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***In vitro* and *in vivo* Evaluation of Individually Compost Fungi for Potato *Fusarium* Dry Rot Biocontrol**

¹M. Daami-Remadi, ²H. Jabnoun-Khiareddine, ²F. Ayed, ²K. Hibar,
³I.E.A Znaïdi and ²M. El Mahjoub

¹National Institute of Agronomic Research of Tunisia
PRRDA-CE 4042 Chott-Mariem, 4042, Sousse, Tunisia

²High School of Horticulture and Breeding of Chott-Mariem, 4042, Sousse, Tunisia

³Technical Centre of Biological Agriculture, 4042, Chott-Mariem, Tunisia

Abstract: Eight thermo-resistant fungi isolated from compost extracts are tested, for the first time in Tunisia, for their antagonistic activity, *in vitro* by dual culture with *Fusarium* spp. on PDA and *in vivo* by wound treatment prior to tuber inoculation. *In vitro* experiments showed that all tested compost fungi significantly inhibited mycelial growth of *F. graminearum*, *F. sambucinum*, *F. solani* and *F. oxysporum* f.sp. *tuberosi*, observed after incubation at 25°C during 6 days, revealing a higher competition. Light microscopic studies of tested pathogen mycelium, removed from the confrontation zone of both microorganisms, showed multiple mechanisms of action including mycoparasitism, lysis, early formation of chlamydo spores and induction of mycelial cords via anatomosis between mycelial filaments. Tuber (cv. Spunta) treatment, 24 h before their inoculation by individually thermoresistant fungi suspensions, reduced development of dry rot after incubation for 21 days at 25-27°C, comparatively to the untreated control and this for the majority of the *Fusarium* complex tested.

Key words: *Solanum tuberosum* L., mycoparasitism, lysis, inhibition, thermo-resistant fungi

INTRODUCTION

In Tunisia, a complex of *Fusarium* infects potato tubers during storage (Daami-Remadi and El Mahjoub, 1996a, 2004; Priou and El Mahjoub, 1999; Chérif *et al.*, 2001; Triki *et al.*, 2001). Given that potato traditional storage is important in Tunisia, absence of registered fungicides (Anonymous, 2003) and resistant cultivars to *Fusarium* species (Daami-Remadi and El Mahjoub, 1996b; Slininger *et al.*, 1996; Vitale *et al.*, 2004) together with diversity of the pathogen and its soilborne origin justify necessity of tuber treatment. However, benzimidazoles fungicides, used in other countries for disease control, showed variable interaction with *Fusarium* spp. In fact, *in vitro* screening for benzimidazoles resistance showed that *F. solani*, *F. oxysporum* f.sp. *tuberosi* and *F. graminearum* isolates are susceptible to these fungicides whereas *F. sambucinum* isolates are resistant (Daami-Remadi and El Mahjoub, 2006). Furthermore, chemicals loss their utility due to security regulations, none target effects and development of fungicide resistance.

Compost, used as fertilizer or for physical soil structure optimisation, is suppressive for diseases caused by nematodes, bacteria, soilborne fungi, in several pathosystems (Hoitink and Fahy, 1986; Zhang *et al.*, 1998; Al-Dahmani *et al.*, 2003). In fact, culture substrates amended with composts are able of suppressing diseases caused by *Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani* and other pathogens (Kai *et al.*, 1990; Hoitink *et al.*, 1991; De Brito Alvarez *et al.*, 1995; Cao and Forrer, 2001; El-Masry *et al.*, 2002; Lyons *et al.*, 2002; Scheuerell and Mahaffee, 2004).

Inhibition induced by composts resulted from a combination of chemical and biological mechanisms. Biological factors included populations of compost, microbial competition for nutrients with pathogen (Chen *et al.*, 1988a,b), production of antibiotics, production of extra cellular lytic enzymes, parasitism and predation and host resistance induction (Zhang *et al.*, 1998; Pharand *et al.*, 2002). A number of thermophilic fungi have been isolated from self-heating materials and other sources. Kashimoto *et al.* (1972) isolated eighty one fungi capable of growing at 45°C.

Microorganisms and thermo-resistant components are, in part, responsible of the suppressive quality of compost, observed even after autoclaving (Serra-Wittling *et al.*, 1996). Acetonic extracts of compost have a strong antifungal activity against *F. oxysporum* f.sp. *cucumerinum*. These extracts stay relatively stable after heating at 100°C and also active against *Gibberella zeae*, *Helminthosporium sigmoideum* and *Glomerella cingulata* (Kai *et al.*, 1990).

A multitude fungal and bacterial species are isolated from composts showed antagonistic activity against pathogens taxonomically different such as Deuteromycetes, Oomycota and others (Hoitink and Fahy, 1986; Elad and Shteinberg, 1994; Tuitert *et al.*, 1998; Cao and Forrer, 2001; Lazarovits, 2001; Boulter *et al.*, 2002a, b; El-Masry *et al.*, 2002; Pharand *et al.*, 2002; Muhammad and Amusa, 2003) and some of them are applicable at the post-harvest phase (Mari and Guizzardi, 1998).

Preliminary double culture of some compost extracts with *Fusarium* spp. showed inhibition of pathogen mycelial growth whereas in *Fusarium* spp. potato pathosystem, application of these extracts, in co-inoculation with pathogen, does not significantly inhibited dry rot development on tubers (Daami-Remadi, unpublished data). However, multiplication of these compost extracts in culture media and their incorporation into culture substrate limited development of *Fusarium* crown and root rot on inoculated tomato plants (Hibar *et al.*, 2006). Major objective of the current study is the screening, for the first time in Tunisia, following similar methods of confrontation *in vitro* and application *in vivo*, of effective antagonists from compost extracts for *Fusarium* spp. biocontrol. Mechanisms employed by compost fungi for *in vitro* pathogen inhibition are also investigated.

MATERIALS AND METHODS

Pathogens: *F. solani*, *F. graminearum*, *F. sambucinum* and *F. oxysporum* f.sp. *tuberosi* are isolated from tubers of cv. Spunta showing typical symptoms of dry rot. These *Fusarium* spp. are grown at 25°C on PDA for one week. They are stored at -20°C in 20% glycerol solution for long term preservation.

Potato cultivars: Tubers cv. Spunta, the most cultivated in Tunisia, is used in this current study. They are obtained from the Technical Centre of Potato of Tunisia, stored in darkness at 6°C and bought to room temperature three hours before use.

Thermo-resistant compost fungi: The thermo-resistant tested fungi are obtained from several compost extracts,

composed of variable fractions of bovine, ovine and fowl manures. They are separately isolated by selective heating. Fractions of 2 mL of compost extracts were maintained, for an hour, in a bain-marie preheated at temperatures varying from 50 to 80°C. These thermo-treated fractions are then individually incorporated to 200 ml of PDA in surfusion amended with 60 mg of streptomycin-sulphate. Different cultures are incubated at 25°C for 10 to 15 days before individual fungi isolation. This technique presents advantage of reducing fungal population by increasing preheating temperatures.

Three isolates of *Penicillium* sp. (TO1, TO3 and TO4), one of *Trichoderma* sp. (TO6) and four of *Aspergillus* spp. (TO2, TO5, TO7 and TO8) are used. They grow and fructify at these culture conditions (on PDA and at 25°C) and are maintained for testing their antagonism against *Fusarium* spp. Cultures of 15 days are used for confrontation tests.

In vitro activity of thermo-resistant compost fungi against Fusarium spp: The technique used for *Fusarium* spp. x thermo-resistant fungi confrontation is the dual culture on PDA. Two mycelial discs (6 mm), one of the antagonist and the other of the pathogen, were placed equidistant of 3 cm in diametrical axis. For untreated plates, an agar disc of *Fusarium* is placed at the center of the Petri dish. All cultures are then incubated at 25°C during 6 days. This incubation temperature is chosen because it permits an optimum growth for all the tested *Fusarium* spp. (Daami-Remadi, 1996).

The mean diameter of pathogen colonies is measured after 6 days of incubation at 25°C and any morphological alteration of colonies, in comparison to untreated control, is also noted. Damage caused by thermo-resistant compost fungi to the pathogen mycelium, removed from confrontation zone of both microorganisms (pathogen and antagonist), is observed under light microscope, in comparison to untreated controls.

Statistical analyses (ANOVA) are performed following a completely randomised factorial design where treatments (compost fungi and untreated control) and *Fusarium* spp. are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

In vivo activity of thermo-resistant compost fungi against Fusarium spp.: Tubers (cv. Spunta) are superficially disinfected with a solution of 10% sodium hypochlorite, for 5 min and then rinsed abundantly with sterile distilled water. Container and alveolus plaques used for inoculated tubers incubation, are washed before use, dipped for 24 h in sodium hypochlorite solution then rinsed with sterile distilled water.

As *Fusarium* spp. are wound tuber pathogens, thermo-resistant compost fungi are applied by injecting 100 µL of a fungal suspension (10^8 spores. mL⁻¹) at sites of inoculation 24 h before pathogen application. Dimension of inoculation sites is of 6 mm diameter and depth. Inoculation technique consists of depositing an agar disc (6 mm diameter) colonized by pathogen at occasioned wounds. Tuber incubation is realized at 25-27°C for 21 days at high relative humidity. Every elementary treatment is repeated twenty times (ten tubers x two wounds).

After incubation period, tubers were cut longitudinally via sites of inoculation. Parameters of dry rot induced (maximal width (w) and depth (d)) are noted. The pathogen penetration within tubers is calculated following formula of Lapwood *et al.* (1984) where:

$$\text{Penetration (mm)} = (w/2 + (d - 6))/2$$

Statistical analyses (ANOVA) are performed following a completely randomised factorial design where treatments (compost fungi and untreated control) and *Fusarium* spp. are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

RESULTS

In vitro interactions of *Fusarium* spp. with thermo-resistant compost fungi: Mean colony diameter of four *Fusarium* species, noted after 6 days of incubation at 25°C, depends upon pathogens tested and treatments realized. A significant interaction is observed between both fixed factors ($p \leq 0.05$).

All thermo-resistant compost fungi, tested in dual culture with the pathogens on PDA, have significantly inhibited, with variable degrees, mycelial growth of *Fusarium* spp. comparatively to the untreated control (Table 1).

Fusarium sp. x compost fungi interaction is well observed when mycelial growth of each *Fusarium* species examined following all treatments (Table 1). In fact, in the case of *F. sambucinum*, mycelial growth inhibition varied from 33% (treatment by TO8) to 64% (treatment by TO4). Inhibition of *Fusarium* radial growth exceeded 40% for the entire biological treatments used showing a competitive activity of thermo-resistant compost fungi tested and this following the mentioned culture and incubation conditions.

In fact, a covering of *Fusarium* spp. colonies by that of thermo-resistant compost fungi is also noted in several interactions such as TO2 and TO5 confrontation.

Competition is not the sole mechanism employed by thermo-resistant compost fungi in antagonism of *Fusarium* spp. In fact, light microscopic studies of mycelial *Fusarium* spp. filaments, removed from the zone of confrontation with antagonists, revealed presence of multiple morphological disruptions.

Certain thermo-resistant compost fungi showed a typical mycoparasitism on *Fusarium* spp. (such as TO5, TO6 and TO2) where rolling up of their mycelial filaments around those of *Fusarium* spp. are intensively observed along the entire confrontation zone (Fig. 1). This mycoparasitism is also associated with a severe lysis of parasitized mycelium, a reduction of pathogen sporulation, an early chlamydo-spores formation (Fig. 2), an induction of mycelial cords and a discoloration of the pathogen colony along confrontation zone. It is to note that in the absence of a typical mycoparasitism, thermo-resistant compost fungi showed a diversity of pathogen inhibition mechanisms (Fig. 1 and 2).

In vivo interactions of *Fusarium* spp. with thermo-resistant compost fungi: The effect of tuber's (cv. Spunta) treatment, 24 h before inoculation with suspensions of thermo-resistant compost fungi is assessed on dry rot development. Mean penetration,

Table 1: Effect of some thermo-resistant compost fungi on mean radial growth (cm) of *Fusarium* spp. in comparison to untreated control (PDA, incubation at 25°C for 6 days)

Treatments	Control	TO1	TO2	TO3	TO4	TO5	TO6	TO7	TO8
<i>F. graminearum</i>	7.9	5.36	4.67	4.86	4.71	5.71	5.06	5.93	6.24
<i>F. sambucinum</i>	7.54	4.58	3.45	3.02	2.67	3.64	3.16	3.87	5.01
<i>F. oxysporum</i>	7.93	3.52	2.83	3.63	2.96	3.19	2.47	3.27	4.48
<i>F. solani</i>	7.36	4.01	3.31	3.22	3.01	3.06	3.36	4.66	4.25

TO1, TO3, TO4: *Penicillium* sp., TO6: *Trichoderma* sp., TO2, TO5, TO7 and TO8: *Aspergillus* spp., LSD (Treatments x *Fusarium* spp.) = 0.42 cm ($p \leq 0.05$)

Table 2: Effect of tuber treatment, 24 h prior inoculation, by thermo-resistant compost fungi conidial suspensions on dry rot development occasioned by *Fusarium* spp., in comparison to untreated controls, as measured by the mean pathogen penetration (mm)

Treatments	Control	TO1	TO2	TO3	TO4	TO5	TO6	TO7	TO8
<i>F. graminearum</i>	22.66	6.94	5.52	8.36	10.61	7.36	9.52	8.02	6.11
<i>F. sambucinum</i>	14.72	5.15	12.85	10.97	5.725	6.32	6.7	6.22	11.67
<i>F. oxysporum</i>	14.82	4.65	4.65	4.275	5.575	4.62	4.37	4.47	3.97
<i>F. solani</i>	10.02	4.82	4.5	4.825	5.225	4.2	4.3	4.15	3.85

Tubers cv. Spunta incubated for 21 days at 25-27°C TO1, TO3, TO4: *Penicillium* sp., TO6: *Trichoderma* sp., TO2, TO5, TO7 and TO8: *Aspergillus* spp., LSD (Treatments x *Fusarium* spp.) = 1.84 mm ($p \leq 0.05$)

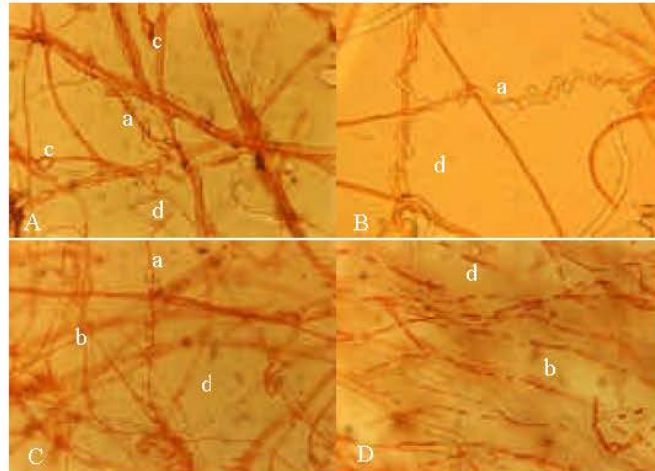


Fig. 1: Multiples interactions between *Fusarium* species and a thermoresistant compost fungus (TO5) observed at the confrontation zone after 6 days of incubation at 25°C. A: *F. solani*, B: *F. graminearum*, C: *F. oxysporum* f.sp. *tuberosi*, D: *F. sambucinum*, TO5: *Aspergillus* spp., (a) Mycoparasitism, (b) lysis of pathogen mycelium, (c) early formation of chlamydospores, (d) reduction in pathogen sporulation

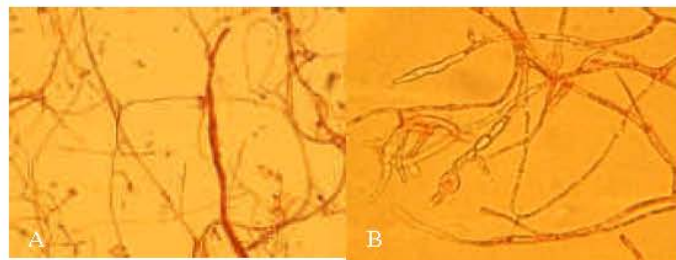


Fig. 2: Severe lysis of *F. solani* (A) and *F. graminearum* (B) mycelium observed at the confrontation zone of their dual culture with *Penicillium* sp. isolates TO4 and TO3 respectively after 6 days of incubation at 25°C

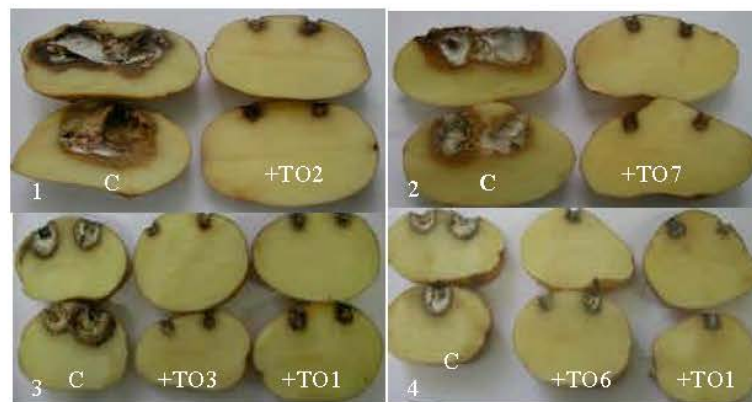


Fig. 3: Inhibition of dry rot development on potato tubers (cv. Spunta) treated, 24 hours prior inoculation by *Fusarium* species, with conidial suspensions of individual thermoresistant compost fungi (after 21 days of incubation at 25-27°C). 1: *F. graminearum*, 2: *F. oxysporum* f.sp. *tuberosi*, 3: *F. solani* and 4: *F. sambucinum*, TO1 and TO3: *Penicillium* sp., TO2 and TO7: *Aspergillus* spp., TO6: *Trichoderma* sp., C: Untreated control

noted after 21 days of incubation at 25-27°C, depends upon treatments applied and *Fusarium* species used for tuber inoculation (Table 2). A significant interaction is observed between both fixed factors ($p \leq 0.05$).

This pathogen x biological treatments interaction is well illustrated in *F. sambucinum* where occasioned inhibition varied from 12% by TO2 treatment to 65% by that of TO1, whereas inhibition percentage reached, with these both treatments, varied from 51% to 69% against the other tested *Fusarium* species.

Table 2 showed inhibitory activities of thermo-resistant compost fungi on *in vivo* development of *Fusarium* spp. causing tubers dry rot. In fact, disease induction is inhibited, following these methods of confrontation and incubation on tubers, by 50 to 75%.

Furthermore, certain tested compost fungi are efficient against all *Fusarium* species such as TO1, TO5, TO6 and TO7. Other tested compost fungi showed lower efficacy, due to specific pathogen x antagonist interaction, against at least one *Fusarium* species, this is the case for TO2, TO3, TO4 and TO8 cultures (Fig. 3).

DISCUSSION

In the current study, *Fusarium* potato dry rot biocontrol is essayed by using an indigenous and original biological material which is thermo-resistant compost fungi. Diverse interactions are noted, *in vitro* and *in vivo*, between biological potential used and *Fusarium* spp. Originality of this study resides in multitudes of antagonist x pathogen interactions and use for the first time in Tunisia of fungi isolated from several compost extracts. Furthermore, confrontations, *in vitro* and *in vivo*, are realized against the entire *Fusarium* complex representing actually fusarial microflora responsible of tuber dry rot in Tunisia (Daami-Remadi and El Mahjoub, 2004).

Thermo-resistant compost fungi tested showed a significant reduction *in vitro* and *in vivo* growth of four *Fusarium* species. Their antagonistic activity is verified not only by competition on culture medium but also by a diversity of mechanisms of action characteristic of biological agents. In fact, compost suppressive activity is reported to be due to a multitude of microorganisms present and Ben-Yephet and Nelson (1999) mentioned that their frequency is variable upon initial compost composition and its degree of maturity. Suppressing qualities of composts are generally attributed to microorganisms and also to abiotic factors (Boulter *et al.*, 2002b). Microorganisms and thermo-resistant components are, in part, responsible of suppressive quality of compost, observed after autoclaving (Serra-Wittling *et al.*,

1996). Consequently, presence in compost extracts used in the current study of thermo-resistant antagonists against *Fusarium* spp. is justified. Mechanisms of biocontrol based on competition, antibiosis and hyperparasitism are described in culture substrates amended with compost (Hoitink *et al.*, 1991).

Nelson *et al.* (1983) found that *Trichoderma* sp. are the most frequent and efficient antagonists present in composts. Kwok *et al.* (1987) identified different bacteria such as *Bacillus cereus*, *Pseudomonas* sp. and *Flavobacterium balustinum* able of suppression of *Rhizoctonia solani* individually or in synergistic interaction with *Trichoderma* sp.

El-Masry *et al.* (2002) have isolated from compost several bacterial microorganisms such as *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and *Corynebacterium* sp., fungal microorganisms such as *Aspergillus* sp., *Rhizopus* sp. and *Drechslera* sp. and diverse Actinomycetes.

Aspergillus niger, *Trichoderma harzianum*, *Bacillus cereus* and *B. subtilis* are isolated from composts (Muhammad and Amusa, 2003). *T. harzianum*, *B. subtilis* and at a lesser degree *B. cereus* inhibited pathogens such as *Sclerotium rolfsii*, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Helminthosporium maydis*. *Penicillium duponti*, reported to be a saprophyte on the plant debris and thermophilous, is closely related to thermophilous floras known from various composts (Apinis and Pugh, 1967).

All *in vitro* confrontations are realized on PDA and incubated at 25°C, temperature favourable for *Fusarium* spp. development (Daami-Remadi, 1996) and for the antagonists tested (Triki *et al.*, 1996; Daami-Remadi, 2001; Sadfi *et al.*, 2001; Chérif *et al.*, 2002). Thermo-resistant fungi are isolated and cultivated on PDA; they grow, fructify and exerted their antagonism under these culture conditions (mycoparasitism and morphological disruptions). Although competition on culture medium can even occur between non antagonist agents, microscopic studies realized for all biological treatments, revealed several types of mechanisms in their parasitic reactions.

Antagonists are able of controlling disease by competition, induced resistance and mycoparasitism which is not frequently detected in Petri dishes (Schisler *et al.*, 1997). However, the present study showed cases of typical mycoparasitism and that for some thermo-resistant fungi. Some antagonists are also able of increasing phytoalexin production and decreasing ability of isolates to detoxifying these components by making them less virulent (Schisler *et al.*, 1998).

Suppressive mechanisms of compost include one or a combination of competition for nutrients, antibiosis, lytic and other extracellular enzyme production, parasitism, predation and host-mediated induction of resistance (Boulter *et al.*, 2000). El-Masry *et al.* (2002) found that compost water extract produced clear inhibition zones against all the tested fungi and the presence of protease, chitinase, lipase and β -1, 3 glucanase (lysogenic enzymes) indicates a possible role in fungal degradation. This phenomenon could explain the strong lysis observed at the zones of confrontation of thermo-resistant compost fungi and *Fusarium* spp.

Aspergillus niger is a filamentous fungus commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation (Kiran *et al.*, 2004). Our results revealed that *Aspergillus* isolates obtained from water compost extracts exerted typical mycoparasitism against *Fusarium* spp., this joins results of Venkatasubbaiah and Safeeulla (1984) who found that *Aspergillus niger* (van Tiegh) isolated from the rhizosphere of coffee seedlings was antagonistic to the collar rot pathogen, *Rhizoctonia solani* Kuhn, *in vitro* and hyperparasitised *R. solani* completely in dual culture. The mode of hyphal interaction and parasitism of *R. solani* by *A. niger* indicated the direct growth of the mycoparasite towards its host and as a consequence the host hypha became vacuolated, collapsed and finally disintegrated. Furthermore, they observed that seed treatment with *A. niger* spores increased germination and reduced the incidence of collar rot disease under greenhouse conditions. Furthermore, in Arizona, Cotty and Bayman (1993) used an atoxigenic *Aspergillus flavus*, for the biocontrol of the toxigenic strains of this taxon.

Wickremasinghe *et al.* (1999) found that crude aqueous extracts of *Aspergillus fumigatus* and *Trichoderma harzianum* showed antifungal activity against three plant pathogens at fairly high concentrations.

Aspergillus giganteus was found to excrete an antifungal protein (AFP) which exerts growth inhibitory effects on various filamentous fungi mainly from the genus *Fusarium* (Theis *et al.*, 2003). This protein was found to act in a dose-dependent manner: it was fungistatic when applied at concentrations below the minimal inhibitory concentration, but fungicidal at higher concentrations. Following an *in vivo* model system, Theis *et al.* (2005) found that AFP prevented the infection of tomato roots (*Lycopersicon esculentum*) by the plant-pathogenic fungus *Fusarium oxysporum* f.sp. *lycopersici*. *In vitro* antifungal essays revealed that AFP was capable to inhibit mycelial growth, morphological anomalies as well as conidial germination of *Botrytis cinerea* and its application to geranium leaves completely prevented fungal growth (Moreno *et al.*, 2003).

The species-specificity of AFP resides in the outer layer and cell wall of sensitive fungi and that the plasma membrane might represent the primary target for antifungal activity. Due to its fungicidal mode of action and its high temperature stability, AFP might be a promising candidate for use in food preservation and for the generation of pathogen resistant plants, as AFP is highly specific to diverse plant-pathogenic fungi, e.g., *Fusarium* spp. Furthermore, as chitin is the main structural component of fungal cell walls, it is most likely that binding of AFP to the cell wall is due to its affinity to chitin (Theis *et al.*, 2005).

A novel antifungal peptide was isolated from the culture supernatant of the filamentous fungi, *Aspergillus niger*. The peptide shows some degree of sequence homology to cysteine-rich antifungal peptides reported from the extracellular media of *Aspergillus giganteus* and *Penicillium chrysogenum*. This antifungal peptide exhibited potent growth inhibitory activities against yeast strains as well as filamentous fungi (Lee *et al.*, 1999). An acid protease excreted by *Penicillium duponti* K1014, isolated from compost, was stable at 50 and 60°C for 1 h (Kashimoto *et al.*, 1972).

Thermo-resistant fungi used in the current study reduced tuber dry rot incidence occasioned by *Fusarium* species susceptible to benzimidazoles (case of *F. oxysporum* f.sp. *tuberosi*, *F. solani* and *F. graminearum*) but also that induced by *F. sambucinum* resistant to these fungicides. These results join, in part, that obtained by Schisler *et al.* (1998) which found five bacterial antagonists able of controlling 10 strains of *Gibberella pulicaris* (*F. sambucinum*) even those resistant to thiabendazole; but the majority of these bacteria are Gram negative such as *Pseudomonas*, *Enterobacter* and *Pantoea*.

Biocontrol *in vivo* experiments are realized on entire tubers and the obtained results reflected interactions between tuber defence, antagonists and pathogens. Schisler *et al.* (1998) also used entire tubers for testing their bacterial antagonists: *Peudomonas*, *Enterobacter* and *Pantoea*. This method permits, according to these authors, selection of antagonist microorganisms, able of surviving in potato stores and simulation of natural conditions of wounds.

Furthermore, antagonists applied before tuber inoculation seem to colonise unsterilized tissues, inhibit *Fusarium* spp. setting up and consequently, dry rot development. Chérif *et al.* (2002) also applied their *Bacillus* isolates in pre-inoculation for promoting antagonist's installation and colonization of inoculation sites. Metabolites secreted by different antagonists have a direct effect on dry rot of potato (Slinginger *et al.*, 2003).

F. sambucinum requires presence of wounds for infecting potato tubers as periderm formation necessitates 5 days to several weeks. Thus, antagonist has to stay viable and inhibit pathogen development just during this period. Probability of succeeding biological product development is more important in a pathosystem where pathogen acts in a microenvironment, favourable for introduced antagonists, characterized by a constant temperature of storage and a high relative humidity (Schisler *et al.*, 1997).

Antagonist use requires its study and its interactions with pathogen, plant, microbial flora and environment (Emmert and Handelsman, 1999). In the present study, all successful elements are respected. In fact, *Fusarium* species used are representative of fungi complex associated to potato. These interactions between microorganisms are studied *in vitro* and *in vivo* and a potato cultivar susceptible to *Fusarium* spp. is used and chosen for plant interaction. Furthermore, environmental factors are controlled because tuber incubation is realized at 25-27°C and under a relative humidity exceeding 90%. The obtained results are promising and inhibitions noted, just 21 days after incubation, showed limited dry rot development by majority of biological treatments tested.

Although some authors reported divergence between *in vitro* and *in vivo* results (Schisler *et al.*, 1997; Schisler *et al.*, 2000a; Schisler *et al.*, 2000b), the present study shows, a certain convergence and that for all antagonists tested. Furthermore, a diversity of *Fusarium* spp. species caused dry rot and frequently at least one antagonist tested inhibited the totality of them *in vitro* and *in vivo*. This shows importance of antagonist potential employed, that is naturally colonising mature compost and applicable to the post-harvest phase. These thermo-resistant compost fungi are able of supporting environmental conditions of traditional potato storage.

Although several modes of action are employed by these antagonists, a biochemical and toxicological detailed studies of these thermo-resistant compost fungi and their extracellular media are indispensable to go further into their mechanisms of action and for promoting their activity, establishing their security and for envisaging more important experiments in natural conditions.

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