7-day-old culture of Foa on PDA was placed in the center of the plate. Plates without bacteria were used as control. Plates were incubated at 27±2°C for 5 days, after which the length of hyphal growth toward the bacteria (Ri) and that on a control plate (Rc) were measured. The relative growth inhibition was expressed as [(Ri-Rc)/Rc × 100]. The isolates that caused significant inhibition of the pathogen growth were also examined for gram staining, endospore formation and fluorescent pigment on pseudomonas agar F (PAF) and hypersensitive reaction on White Burley tobacco leaves (Grant and Holt, 1977). Regarding the fungal isolates, the same dual culture assays were applied to assess their antagonistic activity against Foa, except that the PDA plates were inoculated with a plug from leading edge of 7-day-old culture of the fungal isolate and the pathogen at the same time.

Statistical analyses: The collected data were subjected to analyses of variance (ANOVA) using SAS software (SAS Institute, version 8.2, Cary, NC, USA) and the significance of differences among treatments was recorded at $p<0.05$ by means Duncan's Multiple Range test.

RESULTS

Compost effect on date palm seedling protection against Foa: The infestation of substrate mixture containing peat and vermiculite with *F. oxysporium* f. sp. *lbedinus* caused important losses of date palm seedlings at least 90% died as compared to noninfested substrate S1 (data not shown). However, the addition of mature compost to this substrate has significantly protected the date palm seedlings against Foa. Indeed, 70% of date palm seedlings cultivated in substrate S2 containing 50% of compost (1v:1v) remain unhurt.

Substrate mixtures effect on date palm seedlings protection against Foa: Table 1 show that the mortality rate of date palm seedling was significantly influenced by culture substrate compound independently of date palm cultivar. The highest mortality due to *F. oxysporium* f. sp. *albedinus* was observed in substrate mixture S3 containing 100% of soil. However, the addition of mature compost reduces significantly the seedlings mortality at

Table 1: Mortality rate (%) of the date palm seedlings cultivars obtained after ten months of experiment in different substrate mixtures infested with *Fusarium oxysporum* f. sp. *albedinis* at 10^5 cfu g^{-1} of dry substrate

	Date palm cultivars		
Substrate mixtures	Bouskri	Aguellide	Ennajda
S3	100a	100a	50a
S4	40b	50b	20b
S5	20c	40c	20b

S3: 100% soil; S4: Mixture containing compost and soil (1v:2v) and S5: Mixture containing compost and soil (1v:1v). In the same column, the treatment having the same letter(s) are not significantly different according to Duncan´s multiple ranges test ($p<0.05$)

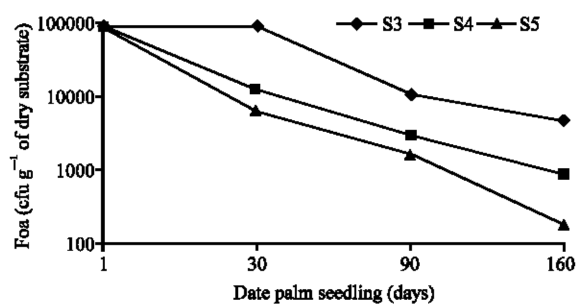

Fig. 1: Evolution of *Fusarium oxysporum* f. sp. *albedinis* in substrate mixtures of the date palm seedlings. S3: 100% soil; S4: mixture containing compost and soil (1v:2v); S5: mixture containing compost and soil (1v:1v)

least 60 and 80% for substrate mixtures S4 and S5 containing compost and soil at ratio of (1v:2v) and (1v:1v) respectively as compared to the control S3 (100% soil) in the case of the Bouskri cultivar and from 50 to 60% in the case of the other tested cultivars.

The date palm seedlings conducted in noninfested substrate mixtures containing compost and soil shown to be no infected with Foa and no died seedlings has been observed. Once again, these results confirm, the fast elimination of Foa during the composting process and also its suppressive effect against Foa which considered being highly virulent because in absence of the compost, the mortality rate reached 100% in susceptible cultivars (Bouskri and Aguellide) and 50% in resistant cultivar (Ennajda).

Figure 1 shows that the population density of Foa in different culture substrates is significantly dependent on the ratio of compost in the substrate mixture. The Foa density remains unchanged after 30 days of planting of date palm seedlings in different infested substrate mixtures without the compost. The addition of the compost reduce significantly the Foa density at least 88 and 94%, respectively in substrate mixtures containing compost and soil at ratio of (1v:2v) and (1v:1v). This reduction continues until the eradication of the fungus. In contrast, without the compost, its density remains relatively higher after 160 days of culture; it is about 5×10^3 cfu g^{-1} of dry substrate.

Screening of bacteria and fungi from mature compost for antagonism against Foa: The mature compost of date palm by products contains a diversified and rich population of microorganisms. It shelters approximately 5.4×10^7 cfu g^{-1} of dry compost. Based on the microbiological and morphological characteristics such as color, size and the general aspect of colonies, more than about twenty kinds of fungi and about fifty different colonies of bacteria have been distinguished. The microflora of the compost is mainly constituted by fungi belonging to the genera *Aspergillus* and *Penicillium* and bacteria which belong mainly to the genus *Bacillus*.

The ability of some bacterial and fungal isolates to inhibit mycelial growth of Foa was tested in *in vitro* dual culture assays and the results showed that the mycelial growth of Foa on PDA medium is slowed down in the presence of some isolated micro-organisms from the compost (Table 2). The inhibition rate varies significantly from one microorganism to the other one and ranged from 20 to 40% in the presence of *Bacillus* isolates and from 40 to 70% in the presence of fungi.

DISCUSSION

Biological control of soilborne plant pathogens takes into account three essential factors: the host, the pathogen and the environment. The soil and the peat used in this study are considered as receptive substrates against *Fusarium oxysporum* f. sp. *albedinis* because they permit it to perform its pathogenic activity without any difficulty (Table 1). Substrates mixtures containing the mature compost of date palm by-products show a significant suppressive effect against Bayoud. Its presence in soil or in peat reduces significantly the disease in date palm seedlings. Similar results were reported by Cohen *et al*. (1998) which showed that the severity of the disease caused by *Fusarium oxysporum* was reduced up to 95% in the cotton culture amended with mature compost from municipal solid residues. Weltzein (1991) also obtained encouraging results against gray decay of tomato by using the compost based on bovine fertilizer. The compost can act directly against plants diseases through its microbial biodiversity. This richness in microflora maybe consisted of important populations of antagonistic microorganisms, with great potentials to protect actively plants through creation of unfavourable environment for pathogens (Bordeleau, 1999; Zhang *et al*., 1998). Even in absence of the plant, the compost can act directly on the pathogens by inhibiting their development. Indirectly the compost can also operate on the plant living conditions by improving the soil structure as a contribution to stabilize the humus complex. So, plants growing in such conditions are less stressed and consequently more resistant to the diseases (Linderman and Gilbert, 1973) as the resistance can be induced by the presence of antagonistic microorganisms (El Hassni *et al*., 2007).

The reduction of *Fusarium* density observed in mixtures substrates containing the compost with/without host plant (Fig. 1) can explained the direct effect of compost of date palm by-products on Bayoud. The suppressive effects of compost are frequently mentioned in literature (Fuchs, 2003; Veeken *et al*., 2005; Pérez-Piqueres *et al*., 2006). Zinati (2000) indicated that compost can substitute the methyl bromide used as preventive fungicide treatment in extensive horticultural culture. This fungicide was often used to disinfect soil from Foa. The effect of compost on some pathogens is generally biological and is supported by the high microbial diversity occurring on compost (De Ceuster and Hoitink, 1999; Postma *et al*., 2003) or can have also a physico-chemical origin due to the presence of inhibiting substances (Ezelin De Souza, 1998). Accordingly, Serra-Wittling *et al*. (1996) showed that compost prepared from Municipal solid waste reduce both the inoculum density and the attack severity of *Fusarium oxysporum* f. sp. *lini* of flax. This inhibition could have a physicochemical or biological origin.

Znaidi (2002) showed that compost rich in lignocellulosic substances are the most effective in inhibiting effect of several species of *Fusarium*. Accordingly, the protective effect of date palm by-product compost against vascular fusariose of date palm seedlings (Table 1 and 2) can be attributed to the presence of lignocellulosic substances and/or in its richness with antagonistic microorganisms.

The date palm tree by-products compost shows a suppressive effect in a substrate, even without host plant, by preventing the development of pathogen populations. Ezelin-De Souza (1998) underlined a similar effect of compost of bagasse on the population dynamics of *Fusarium solani*. These results highlight the effect of the compost microflora in eliminating Foas and consequently protect the date palm seedlings. Indeed, autoclaving destroyed the microflora present in the compost and make it more dangerous as the pathogenic fungi finds accurate conditions for its proliferation without any form of competition and the presence of essential nutrients and good physiochemical conditions. Under these conditions,

Table 2: Inhibition rate (%) of mycelial growth of *Fusarium oxysporum* f. sp. *albedinis* by some selected microorganisms isolated from mature compost of date palm by-products

Microorganisms	Isolates	Mycelial growth inhibition rate (%)
Aspergillus	AC01*	60±8
	AC02	40±3
	AC03	70±0
	AC04	65±1
Penicillium	PC01	53±3
	PC02	68±7
	PC03	53±6
Bacillus	*B. subtilis* BC01	29±3
	B. cereus BC07	34±4
	B. subtilis BC08	45±5
	B. pumilis BC16	18±2
	B. pumilis BC19	25±1
	B. subtilis BC21	28±4
	B. subtilis BC33	39±1
	B. cereus BC34	37±1
	B. subtilis BC44	34±4
	B. subtilis BC45	27±6

*A: *Aspergillus*; P: *Penicillium*; B: *Bacillus* and C: Compost

the pathogenic fungi develop quite easily and the suppressive effect of the compost is ruined as observed by Fuchs (2003). The older compost operates probably much more by its natural microbial activity than by stimulating microbial activity of the substrate (Yao *et al*., 2006; Pérez-Piqueres *et al*., 2006). These results can be explained by the fact that the compost stimulates the biological activity in the soil as part of organic matter it nourish the microorganisms present in the soil and therefore support both their growth and development (Bailey and Lazarovits, 2003). Moreover, the compost feeds the soil in an important and diversified microbial flora while enriching its biodiversity and consequently its suppressive capacity against pathogens (Gomez *et al*., 2006). Therefore, the effectiveness of the date palm by-product compost is due primarily to the biological antagonists such as bacteria and fungi as yet their presence is a sign of maturity. In fact, the young composts which are rich in lignin are likely able to exhibit inhibiting chemical compounds, whereas the inhibiting effect of the mature compost is primarily assured by its microbial population. The beneficial effect of the compost attributed to biological activity is due, either to the overall microorganism population of the soil (general control) or, to the presence of antagonistic microorganisms (specific control) (Hoitink *et al*., 1997). For general resistance, microorganisms compete with the pathogens for nutrient sources and/or vital space. This system is effective for the pathogens depending on external sources of carbon as the fusariose causative agents. For specific resistance, antagonistic microorganisms constitute the initial infectious population with no harmful effects on the plant, or exhibit antibiotic substances which inhibit pathogens development. Van Bruggen and Duineveld (1995) reported that the microorganisms present in the composts are excellent competitors for easily available carbon sources, involving a fungicidal effect against pathogens depending on this carbon source.

In the case of biological control of the vascular fusarioses, several studies reported the use of bacteria such as, *Bacillus*, *Pseudomonas fluorescens* and fungi such as *Penicillium*, nonpathogenic *Fusarium* and *Trichoderma* (Postma *et al*., 2003). Sedra *et al*. (1990) attributed the resistance of the soil from Marrakech to the presence of *Pseudomonas fluorescens*, nonpathogenic *Fusarium* and actinomycota. The suppressive effect of the mature compost of date palm by-product against Foa can be explained by its richness in microorganisms belonging to the genus *Aspergillus*, *Penicillium* and *Bacillus* (Table 2). According to Trankner (1992), these genuses are among the active components of the compost microflora.

These inhibitions could be explained by the presence of fungicidal substances secreted during the developing phase of these microorganisms. The intensity of inhibition depends on the type of antagonistic microorganism. This difference in activity against fungal pathogens can be explained by the quality or the quantity of secreted toxic substances. The suppressive effect exerted by microbial flora of this compost on Foa can be attributed on one hand to the high fungistatic and antibiotic activities which impact directly on the parasite and on the other hand, with the start of the induced resistance for date palm seedlings (El Hassni *et al*., 2007).

In summary, present research is the first investigation highlighting the suppressive effect of mature compost of date palm by-products against Bayoud diseases in North Africa. Our results proved that the date palm by-products compost seems to be an effective biological tool able to limit the disease propagation and to control the severity of the vascular fusariose in palm plantations of the oasis ecosystem of North Africa. The use of this compost on the infested soil with Foa considerably reduces the population of this fungus through biological mechanisms in respect to the vulnerability of oasis ecosystem. Furthermore the use of this compost in the agricultural systems of this area can provide a tool for recycling all agricultural waste, to alleviate the production costs in vegetable cultivation by decreasing the losses due to diseases, to lower the use of chemicals substances and promote production of chemical residues free foods.

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