Dynamic Soil, Dynamic Plant ©2009 Global Science Books

Biocontrol of Tomato Verticillium Wilt by Using Indigenous *Gliocladium* spp. and *Penicillium* sp. Isolates

Hayfa Jabnoun-Khiareddine[1] • Mejda Daami-Remadi[2*] • Fakher Ayed[3] • Mohamed El Mahjoub[1]

[1] Institut Supérieur Agronomique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia
[2] Centre Régional des Recherches en Horticulture et Agriculture Biologique, 4042, Chott-Mariem, Sousse, Tunisia
[3] Centre Technique de l'Agriculture Biologique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

Corresponding author: * **daami_rm@yahoo.fr**

ABSTRACT

Endogenous *Gliocladium* spp. and *Penicillium* sp. isolates were tested *in vitro*, *in vivo* and *in situ* for their antagonistic activity against *Verticillium* spp. causing tomato vascular wilt in Tunisia. *Gliocladium catenulatum*, *G. roseum* and *Penicillium* sp. isolates reduced the radial growth of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* in comparison to the untreated controls. Antagonistic potential of *Gliocladium* spp. and *Penicillium* sp. against tested wilt agents showed intra- and inter-specific variations. Additionally to the sclerotinization inhibitory activity and to the reduced abundance of resting structures of *Verticillium* spp. observed, compared to untreated controls, antagonists tested caused several alterations of *Verticillium* spp. mycelium at the confrontation zone. The germination of *V. dahliae* microsclerotia, exposed for 30 min to liquid cultures of antagonists tested and incubated at 20°C, was completely suppressed compared to the control microsclerotia treated with sterile distilled water. Furthermore, germinating microsclerotia dual cultured with *Gliocladium* spp. and *Penicillium* sp. became inable to germinate and mature microsclerotia progressively lost their typical dark colour. All tomato cv. 'Ventura' plants, when treated at planting with *Gliocladium* spp. and *Penicillium* sp. spore suspensions and inoculated with *V. dahliae*, showed after 60 days of culture under growth chamber conditions, reduced severity of Verticillium wilt in comparison to inoculated and untreated control plants. Plants treated with antagonists tested showed increased height and root and stem fresh weights in comparison to the inoculated and untreated control. The discoloration index, noted on tomato plants treated at planting by *G. catenulatum, G. roseum* and *Penicillium* sp. and grown under greenhouse conditions, was significantly reduced compared to the untreated control. Plants treated with *Penicillium* sp. showed, after 90 days of culture, an increase of more than 40% of their roots and stem fresh weights in comparison to the untreated control.

Keywords: disease severity, inhibition, inoculation, *Lycopersicon esculentum* Mill., microsclerotia, mycelial growth, plant growth, sclerogenesis, wilt

INTRODUCTION

In Tunisia, tomato (*Lycopersicon esculentum* Mill.) can be infested by at least three *Verticillium* species such as, *V. albo-atrum*, *V. tricorpus* and mainly *V. dahliae* (Hajlaoui *et al.* 2003; Jabnoun-Khiareddine *et al.* 2005, 2006, 2007). Economic losses caused by these pathogens can reach 50%, especially on tomato cultivars severely infected by the emergent race 2 of *V. dahliae* (Daami-Remadi *et al.* 2006; Jabnoun-Khiareddine *et al.* 2007). In Tunisia, as many countries around the world, Verticillium wilt control is still based on the use of resistant cultivars (Bender and Shoemaker 1984; Harrington and Dobinson 2000; Kawchuk *et al*. 2001). However, this control can be difficult due to emergence of new races, such as race 2 of *V. dahliae*, to which resistant cultivars are absent (Fradin and Thomma 2006).

In view of health and environmental concerns about chemical control methods which were largely used for Verticillium wilt control (Aghighi *et al.* 2004), more attention was focused on the environmental safe methods such as steam sterilization, solarization and cultural practices (Concibido *et al.* 1994; Lynch *et al.* 1997; Jansky and Rouse 2000; Pegg and Brady 2002; Jansky *et al.* 2004; Uppal *et al.* 2007). Bio-suppression of *Verticillium* was achieved by several rhizosphere-colonizing fungi and bacteria of the genera *Talaromyces*, *Trichoderma*, *Penicillium*, *Gliocladium* and *Bacillus* (Matta and Garibaldi 1977; Hall and Scheiber 1984; Millar *et al*. 1984; Henni 1987; Kim *et al*. 1988; Globus and Muromtsev 1990; Ordentlich *et al*. 1990; Berg *et al*. 1999; Alström 2000; D'Ercole *et al*. 2000; Larena *et al*. 2003; Stinson *et al.* 2003; Aghighi *et al.* 2004).

As plant species are colonized by their native antagonists, bacteria and/or fungi, it is possible to increase the antagonistic potential against target pathogens by introducing these microorganisms as biocontrol agents (Berg *et al.* 2000, 2005).

Trichoderma, *Gliocladium* and *Penicillium* species are important biocontrol agents due to hyperparasitism, competition for infection sites and capacity to produce several antibiotic metabolites (D'Ercole *et al.* 2000). *Gliocladium* species were largely used as antagonists of *Verticillium* spp. and particularly *G. roseum* was successfully used for the control of *V. dahliae* and reduction of microsclerotia viability (Marois *et al.* 1984; Keinath *et al.* 1991; Fravel 1996). Furthermore, an endophytic *Gliocladium* sp. isolate, obtained from *Eucryphia cordifolia*, was shown to produce a mixture of organic and volatile compounds with inhibitory activity against *V. dahliae* (Stinson *et al.* 2003). Larena *et al.* (2003) used *Penicillium oxalicum* for reduction of tomato vascular wilts caused by *V. dahliae* and *Fusarium oxysporum* f. sp. *lycopersici* both under growth chamber and field conditions. Moreover, *Verticillium* fungal antagonists in Rostock region were found to be dominated by *Penicillium* (Costa *et al.* 2006).

Isolation of potential biocontrol agents against the Verticillium wilt pathogen was reported to be possible from several pathosystems including *V. dahliae*. In fact, from the colza-*V. dahliae* pathosystem, Alström (2000) isolated,

Received: 24 July, 2009. Accepted: 3 December, 2009.

Original Research Paper

among other microorganisms, *Trichoderma* and *Gliocladium* which are able to produce antimicrobial metabolites and have an important competitive capacity. Narisawaa *et al.* (2002) isolated an unidentified species of *Fusarium*, *Penicillium* and *Trichoderma* showing antagonistic activity against *V. dahliae*.

As native associate microorganisms, natural rhizosphere and plant's subterranean parts colonizers are likely more promising for an efficient *Verticillium* biocontrol, the present study focused on the use of endogenous *Gliocladium* spp. and *Penicillium* sp., colonizing naturally vegetable roots, for the biocontrol of the tomato Verticillium wilt in a growth chamber and under greenhouse conditions. The effect of these antagonists on pathogen mycelial growth and resting structures viability was also studied.

MATERIALS AND METHODS

Plant material

Verticillium susceptible (*ve*) tomato seeds cv. 'Ventura' used were gratefully provided by the laboratory of seeds and plants control of the General Direction of the Protection and Control of the Agricultural Product Quality, Tunisia.

Tomato seeds were superficially disinfected by immersion in absolute ethanol for 2 min and rinsing in sterile distilled water (SDW). Seeds were sown in alveolus plates filled with previously sterilised peat. Seedlings were grown in a growth chamber at 24-26°C with 12-h photoperiod and 70% humidity. They were watered daily and fertilized twice a week with a standard nutrient solution according to Pharand *et al.* (2002). Experiments were performed with 4-week-old tomato plants.

Pathogens

Three *Verticillium* species, *V. dahliae*, *V. albo-atrum* and *V. tricorpus*, were tested in the present study. They were obtained from different host plants (**Table 1**) showing typical wilt symptoms and vascular discoloration. *Verticillium* spp. isolates were cultured at 20°C on PDA (Potato Dextrose Agar) medium added with 300 mg/l of streptomycin sulphate (Pharmadrug Production, Gmbh-Hamburg, Germany). Liquid cultures used for substrate inoculation were prepared on PDB (Potato Dextrose Broth) and incubated at 20°C during 4 to 5 days. The spore suspension concentration used was adjusted to 10^7 spores/ml by a Malassez cystometer. For their long term preservation, pathogen isolates were stored at -20°C in a 25% glycerol solution.

For assessment and qualification of *Gliocladium* spp. and *Penicillium* sp. effects on *V. dahliae* microsclerotia, pathogen inoculum was prepared on autoclaved perlite inoculated with a mixture of liquid cultures (100 ml/flask) of *V. dahliae* isolates and incubated at room temperature during three months before use.

Gliocladium spp.

Gliocladium catenulatum and *G. roseum* isolates were obtained from roots of several *Verticillium* host-plants (Tomato, eggplant, potato and melon) and the monoconidial cultures were identified according to the Kubicek and Harman (1998) key. *Gliocladium* spp. isolates (three of G. *catenulatum* and two of *G. roseum*) were cultured at 25°C on PDA and stored at -20°C in a 10% glycerol solution until use. Liquid cultures were prepared on PDB and the spore concentration used was adjusted to 10^7 spores/ml.

Penicillium spp.

Two *Penicillium* sp. isolates, showing antibiosis zones in contact of developing *Verticillium* and associate fungi colonies during pathogen isolations, were used in the present study against three *Verticillium* species. They were cultured at 25°C on PDA and stored at -20°C in a 10% glycerol solution until use. Liquid cultures were prepared on PDB and the spore concentration used was adjusted to 10^7 spores/ml.

Table 1 *Verticillium* spp. isolates tested and their origins.

Verticillium species	Isolates	Original plant hosts	Origins
V. dahliae	Vd13	Tomato	Chott Mariem
	Vd66	Tomato	Téboulba
	Vd95	Tomato	Chott Mariem
	Vd14	Potato	Nabeul
	Vd20	Potato	Chott Mariem
	Vd28, Vd29	Potato	Sidi Bou Ali
	Vd82	Potato	Nabeul
	Vd64	Melon	Chott-Mariem
	Vd8	Eggplant	Téboulba
V. tricorpus	Vt1	Tomato	Chott-Mariem
	Vt2	Potato	Sidi Bouali
	Vt15	Melon	Mahdia
V. albo-atrum	Vaa1, Vaa3	Tomato	Chott-Mariem
	Vaa2	Potato	Chott-Mariem

Effect of *Gliocladium* spp. and *Penicillium* sp. on mycelial growth of *Verticillium* spp.

The pathogen × antagonist *in vitro* interaction was studied following the dual culture method on PDA added with streptomycin sulphate (300 mg/l). In fact, three agar discs (diameter 6 mm) colonized by the pathogen were plated at 2 cm from the edge of the Petri dish (diameter 9 cm) and equidistantly spaced from each other by 3 cm. *Gliocladium* spp. and *Penicillium* sp. isolates were placed at the centre of the Petri dish. Untreated control plates were plated with pathogen discs only.

The mean diameter of the pathogen developing colonies was noted after 9 days of incubation at 20°C. These *in vitro* dual culture studies were completed by several macroscopic and microscopic observations of colonies confronted with antagonists for elucidating and qualifying the damages occasioned on pathogen mycelium in comparison to untreated controls.

Statistical analyses were performed following a completely randomised factorial design where treatments (*Gliocladium* spp. and *Penicillium* sp. isolates and untreated controls) and *Verticillium* spp. isolates were the fixed factors. Six replicates were used per elementary treatment and means were separated using Fisher's protected LSD test (at $p \leq 0.05$).

Effect of *Gliocladium* spp. and *Penicillium* sp. on *V. dahliae* microsclerotia

Gliocladium spp. and *Penicillium* sp. isolates were tested for their ability to inactivate the microsclerotia germination. In fact, 3 g of a perlite previously colonized by *V. dahliae* was soaked for 30 min in the liquid culture of each antagonist tested (10^7 spores/ml), washed with SDW and air dried at room temperature. The treated perlite was crushed in mortar with 10 ml of SDW. The obtained suspension of microsclerotia was sprayed on 2% agar medium (1 ml per Petri dish). Colonized perlite soaked in SDW only served as untreated control.

After 10 days of incubation at 20°C, the different cultures were observed for eventual *V. dahliae* development from plated microsclerotia. The microsclerotia viability was estimated via the formation or not of secondary microsclerotia (Nagtzaam *et al.* 1998).

Antagonists were also tested against *V. dahliae* germinating microsclerotia by dual culture of *Gliocladium* spp. and *Penicillium* sp. isolates with microsclerotia in the same Petri dish. The suspension of microsclerotia, obtained by crushing 1 g of colonized perlite with 5 ml of SDW, was filtered and 200 µl was sprayed on the surface of the Petri dishes containing PDA medium. After incubation at 20°C during 7 days, agar discs (diameter 6 mm) of antagonist's isolates were plated at the centre of the colonized Petri dishes.

The antagonist × germinating microsclerotia interaction was qualified, after 20 to 35 days of incubation at 20°C, via some macroscopic and microscopic criteria.

Effect of *Gliocladium* spp. and *Penicillium* sp. on Verticillium wilt development and severity under growth chamber conditions

A mixture of 10 *V. dahliae* isolates obtained from different host-plants and regions (**Table 1**) was used for tomato inoculation. Furthermore, due to the presence of intra-specific variations of the antagonist potential previously recorded *in vitro*, a mixture of isolates of each *Gliocladium* and *Penicillium* species was tested *in vivo* in comparison to untreated and inoculated or non controls.

Antagonists tested were applied by watering the culture substrate contained in each alveolus plate with 3 ml of mixed liquid culture of the antagonist used (adjusted to 10^7 spores/ml). Control peat was watered by a similar quantity of SDW. Disinfected tomato seeds were sowed in these treated alveolus plates and placed in a growth chamber (adjusted to 12-h photoperiod, 12-25 ± 2°C temperature and 70-90% relative humidity) during three weeks.

Before the final transplanting, roots were dipped for 30 min in the similar mixed antagonist suspension used for peat treatment. Treated plants were transplanted individually in pots (diameter 10 cm) containing a mixture of peat and perlite infested with *V. dahliae* microsclerotia (50 microsclerotia/g of mixed substrate).

Ten plants were used per elementary antagonistic treatment. Ten untreated plants dipped in SDW only were transplanted in sterile substrate or in substrate infested with *V. dahliae* served as untreated and inoculated or non controls, respectively.

Plants were maintained in a growth chamber at 15-30°C during 60 days and regularly watered and fertilized with a nutritious solution (Pharand *et al*. 2002).

Verticillium wilt severity was estimated via the leaf damage index and according to 0-4 scale (Jabnoun-Khiareddine *et al.* 2007). Plant height and the roots and stem fresh weights were noted on all tomato plants. Furthermore, the presence of pathogen in the stem was also verified by re-isolation on PDA.

Statistical analyses were performed, for all parameters measured, following a completely randomised design where treatments (*Gliocladium* spp. and *Penicillium* sp. isolates and untreated control) were the sole fixed factor. Ten replicates were used per elementary treatment and means were separated using Fisher's protected LSD test (at $p \leq 0.05$). The whole experiment was repeated twice but only the data of one essay is presented in the present study.

Effect of *Gliocladium* spp. and *Penicillium* sp. on Verticillium wilt development and severity under greenhouse conditions

Efficiency of *Gliocladium* spp. and *Penicillium* sp. against *V. dahliae* was also tested in a large-scale essay conducted, during the 2006-2007 agricultural campaign, in a field with tomato Verticillium wilts history for several years ago at the domain of the Higher Agronomic Institute of Chott-Mariem. The infectious potential of this naturally infested field was estimated at 34 microsclerotia/g of soil, according to the method of Nadakavukaren and Horner (1959) previously described in Jabnoun-Khiareddine *et al.* (2007).

Tomato seeds cv. 'Ventura' were sown in culture substrate treated (as previously described) or not with *Gliocladium* spp. and *Penicillium* sp. At the 3-4 leaf true stage, plants were dipped for 30 min, just before transplanting, in a conidial suspension of the tested antagonists. Control plants were dipped in SDW. Plants were transplanted directly in the soil under a plastic greenhouse following six lines 80 cm a part with an inter-plant spacing of 40 cm. Plants were irrigated via a drip irrigation system. Fertilization and the other cultural practices were the most commonly used for tomato farming in the region.

Antagonist's efficacy was assessed, 90 days post-transplanting, via the roots and stem fresh weights and the Verticillium wilt severity. In fact, wilt severity was visually estimated via a vascular discoloration index calculated based on a 0-5 scale (Jabnoun-Khiareddine *et al*. 2009).

Stem segments showing or not vascular discoloration symptoms were used for fungal isolation on PDA at 20°C.

Statistical analyses were performed, for all parameters measured, following a randomised complete block design with 3 blocks and 7 replicates (plants) per block and per elementary treatment. Treatments (*Gliocladium* spp., *Penicillium* sp. and untreated control) were randomly arranged into blocks. Means were separated using Fisher's protected LSD test (at $p \leq 0.05$). The whole experiment was repeated during two years (2006 and 2007) and only the data of the second year was considered.

RESULTS

Endogenous *Gliocladium* spp. and *Penicillium* sp. isolates were tested *in vitro, in vivo* and *in situ* for their antagonistic activity against *Verticillium* spp. causing tomato vascular wilt in Tunisia.

Effect of *Gliocladium* spp. and *Penicillium* sp. on mycelial growth of *Verticillium* spp. *in vitro*

Mean diameter of *Verticillium* spp. colonies, formed after 9 days of incubation at 20°C, depended on antagonistic treatments tested and on pathogen's isolates; a significant interaction was observed between both fixed factors at $p \leq 0.05$. In fact, *Gliocladium* spp. and *Penicillium* sp. isolates reduced the radial growth of the Verticillium wilt agents in comparison to their relative untreated controls (**Table 2**). The most important mycelial growth reduction, of about 60% compared to the untreated control, was recorded in the case of the isolate Vaa1 of *V. albo-atrum* treated with the isolates Gc1 of *G. catenulatum* and pc7 of *Penicillium* sp.

Table 2 Effect of *Gliocladium* spp. and *Penicillium* sp. isolates on the mean colony diameter (cm) of *Verticillium* spp. isolates observed after 9 days of incubation at 20°C in comparison to the untreated controls

***Verticillium* isolates**	**Co.**	***G. catenulatum***			***G. roseum***		***Penicillium* sp.**		**Mean[a*]**
		Gc1	**Gc2**	**Gc3**	**Gr1**	**Gr2**	**pc7**	**pc1**	
V. dahliae									
d82	3.62	2.38	1.88	2.32	2.78	2.88	1.63	2.09	2.45 c
Vd13	3.41	3	2.91	3.08	2.71	2.8	1.9	2.5	2.79 b
Vd66	3.28	2.83	1.79	2.51	2.28	2.92	1.83	2.08	2.44 c
V. tricorpus									
Vt1	3.95	2.73	3.77	2.96	3.56	3.26	2.62	3.28	3.26 a
Vt3	4.06	3.33	3.19	1.7	2.94	2.38	2.01	3.49	2.89 b
Vt15	3.93	3.05	2.33	2.74	2.93	3.54	2.23	3.08	2.98 b
V.albo-atrum									
Vaa1	4.32	1.74	2.23	2.53	2.48	2.2	1.83	3.28	2.58 c
Vaa2	4.07	3.48	3.58	3.43	3.22	3.18	2.83	2.5	3.28 a
Vaa3	3.14	1.64	2.62	2.85	2.72	2.3	1.57	2.63	2.43 c
Mean[b*]	3.75 a	2.68 b	2.7 b	2.68 b	2.85 b	2.83 b	2.05 c	2.77 b	

LSD (*Verticillium* spp. isolates x Treatments tested) = 0.48 cm at $p \leq 0.05$.

[a] Mean colony diameters per *Verticillium* spp. isolates independently of antagonistic treatments tested

[b] Mean colony diameters of *Verticillium* spp. (independently of isolates used) per antagonistic treatment tested.

[*] For *Verticillium* spp. isolates and antagonistic treatments tested, values (means) affected with the same letter are not significantly different at $p \leq 0.05$.

Co: untreated control; Gc1, Gc2 and Gc3: *G. catenulatum* isolates; Gr1 and Gr2: *G. roseum* isolates; Pc1 and Pc7: *Penicillium* sp. isolates; Vd82, Vd13 and Vd66: *V. dahliae* isolates; Vt1, Vt3 and Vt15: *V. tricorpus* isolates; Vaa1, Vaa2 and Vaa3: *V. albo-atrum* isolates.

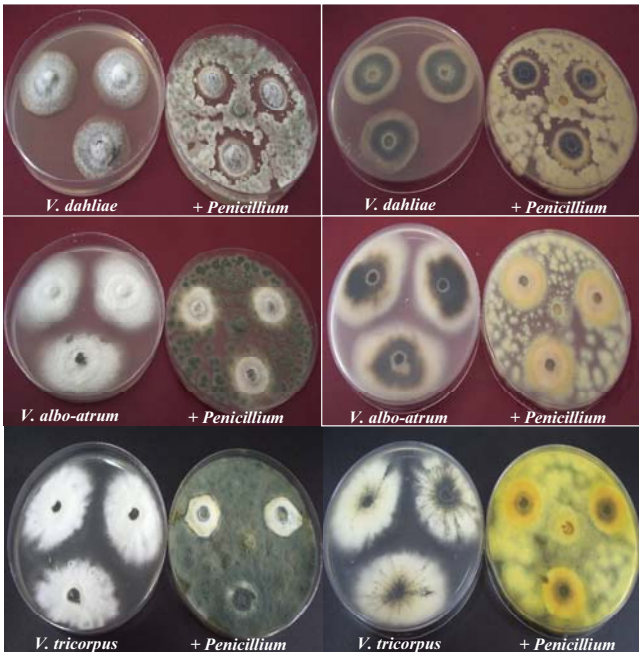

Fig. 1 Competitive potential of *Penicillium* sp. against three *Verticillium* species observed after 9 days of dual culture on PDA at 20°C in comparison to the untreated controls.

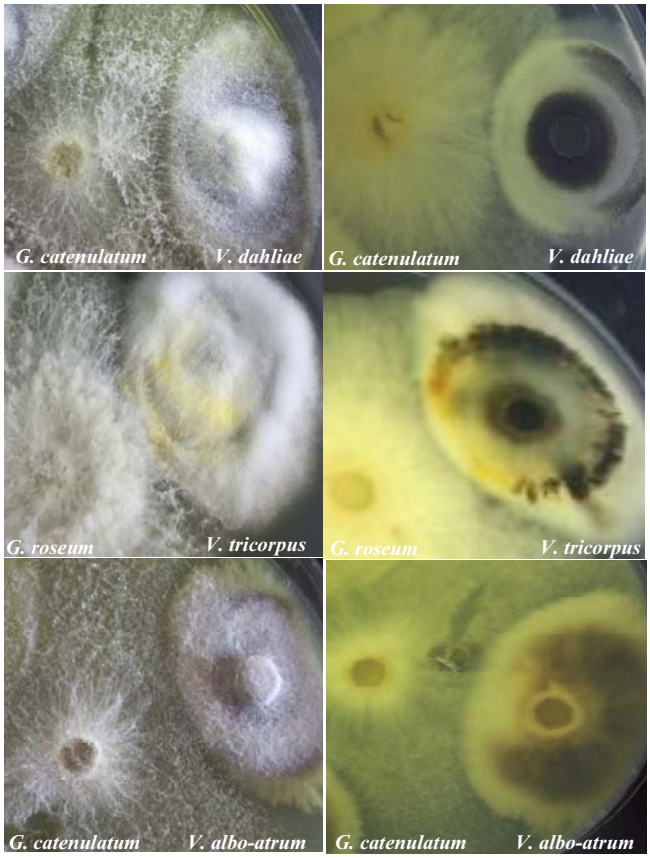

Fig. 2 Inhibition of formation of resting structures of *Verticillium* spp. occasioned by two *Gliocladium* species observed after 9 days of incubation at 20°C.

The mycelial growth reduction of *Verticillium* spp. isolates varied depending on the antagonistic species tested and between the *Gliocladium* spp. and *Penicillium* sp. isolates used; thus, intra- and inter-specific variations were noted within these endogenous fungi regarding their antagonistic potential against the target pathogens. In fact, for the same *Verticillium* isolate, as is the case of *V. dahliae* Vd82, reduction varied from 20 (treatment with *G. roseum* Gr2) to

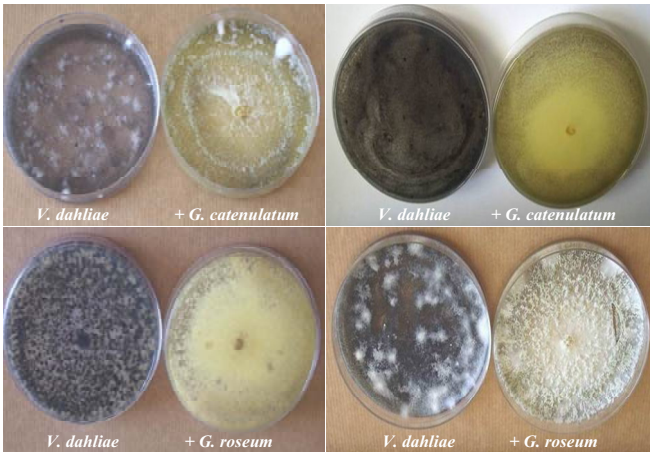

Fig. 3 Melanin bleaching of *V. dahliae* colonies treated with two *Gliocladium* species compared to untreated control observed after 20 days of incubation at 20°C.

55% (treatment with *Penicillium* sp. pc7). Furthermore, for the same antagonistic treatment, as is the case of *G. catenulatum* Gc1, mycelial growth inhibition, in comparison to the untreated control, varied from 12 (*V. dahliae* Vd13 isolate) to 60% (*V. albo-atrum* Vaa1 isolate). In the case of *G. roseum* Gr2, mycelial growth inhibition of *V. tricopus* isolates ranged between 5 and 41%.

Verticillium spp. colonies, observed 9 days after dual culture (**Fig. 1**) showed the important competitive potential of the majority of *Gliocladium* spp. and *Penicillium* sp. isolates used as only the isolate pc7 of *Penicillium* sp. induced an antibiosis zone.

Figs. 1 and **2** illustrated not only the antagonist's competitive potential but also the significant reduction abundance in resting structures of *Verticillium* spp. isolates compared to the untreated controls. Furthermore, 9 days after the dual culture *Verticillium* spp.-*Gliocladium* spp. or -*Penicillium* sp., the dark typical colour (of melanin) of the resting structures, microsclerotia, dark mycelium and chlamydospores, disappeared progressively and turned to light brown. This phenomenon was not observed on untreated controls. Additionally to this sclerotinization inhibitory activity and discoloration, antagonists tested caused several alterations of *Verticillium* spp. mycelium, at the confrontation zone, expressed by an important lysis and deformation (zigzag), formation of mycelial cords and reduction of mycelium density and sporulation compared to the untreated controls.

Effects of *Gliocladium* spp. and *Penicillium* sp. on *V. dahliae* microsclerotia

Germination of *V. dahliae* microsclerotia, exposed for 30 min to liquid cultures of antagonists tested and incubated at 20°C, was suppressed in contact of treatments applied compared to the control microsclerotia treated with SDW only. In fact, after 10-30 days of incubation at 20°C, *V. dahliae* microsclerotia confronted with *Gliocladium* spp. and *Penicillium* sp. lost their germinative potential and consequently their viability.

Dual culture on PDA of *Gliocladium* spp. or *Penicillium* sp. with mature and viable *V. dahliae* microsclerotia revealed an important space colonizing potential of antagonists tested.

Furthermore, for colonizing a media already colonized by *V. dahliae*, antagonists inhibited the pathogen sclerogenesis as treated germinating microsclerotia became incapable of forming secondary microsclerotia and mature microsclerotia treated with antagonists progressively lost their typical dark colour and turned to brown and finally to light (**Fig. 3**). In addition, *Gliocladium* spp. and *Penicillium* sp. also degraded the cell wall of microsclerotia which lost

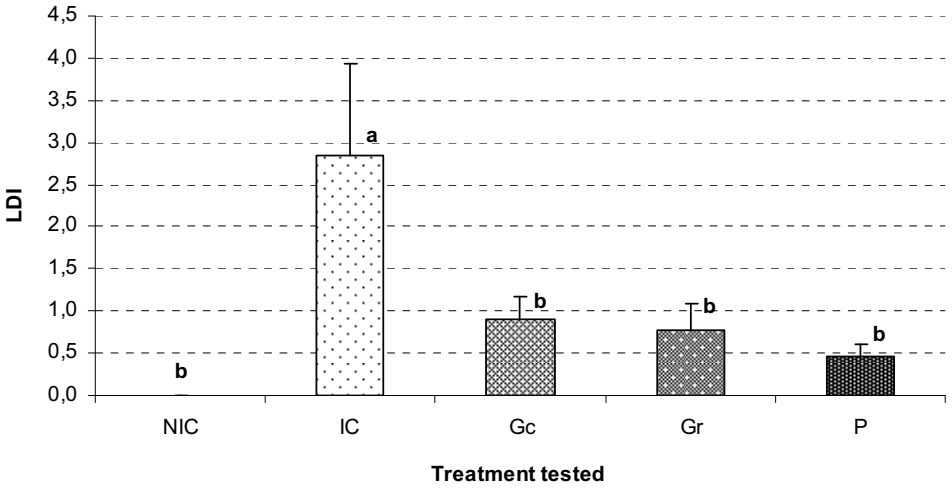

Fig. 4 Effect of treatments at planting with *Gliocladium* spp. and *Penicillium* sp. on Verticillium wilt severity observed on tomato cv 'Ventura' plants 60 days after their transplanting in a culture substrate infested with *V. dahliae* in comparison to the controls. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). NIC: untreated and uninoculated control, IC: untreated and inoculated control, Gc: inoculated and treated with *G. catenulatum*, Gr: inoculated and treated with *G. roseum*, P: inoculated and treated with *Penicillium* sp., 15°C <T< 30°C.

their typical mass form and became totally disintegrated after 20 days of dual culture.

Effects of *Gliocladium* spp. or *Penicillium* sp. on tomato Verticillium wilt development under growth chamber conditions

The efficiency of *Gliocladium* spp. or *Penicillium* sp. against *V. dahliae in vivo* was evaluated on wilt severity and plant growth compared to untreated but inoculated or non controls (IC and NIC respectively).

Leaf damage index

All tomato plants, treated at planting with *Gliocladium* spp. or *Penicillium* sp. spore suspensions and transplanted in culture substrate infested with *V. dahliae*, showed typical Verticillium wilt symptoms but uninoculated and untreated (NIC) plants were symptomless. However, disease severity, estimated via the leaf damage index (LDI), depended significantly on treatments tested. In fact, the LDI, recorded 60 days post-planting on cv. 'Ventura' tomato plants treated with *G. catenulatum*, *G. roseum* and *Penicillium* sp., was significantly comparable to that noted on uninoculated and untreated control (NIC) plants and it was reduced by more than 68% in comparison to the inoculated and untreated control (IC) (**Fig. 4**).

Plant height

Tomato plant height, noted 60 days post-planting (**Fig. 5**), depended significantly on treatments tested. In fact, plants treated with *G. catenulatum* and *Penicillium* sp., showed an increase of about 27% of their height in comparison to the inoculated and untreated control (IC). This increased plant height was significantly comparable to that noted on uninoculated and untreated control (NIC) plants.

Root fresh weight

Tomato root fresh weight, noted 60 days post-planting, did not significantly depend on treatments tested. However, plants treated with *G. catenulatum*, *G. roseum* and *Penicillium* sp. showed a slight increase of about 25% (**Fig. 6**), even statistically insignificant, of their root fresh weight in comparison to the inoculated and untreated control (IC) and

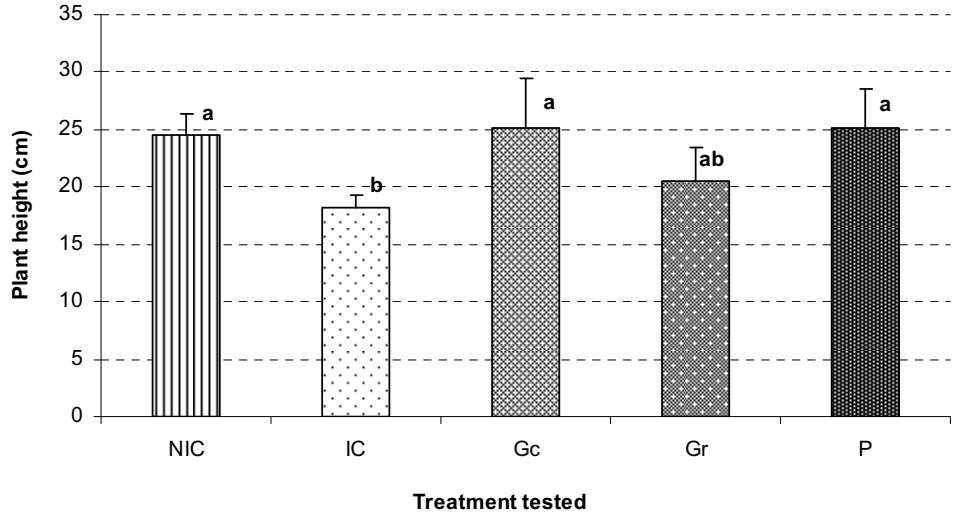

Fig. 5 Effect of treatments at planting with *Gliocladium* spp. and *Penicillium* sp. on tomato cv. 'Ventura' plant height noted 60 days after transplanting in a culture substrate infested with *V. dahliae* in comparison to the controls. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). NIC: untreated and uninoculated control, IC: untreated and inoculated control, Gc: inoculated and treated with *G. catenulatum*, Gr: inoculated and treated with *G. roseum*, P: inoculated and treated with *Penicillium* sp., 15°C <T< 30°C.

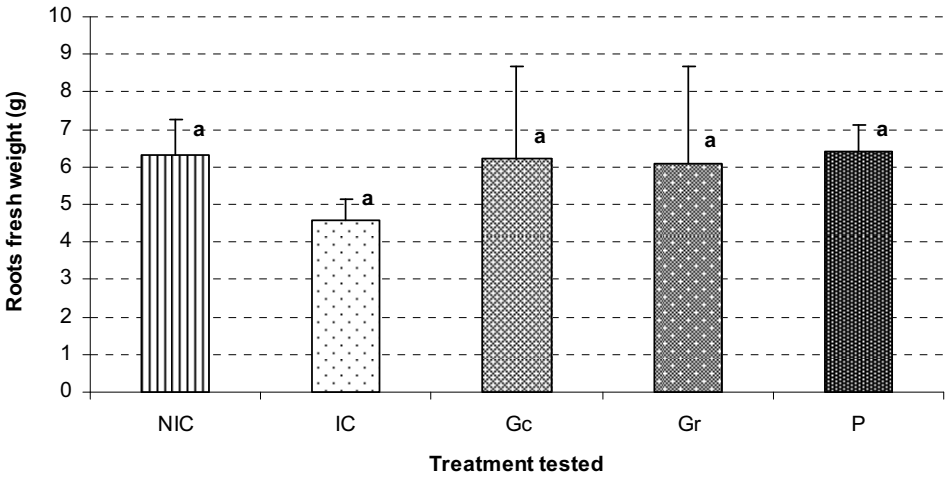

Fig. 6 Effect of treatments at planting with *Gliocladium* spp. and *Penicillium* sp. on tomato cv. 'Ventura' root fresh weight noted 60 days after transplanting in a culture substrate infested with *V. dahliae* in comparison to the controls. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). NIC: untreated and uninoculated control, IC: untreated and inoculated control, Gc: inoculated and treated with *G. catenulatum*, Gr: inoculated and treated with *G. roseum*, P: inoculated and treated with *Penicillium* sp., 15°C <T< 30°C.

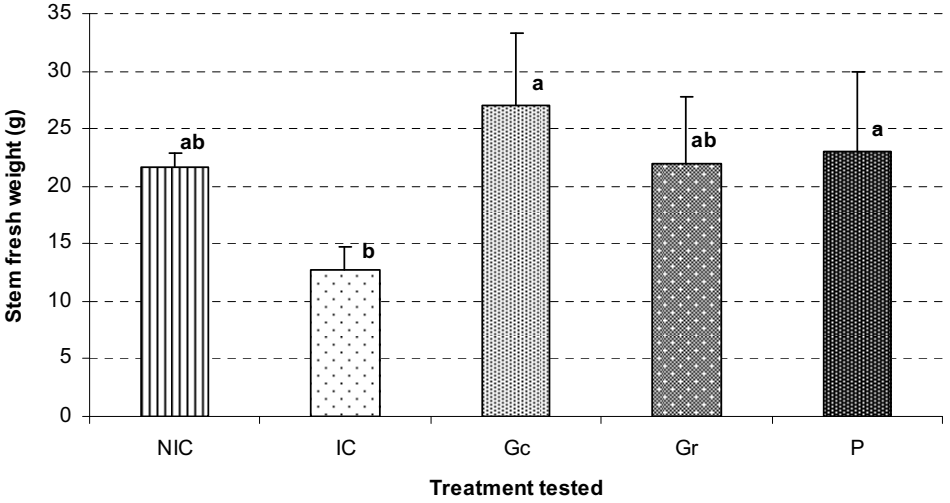

Fig. 7 Effect of treatments at planting with *Gliocladium* spp. and *Penicillium* sp. on tomato cv. 'Ventura' stem fresh weight noted 60 days after transplanting in a culture substrate infested with *V. dahliae* in comparison to the controls. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). NIC: untreated and uninoculated control, IC: untreated and inoculated control, Gc: inoculated and treated with *G. catenulatum*, Gr: inoculated and treated with *G. roseum*, P: inoculated and treated with *Penicillium* sp., 15 <T< 30°C.

which was comparable to that noted on uninoculated and untreated (NIC) plants.

Stem fresh weight

Tomato stem fresh weight, noted 60 days post-planting, depended significantly on treatments tested. In fact, plants treated with *G. catenulatum, G. roseum* and *Penicillium* sp. showed increased stem fresh weight, by more than 41% in comparison to the inoculated and untreated control (IC) plants, which was significantly comparable to the untreated and uninoculated (NIC) plants. Plants treated with *G. roseum* showed a stem fresh weight significantly similar to that noted on inoculated and untreated control plants (**Fig. 7**).

For all treatments tested, except for the untreated and uninoculated control (NIC), a vascular discoloration was observed on roots, collar and the stem base and the pathogen presence was confirmed after re-isolation on PDA from collar and stem.

Effects of *Gliocladium* spp. or *Penicillium* sp. on tomato Verticillium wilt development under greenhouse conditions

The inhibitory activity of *Gliocladium* spp. and *Penicillium* sp. against *V. dahliae*, previously assessed *in vitro* and *in vivo*, was also evaluated *in situ*, in a naturally infested soil (infectious potential estimated at 34 microsclerotia/g), on wilt severity and plant growth in comparison to the untreated control.

Discoloration index

The Verticillium wilt severity, estimated via a discoloration index noted 90 days after transplanting, depended significantly on treatments tested. In fact, the discoloration index noted on tomato plants treated at planting by *G. catenulatum*, *G. roseum* and *Penicillium* sp. was significantly reduced by more than 54% compared to the untreated and infested control (IC) (**Fig. 8**). The most important reduction of this parameter, of about 74% in comparison to the untreated control, was recorded on tomato plants treated with *G. roseum*.

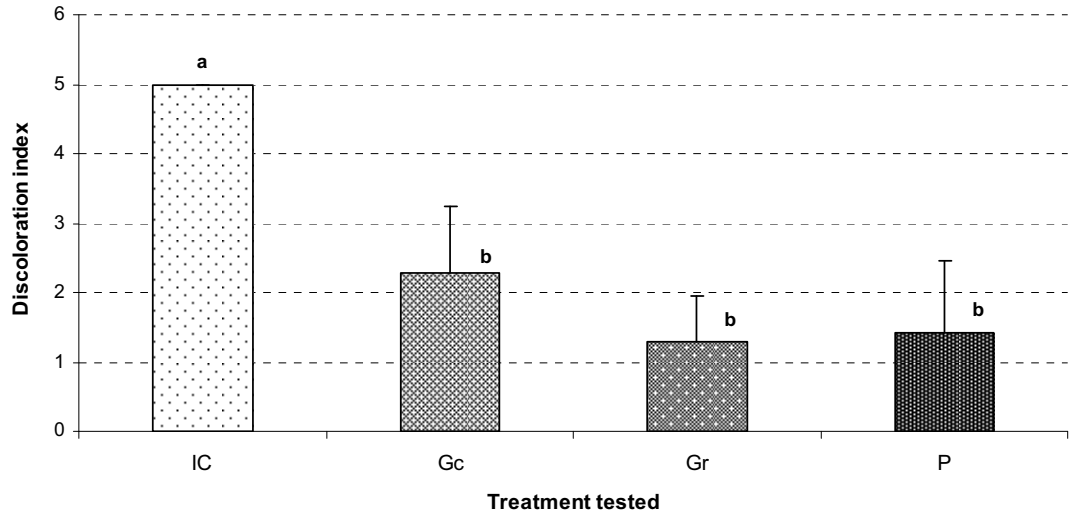

Fig. 8 Effect of treatments of tomato plants cv. 'Ventura' at planting with *Gliocladium* spp. and *Penicillium* sp. on the discoloration index noted 90 days after their transplanting in a naturally infested soil with *V. dahliae* in comparison to the untreated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). IC: infested and untreated control, Gc: infested and treated with *G. catenulatum*, Gr: infested and treated with *G. roseum* and P: infested and treated with *Penicillium* sp.

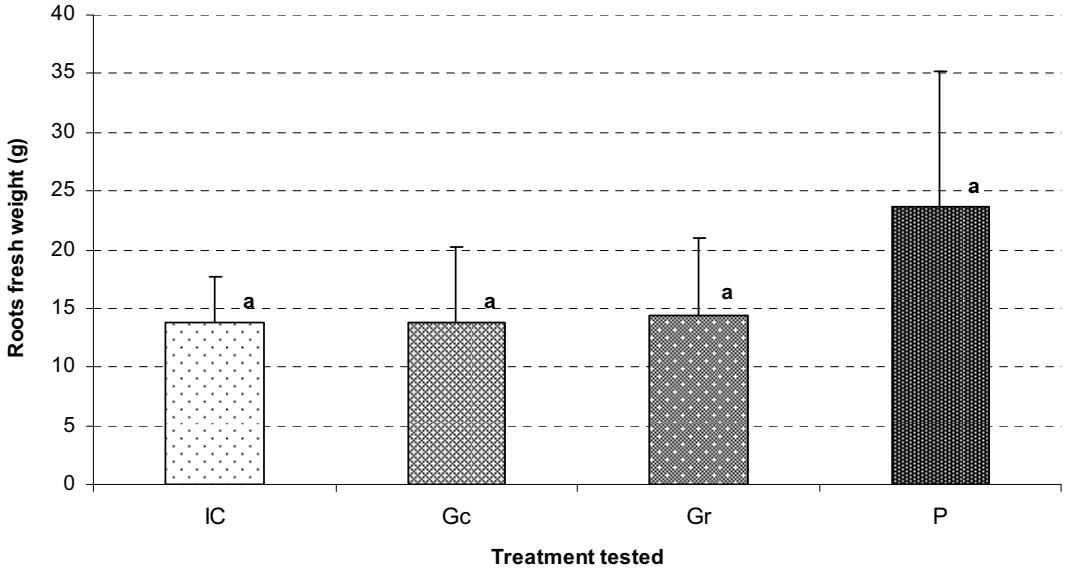

Fig. 9 Effect of treatments of tomato plants cv. 'Ventura' at planting with *Gliocladium* spp. and *Penicillium* sp. on the roots fresh weight noted 90 days after their transplanting in a naturally infested soil with *V. dahliae* in comparison to the untreated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). IC: infested and untreated control, Gc: infested and treated with *G. catenulatum*, Gr: infested and treated with *G. roseum* and P: infested and treated with *Penicillium* sp.

Root fresh weight

Tomato root fresh weight, noted 90 days post-planting (**Fig. 9**), did not significantly depend on treatments tested. However, plants treated with *Penicillium* sp. showed an increase of about 42%, even statistically insignificant, of their roots fresh weight in comparison to the untreated and infested control plants (IC).

Stem fresh weight

Tomato stem fresh weight, noted 90 days post-planting (**Fig. 10**), did not significantly depend on treatments tested. However, plants treated with *G. catenulatum*, *G. roseum* and *Penicillium* sp. showed an increase by more than 16%, even statistically insignificant, of their stem fresh weight in comparison to the untreated control (IC). Plants treated with *Penicillium* sp. showed an increase, of about 42%, of their stem fresh weight in comparison to the untreated and infested control.

DISCUSSION

Biocontrol of *Verticillium* via enhancement of microbial interactions in the rhizosphere

Endogenous agents, considered ecologically more adaptable, were extensively used for the biocontrol of soil-borne plant pathogens (Narisawaa *et al.* 2000) as they are able to mineralize nutrients, present at the root system zone, and to affect the competitive ability of target microorganisms such as *V. dahliae* (Alström 2000). In fact, several bacterial and fungal agents such as *Trichoderma*, *Gliocladium*, *Penicillium* and *Bacillus* were reported to be potential antagonists of *V. dahliae* (Narisawaa *et al.* 2002; Stinson *et al.* 2003). In a previous study, Jabnoun-Khiareddine *et al.* (2009) obtained promising and original results with endogenous *Trichoderma* spp. isolates for tomato Verticillium wilt biocontrol under growth chamber and greenhouse conditions. In the present study, the antagonistic potential of endogenous *Gliocladium* spp. and *Penicillium* sp. isolated from several vegetable plants was investigated.

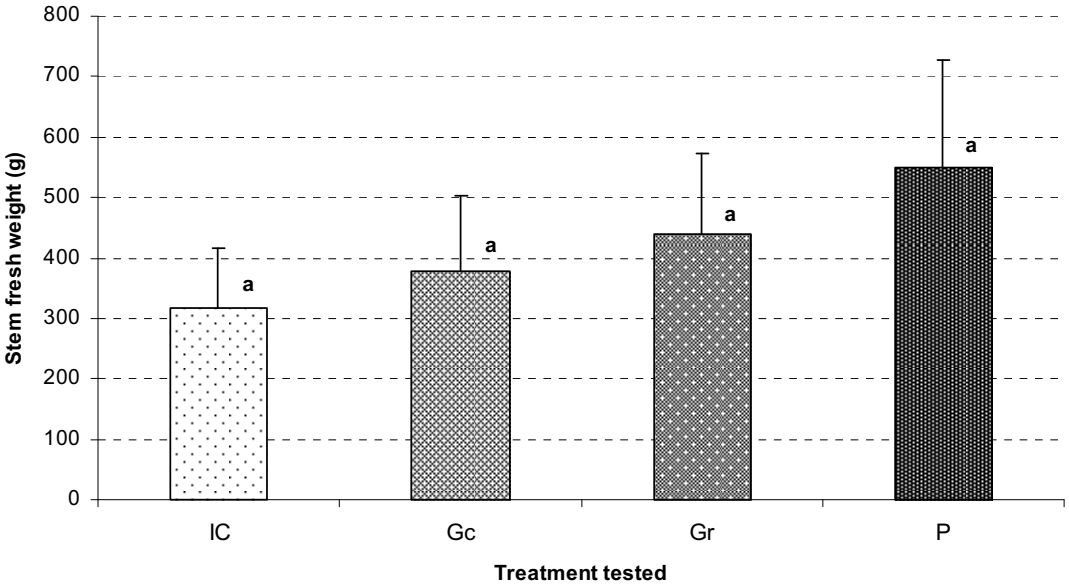

Fig. 10 Effect of treatments of tomato plants cv. 'Ventura' at planting with *Gliocladium* spp. and *Penicillium* sp. on the stem fresh weight noted 90 days after their transplanting in a naturally infested soil with *V. dahliae* in comparison to the untreated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). IC: infested and untreated control, Gc: infested and treated with *G. catenulatum*, Gr: infested and treated with *G. roseum* and P: infested and treated with *Penicillium* sp.

Effects of *Gliocladium* spp. and *Penicillium* sp. on pathogen mycelial growth

In vitro dual cultures of the endogenous agents tested were conducted with three *Verticillium* species representing the parasitic complex actually involved in the tomato Verticillium wilt development in Tunisia (Jabnoun-Khiareddine *et al*. 2005, 2007). In fact, *Gliocladium catenulatum*, *G. roseum* and *Penicillium* sp. isolates tested reduced the radial growth of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* in comparison to their relative untreated controls.

This mycelial growth reduction was considered as a reliable character for microbial antagonism against fungal microorganisms (Hall and Schreiber 1984; El Abyad *et al*. 1993). Moreover, present results are in agreement with previous records on the antagonistic effect of *Penicillium* sp. against *Verticillium* (Larena *et al.* 2003; Regragui 2005).

The mycelial growth reduction, noted in the present study, was mainly due to the important competitive potential of *Gliocladium* spp. and *Penicillium* sp. isolates, as antibiosis zone was noted with one *Penicillium* sp. isolate only. These antagonists tested caused several alterations of *Verticillium* spp. mycelium at the confrontation zone. Similar results were reported by Dutta (1981) who found that *Trichoderma viride* and *Gliocladium* spp. suppressed the mycelial growth of *V. albo-atrum* by penetration and invasion whereas *Penicillium chrysogenum* inhibited pathogen growth via an antibiosis mechanism. Furthermore, although hyphal coiling of *Gliocladium* and *Penicillium* around *Verticillium* spp. hypha was not observed, their attachment to pathogen's mycelium was noted. Huang (1978) reported that *G. catenulatum* inhibited *Sclerotinia sclerotiorum* and *Fusarium* spp. via direct contact without penetration and caused disintegration of affected cells. However, other authors showed that *G. catenulatum* isolates were able to coil around and to penetrate *Rhizoctonia solani* (Turhan 1990) and *Botrytis cinerea* hyphae (Simay 1988). Moreover, *Gliocladium* species were shown to produce hydrolytic enzymes such as chitinases and β-1,3-glucanases (Mcquilken *et al.* 2001) as well as a mixture of organic volatile compounds with antifungal activities (Stinson *et al.* 2003).

Effects of *Gliocladium* spp. and *Penicillium* sp. on the sclerogenesis process

Additionally to the inhibitory activity of the sclerotinization process and to the reduced abundance of resting structures of *Verticillium* spp., the present study showed intra- and inter-specific variations of the antagonistic potential of *Gliocladium* spp. and *Penicillium* sp. used. Furthermore, the germination of *V. dahliae* microsclerotia, exposed for 30 min to liquid cultures of antagonists tested and incubated at 20°C, was completely suppressed compared to the control microsclerotia treated with SDW only. Furthermore, germinating microsclerotia dual cultured with *Gliocladium* spp. and *Penicillium* sp. isolates became incapable of germinating, and mature microsclerotia progressively lost their typical dark colour. Similar results were reported in Keinath *et al.* (1991) studies where three different isolates of *G. roseum* had reduced by 100% the viability of *V. dahliae* microsclerotia when added to a non sterile soil. In the same way, Hadar and Gorodecki (1991) found that germination of *Sclerotium rolfsii* sclerotia, added to peat previously mixed with *Penicillium* sp. mycelium during 7 days was reduced by 90%.

Additionally to their effect on germination of microsclerotia, antagonists tested in the present study were also able to affect the resting structures of *V. dahliae* during their different development stages. In fact, *Gliocladium* spp. tested were shown to be highly competitive on colonized PDA contrary to *Penicillium* sp. isolates. In fact, for colonizing a media previously invaded by *V. dahliae*, *Gliocladium* spp. isolates caused important damage to pathogen microsclerotia affecting their structure and/or their form. These altered microsclerotia progressively lost their typical dark melanized colour, progressively bleached and became finally transparent. Treated microsclorotia lost their form, became degraded and disintegrated due to the cell wall lysis. These results revealed the probable involvement of a melanolytic activity displayed by *G. roseum* and *G. catenulaum* isolates against *V. dahliae* microsclerotia. A similar effect was observed by Keinath *et al.* (1991) on microsclerotia exposed to *G. roseum*.

The degradation of fungal melanin was also reported by several authors. In fact, El Bassam *et al.* (2002) found that *Microsphaeropsis ochracea* became attached to *Venturia inequalis* melanin via the formation of lytic compounds. De-Cal and Melgarejo (1994) also reported similar melanin attraction exerted by *Penicillium frequentans* against *V. inequalis*. Furthermore, *Trichoderma* spp., *Penicillium* spp., *Fusarium* spp., *Coniothyrium minitans*, *Gliocladium virens* and *Sporidesmium schrotivorum* were able to colonize, degrade and destroy *Sclerotinia* spp. and *Sclerotium* spp. sclerotia as shown for *V. dahliae* microsclerotia (Artigues and

Davet 1984; Gracia-Gracia *et al.* 1997; Tsahouridou and Thanassoulopoulos 2001).

Besides the inhibition of microsclerotia germination and the melanin degradation, antagonistic activity of *Gliocladium* spp. was also expressed by a delay in the sclerogenesis process and consequently, the formation of new microsclerotia. In fact, these antagonists inhibited melanin formation and reduced its level in the recently formed microsclerotia. Similar conclusions were reported by Tjamos (2000) and Madi *et al.* (1997) during the *Talaromyces flavus* × *V. dahliae* interaction. Moreover, Madi *et al.* (1997) suggested that the prevention of the melanization process can affect microsclerotia survival in the soil by increasing their vulnerability to ultraviolet radiation and to microbial antagonists.

Effects of *Gliocladium* spp. and *Penicillium* sp. on Verticillium wilt severity

The *in vivo* and *in situ* evaluation of endogenous fungi tested showed that all cv. 'Ventura' tomato plants, treated at planting with *Gliocladium* spp. and *Penicillium* sp. spore suspensions and inoculated with *V. dahliae*, showed after 60 days of culture under growth chamber conditions, reduced Verticillium wilt severity, in comparison to inoculated control plants. Moreover, under greenhouse conditions, the discoloration index noted on tomato plants treated prior their plantation in a naturally infested soil was significantly reduced compared to untreated control. Similar Fusarium and Verticillium wilt severity reduction was noted when *Penicillium oxalicum* was watered, as spore suspension, to tomato plants 7 days prior to transplanting (Sabuquillo *et al.* 2005, 2006).

Although Keinath *et al.* (1991) found that *G. roseum* and *G. catenulatum* were more efficient when added to soil at high rates (1%), endogenous antagonists, tested in the present study, were shown to be efficient even when added to the culture substrate (nursery stage) and applied by plant watering with spore suspension prior transplanting. This method of antagonist application led to a significant reduction of Verticillium wilt severity both under growth chamber (artificial inoculation) and greenhouse conditions (natural infection). The application method of biocontrol agents, the treatments number (applications) and the adaptation potential of selected strains, to different environments, were shown to be crucial factors for efficiency improvement (Minuto *et al.* 2006). Introduction of antagonists prior transplanting into culture substrate favoured their establishment around subterranean plant parts (subterranean plants tissues and rhizosphere); thus, pathogen internal and external progress was prevented and its spread reduced (D'Ercole *et al.* 2000). In the same way, Dutta (1981) found that application of *Trichoderma viride*, *Penicillium chrysogenum*, *Penicillium* sp. and *Gliocladium* spp. culture filtrates, via root dipping method, was more efficient for Verticillium wilt biocontrol. In the present study, previous substrate colonization and root dipping with antagonist's spore suspension inhibited the *in vivo* expression of *V. dahliae* and consequently wilt severity. Furthermore, the period between antagonistic treatment and the subsequent infection occurrence seemed to be necessary for improvement of biocontrol agent's efficacy. This period depended on *Verticillium* × host-plant interactions and on biocontrol agents. In fact, a short period was shown to be necessary for protection of lucerne with *G. roseum* and of cotton with *Talaromyces flavus* (Millar *et al.* 1984; Murray *et al*. 1997).

Effects of *Gliocladium* spp. and *Penicillium* sp. on plant growth

Increased height and root and stem fresh weights were noted on tomato plants treated with antagonists tested in comparison to the inoculated and untreated control plants under growth chamber conditions. Moreover, even under greenhouse conditions, plants treated with *Penicillium* sp. showed, in the present study and after 90 days of culture, an increase of more than 40% of their roots and stem fresh weights in comparison to the untreated control. Similarly, Dutta (1981) found that application of *Trichoderma viride*, *Penicillium chrysogenum*, *Penicillium* sp. and *Gliocladium* spp. culture filtrates, via root dipping method, enhanced plant vigour and height and increased yield. Moreover, *Penicillium oxalicum* was also shown to induce resistance in tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* (Sabuquillo *et al.* 2005, 2006).

Finally, given the significant reduced Verticillium wilt severity, the enhanced plant growth achieved and the important damage occasioned on pathogen resting structures by using *Gliocladium* spp. and *Penicillium* sp. isolates, these endogenous fungi-*Verticillium* spp. interactions should be elucidated via histopathological studies (for resistance induction assessment), via surveys of soil infectious potential (microsclerotia population in the soil) before and after treatments, disease incidence and consequently impact on yield of several vegetable crops including tomato.

ACKNOWLEDGEMENTS

We thank the Regional Center of Research in Horticulture and Organic Agriculture (CRRHAB) and the Higher Agronomic Institute of Chott-Mariem (ISA-CM) for financial contribution. We also thank Mouna Gueddess-Chahed and Aymen Youssef for technical assistance.

REFERENCES

Aghighi S, Shahidi Bonjar GH, Saadoun I (2004) First report of antifungal properties of a new strain of *Streptomyces plicatus* (strain 101) against four Iranian phytopathogenic isolates of *Verticillium dahliae*, a new horizon in biocontrol agents. *Biotechnology* **3**, 90-97

Alström S (2000) Root colonizing fungi from oilseed rape and their inhibition of *Verticillium dahliae*. *Journal of Phytopathology* **148**, 417-423

Artigues M, Davet P (1984) Comparaison des aptitudes parasitaires de clones de *Trichoderma* vis-à-vis de quelques champignons à sclérotes. *Soil Biology and Biochemistry* **16**, 413-417

Bender CG, Shoemaker PB (1984) Prevalence of Verticillium wilt of tomato and virulence of *Verticillium dahliae* race 1 and race 2 isolates in Western North Carolina. *Plant Disease* **68**, 305-309

Berg G, Frankowski J, Bahl H (1999) Biocontrol of Verticillium wilt in oilseed rape by chitinolytic *Serratia plymuthica*. *Proceeding of 10th International Rapeseed Congress*, 26th-29th September, Canberra, Australia, p 369 (Abstract)

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiology Ecology* **51**, 215-229

Berg G, Kugler A, Wellington EHM, Smalla K (2000) Successful strategy for the selection of new strawberry-associated rhizobacteria antagonistic to Verticillium wilt. *Canadian Journal of Microbiology* **46**, 1128-1137

Concibido VC, Secor GA, Jansky SH (1994) Evaluation of resistance to Verticillium wilt in diploid, wild potato interspecific hybrids. *Euphytica* **76**, 145-152

Costa R, Salles JF, Berg G, Smalla K (2006) Cultivation-independent analysis of *Pseudomonas* species in soil and in the rhizosphere of field-grown *Verticillium dahliae* host plants. *Environmental Microbiology* **8**, 2136-2149

D'Ercole N, Nipoti P, Di Pillo L, Gavina F (2000) *In vitro* and *in vivo* tests of *Trichoderma* spp. as a biocontrol agent of *Verticillium dahliae* kleb. in eggplants. In: Tjamos EC, Rowe RC, Heale JB, Fravel DR (Eds) *Advances in Verticillium: Research and Disease Management*, APS Press, St. Paul, MN, USA, pp 260-263

Daami-Remadi M, Jabnoun-Khiareddine H, Barbara DJ, Ayed F, El Mahjoub M (2006) First report of *Verticillium dahliae* race 2 in Tunisia. *Plant Pathology* **55**, 816

De-Cal A, Melgarejo P (1994) Effect of *Penicillium frequentans* and its antibiotics on unmelanized hyphae of *Monilinia laxa*. *Phytopathology* **84**, 1010-1014

Dutta BK (1981) Studies on some fungi isolated from the rhizosphere of tomato plants and the consequent prospect for the control of Verticillium wilt. *Plant and Soil* **63**, 209-216

El-Abyad MS, El-Sayed MA, El-Shanshoury AR, El-Sabbagh SM (1993) Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp. *Plant and Soil* **149**, 185-195

El Bassam S, Benhamou N, Carisse O (2002) The role of melanin in the antagonistic interaction between the apple scab fungus *Venturia inequalis* and *Microsphaeropsis ochracea*. *Canadian Journal of Microbiology* **48**, 349-358

Fradin EF, Thomma BPHJ (2006) Physiology and molecular aspects of

Verticillium wilt diseases caused by *V. dahliae* and *V. albo-atrum*. *Molecular Plant Pathology* **7**, 71-86

Fravel DR (1996) Interaction of biocontrol fungi with sublethal rates of metham sodium for control of *Verticillium dahliae*. *Crop Protection* **15**, 115-119

Globus GA, Muromtsev GS (1990) The use of *Gliocladium roseum* as antagonist for defence of cotton from phytopathgenic fungi. *Proceedings of the 5th International Verticillium Symposium*, 25-30 June, Leningrad, USSR, p 90 (Abstract)

Gracia-Garza JA, Reeleder RD, Paulitz TC (1997) Degradation of sclerotia of *Sclerotinia* and *Sclerotium* by fungus gnats *(Bradysia coprophila)* and the biocontrol fungi *Trichoderma* spp. *Soil Biology and Biochemistry* **29**, 123-129

Hadar Y, Gorodecki B (1991) Suppression of germination of sclerotia of *Sclerotium rolfsii* in compost. *Soil Biology and Biochemistry* **23**, 303-306

Hajlaoui MR, Gargouri S, Guermech A, Hamza N (2003) Emergence en Tunisie d'un nouveau pathotype de *Verticillium albo-atrum* pouvant attaquer les cultivars de tomate possédant le gène de résistance *Ve*. *Bulletin OEPP/EPPO* **33**, 343-345

Hall TJ, Schreiber LR (1984) Effect of *Bacillus* on Verticillium wilt in silver Maple. *Phytopathology* **74**, 805

Harrington MA, Dobinson KF (2000) Influences of cropping practices on *Verticillium dahliae* populations in commercial processing tomato fields in Ontario. *Phytopathology* **90**, 1011-1017

Henni JE (1987) Evaluation de l'efficacité de certains champignons antagonistes vis-à-vis de *Verticillium dahliae*. *Cryptogamie, Mycologie* **8**, 203-207

Huang HC (1978) *Gliocladium catenulatum*: hyperparasite of *Sclerotinia sclerotiorum* and *Fusarium* species. *Canadian Journal of Plant Pathology* **56**, 2243-2246

Jabnoun-Khiareddine H, Daami-Remadi M, El Mahjoub M (2005) Emergence en Tunisie de nouveaux pathotypes de *Verticillium tricorpus* pouvant attaquer la tomate, l'aubergine et la pomme de terre. *Bulletin EOPP/OEPP* **35**, 497-503

Jabnoun-Khiareddine H, Daami-Remadi M, Hibar K, Ayed F, El Mahjoub M (2006) Pathogenicity of Tunisian isolates of three *Verticillium* species on tomato and eggplant. *Plant Pathology Journal* **5**, 199-207

Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, Jebari H, El Mahjoub M (2007) Incidence of Verticillium wilt of melon in Tunisia. *The African Journal of Plant Science and Biotechnology* **1**, 10-15

Jabnoun-Khiareddine H, Daami-Remadi M, El Mahjoub M (2009) Biological control of tomato Verticillium wilt by using indigenous *Trichoderma* spp. In: Daami-Remadi M (Ed) *Tunisian Plant Science and Biotechnology I. The African Journal of Plant Science and Biotechnology* **3 (Special Issue 1)**, 26-36

Jansky SH, Rouse DI (2000) Identification of potato interspecific hybrids resistant to Verticillium wilt and determination of criteria for resistance assessment. *Potato Research* **43**, 239-251

Jansky SH, Rouse DI, Kauth PJ (2004) Inheritance of resistance to *Verticillium dahliae* in diploid interspecific potato hybrids. *Plant Disease* **88**, 1075-1078

Kawchuk LM, Hachey J, Lynch DR, Kulcsar F, van Rooijen G, Waterer DR, Robertson A, Kokko E, Byers R, Howard RJ, Fischer R, Prüfer D (2001) Tomato *Ve* disease resistance genes encode cell surface-like receptors. *Proceedings of the National Academy of Science USA* **98**, 6511-6515

Keinath AP, Fravel DR, Papavizas GC (1991) Potential of *Gliocladium roseum* for biocontrol of *Verticillium dahliae*. *Phytopathology* **81**, 644-648

Kim KK, Fravel DR, Papavizas GC (1988) Identification of a metabolite produced by *Talaromyces flavus* as glucose oxidase and its role in biocontrol of *Verticillium dahliae*. *Phytopathology* **78**, 488-492

Kubicek CP, Harman GE (1998) *Trichoderma and Gliocladium. Basic Biology, Taxonomy and Genetics* (Vol 1), Academic Press, London, 278 pp

Larena I, Sabuquillo P, Melgarejo P, De Cal A (2003) Biocontrol of Fusarium and Verticillium wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. *Journal of Phytopathology* **151**, 507-512

Lynch DR, Kawchuk LM, Hachey J, Bains PS, Howard RJ (1997) Identification of a gene conferring high levels of resistance to Verticillium wilt in *Solanum chacoense*. *Plant Disease* **81**, 1011-1014

Madi L, Katan T, Katan J, Henis Y (1997) Biological control of *Sclerotium rolfsii* and *Verticillium dahliae* by *Talaromyces flavus* is mediated by different mechanisms. *Phytopathology* **87**, 1054-1060

Marois JJ, Fravel DR, Papavizas GC (1984) Ability of *Talaromyces flavus* to occupy the rhizosphere and its interaction with *Verticillium dahliae*. *Soil Biology and Biochemistry* **16**, 387-390

Matta A, Garibaldi A (1977) Control of *Verticillium* of tomato by preinoculation with avirulent fungi. *Netherlands Journal of Plant Pathology* **83**, 457-462

Mcquilken MP, Gemmell J, Lahdenpera ML (2001) *Gliocladium catenulatum* as a potential biological control agent of damping-off in bedding plants. *Journal of Phytopathology* **149**, 171-178

Millar RL, Kalb DW, Keinath AP (1984) Biological and chemical control of Verticillium wilt of alfafa. *Phytopathology* **74**, 805

Minuto A, Sapadaro D, Garibali A, Gullino ML (2006) Control of soilborne pathogens of tomato using a commercial formulation of *Streptomyces griseoviridis* and solarization. *Crop Protection* **2**, 468-475

Murray FR, Lewellyn WJ, Peacock WJ, Denis ES (1997) Isolation of the glucose oxidase gene from *Talaromyces flavus* and characterization of its role in the biocontrol of *Verticillium dahliae*. *Current Genetics* **32**, 367-375

Nadakavukaren MJ, Horner CE (1959) An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. *Phytopathology* **49**, 527-528

Nagtzaam MPM, Bollen GJ, Termorshuizen AJ (1998) Efficacy of *Talaromyces flavus* alone or in combination with other antagonists in controlling *Verticillium dahliae* in growth chamber experiments. *Journal of Phytopathology* **146**, 165-173

Narisawaa K, Kawamata H, Currah RS, Hashiba T (2002) Suppression of Verticillium wilt in eggplant by some fungal root endophytes. *European Journal of Plant Pathology* **108**, 103-109

Narisawaa K, Ohkib KT, Hashibab T (2000) Suppression of clubroot and *Verticillium* yellows in Chinese cabbage in the field by the root endophytic fungus, *Heteroconium chaetospira*. *Plant Pathology* **49**, 141-146

Ordentlich A, Nachmias A, Chet I (1990) Integrated control of *Verticilllum dahliae* in potato by *Trichoderma harzianum* and captan. *Crop Protection* **9**, 363-366

Pegg GF, Brady BL (2002) *Verticillium Wilts*, CABI Publishing, Oxford, UK. 541 pp

Pharand B, Carisse O, Benhamou N (2002) Cytological aspects of compost-mediated induced resistance against Fusarium crown and root rot in tomato. *Phytopathology* **92**, 424-438

Regragui A (2005) Contribution à l'étude de l'influence de la salinité sur le couple tomate-*Verticillium*: Conséquences physiologiques et impact sur la bioprotection des tomates contre la Verticilliose. PhD thesis, Université Mohammed V-Agdal, Rabat, Morocco, 231 pp

Sabuquillo P, De Cal A, Melgarejo P (2005) Dispersal improvement of a powder formulation of *Penicillium oxalicum*, a biocontrol agent of tomato wilt. *Plant Disease* **89**, 1317-1323

Sabuquillo P, De Cal A, Melgarejo P (2006) Biocontrol of tomato wilt by *Penicillium oxalicum* formulations in different crop conditions. *Biological Control* **37**, 256-265

Simay EI (1988) Occurrence of hyperparasitism of *Botrytis cinerea* Pers. by *Gliocladium catenulatum*. *Acta Phytopathologica Entomologica Hungarica* **23**, 133-135

Stinson M, Ezra D, Hess WM, Sears J, Strobel G (2003) An endophytic *Gliocladium* sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. *Plant Science* **165**, 913-922

Tjamos EC (2000) Strategies in developing methods and applying techniques for the biological control of *Verticillium dahliae*. In: Tjamos EC, Rowe RC, Heale JB, Fravel DR (Eds) *Advances in Verticillium: Research and Disease Management*, APS Press, St. Paul, MN, USA, pp 249-252

Tsahouridou PC, Thanassoulopoulos CC (2001) *Trichoderma koningii* as a potential parasite of sclerotia of *Sclerotium rolfsii*. *Cryptogamie Mycologie* **22**, 289-295

Turhan G (1990) Further hyperparasites of *Rhizoctonia solani* as promising candidates for biological control. *Journal of Plant Disease and Protection* **97**, 208-215

Uppal AK, El Hadrami A, Adam LR, Tenuta M, Daayf F (2007) Pathogenic variability of *Verticillium dahliae* isolates from potato fields in Manitoba and screening of bacteria for their biocontrol. *Canadian Journal of Plant Pathology* **29**, 141-152