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Raoudha Khanfir Ben Jenana, Rabiaa Haouala, Mohamed Ali Triki, Jean-Jacques Godon, Khaled Hibar, Mohamed Ben Khedher, Belgacem Henchi

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COMPOSTS, COMPOST EXTRACTS AND BACTERIAL SUPPRESSIVE ACTION ON *PYTHIUM APHANIDERMATUM* IN TOMATO

RAOUDHA KHANFIR BEN JENANA[1*], RABIAA HAOUALA[1], MOHAMED ALI TRIKI[2], JEAN-JAQUES GODON[3], KHALED HIBAR[4], MOHAMED BEN KHEDHER[5] AND BELGACEM HENCHI[6]

[1]*Institut Supérieur de Biotechnologie, Monastir, Tunisia,*
[2]*Institut de l'Olivier, Sfax, Tunisia,*
[3]*INRA, UR050, Laboratoire de Biotechnologie de l'Environnement, Narbonne, F-11100, France,*
[4]*Ecole Supérieure d'Horticulture, Chott Mariem, Tunisia,*
[5]*Centre Technique de l'Agriculture Biologique, Chott Mariem, Tunisia and*
[6]*Faculté des Sciences, Tunis, Tunisia*

Abstract

The effects of three composts (C_1, C_2 and C_3) produced from Solid Olive Mill wastes (SOMW), *Posidonia oceanica* (Po) and Chicken Manure (CM), at different proportions, were tested on *Pythium aphanidermatum*. To evaluate the fungal pathogen inhibition, *In vitro* and *In vivo* tests were carried out. *In vitro* tests aimed to study the inhibitive effect of pure compost extracts and the isolated antagonist bacteria. *In vivo* tests drench and dip root inoculations were done on tomato seedlings. Pure extracts inhibited the fungal pathogen growth. Isolated bacteria also showed an antagonistic action on the mycelial growth of *P. aphanidermatum* and the 16sRNA identification showed that *Bacillus subtilis* and *B. thuringiensis* had the highest inhibition. *In vivo* tests showed that drench inoculated tomato seedlings sown in substrates with composts resisted to *P. aphanidermatum*. Root dip inoculated seedlings had a more sustained growth in substrates mixed with composts. Results showed that tested composts acted by both their chemical composition and microorganisms and could be used at appropriate proportions as biological fertilizers.

Introduction

Damping-off and root rot caused by the *Pythium* are considered among the most devastating diseases of greenhouse-grown crops. This pathogen affects nearly every crop grown in every part of the world (Ben Yephet & Nelson 1999). The main causal agent of the damping-off and root rot is *P. aphanidermatum* Edson (Fitzp.). In spite of its devastating effect, chemical fight (Folman *et al*., 2004) and physical control (Benhamou *et al*., 1997) of this fungal pathogen are very difficult to realize. Some authors studied the fight by organic amendment (Shuler *et al*., 1989; Paulitz & Baker, 1987; Kao & Ko, 1986). Biological control of *Pythium* is a promising approach, seeing that it is comparatively benign towards the environment (Paulitz & Bélanger, 2001; Rattink, 1992). Many factors may affect suppression of diseases in compost amended growth media caused by *Pythium spp*. These factors include compost type (Craft & Nelson 1996; Ringer *et al*., 1997), organic matter quality (Boehm & Hoitink 1992; Boehm *et al*., 1997) and quantity (Erhart & Burian, 1997) and associated levels of microbial activity (Theodore & Toribio, 1995; Craft & Nelson, 1996). In previous studies, damping-off of cucumber was suppressed with composts prepared from cattle manure (Mandelbaum *et al*., 1988, Mandelbaum & Hadar, 1990), grape marc (Mandelbaum *et al*., 1988), licorice roots (Hadar & Mandelbaum 1986), municipal biosolids (Lumsden *et al*., 1983) and sugarcane residues (Teodore & Toribio, 1995).

[*]Corresponding author. Email: bjnkh@yahoo.fr
Phone: +216 73 340 696, Fax: +216 73 244 624

Several composts from vegetal material were assessed by many authors. Seagrass and seaweed residues were tested with yard waste for horticultural purposes by Orquín *et al*., (2001). Roig *et al*., (2001) studied the composting of the solid fraction of olive mill wastewater. More than one compost from animal material were also studied in the literature e.g., pig manure (Atiyeh *et al*., 2000), mink manure (Ferguson 2001), racetrack manure, (Warman &Termeer 1996) and chicken manure (Hachicha *et al*., 2006; Kogbe, 1980).

Larbi (2006) concluded that most of the tested composts protected cucumber seedlings against damping-off caused by *Pythium ultimum*. According to this author, during the composting process, an antagonist microbial population might be developed to enable compost to protect plants against telluric diseases. High quality composts can be successfully used as a biological control means particularly in vegetable crops (Fuchs, 2002; Fuchs & Larbi 2004) as well horticultural crops especially in soilless cultures (Inbar *et al*., 1993). Generally, the main protection mechanism against phytopathogens seems to be clearly dependant on compost microbial activity (Hoitink *et al*., 1997; Tilston *et al*., 2002). Specific microorganism importance is established by several authors: *Trichoderma asperellum* against *Fusarium* on tomato (Cotxarrera *et al*., 2002), *Acromonium* sp., against *Phytophthora nicotianae* (Widmer *et al*., 1998) and *Bacillus subtilis* against *Pythium* sp., (Theodore & Toribio 1995).

Posidonia (*Posidonia oceanica* Delile) is a Mediterranean marine phanerogam. Leaves accumulated in litter are rejected in enormous quantities on the Tunisian beaches. Not many studies have been conducted on the Posidonia's antibiotic activity (Cariello & Zanetti, 1997; Bernard & Clément 1983; Bernard & Pesando 1989). Chicken manure accumulation is accompanied by serious environmental problems. For instance, the underground water is affected by nitrates and phosphates; air is affected by the ammonia emission and soil by accumulated heavy metals and phosphorus (Walker & Bernal 2007). The solid fraction of olive mill wastewater is accumulated each year in the Tunisian oil mills and is very rich with organic matter. Despite its various uses, the available quantities are still huge (Fourati *et al*., 2001; Nefzaoui & Ben Dhia, 1978; Nefzaoui & Zidani, 1987).

Aqueous compost extract (compost tea) is rich with nutrients and microorganisms. It can stimulate growth, protect plants from diseases and helps suppress soil borne pathogens (Quarles, 2001). During the two last decades, many authors found out that compost extracts are efficient against phytopathogens (Elad & Shteinberg, 1994; Yohalem *et al*., 1996; Reuveni *et al*., 2002; Al-Dahmani *et al*., 2003).

Within the framework of evaluating three new types of compost made from *Posidonia oceanica*, Chicken manure and solid olive mill wastes, we tested in this study their suppressive effect on *Pythium aphanidermatum.* To observe and evaluate the antagonistic effect of the composts, their extracts and bacteria present in these extracts, the current study has been conducted *In vitro* and *In vivo*. The *In vitro* test consisted of the confrontation of the compost extracts with the fungal pathogen. The bacterial strains that had been isolated and purified from the compost extracts were then confronted to the fungal pathogen. The bioassay was conducted on *P. aphanidermatum* inoculated tomato (*Solanum lycopersicum* L.) seedlings.

Materials and Methods

Composition of the original composts: In this study, we used three composts (C_1, C_2 and C_3) produced in the Technical Center of the Organic Agriculture of Chott Meriem, Tunisia with the following compositions: i) C_1: 50% solid olive mill wastes (SOMW),

20% *Posidonia oceanica* (Po) and 30% Chicken Manure (CM); ii) C_2: 35% (SOMW), 35% (Po) and 30% (CM); iii) C_3: 20% (SOMW), 50% (Po) and 30% (CM) (Table 1). The percentages are the proportions in weight. The total weight of each compost pile is 20 tons. Posidonia was collected from Hergla beaches 20 km North from Sousse, Tunisia and abundantly washed to eliminate accumulated salts from the leaf surface as suggested by Saïdane *et al*., (1979).

Preparation of pure compost extract: The pure extracts, E_1, E_2 and E_3 corresponding to C_1, C_2 and C_3 respectively, were prepared according to the steepage procedure of Weltzein (1992) modified by Znaïdi (2002). Each compost was suspended in tap water (1:5 vol/vol) in a loosely covered 10-liter plastic container. The suspension was incubated for 6 days at 15 to 20°C and daily stirred 5 to 10 minutes. Extracts were then filtered through a 2-mm screen to remove large particles. After a 8000 rpm centrifugation (Sigma) for 15 minutes, surfactant was filtered through a 0.2 µm filter (Schleicher & Schuell, D-37582).

Isolation and purification of bacterial colonies: From 1 ml of each compost extract, decimal dilutions from 10^{-2} to 10^{-4} were made. In sterile Petri plates, 5 ml of each solution were poured on a Nutrient Agar medium. To avoid fungal development Cycloheximide antibiotic (100µg ml^{-1}) was added to the medium. After a 48 hour incubation at 28°C, the most numerous bacterial colonies with visually different morphology were picked out and isolated. These isolates were transferred to other dishes on LB (Luria Bertani) medium (Tryptone 10g, yeast extract 5g, NaCl 10g). After a 48 hour incubation at 28°C, the Petri dishes were removed and the isolates put in Appendorf tubes in 1ml of the solution (50% LB and 50% Glycerin) already autoclaved for 20 minutes at 120°C. These tubes were then preserved at -20°C for identification of bacteria.

Molecular analysis of 16S rDNA: Bacterial colonies were suspended into 35µl ultra-pure water and were subjected to four freeze-thaw cycles in order to lyse the cells (Dunbar *et al*., 1996). Bacterial 16S rDNA genes were amplified by PCR using the universal reverse primer W02 (5'-GNTACCTTGTTACGACTT-3') and the bacterial forward primer W18 (5'-GAGTTTGATCMTGGCTCAG-3'), *Escherichia coli* position 1509-1492 and 9-27, respectively (Brosius *et al*., 1981). Each reaction tube contained 1 µl of each primer, 1×RED Taq PCR buffer (Sigma, St. Louis, MO. USA), 200ng (each) desoxynucleotide triphosphate and 1U of Taq DNA polymerase (Sigma) in a total volume of 50 µl. The reaction mixture was placed in a thermocycler (Applied biosystems) at 94°C. After an initial denaturation at 94°C for 2min, 25 temperature cycles were performed for bacteria (94°C for 1min, 50°C for 1 min, and 72°C for 1min). Products were electrophoresed on a 0.7% agarose gel and viewed by ethidium bromide staining. After sequencing (Millegen, Labège, France), each sequence was compared using BLASTIN (Altschul *et al*., 1990). The nucleotide sequence data reported in this paper is available in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers from EU515232 to EU515236.

Fungal pathogen: *P. aphanidermatum* pathogen was isolated from diseased tomato roots by the laboratories of phytopathology of the Olive Institute in Sfax (Tunisia).

Table 1. Compositions (%) of the original composts C_1, C_2 and C_3.

	Posidonia oceanica (Po)	Chicken manure (CM)	Solid olive mill wastes (SOMW)
C_1	20	30	50
C_2	35	30	35
C_3	50	30	20

The *In vitro* assay

Effect of pure compost extracts: Compost extracts sterilization aims to check whether microorganisms are responsible for the seedlings protection (Larbi, 2006) or not.

Hundred µl of each pure extract were pipetted into pits of 6 mm diameter on PDA medium. In each Petri plate, a 6 mm diameter disk of the pathogen mycelial culture was placed 4 cm from the pit. Control plates were prepared by replacing the quantity of pure extracts by the same quantity of sterile distilled water (Hibar, 2002). After a 72 hour incubation at 26°C, pathogen mycelial growth was measured in each plate and the growth on PDA containing compost extracts was compared with the control. Each treatment was replicated three times. The inhibitive effect of the composts' extracts on mycelial growth of *P. aphanidermatum* was measured in terms of percentage growth inhibition (I %) and calculated according to the method of Hmouni *et al*., (1996). Percentage growth inhibition was determined as = 100 × (1-Cn/Co), where Cn is the average diameter increase of pathogen mycelium in each treatment and Co is the average diameter increase in the control plate.

The antagonist effect of bacterial isolates: The confrontation by direct contact consists of placing on PDA medium at 3 cm radius, diametrally opposed the isolated bacteria and *P. aphanidermatum*. The confrontation was done under sterile conditions. The Petri plates were then placed in an incubator at 28°C for 72h. Control was prepared by placing the pathogen without bacteria. Bacteria inhibition rates were obtained by the calculation of mycelium growth inhibition percentage according to Hmouni *et al*., (1996). Mean value of three replications is considered.

The bioassay

Seedling preparation: Tomato seeds (*Solanum lycopersicum*, L. cv. Rio Grande) were disinfected in absolute ethanol for 5 minutes, and rinsed with sterile distilled water to eliminate the pesticides residues used in seeds treatment (Benhamou *et al*., 1997). After drying, seeds were placed under sterile conditions in Petri plates on filter papers soaked with sterile distilled water. Seed germination was assessed after 4 days in a growth chamber at 20°C (Hibar *et al*., 2005). Twenty germinated seedlings were transferred to alveolus plates filled with the following substrates: i) So: 50% sterilized peat and 50% sterilized perlite (for inoculated and non inoculated control (IC and NIC respectively)), ii) S_1: 1/5 C_1 and 4/5 So (v/v), iii) S_2: 1/5 C_2 and 4/5 So (v/v); iv) S_3: 1/5 C_3 and 4/5 So (v/v).

Preparation of the inoculum solution: The fungal pathogen was cultivated on PDA medium (Ben Yephet & Nelson, 1999). After ten days of incubation at 28°C, oospores suspension was scraped from the surface of the medium using a sterile blade and placed in a conical 100ml tube containing sterile distilled water. A concentration of 10^3 oospores ml^{-1} was determined using a Malassez cell (Hibar *et al*., 2006).

Table 2. Inhibition of *P. aphanidermatum* mycelial growth caused by compost extracts after 72 hours at 26°C on PDA medium.

Compost extract	Inhibition rate (I %)
E_1	$22,81^a$ (± 0.3)
E_2	16.02^b (± 0.3)
E_3	14.56^b (± 0.25)

Values followed by different letters are significantly different at $p<0.05$. Values between parentheses represent the standard deviations. E_1: Extract of Compost C_1; E_2: Extract of Compost C_2; E_3: Extract of Compost C_3.

Drench inoculation: Two-leaf germinated seedlings were transferred in alveolus plates containing the substrates mentioned above (So, S_1, S_2, and S_3). Three days after their transplanting, seedlings were drenched with 100 ml of the inoculum solution for the three treatments and the inoculated control (IC). For non-inoculated control (NIC) distilled water were used. The experiment was carried out in a growth chamber at 24°C with a 12-hour-photoperiod and 80% humidity. Seedlings were watered daily. As a measure of disease severity, seedling stands were counted 25 days after inoculation. Low percent-seedling stands indicated high disease severity, whereas high percent-seedling stands indicated high disease suppressiveness. Percent-seedling stands were calculated and the mean value of five replications was considered (Ben-Yephet & Nelson 1999). Means of the percent-seedling stands of five replications were calculated.

Root dip inoculation: At two-leaf-stage, seedling roots of 20 inoculated seedlings were cut at 1cm from the tips, soaked in inoculum solution for 1 hour and transferred to alveolus plates filled with the following substrates: So (for IC and NIC), S_1, S_2, and S_3. Transferred seedlings were daily watered and 30 days after their inoculation, the seedling top lengths were measured and the mean value of five replications was calculated.

Experimental setup and statistical analysis: Each experiment was replicated five times. For the bioassay, 20 plants per treatment were used. The experiments were analyzed using the DUNCAN test with factorial treatment structure and the analysis of the treatment effect was performed by using the general linear model procedure of SPSS (SPSS 10.0). The means were separated on the basis of least significant differences at 5% probability level.

Results

In vitro assay

Compost tea inhibition: Results showed that all compost extracts had an inhibiting effect on *P. aphanidermatum* development and percentage inhibition varied between 14.56% and 22.81% (Table 1). Mycelial growth measured in the different Petri plates showed that there is no significant difference between the suppressive effects of extracts E_2 and E_3 whereas the inhibitive effect of E_1 is significantly different from E_2 and E_3. Inhibition rates were 22.81%, 16.02%, and 14.56 % for E_1, E_2 and E_3 extracts respectively (Table 2).

Table 3. 16S rDNA molecular analysis and antagonist effects of bacterial isolates on *P. aphanidermatum*.

Extracts	Cultivated isolates	Antagonist effect (%)*	Gen Bank accession number	Nearest blast-determined neighbor	
				Species	Similarity (%)**
E_1	E_{1-1}	10^a			
	E_{1-2}	10^a			
	E_{1-3}	0^b			
	E_{1-4}	0^b			
	E_{1-5}	38^c	EU515233	*Bacillus subtilis*	100
	E_{1-6}	0^b			
	E_{1-7}	0^b			
	E_{1-8}	34^d	EU515234	*Achromobacter xylosoxidans*	100
E_2	E_{2-1}	0^b			
	E_{2-2}	0^b			
	E_{2-3}	35^d	EU515236	*Pseudomonas pseudoalcaligenes*	100
	E_{2-4}	28^e			
	E_{2-5}	35^d	EU515232	*Pseudomonas fluorescens*	100
	E_{2-6}	0^b			
E_3	E_{3-1}	0^b			
	E_{3-2}	0^b			
	E_{3-3}	37^c	EU515235	*Bacillus thuringiensis*	100
	E_{3-4}	0^b			
	E_{3-5}	10^a			
	E_{3-6}	8^a			
	E_{3-7}	0^b			

E_1: Extract of Compost C_1; E_2: Extract of Compost C_2; E_3: Extract of Compost C_3.
Values followed by different letters are significantly different at $p<0.05$.
* Antagonist effect based on five replicates per treatment.
**Percentage of 16S rDNA sequence similarity between the representative isolate and its nearest neighbor from Gen Bank database.

Antagonistic effect of bacterial isolates: To reveal the microbiological role of the extracts suppressive effect, bacterial colonies of the extracts were purified after selection based on macroscopic aspect. The observations of these colonies showed at least 8 isolates in extract E_1, 6 isolates in extracts E_2 and 7 isolates in E_3. Confrontation bacteria-fungal pathogen demonstrated that E_1 contains 4 types of suppressive bacteria to *P. aphanidermatum* (E_{1-1}; E_{1-2}; E_{1-5} and E_{1-8}). Isolates E_{1-1} and E_{1-2} inhibited 10% the development of the fungal pathogen while isolate E_{1-8} gave an inhibition rate of 34 %, and isolate E_{1-5} produced the highest inhibitive effect with a 38% rate (Table 3). The antagonist effects of both E_{1-1} and E_{1-2} were significantly different from the ones of E_{1-5} and E_{1-8}. Concerning E_2, 3 isolates of bacteria had a suppressive effect on the fungal pathogen development (E_{2-2}; E_{2-4} and E_{2-5}) with a rate of 28% of inhibition for isolate E_{2-4} and a rate reaching 35% for isolates E_{2-3} and E_{2-5}. E_3 showed 3 bacterial isolates with a suppressive effect on *P. aphanidermatum* (E_{3-3}; E_{3-5} and E_{3-6}). The inhibiting effects of these isolates were 37%, 10% and 8%, respectively. Identification of bacteria with a suppressive effect higher than 30% showed that the most important inhibition was obtained by *Bacillus subtilis* (E_{1-5}) and *Bacillus thuringiensis* (E_{3-3}) with inhibition rates of 38 and 37% respectively. *Pseudomonas pseudoalcaligenes* (E_{2-3}) and *P. fluorescens* (E_{2-5}) suppressed at 35% the fungal pathogen, while *Achromobacter xylosoxidans* (E_{1-8}) had an antagonist effect of 34% (Table 3).

In vivo assay

Drench inoculation: Values recorded after 25 days of inoculation showed that all (100%) of the non-inoculated seedlings (NIC) survived, while 10% survived in the inoculated, untreated control. Seedling stand percentage in S_1 was significantly higher than in the S_2 and S_3 treatments, and all were higher than inoculated, untreated control (Table 4). The percentages of seedlings stand sown in the composts were 80% for S_1 and 55% for S_2 and S_3.

Root dip inoculation: Values recorded 30 days after inoculation showed that 100% of the seedlings in the non-inoculated control (NIC) were dead, whereas seedlings planted in the substrates showed a resistance against the inoculated pathogen and abundant root systems. Mean seedling lengths were approximately: 12 cm, 15 cm, 20 cm, 8 cm and 18 cm in S_1, S_2, S_3, So-IC and So-NIC respectively (Table 5). Furthermore, we noticed that seedlings in S_3 are higher than those in So-NIC (Fig. 1).

P. aphanidermatum was re-isolated from diseased plants. Microscopic examination of the re-isolated colonies did not indicate differences between re-isolated colonies and those used for inoculation. We note also that no pathogen was re-isolated from non-inoculated control plants.

Discussion

In vitro tests showed antagonist effect on *P. aphanidermatum*. However, the suppressiveness of isolated bacteria was more accurate than this of pure composts extracts. Our results confirm that plant protection was not due essentially to extract microbial populations, which is confirmed by other authors reporting that the antifungal activity of composts extracts persists even after sterilization (Achimu & Schlösser, 1991; Elad & Shtienberg, 1994; Cronin *et al*., 1996; Zhang *et al*., 1998). On the other hand, according to McQuilken *et al*., (1994) and Weltzien (1992), sterilization of composts extracts eliminates their antagonistic effect. Different mechanisms are supposed to contribute to the suppression of soil-borne plant pathogens: parasitism, production of antifungal compounds, competition for nutrients and colonization sites, and inducing systemic resistance in plants against pathogens (Fravel, 1988; Han *et al*., 2000; Lugtenberg *et al*., 2001; Bergsma-Vlami *et al*., 2005; Dahiya, 2005; Kobayashi *et al*., 2005).

Bacteria identified in our study are *Bacillus subtilis, Achromobacter xylosoxidans, Pseudomonas pseudoalcaligenes, Pseudomonas fluorescens* and *Bacillus thuringiensis.* Nielsen & Sørensen (1997) reported that *Bacillus polymyxa* synthesizes antibiotics such as Fusaricidins B, C and D or proteolytic and hydrolytic enzymes (cellulase, mannanase and xylanase) which destroy the cellular membrane of the following fungal pathogens: *Aphanomyces cochleoïdes, Pythium ultimum* and *Rhizoctonia solani.* On the other hand, Raupach *et al*., (1996) reported that *Bacillus subtilis* induced systemic resistance in cucumber and tomato against mosaic cucurmovirus using plant growth-prometting rhizobacteria *(PGPR). Pseudomonas fluorescens* produces antifungal compounds (Notz *et al*., 2001) and antibiotics (Delaney *et al*., 2001; Thomashow & Weller 1988). *Pseudomonas* spp. produces different types of metabolites, such as volatiles, toxins, cyclic lipopeptides, enzymes and antibiotics, which enable the genus to compete effectively, and microbial fitness to survive in most environments (Haas & Keel, 2003).

Table 4. Mean percentages of tomato seedlings stand sown in tested substrates.

Substrate	Seedlings stand percentage (%)
S_1	80^a (±1.73)
S_2	55^b(±1.00)
S_3	55^b(±2.64)
So-IC	10^c (±1.80)
So-NIC	100^d

So-IC: 50% sterilized peat and 50% sterilized perlite for inoculated control, So-NIC: 50% sterilized peat and 50% sterilized perlite for non-inoculated control; S_1: 1/5 C_1 and 4/5 So (v/v); S_2: 1/5 C_2 and 4/5 So (v/v); S_3: 1/5 C_3 and 4/5 So (v/v).
Values followed by different letters are significantly different at $p<0.05$. Values between parentheses represent the standard deviations. Mean values of five replications are considered.

Table 5. Mean seedling lengths of tomato sown in tested substrates 30 days after their inoculation.

Substrates	S_1	S_2	S_3	So-IC	So-NIC
Mean seedling lengths	11.87^c (±1.58)	14.85^d (±2.47)	20.08^e (±1.53)	7.9^a (±1.56)	17.8^b (±1.29)

Mean seedling lengths in S_1: (1/5 C_1 and 4/5 C (v/v)); S_2: (1/5 C_2 and 4/5 C (v/v)); S_3: (1/5 C_3 and 4/5 C (v/v)); IC: Inoculated Control (50% sterilized peat and 50% sterilized perlite); and NIC: Non Inoculated Control (50% sterilized peat and 50% sterilized perlite). Different letters show significant differences at $p<0.05$.Values between parentheses represent the standard deviations.

Fig. 1. Seedlings transplanted in the tested substrates 30 days after inoculation. So-IC: 50% sterilized peat and 50% sterilized perlite (Inoculated Control), So-NIC: 50% sterilized peat and 50% sterilized perlite (Non Inoculated Control); S_1: 1/5 C_1 and 4/5 So (v/v); S_2: 1/5 C_2 and 4/5 So (v/v); S_3: 1/5 C_3 and 4/5 So (v/v).

In vitro and *In vivo* production of antibiotics by numerous antagonistic bacterial strains has been demonstrated (Bergsma-Vlami *et al*., 2005). The *In vivo* test results showed that the investigated composts have a suppressive effect on *P. aphanidermatum*. Furthermore, inoculated seedlings had better stand and growth than non inoculated control. Results differed as per substrates. Indeed, seedlings stand percentage in S_1 (80%) is significantly higher compared to seedlings stand percentage registered in S_2 and S_3 (55%). These values are higher than those obtained by Ben-Yephet & Nelson (1999), who reported that between 24 and 28°C, municipal biosolids compost and a leaf compost give a mean seedling stand of cucumber between 50 and 54.2%. These authors found out that, in a peat and compost media, tomato seedlings inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* show lower level of contamination compared to peat growing control. On the other hand, Hibar *et al*., (2005) demonstrate that the transplantation of inoculated tomato seedlings in compost extract treated peat inhibited *Fusarium oxysporum* effect. In the same study, the authors show significant difference between inoculated control roots and those of seedlings inoculated and treated with compost extracts. Suppression of damping-off caused by *P. aphanidermatum* by different types of composts is widely reported in literature. Damping-off of cucumber caused by *P. aphanidermatum* is suppressed by composts prepared from separated cattle manure (Mandelbaum & Hadar, 1990; Mandelbaum *et al*., 1988), grape marc (Mandelbaum *et al*., 1988), licorice roots (Hadar & Mandelbaum, 1986), sugarcane residues (Theodore & Toribio 1995). Nevertheless, Lumsden *et al*., (1983) reported that municipal biosolids compost does not inhibit damping-off of bean.

Bailey & Lazarovits (2003) reported that composts are an important source of nutrients usable by the microorganisms. As a consequence, composts amendments generally enhance the development of the microflora and increase the global activity of the soils. This is an explanation of the better growth in presence of composts. These findings are confirmed by the *In vivo* tests results showing that inoculated tomato seedlings grow better in media containing compost than non-inoculated seedlings in peat and perlite growing media. Root dip inoculation test showed that seedlings transplanted in substrate S_3 are longer than non-inoculated control with 11% of stimulation. This result confirms those of authors testing several types of composts on tomato seedlings. Levy & Taylor (2003) mentioned that mink farm compost and horse manure compost stimulate root and shoot growth of tomato Meunchang *et al*., (2006) reported that sugar mill by-products compost may be a good soil amendment to promote tomato growth. Benefic effect of composts on plant growth and yield was reported by more than one author. Manios & Kapetanios (1992) studied composted municipal solid waste in greenhouse tomato production. Lulakis & Petsas (1995) studied the effect of vine-cane compost on tomato seedlings. The results found in this work comply with those reported in previous studies on suppressive activity of composts. To our knowledge, this is the first study on such composts against *P. aphanidermatum*. Thus, a biological control system requires a better knowledge of the inhibitive agent action mechanism. Further researches on the identified bacteria might be conducted in order to determine their suppressiveness mechanism.

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