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Soil-borne and Compost-borne *Aspergillus* Species for Biologically Controlling Post-harvest Diseases of Potatoes Incited by *Fusarium sambucinum* and *Phytophthora erythroseptica*

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Abstract

Nine isolates of *Aspergillus* spp., isolated from soil and compost were tested *in vitro* and *in vivo* for their antifungal activity against *Fusarium sambucinum* and *Phytophthora erythroseptica*, the causal agents of the Fusarium dry rot and pink rot of potato tubers. Tested using the dual culture method, the pathogen growth of *F. sambucinum* and *P. erythroseptica* was inhibited by 27 to 68% and 16 to 25% by all *Aspergillus* species, respectively. The highest inhibitory activity against both pathogens was induced by the isolate CH12 of *A. niger*. A significant reduction of the mycelial growth of both pathogens tested using the inverse double culture method involves the presence of volatile antifungal metabolites. Their effectiveness was also evaluated as tuber treatment prior to inoculation with the pathogens. The highest effectiveness in reducing Fusarium dry rot severity was recorded on tubers treated with the isolate CH12 of *A. niger*. This study also revealed that the efficacy of *Aspergillus* spp. as biocontrol agents may be enhanced by varying the timing of their application. In fact, the lesion diameter of dry and pink rots was reduced by 54-70 and 52% with preventive application, respectively. However, this parameter decreased by 21-48 and 47% when the *Aspergillus* spp. were applied simultaneously with pathogens, respectively. Similarly, diseases' severity, estimated based on average penetration of *F. sambucinum* and *P. erythroseptica*, was reduced by 57-77 and 55% with preventive treatments and by 29-68 and 44% with simultaneous application, respectively. This study reveals that *Aspergillus* spp., isolated from compost and soil, exhibits an interesting antifungal activity toward *F. sambucinum* and *P. erythroseptica* and may represent a potential source of biopesticide. Testing of their culture filtrates, their organic extracts and their toxicity may give additional information on their safe use as biocontrol agents.

Keywords: *Aspergillus* spp; Biological control; Dry rot; *Fusarium sambucinum*; Mycelial growth; *Phytophthora erythroseptica*; Pink rot

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world [1-3]. In Tunisia, it is one of the strategic crops occupying about 16% of all Tunisian cultivated areas [4]. However, potato production is threatened by several fungal diseases resulting in considerable yield losses [5,6]. These diseases can affect potato at any growth stage or even during storage, where potato tubers may exhibit diverse types of rots. Dry rot induced by different *Fusarium* species, Pythium leak caused by *Pythium* spp. and pink rot incited by *Phytophthora erythroseptica* are responsible for important tuber storage losses in the world and in Tunisia [7-13].

Significant losses in storage with estimates of up to 100% have been reported both in developed and developing countries when disease management is neglected [14]. Losses associated with dry rot have been estimated to range from 6 to 25%, and occasionally losses as great as 60% have been reported during long-term storage [15]. The primary sources of inoculum are contaminated or infected seed tubers and infested soil [5]. Tuber infection by *F. sambucinum* occurs through wounds produced during planting, harvesting or transport. Dry rot causes a dry and crumbly decay with abundant white, yellow or carmine-coloured mycelium depending on *Fusarium* species [15]. For pink rot, the oomycete, *P. erythroseptica* infects potato tubers through stolons or lenticels via zoospores and through cracks and cuts made during harvest and handling operations [5,16-17]. The infected tuber flesh becomes soft, spongy and watery with a light brown color. A clear liquid came out from a cut tuber and the surface acquires a pink coloration which turns brown and darkens after a few hours [18]. Thus, the health of seed tubers, the management practices during the growing period, the harvesting and handling practices and the environmental conditions maintained throughout storage are key factors affecting tuber infection by these pathogens [19].

Currently, the primary control for these diseases in storage facilities includes elimination of infected tubers prior to storage and storage management using forced air ventilation, and controlled temperature and humidity feedback systems [20]. In fact, there is a shortage of postharvest fungicides to completely manage these pathogens [21]. The control of potato dry rot has been achieved, for many years, by postharvest application of thiabendazole, a Benzimidazole fungicide [5]. However, *F. sambucinum* resistant to this fungicide and other benzimidazole fungicides were discovered in many parts of the world, leading to reduced effectiveness in controlling dry rot [15]. For pink

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rot, chemical control is limited to a few compounds. In fact, fungicides containing metalaxyl and mefenoxam were used effectively to control pink rot in the 1990's. However, metalaxyl-resistant isolates of *P. erythroseptica* are now widespread and this may lead to failure of these chemicals to control pink rot [22]. Phosphorous acid and many other disinfectants are currently registered for postharvest management of pink rot. However, use of these products does not completely control storage pathogens [23].

Furthermore, all commonly grown potato cultivars are susceptible to potato pink and dry rots. Therefore, lack of available post-harvest fungicides and disease-resistant cultivars has prompted the search for new and efficient alternative methods as seed tuber and/or postharvest treatments to reduce incidence and severity of dry and pink rots.

Recent studies indicate that generally recognized as safe (GRAS) compounds and microbial antagonists could eventually be integrated into dry rot management strategies [24]. In fact, many biocontrol agents have been explored to control potato postharvest diseases such as *Pseudomonas* spp., *Enterobacter* spp., *Trichoderma* spp, *Aspergillus* spp. which reduced the severity of dry and pink rots caused by *F. sambucinum* and *Phytophthora* spp., respectively [25-27]. *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. fumigatus* have been shown to control potato postharvest diseases caused by *Pythuim ultimum* [28-30], *Fusarium sambucinum* [31] and *Phytophthora* sp. [32]. These *Aspergillus* species used as biological control agents against many fungal pathogens such as *F. oxysporum*, *Sclerotinia sclerotiorum*, *Pythium* spp. act through mycoparasitism, competition, mycelial lysis and antibiosis via the synthesis of volatile and/or non-volatile metabolites [11,33-34]. *Aspergillus* species are ubiquitous in most agricultural soils and they generally produce a variety of secondary metabolites exhibiting inhibitory effects on several soil-borne microorganisms [35]. Moreover, many *Aspergillus* species are considered among the potential thermotolerant antagonists surviving after soil solarisation such as *A. terreus*, *A. ochraceus* and *A. fumigatus* [36]. They are also among the most abundant in composts [37-39] and they play a significant role in the composting process as well as in suppressing the growth of pathogenic fungi [40-42]. In recent studies, many atoxigenic *Aspergillus* spp. strains, mostly isolated from soil, were used as effective biocontrol agents against *Aspergillus* toxigenic strains [43]. In fact, naturally occurring populations of atoxigenic strains are considered as potentially important reservoirs for the selection of strongest biocompetitors [43]. Furthermore, Frisvad and Larsen [44] mentioned that the genus *Aspergillus* is rich in species and these species are able to produce a large number of extrolites, including secondary metabolites, bioactive peptides/proteins, lectins, enzymes, hydrophobins and aegerolysins.

Therefore, the objective of the present study was to evaluate the *in vitro* and *in vivo* antifungal potential of *Aspergillus* species (*A. niger*, *A. terreus*, *A. flavus*, *Aspergillus* sp.) isolated from compost, solarised and non-solarised soils to control *F. sambucinum* and *P. erythroseptica*. The timing of their application as tuber treatment was also tested to optimize their suppressive effects toward potato Fusarium dry rot and pink rot.

Materials and Methods

Plant material

Apparently healthy and undamaged potato tubers (*Solanum tuberosum* L.) cv. Spunta were used in this study. This cultivar was known by its susceptibility to Fusarium dry rot and pink rot [7,11].

Pathogens

The isolates of *F. sambucinum* and *P. erythroseptica* used in this study were obtained from potato tubers showing typical symptoms of dry rot and pink rot, respectively. They were cultured on Potato Dextrose Agar (PDA) and incubated at 25°C for seven days before use.

Biocontrol agents

Four *Aspergillus* species namely *A. niger*, *A. terreus*, *A. flavus* and *Aspergillus* sp., isolated from soil or compost, were used in this study (Table 1). They were cultured on PDA medium and incubated at 25°C for seven days before being used in the bioassays.

Assessment of the *in vitro* antifungal activity of *Aspergillus* spp. against *Fusarium sambucinum* and *Phytophthora erythroseptica*

The antagonism of *Aspergillus* spp. isolates against *F. sambucinum* and *P. erythroseptica* was tested *in vitro* using the dual culture method and the inverse double technique on PDA medium. The first technique consists in depositing equidistantly two agar plugs (diameter 6 mm) colonized by the pathogen (removed from a 7-days-old culture at 25°C) or the antagonist (removed from a 7-day-old culture at 25°C) in the same Petri dish containing PDA medium supplemented with streptomycin sulfate (300 mg/L) [45].

The second method consists in transplanting the antagonist and the pathogen in two separate Petri dish containing PDA medium supplemented with streptomycin sulfate (300 mg/L). Thereafter, a 6 mm agar plug of the antagonist, removed from the margin of an actively growing culture, was placed in the centre of the bottom dish whereas the agar plug (diameter 6 mm) colonized by the pathogen (removed from a 7-days-old culture at 25°C) was placed in the centre of the top Petri dish. Both dishes were sealed with parafilm layers to prevent loss of volatile substances [46]. The two pathogens (*F. sambucinum* and *P. erythroseptica*) were thus exposed to the influence of the volatile substances released from isolates of *Aspergillus* spp. tested. Control cultures are challenged by the pathogen only in the PDA medium without transplanting antagonists, for the two confrontation methods.

The diameter of the pathogen colony (treated and control) was measured after 7 and 4 days of incubation at 20°C for *F. sambucinum* and *P. erythroseptica*, respectively. The mycelial growth inhibition percentage was calculated according to the following formula:

$I\ \% = [(C2-C1)/C2] \times 100$ with C2: Mean diameter of the control colony and C1: Mean pathogen colony diameter in the presence of the antagonist [33].

Assessment of the *in vivo* antifungal activity of *Aspergillus* spp. against *Fusarium sambucinum* and *Phytophthora erythroseptica*

Apparently healthy potato (cv. Spunta) tubers were used in this study. They were washed under running tap water to remove excess soil, dipped into 10% sodium hypochlorite solution for 5 min, rinsed twice with sterile distilled water (5 min each) and air-dried.

Tubers were wounded at two sites along the tuber longitudinal axis by a disinfected Pasteur pipette occasioning wounds of 6 mm in diameter and in depth, which serve as infection sites. Tuber inoculation was made by deposing in each wound an agar plug (6 mm diameter) colonized by the pathogen removed from a 7-day-old culture at 25°C.

Conidial suspensions (10^6 CFU/mL) of the biocontrol agents, tested individually, were applied by injecting 100 µl in each wound either

simultaneously with the pathogen or 24 h before. The positive control was inoculated with the pathogen only whereas the negative control was uninoculated and untreated with any of the antagonists tested.

All tubers were incubated at high relative humidity for 21 days at 20°C and for 7 days at 25°C for *F. sambucinum* and *P. erythroseptica*, respectively. After this incubation period, tubers were cut longitudinally via sites of inoculation and lesion diameter, width (l) and depth (p) of the occasioned rot were measured. The penetration of the pathogen was calculated using the following formula:

P (mm)=[(l/2) + (P-6)]/2 [47].

The rate of reduction for the lesion diameter of dry and pink rots and the penetration of the pathogens were calculated using the following formula:

I %=[(C2-C1)/C2] * 100 with C2: lesion diameter of the rot or penetration of the pathogen in the untreated control and C1: lesion diameter of the rot or penetration of the pathogen in the presence of the antagonist [33].

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using SPSS 16.0. For the *in vitro* tests, each individual treatment was repeated three times and the essays were analyzed in a completely randomized model. The *in vivo* essay was analyzed in a completely randomized factorial model with two factors (antagonistic treatments and timing of application) and each individual treatment was repeated six times. Means were separated using Student-Newman-Keul's (SNK) test (at $P \leq 0.05$).

Results

Effect of *Aspergillus* spp. on the mycelial growth of *Fusarium sambucinum* and *Phytophthora erythroseptica*

Analysis of variance showed a highly significant (at $P \leq 0.05$) inhibitory effect of the antagonists tested on the average colony diameter of *F. sambucinum* and *P. erythroseptica*, recorded after 7 and 4 days of incubation, respectively. Tested using the dual culture method, *F. sambucinum* and *P. erythroseptica* growth at 20°C were inhibited by 27 to 68% and by 16 to 25% by all *Aspergillus* isolates, respectively. The highest inhibitory activity against pathogens, as compared to the untreated control, was induced by the isolate CH12 of *A. niger* (Figures 1 and 2). An important inhibition of *F. sambucinum* by 37, 32 and 35% was also induced by the isolates CH1 and MC2 of *A. niger* and MC5 of *A. flavus*, respectively. The isolates CH2 and MC8 of *A. terreus*, CH8, CH4 and CH3 of *Aspergillus* sp. reduced the colony diameter of *F. sambucinum* by 31, 30, 29, 28 and 27%, respectively. *A. niger* was also active against *P. erythroseptica*, causing an inhibition of 24 and 22%, by the isolates MC2 and CH1, respectively. An inhibition of *P. erythroseptica* mycelial growth by 20 to 21% was achieved using the isolates CH2 of *A. terreus,* MC5 of *A. flavus,* and MC8 of *A. terreus*. The isolates of *Aspergillus* sp. (CH8, CH4 and CH3) were less active against *P. erythroseptica* with an inhibition of 19, 18 and 16%, respectively (Figure 1).

Tested using the inverse double culture method, a significant reduction of *F. sambucinum* mycelial growth, by 27.84 and 27.45% compared to the untreated control was induced by the isolates CH2 of *A. terreus* and MC5 of *A. flavus*, respectively (Figures 3a and 4). All *Aspergillus* spp. tested had significantly inhibited *P. erythroseptica* by distance culture method from 9.24 to 23.09% compared to the untreated control. The highest reduction of *P. erythroseptica* mycelial growth was achieved using the isolates CH1, CH12, MC2 of *A. niger* and MC5 of *A. flavus* (Figures 3b and 4). An inhibition varying from 15.70 to 17.78% was caused by *Aspergillus* sp. isolates (CH3, CH4 and CH8) and MC8 of *A. terreus*. The isolate CH2 of *A. terreus* showed the lowest inhibition of *P. erythroseptica* at distance compared to the untreated control (Figure 3b).

Effect of the application timings of *Aspergillus* spp. on potato dry rot and pink rot severity

Results analyzed by ANOVA revealed a significant variation in Fusarium dry rot severity, recorded after 21 days of incubation at 20°C, depending upon treatments tested, timings of their application and their interaction. The lesion diameter of dry rot was reduced by 54 to 70%, as compared to the untreated control, with preventive application. However, this parameter decrease varied from 21 to 48% only when the *Aspergillus* spp. were applied simultaneously with the pathogen (Figures 5a and 8). Disease severity, estimated based on average penetration of *F. sambucinum*, was reduced by 57 to 77% with preventive treatments compared with 29 to 68% obtained with simultaneous application (Figures 5b and 8).

The highest effectiveness of *Aspergillus* spp. in reducing Fusarium dry rot severity, as compared to the inoculated and untreated control tubers, was recorded on potato tubers treated with the isolate CH12

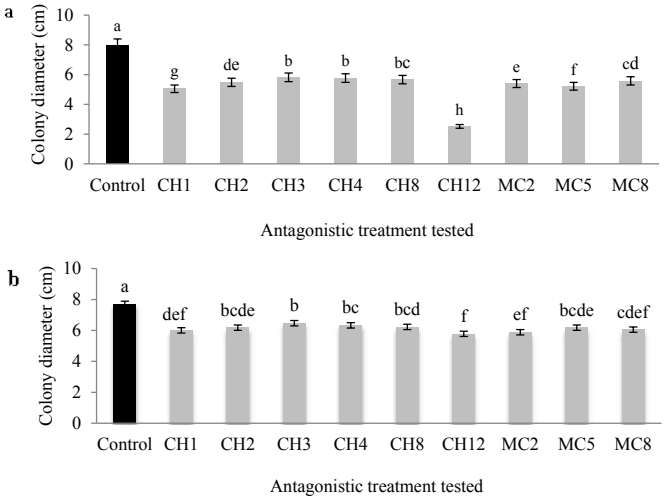

Figure 1: Effect of *Aspergillus* spp. on the mycelial growth of *Fusarium sambucinum* (a) and *Phytophthora erythroseptica* (b) noted after 7 and 4 days of incubation at 20°C, respectively, using the dual culture method. CH1, CH12, MC2: *A. niger*; CH2, MC8: *A. terreus*; CH3, CH4, CH8: *Aspergillus* sp.; MC5: *A. flavus*. Bars affected with the same letter are not significantly different according to Student-Newman-Keul's test at $P \leq 0.05$.

Figure 2: Effect of *Aspergillus niger* (isolate CH12) on the mycelial growth of *Fusarium sambucinum* and *Phytophthora erythroseptica* noted after 7 and 4 days of incubation at 20°C, respectively, as compared to the untreated controls.

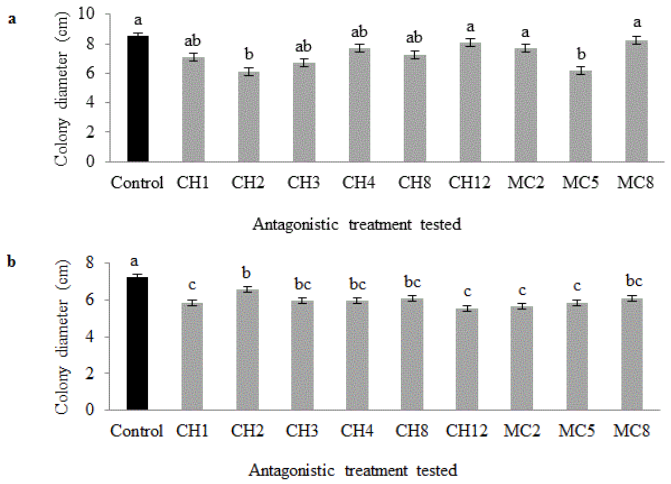

Figure 3: Effect of *Aspergillus* spp. on the mycelial growth of *Fusarium sambucinum* (a) and *Phytophthora erythroseptica* (b) noted after 7 and 4 days of incubation at 20°C, respectively, tested using the inverse double culture method. CH1, CH12, MC2: *A. niger*; CH2, MC8: *A. terreus*; CH3, CH4, CH8: *Aspergillus* sp.; MC5: *A. flavus*. Bars affected with the same letter are not significantly different according to Student-Newman-Keul's test at $P \leq 0.05$.

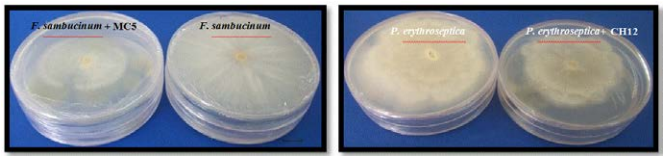

Figure 4: Effect of volatile metabolites produced by *Aspergillus* spp. (MC5: *A. flavus*; CH12: *A. niger*) on the mycelial growth of *Fusarium sambucinum* and *Phytophthora erythroseptica*, noted after 7 and 4 days of incubation at 20°C, respectively, compared to the untreated controls.

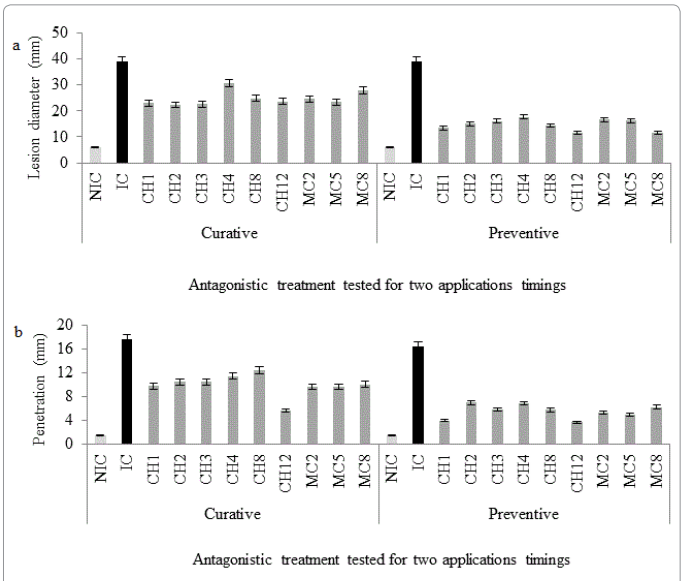

Figure 5: Effect of the application timing of *Aspergillus* spp.-based treatments on potato tubers on the lesion diameter (a) and the average penetration (b) of *Fusarium sambucinum* noted after 21 days of incubation at 20°C. CH1, CH12, MC2: *A. niger*; CH2, MC8: *A. terreus*; CH3, CH4, CH8: *Aspergillus* sp.; MC5: *A. flavus*. NIC: Uninoculated and untreated control; IC: Inoculated and untreated control. LSD (Timings of application × Antagonistic treatment tested): 5.8 mm (a) or 2.28 mm (b) at $P \leq 0.05$.

of *A. niger* in both application timings. This isolate reduced the lesion diameter by 55% and the average penetration of the pathogen by 73%, as compared to the inoculated and untreated control tubers (Figures 5 and 9).

Analysis of variance showed a significant (at $P \leq 0.05$) variation in pink rot severity, recorded after 7 days of incubation at 25°C, depending upon treatments tested and the timings of their application. The interaction between these two factors did not show significant variation on the reduction of pink rot severity. Whatever the timing of their application, a reduction of the lesion diameter of pink rot varied between 83 and 88% with all biological treatments tested compared to the inoculated and untreated control (Figure 6a). All isolates of *Aspergillus* spp. tested had also limited the average penetration of *P. erythroseptica* by about 82 to 91% compared to the inoculated and untreated control (Figure 6b).

A significant decrease in pink rot severity was also achieved using the antagonists tested preventively. The lesion diameter of pink rot was reduced by 52% with preventive application as compared to 47% when the *Aspergillus* spp. were applied simultaneously with the pathogen (Figure 7a). All antagonists tested and applied 24 h prior to inoculation with *P. erythroseptica* led to 55% decrease in the average penetration of the pathogen compared to 44% obtained with simultaneous application (Figures 7b).

Discussion

In recent years, intense research efforts have been devoted to the development of antagonistic microorganisms to control potato diseases such as *Penicillium*, *Trichoderma*, *Aspergillus*, *Gliocladium*, etc [11,48]. In fact, several studies have promoted the antagonistic activity of *Aspergillus* species which have been identified in compost- or residue-amended substrates, in solarised and non-solarised soils, in the rhizosphere, in decaying plant material, in stored grains, etc. Furthermore, some of them are essential component of many compost

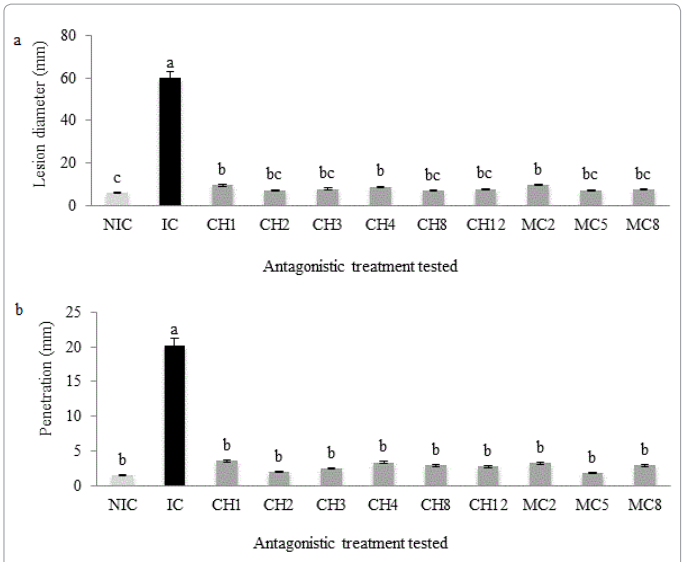

Figure 6: Effect of *Aspergillus* spp. based-treatments of potato tubers on the lesion diameter (a) and the average penetration of *Phytophthora erythroseptica* (b), noted after 7 days of incubation at 25°C. CH1, CH12, MC2: *A. niger*; CH2, MC8: *A. terreus*; CH3, CH4, CH8: *Aspergillus* sp.; MC5: *A. flavus*. NIC: Uninoculated and untreated control; IC: Inoculated and untreated control. Bars affected with the same letter are not significantly different according to Student-Newman-Keul's test at $P \leq 0.05$.

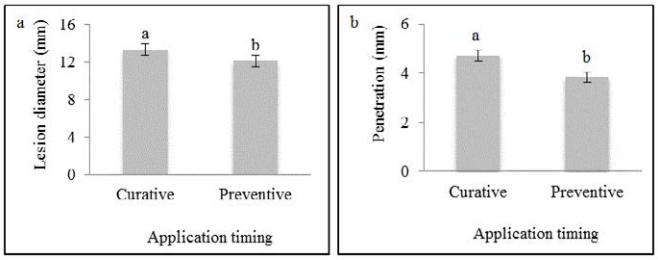

Figure 7: Effect of the application timing of *Aspergillus* spp.-based treatments on potato tubers on the lesion diameter (a) and the average penetration (b) of *Phytophthora erythroseptica*, noted after 7 days of incubation at 25°C. Bars affected with the same letter are not significantly different according to Student-Newman-Keul's test at $P \leq 0.05$.

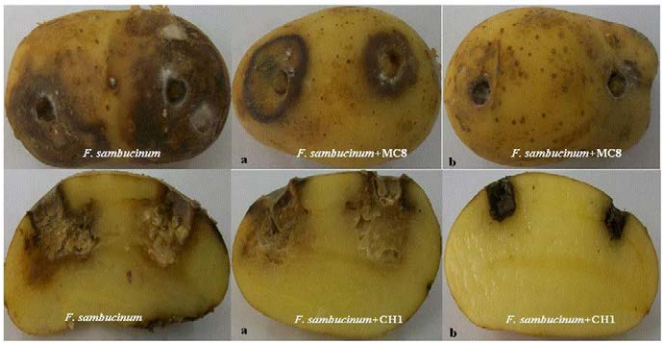

Figure 8: Effect of *Aspergillus* spp. (MC8: *A. terreus*; CH1: *A. niger*) applied simultaneously with pathogens (a) or 24 h before (b) on the severity of dry rot, noted after 21 ays of incubation at 20°C, as compared to the inoculated and untreated controls.

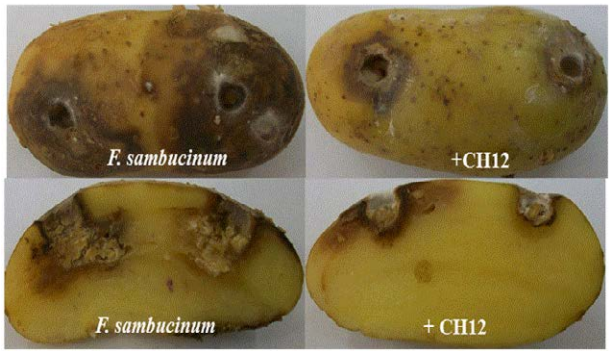

Figure 9: Reduction of Fusarium dry rot severity induced by the isolate CH12 of *Aspergillus niger* as compared to the inoculated and untreated control.

microbiota [28,49-52]. Moreover, *Aspergillus* species have been reported as endophytes with antifungal activity [53-55] and able to produce several metabolites such as phenolic and bioactive flavonoid compounds.

In the current study, the use of naturally occurring *Aspergillus* species, isolated from compost, solarised and non-solarised soils, to control potato dry and pink rots caused respectively by *F. sambucinum* and *P. erythroseptica*, was evaluated *in vitro* and *in vivo*.

Tested using dual culture method, all the nine isolates of *Aspergillus* spp. tested, had inhibited the mycelial growth of *F. sambucinum* and *P. erythroseptica* by 27 to 68% and 16 to 25%, respectively. These findings confirm previous studies showing that native compost and soil-borne *Aspergillus* spp. significantly reduced the *in vitro* growth of *Pythium* spp., causing potato leak as well as that of many *Fusarium* species responsible for potato dry rot in Tunisia [11,29]. Furthermore, results revealed the inhibitory effects of *Aspergillus* species and isolates regardless of their origin of isolation. These findings are in accordance with several previous studies showing the potential antagonistic effects of *Aspergillus* isolated from many substrates. In fact, Barocio-Ceja et al. [56] showed that *Aspergillus* sp. "VC11" isolated from chicken-manure vermicompost inhibited the growth of *F. oxysporum* by 27%, that of *F. subglutinans* by 24% and that of *Rhizoctonia* sp. by 25%. Similarly, Israel and Lodha [57] reported the hyperparasitism of *A. versicolor* isolated from heated (naturally or solarized) cruciferous residue-amended soils against *F. oxysporum cumini*. Moreover, Adebola and Amadi [51] found that three *Aspergillus* species, namely *A. niger*, *A. fumigatus* et *A. repens*, isolated from the rhizosphere of black pod-infected cocoa trees showed ability to inhibit growth of *Phytophthora palmivora in vitro* and that the inhibition zones produced might be due to the production of antifungal metabolites by the test antagonists.

Our *in vitro* results showed that the highest inhibitory activity against both pathogens was induced by *A. niger*. These findings are in accordance with Dwivedi and Enespa [58] who found that among the biocontrol agents tested, *A. niger* isolated from tomato field was most effective and completely inhibited the mycelial growth of *F. solani* and *F. oxysporum* f. sp. *lycopersici*. In the same sense, Tiwari et al. [59] showed that *A. niger* inhibit the growth of all the fungal species tested which belong to ten genera of wood decay fungi (*Trametes*, *Stereum*, *Pycnoporus*, *Phellinus*, *Lenzites*, *Phellinus*, *Earliella Gloeophyllum*, *Flavodon* and *Daedalea*), from 29.2 to 66.7%. In addition, Venkatasubbaiah and Safeeulla [60] found that *A. niger*, isolated from the rhizosphere of coffee seedlings, was antagonistic to *Rhizoctonia solani in vitro* and exhibited hyperparasitism against this collar rot pathogen in dual culture experiments. The compost inhabiting *A. niger* was also able to inhibit the growth of soil-borne pathogens including *Macrophomina phaseolina*, *Pythium aphanidermatum* and *Rhizoctonia solani* [61]. In addition, besides the inhibition of the mycelial growth, an atoxigenic *A. niger* FS10 isolated from Chinese fermented soybean was found to be able to inhibit spore germination and also to degrade the aflatoxin B1 of toxigenic *A. flavus*, and to reduce its mutagenicity and toxicity [62].

The suppressive effects of the microbial agents used in this study against potato pathogens may involve several mechanisms of action. In fact, to antagonize *F. sambucinum* and *P. erythroseptica*, *Aspergillus* isolates had probably deployed mycoparasitism, involving direct contact between the tested antagonist and the pathogens, (ii) production of antibiotic-type secondary metabolites, which spread through the medium, and (iii) competition for nutrients and space [63,64]. In fact, the first study showing that *A. terreus* can parasitize the sclerotia of *Sclerotinia sclerotiorum* under both laboratory and field conditions was reported by Melo et al. [50]. Recently, the work of Hu et al. [65] clearly demonstrates that the mycoparasite characteristics of *Aspergillus* sp. ASP- 4 may be enhanced and related to its ability to produce a range of extracellular enzymes, such as chitinases and other antifungal extrolites which help colonization of *S. sclerotiorum* sclerotia; as well as thought to be the mode of action of *A. terreus* (*Aspergillus* section Terrei). In addition, chitinases have been shown to be produced by several *Aspergillus* spp. [65]. In fact, antifungal activity of crude and purified chitinase produced by *A. niger* LOCK 62 was observed against *F. culmorum*, *F. solani*, and *R. solani*. The growth

inhibition of *F. culmorum* was the strongest both by crude and purified enzymes (70 and 60%, respectively) whereas the growth of *F. solani* was strongly inhibited by crude chitinase (73%) [66]. Recently, Frisvad and Larsen [44] mentioned that *Aspergillus* species are usually very efficient specialized metabolite producers which produces a wide array of small molecule extrolites (secondary metabolites or specialized metabolites), but also other bioactive molecules. Zhang et al. [53] have also isolated an endophytic *A. clavatonanicus* from a twig of Chinese *Taxus mairei* able to produce clavatol and patulin which exhibited inhibitory activity *in vitro* against several plant pathogenic fungi, i.e., *Botrytis cinerea*, *Didymella bryoniae*, *F. oxysporum* f. sp. *cucumerinum*, *R. solani*, and *P. ultimum*. Recently, Patil et al. [54] have identified an isolate of *A. flavus*, as an endophytic fungus from Indian medicinal plant *Aegle marmelos*, able to produce phenolic and bioactive metabolite, characterized to be a flavonoid rutin with excellent biological activities.

Tested using the inverse double culture method, *Aspergillus* spp. isolates exhibited antifungal activity toward *F. sambucinum* and *P. erythroseptica*. The reduction of mycelial growth of both pathogens by *Aspergillus* spp. isolates involved the presence of volatile metabolites produced by each active antagonist tested. In the same sens, Gao et al. [67] revealed the presence of nine volatile metabolites produced by *Aspergillus* spp. (*A. fumigatus*, *A. versicolor*, *A. sydowi*, *A. flavus*, *A. niger*). In addition, Israel and Lodha [57] reported that, apart from releasing antibiotic substances, *A. versicolor* also produces volatile metabolites such as various hydrocarbons, alcohols, ketones, ethers, esters and sulphur-containing compounds. Moreover, volatile metabolites, emitted by *A. niger*, had been shown to inhibit the growth of *Colletotrichum gloeosporioides* [68]. Recently, Thakur and Harsh [69] demonstrates that *A. niger*, isolated from the phylloplane of healthy *Piper longum* plants, possess higher antagonistic efficacy in inhibiting the mycelial growth of *C. gloeosporioides in vitro* by producing volatile metabolites.

Our results make clear that the inhibitory effect of *Aspergillus* spp. against *F. sambucinum* and *P. erythroseptica in vitro* varied depending on species and isolates of the same antagonist. In fact, populations of *A. flavus* in agricultural fields consist of complex communities that exhibit considerable genetic diversity based on phylogenetic and vegetative compatibility group analyses [70,71]. Natural communities of *A. flavus* consist of individuals that vary widely in ability to produce aflatoxin [72]. In the same sense, Khan and Anwer [73] mentioned that the effectiveness of soil aggregate isolates of *A. niger* against *R. solani* varies with the isolate. In fact, the HCN-producing isolates AnC2 and AnR3, belonging to group I, produced greater amounts of siderophore and solubilized phosphorus than the other isolates and caused the greatest inhibition of colonization by *R. solani* in dual culture. They also suppressed the root rot on eggplant and the soil population of *R. solani* in pot soil [73].

The dry and pink rots are serious post-harvest potato diseases that cause important storage losses in Tunisia [7,9]. In the present study, four species of *Aspergillus* were used as antagonistic agents against the causative agents of these two tuber diseases. Results indicate that all the *Aspergillus* spp. tested significantly reduced the lesion diameter of the dry and pink rots as well as the average penetration of *F. sambucinum* and *P. erythroseptica*, whatever the timing of their application. In fact, the antagonistic potential of *Aspergillus* sp. against both diseases confirmed previous local reports on biocontrol of soil-borne pathogens with *Aspergillus* sp. isolated from compost teas [11,28-29]. Furthermore, efficacy of *Aspergillus* species in controlling fungal diseases has been proved. In fact, *Aspergillus niger* has a fair capacity to suppress plant pathogens and to increase the yield of the plants it colonizes [74,73]. In this sens, Khan and Anwer [73] reported that soil application of *A. niger* aggregate isolates, especially AnC2 and AnR3, suppressed *R. solani* infection and also significantly increased eggplant yield. The production of NH3, HCN and siderophore may have contributed to the suppression of *R. solani*. Hu et al. [65] mentioned also that their results strongly suggest that *Aspergillus* sp. ASP-4 has huge potential to be a successful biocontrol agent for Sclerotinia stem rot of oilseed rape. In fact, this antagonist significantly inhibits *S. sclerotiorum* by parasitism of the sclerotia, resulting in a marked decrease in the formation of apothecia in the field. In addition, Dwivedi and Enespa [58] reported that *Aspergillus* species were the most effective antagonists followed by *Penicillium* spp. and *Trichoderma* spp. for controlling the wilt of tomato and brinjal crops. In addition, the use of these bio-agents are not only safe for the farmers and consumers, but also eco-friendly, cost effective, easy to produce and easy to apply the formulations [58]. Furthermore, results obtained by Wang et al. [75] indicated that the ASD strain, *A. flavipes*, could be useful as a potential biocontrol agent against the soil-borne fungus *Phytophthora capsici* and suggest that the increased activity of defence-related enzymes might be part of the mechanism of *A. flavipes* in controlling *P. capsici*. In other studies, Chuang et al. [76] reported that, based on pot experiments, significant increases in plant (*Brassica chinensis* Linn.) dry weight and N and P contents were observed with the addition of isolate 6A of *A. niger*, which has the ability to solubilise the native soil phosphate, which is not available to plant.

The present study also highlighted the importance of the timing of antagonist's application on their effectiveness in dry and pink rots control. It was clearly evidenced that these compost and soil-borne fungi are able to significantly reduce rot development and severity when applied preventively following tuber wounding. In fact, when applied 24 h before inoculation with the pathogen, the *Aspergillus* isolates tested reduced more effectively the severity of Fusarium dry rot and pink rot. Similarly, Aydi et al. [29] recorded a better antagonistic effect of *Aspergillus* spp. when applied 24 h before inoculation with *Pythium ultimum* on potato tubers. Similar findings were reported by Daami-Remadi et al. [28] who used *Aspergillus* spp., *Penicillium* sp. and *Trichoderma* sp. as antagonistic agents against *P. ultimum* and *P. aphanidermatum*. Aydi-Ben Abdallah et al. [32] demonstrated that an important decrease in pink rot severity was achieved using preventive treatments of potato tubers with the culture filtrates and organic extracts from *Aspergillus* spp. In the same sense, applied preventively (24, 48 and 72 h before inoculation with the pathogen), dry rot severity incited by *F. sambucinum* was also limited using *Bacillus* spp. isolates [10] suggesting that this factor (i.e. treatment timing) may have a direct effect on disease control efficiency. In fact, Mohale et al. [77] reported that the timing of application of atoxigenic *Aspergillus* strains relative to the onset of host tissue colonisation by toxigenic strains, the innate competitive ability of the atoxigenic strains in the presence of their toxigenic counterparts are all important for the successful implementation of control strategies.

Conclusion

Aspergillus spp., isolated from soil and compost, exhibited an interesting antifungal activity against *F. sambucinum* and *P. erythroseptica in vitro*, using the dual culture and the inverse double culture methods, showing that these antagonists are able to release volatile and non-volatile metabolites. Thus, these *Aspergillus* species may represent a potential source of biologically active compounds

and constitute a promising alternative to manage these increasingly important diseases.

Our studies demonstrated that *Aspergillus* species are potential native biological control agents against both potato diseases. They have shown great promise in their ability to reduce potato dry and pink rots. Their effectiveness was proved under extremely favourable conditions for *F. sambucinum* and *P. erythroseptica* development, such as use of susceptible potato cultivar (cv. Spunta), tuber wounding before inoculation, incubation under high moisture and disease conducive temperature, altogether have normally favoured pathogen expression and consequently, rot development.

Our results suggest that *Aspergillus* spp. are successful biocontrol agents for potato tuber rots in storage. These *Aspergillus* spp. may be further stimulated or enhanced by soil amendments and fertilizers, which result in the release of volatile chemicals that are toxic to potato pathogens (e.g., ammonia and glucosinolates), but can stimulate the growth and proliferation of *Aspergillus* species and other soil-borne mycoparasites [78,79].

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