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Assessment of the Antifungal Activity of Non-pathogenic Potato-associated Fungi toward *Fusarium* Species Causing Tuber Dry Rot Disease

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Abstract

Twenty isolates of potato-associated fungi belonging to genera *Aspergillus, Penicillium, Colletotrichum*, and *Trichoderma* and recovered from healthy potato organs (stems, roots, and tubers) were screened for their antifungal potential toward *Fusarium sambucinum* and *F. solani* the major agents of dry rot disease in Tunisia. Tested using the dual culture method, all the potato-associated isolates had significantly lowered pathogen growth, noted after 7 days of incubation at 25°C as compared to the untreated control, but with a variable range depending on isolates used and targeted *Fusarium* species. *F. sambucinum* and *F. solani* were inhibited by 23.4 to 71.5% and by 29.2 to 62.1%, respectively, depending on antagonistic treatments tested. The percentage of *Fusarium* spp. inhibition ranged from 30.1 to 47.2% using *Aspergillus* spp. and from 30.1 to 67.3% with *Penicillium* spp. compared to 40.1-50.6% and 40.8% achieved using *Colletotrichum* sp. and *Trichoderma* sp., respectively. Strong hyphal lysis, formation of mycelial cords and early production of chlamydospores are the most frequent stress responses exhibited by both pathogens during their *in vitro* interactions with the potato-associated fungi. Tested as tuber treatment prior to pathogen challenge using a mixed inoculum composed of *F. sambucinum* and *F. solani*, 13 isolates out of the 20 tested led to a significant decrease, by 26.9 to 54.8%, in the mean diameter of dry rot lesion, as compared to the inoculated and untreated control. All tuber treatments had significantly decreased mean rot penetration, in comparison to *Fusarium* spp.-inoculated and untreated control, which was lowered by more than 50% using 14 out of the 20 potato-associated isolates. Thus, the present study clearly demonstrated that fungal isolates, occurring ubiquitously within potato plants, may be promising candidates for *Fusarium* spp. biocontrol and may be other potato diseases.

Keywords: Associated-fungi; Antifungal activity; Dry rot; Dual culture; *Fusarium* spp.; Rot severity

Introduction

Fusarium dry rot (FDR) of potato tubers is particularly prominent in Tunisia leading to partial or total loss of stored tubers depending on inoculum nature, cropping seasons and storage conditions. *Fusarium sambucinum, F. solani, F. graminearum,* and *F. oxysporum* f. sp. *tuberosi* are the most frequently isolated species from diseased tubers [1-3].

This disease in becoming increasingly important due to the absence of resistant cultivars [4-8] and to the long-lasting of their resting structures i.e. chlamydospores in the soil under different environmental conditions which make them more difficult to control. To prevent *Fusarium* spoilage and disease development, the most commonly applied practice used is the dipping of harvested tubers into fungicide suspensions prior to storage [9,10]. Nevertheless, besides the problems relative to environmental pollution and chemical toxicity to humans and animals, resistance to Benzimidazole fungicides used for tuber treatment seems to be widespread among strains of *Fusarium* spp. in the most potato-growing countries including Tunisia [11-13]. In Tunisia, azoxystrobin- and fludioxonil-based fungicides had reduced by more than 50% the development of dry rot caused by *F. graminearum* and *F. sambucinum* including Benzimidazole-resistant strains [14]. Successful biocontrol of potato postharvest diseases, including Fusarium dry rot, was achieved using *Pseudomonas* spp., *Enterobacter* spp., *Bacillus* spp., *Trichoderma* spp, *Aspergillus* spp. [2,15-20]. Furthermore, an interesting approach to post-harvest disease control that has gained attention is the use of biocontrol agents (BCAs) naturally associated to healthy plants or tubers.

In the past few decades, many studies have been focused on the use of plant-associated antagonists for biologically controlling plant diseases. As each plant species is colonized by its autochthonous antagonists, bacteria as well as fungi, it is possible to protect plants from pathogens by introducing these microorganisms as BCAs [21]. These plant-associated microorganisms are able to colonize the internal tissues without visibly inducing harmful effects. They can be isolated from surface-disinfected tissues or extracted from within the plant [22,23]. Many studies revealed that associated fungi play an important role in plant protection against various bioagressors as they are able to synthesize bioactive compounds involved in plant defense [24-26]. Endogenous agents were considered as ecologically more adaptable and able to protect the plant environment from soilborne pathogens' infections [26,27]. The rhizosphere and endorhiza were the main reservoirs for potato-associated bacteria and their antagonistic potential towards the soilborne pathogens *Verticillium dahliae* and *Rhizoctonia solani* has been previously demonstrated [21]. However, there is little information regarding the role of potato-associated

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fungi in plant protection and the knowledge of their potential role in bioprotection of plants needs to be better elucidated.

Therefore, the present work was carried out to assess the *in vitro* antifungal potential of twenty isolates of potato-associated fungi, recovered from healthy potato plants, toward two *Fusarium* species and to evaluate their comparative ability to suppress tuber dry rot disease.

Materials and Methods

Potato cultivars

Potato (*Solanum tuberosum* L.) cv. Spunta tubers, the most grown cultivar in Tunisia, were used in all trials. Tubers were stored at 6°C for two months. Twenty-four hours before use, they were gently washed in running tap water and allowed to dry under ambient conditions. They were then thoroughly superficially disinfected with a 10% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water and air dried.

Fusarium species

Two *Fusarium* species namely *F. solani* and *F. sambunicum* used in the current study were originally isolated from potato tubers showing typical symptoms of dry rot disease. They were gratefully provided by the Laboratory of Plant Pathology of the Regional Centre of Research on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia. They were grown on Potato Dextrose Agar (PDA) medium supplemented with 300 mg/L of streptomycin sulphate. Their virulence was maintained by bimonthly inoculation of freshly wounded and healthy tubers and re-isolation on PDA.

Potato-associated fungi isolation source and culture conditions

The potato-associated fungi used in the present study were originally recovered from visibly healthy plant samples removed from several potato-growing fields in Tunisia (Table 1). They were isolated on PDA medium from surface-sterilized samples of potato organs (roots, stems, tubers). All isolates were purified to single spore cultures and were previously subjected to pathogenicity tests on potato tubers and found to be non pathogenic. Their identification was based on their macro and micro morphological traits [28,29]. Stock cultures were stored at -20°C in a 20% glycerol solution. Tested isolates were grown on PDA at 25°C for one week before being used in the bioassays.

Screening of the antagonistic potential of the potato-associated fungi against *Fusarium* spp.

The 20 fungal isolates were assessed for their antifungal potential against *F. sambucinum* and *F. solani* using the dual culture technique in which the pathogen and the antagonist were plated in the same Petri plate containing PDA supplemented with streptomycin (300 mg/L). Agar plugs (6 mm in diameter), already colonized by the pathogen or the antagonist and removed from 7-day-old cultures, were placed at 2 cm apart from the edge of the Petri plate and equidistant of 5 cm. In control plates, pathogen agar plugs were placed at the center of the plate. Each individual treatment was replicated four times. The whole experiment was repeated twice. Fungal cultures were maintained at 25°C and the mean diameter of pathogen colony was noted after 7 days of incubation.

The percentage of growth inhibition (GI) rate of the pathogens was calculated using the following Whipps' [30] formula: Growth inhibition % = [(C1-C2) / C1] × 100 where C1: Mean diameter of the control colony and C2: Mean colony diameter of pathogen dual cultured with the antagonist.

Morphological alterations of pathogen mycelium, removed from the confrontation zone, were observed under light microscope and described as compared to the untreated control at the end of the experiment.

Screening of the suppressive effects of the potato-associated fungi toward Fusarium dry rot

Preparation of *Fusarium* inoculum

A mixed inoculum composed of *F. sambucinum* and *F. solani*, being the most aggressive *Fusarium* species complex causing dry rot in Tunisia in a previous work [31], was used for tuber inoculation. Pathogen inoculum was prepared by scraping off mycelium from 7-day-old cultures and then, homogenized with sterile distilled water in a blender for 5 min, filtered through double layered cheese cloth

Isolates		Year of isolation	Origin	Organ	Cultivar
***Aspergillus* spp.**					
E.42.11	*A. terreus*	2010	Teboulba	Stem	Spunta
E.5.11	*A. flavus*	2011	Chott-Mariem	Tuber	Spunta
E.41.11	*A. terreus*	2010	Sahline	Stem	Spunta
E.25.11	*A. flavus*	2011	Chott-Mariem	Tuber	Magda
E.37.11	*A. flavus*	2010	Sahline	Tuber	Safrane
E.61.11	*A. nidulans*	2011	Chott-Mariem	Tuber	Spunta
E.60.11	*A. flavus*	2011	Chott-Mariem	Tuber	Spunta
E.13.11	*A. niger*	2011	Chott-Mariem	Tuber	Carrera
E.33.11	*A. niger*	2011	Chott-Mariem	Tuber	Carrera
E.2.11	*A. nidulans*	2011	Chott-Mariem	Tuber	Kenita
***Penicillium* spp.**					
E.47.11	*Penicillium* sp.	2010	Kairouan	Roots	Spunta
E.39.11	*Penicillium* sp.	2010	Teboulba	Tuber	Bellini
E.29.11	*P. polonicum*	2011	Chott-Mariem	Tuber	Evora
E.44.11	*Penicillium* sp.	2010	Kairouan	Stem	Spunta
E.36.11	*P. chrysogenum*	2011	Chott-Mariem	Tuber	Spunta
E.40.11	*Penicillium* sp.	2010	Teboulba	Tuber	Derby
E.68.11	*Penicillium* sp.	2011	Kairouan	Stem	Spunta
***Colletotrichum* spp.**					
E.16.11	*Colletotrichum* sp.	2011	Chott-Mariem	Roots	Challenger
E.8.11	*Colletotrichum* sp.	2011	Chott Mariem	Stem	Spunta
***Trichoderma* sp.**					
E.45.11	*Trichoderma* sp.	2010	Sahline	Tuber	Spunta

Table 1: Potato-associated fungi used for Fusarium dry rot biocontrol and their isolation sources.

and the final conidial suspension was adjusted to 10^7 CFU/mL using a Malassez hemocytometer. Equal volumes of conidial suspensions of *F. solani* and *F. sambucinum* were combined to obtain a mixed inoculum ready for tuber infection.

Preparation of associated fungi inoculum

Potato-associated fungi isolated were cultured on PDA and incubated at 25°C. Liquid cultures were prepared by transferring five plugs (6 mm in diameter) to 150 ml of Potato Dextrose Broth (PDB) and were incubated at 25°C for 10 days in a rotary shaker incubator at 120 rpm. The conidial concentration used was adjusted to 10^7 CFU/mL.

Tuber inoculation and treatment

Each potato tuber was wounded two times along a line joining the two ends. The wounds were immediately treated with the associated fungi to be tested by injecting 100 µL of the conidial suspension and 24 h later, tubers were challenged with 100 µL of a mixed *Fusarium* inoculum. Tubers were either inoculated with the mixed inoculum only or with a same volume of sterile distilled water were used as controls. After inoculation and treatment, all tubers were placed in plastic bags to maintain high humidity and then incubated at 25°C for 21 days.

Dry rot severity assessment

The mean diameter of dry rot lesions was measured at the end of the incubation period. Also, the extent of disease within tubers was evaluated by quartering tubers longitudinally through along the two wounds and measuring for each wounding site the maximum depth (p) and width (l) of the diseased necrotic tissue. Rot penetration (P) was calculated using the following formula [32]:

$$P\ (mm) = [l/2 + (p-6)] / 2.$$

Statistical analyses

Statistical analyses (ANOVA) were performed for mean colony diameter following a completely randomized factorial design where treatments (potato-associated isolates and the untreated control) and the two *Fusarium* species were the two fixed factors. Four replicates were used per individual treatment. For the *in vivo* bioassay, data were analyzed according to a completely randomized design where the antagonistic treatments tested were the sole fixed factor and each individual treatment was replicated six times. Data analysis was performed using SPSS Software version 20 and mean separations were carried out using the Duncan's Multiple Range test (at $P < 0.05$).

Results

Antifungal potential of the potato-associated fungi towards *Fusarium* spp.

Analysis of variance showed that mean diameter of *Fusarium* spp. colonies, formed after 7 days of incubation at 25°C, varied significantly (at $P \leq 0.05$) depending upon *Fusarium* species and antagonistic treatments tested. In fact, as shown in Table 2, all the 20 potato-associated fungi tested had significantly decreased pathogen growth as compared to the untreated control but with a variable range depending on associated isolates used and targeted *Fusarium* species. Combined data of both *Fusarium* species indicated that percentage of pathogen growth inhibition, relative to control, varied from 30.1 to 47.2% using *Aspergillus* spp. isolates and from 30.1 to 67.3% with *Penicillium* spp. compared to 40.1-50.6% and 40.8% achieved using *Colletotrichum* sp. and *Trichoderma* sp. isolates, respectively.

Data given in Table 2 also demonstrated that *F. sambucinum* dual cultured with the potato-associated fungi tested showed 23.4 to 71.5% lower mycelial growth, as compared to control, depending on isolates used. In fact, *F. sambucinum* growth was lowered by 27.7 to 47.5% using *Aspergillus* spp. isolates and by 23.4 to 71.5% using *Penicillium* spp. compared to 33.4-51.8 and 34.7% achieved using *Colletotrichum* sp. and *Trichoderma* sp., respectively. *F. solani* inhibition rate varied from 29.2 to 62.1% depending on antagonistic treatments tested. In fact, *F. solani* growth decrease ranged between 29.2 and 53.9% using *Aspergillus* spp., between 37.3 and 62.1% using *Penicillium* spp. compared to 47.3-48.9% noted using *Colletotrichum* sp. and *Trichoderma* sp. isolates.

It should be highlighted that *Fusarium* spp. growth was inhibited by more than 40% using 14 out of the 20 potato-associated isolates tested. This indicates their interesting antifungal potential and their competitive ability on PDA medium. In fact, an overgrowing of *Fusarium* spp. colonies by the screened isolates was observed in several potato-associated fungi x *Fusarium* spp. interactions with some *Penicillium* sp., *P. polonicum*, *P. chrysogenum*, *Colletotrichum* sp., *Trichoderma* sp., *A. flavus*, *A. nidulans*, and *A. niger* isolates (Figure 1) being the most competitive. In addition, antagonistic potential

***Fusarium* species Antagonistic treatment**		***Fusarium sambucinum***	***Fusarium solani***	**Average per antagonistic treatment****
Control		4.4 (0)*	3.80 (0)	4.09 a (0)
***Aspergillus* spp.**				
E.42.11	*A. terreus*	2.9 (34.0)	2.69 (29.2)	2.81 b (31.6)
E.5.11	*A. flavus*	2.36 (46.3)	2.33 (38.6)	2.34 bc (43.0)
E.41.11	*A. terreus*	2.81 (36.1)	2.00 (47.3)	2.40 bc (41.6)
E.25.11	*A. flavus*	2.62 (40.4)	1.75 (53.9)	2.18 bcd (46.9)
E.37.11	*A. flavus*	3.18 (27.7)	2.56 (32.6)	2.87 b (30.1)
E.60.11	*A. nidulans*	3.12 (39.0)	2.38 (37.3)	2.75 b (33.0)
E.61.11	*A. flavus*	2.75 (37.5)	1.75 (53.9)	2.25 bcd (45.2)
E.13.11	*A. niger*	2.31 (47.5)	2.63 (30.7)	2.47 bc (39.9)
E.33.11	*A. niger*	3.06 (30.4)	2.63 (30.7)	2.85 b (30.6)
E.2.11	*A. nidulans*	2.4 (45.4)	1.94 (48.9)	2.17 bcd (47.2)
***Penicillium* spp.**				
E.47.11	*Penicillium* sp.	3.37 (23.4)	2.38 (37.3)	2.87 b (30.1)
E.39.11	*Penicillium* sp.	1.93 (56.1)	1.63 (57.1)	1.78 cd (56.6)
E.29.11	*P. polonicum*	2.56 (41.8)	2.13 (43.9)	2.34 bc (43.0)
E.44.11	*Penicillium* sp.	2.27 (48.4)	2.06 (45.7)	2.16 bcd (47.4)
E.36.11	*P. chrysogenum*	1.25 (71.5)	1.44 (62.1)	1.34 d (67.3)
E.40.11	*Penicillium* sp.	1.95 (55.6)	2.31 (39.2)	2.13 bcd (48.1)
E.68.11	*Penicillium* sp.	2.25 (48.8)	1.94 (48.9)	2.09 bcd (49.1)
***Colletotrichum* spp.**				
E.16.11	*Colletotrichum* sp.	2.12 (51.8)	1.94 (48.9)	2.03 bcd (50.6)
E.8.11	*Colletotrichum* sp.	2.93 (33.4)	2.00 (47.3)	2.46 bc (40.1)
***Trichoderma* sp.**				
E.45.11	*Trichoderma* sp.	2.87 (34.7)	2.00 (47.3)	2.43 bc (40.8)
Average per *Fusarium* species**		2.5 a	2.1 b	

*Percent growth reduction (in %) as compared to the control was calculated after 7 days of incubation using Whipps' (1987) formula.
**Means followed by the same letter are not significantly different according the Duncan's Multiple range test at $P < 0.05$.

Table 2: Antifungal potential of the potato-associated fungi isolated from healthy stems, roots and tubers toward *Fusarium* species noted after 7 days of incubation at 25°C.

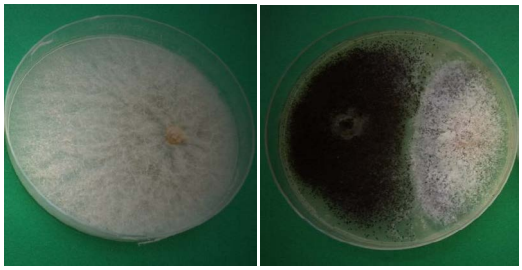

Figure 1: Competitive potential of *Aspergillus niger* over *Fusarium sambucinum* noted after 7 days of incubation on PDA as compared to the control.

exhibited by the potato-associated fungi toward *Fusarium* spp. was also expressed by various hyphal morphological alterations such as coiling around pathogen mycelium, strong lysis, early formation of resting structures, and induction of mycelial cords in response to the exerted biotic stress.

Effects of the potato-associated fungi on Fusarium dry rot severity

The potato-associated fungi tested as tuber treatment, 24 h prior inoculation with conidial suspensions of *Fusarium* spp. (*F. sambucinum* and *F. solani*), were assessed for their ability to suppress dry rot development and severity using two indicator parameters.

Dry rot lesion diameter

The mean diameter of dry rot lesion, noted after 21 days of incubation at 25°C, depended significantly (at $P < 0.05$) upon antagonistic treatments tested. Figure 2 showed that 13 tuber treatments based on E.29.11, E.44.11, E.60.11, E.61.11, E.25.11, E.33.11, E.13.11, E.47.11, E.37.11, E.8.11, E.39.11, E.41.11, and E.36.11 isolates led to a significant decrease by 26.9 to 54.8% in the mean lesion diameter, as compared to the inoculated and untreated control. Furthermore, using the potato-associated isolates E.13.11 (*A. niger*), E.61.11 (*A. flavus*), E.60.11 (*A. nidulans*), and E.41.11 (*A. terreus*), the mean lesion diameter noted on the *Fusarium*-inoculated and treated tubers was significantly similar to that recorded on the untreated and uninoculated control ones (NIC). In fact, E.61.11- and E.13.11-based treatments had reduced this parameter by 54.8%, followed by E.60.11 (51.9%) and E.41.11 (51.5%). However, tubers treated with E.68.11 (*A. nidulans*), E.2.11, E40.11 (*Penicillium* sp.), E.5.11 (*A. flavus*), E16.11 (*Colletotrichum* sp.), E.42.11 (*A. terreus*), and E.45.11 (*Trichoderma* sp.) showed a significantly similar (at $P < 0.05$) mean lesion diameter as the untreated and inoculated control (IC). Thus, and based on this disease scoring parameter, these isolates were found to be ineffective in suppressing dry rot severity.

Mean rot penetration

The mean rot penetration into potato tubers, noted after 21 days of incubation at 25°C, varied significantly (at $P < 0.05$) depending on antagonistic treatments tested. In fact, as shown in Figure 3, all tuber treatments performed using the potato-associated fungi tested led to a significant (at $P < 0.05$) decrease in this parameter in comparison to *Fusarium* spp.-inoculated and untreated control. The mean penetration was lowered by more than 50% using 14 out of the 20 potato-associated isolates tested. The most effective ones in suppressing Fusarium dry rot severity were E.36.11 (*P. chrysogenum*), E.29.11 (*P. polonicum*), E.39.11 and E.44.11 (*Penicillium* sp.), E.41.11 and E.42.11 (*A. terreus*), E.8.11 and E.16.11 (*Colletotrichum* sp.), E.37.11, E.25.11, and E.61.11 (*A. flavus*), E13.11 and E.33.11 (*A. niger*), and E.60.11 (*A. nidulans*) where the mean penetration decrease varied from 54 to 67% and was significantly comparable to that noted on the uninoculated and untreated control tubers. Figure 4 illustrated the variable decrease in dry rot severity depending on potato-associated isolates used as compared to *Fusarium*-inoculated and disease free untreated controls. In fact, tuber treatments based on E.25.11 (*A. flavus),* E.39.11 (*Penicillium* sp.), E.13.11 (*A. niger),* and E.61.11 *(A. nidulans)* exhibited interesting suppressive effects against Fusarium dry rot leading to a 65.3, 65.7, 65.3, and 64.5% lowered disease severity, respectively.

Discussion

The main objective of the present investigation was to select active potato-associated fungi for their eventual use as biocontrol agents for the control of the most predominant *Fusarium* species responsible for Fusarium dry rot disease in Tunisia. In fact, this group of plant-

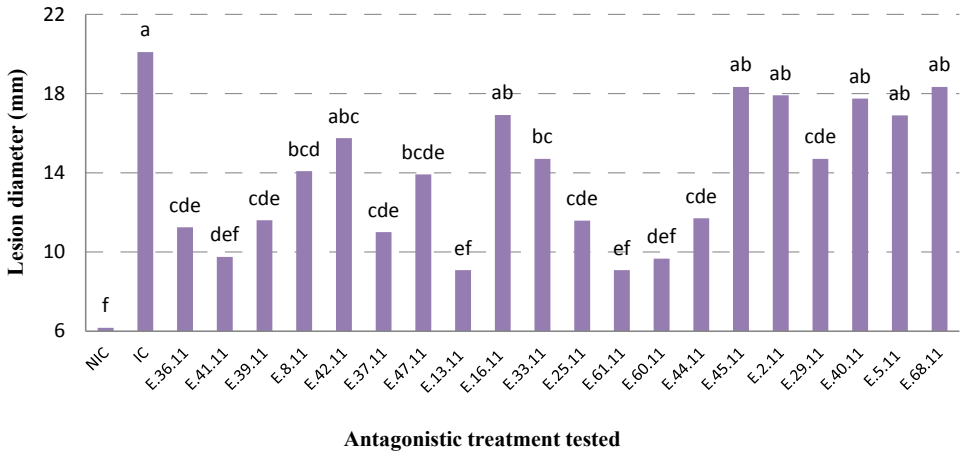

Figure 2: Effect of tuber treatment, 24 h prior inoculation with *Fusarium* spp., with 20 isolates of potato-associated fungi on Fusarium dry rot lesion diameter noted after 21 days of incubation at 25°C as compared to the controls. Bars affected by the same letter are not significantly different according to Duncan's Multiple Range test at $P < 0.05$. IC: Untreated and inoculated control; NIC: Untreated and uninoculated control. Inoculation was performed using mixed inoculum composed of *F. sambucinum* and *F. solani*. *Penicillium* spp: E.36.11, E.29.11, E.37.11, E.16.11, and E.44.11 were isolated from tubers; E.39.11 was isolated from roots and E.45.11, E.40.11 were isolated from stems. *Aspergillus* spp. : E.41.11, E.42.11, E.47.11, E.13.11, E.33.11, E.25.11, E.61.11, E.60.11, E.2.11, E.5.11, E.68.11 were isolated from tubers and E.8.11 was isolated from stems.

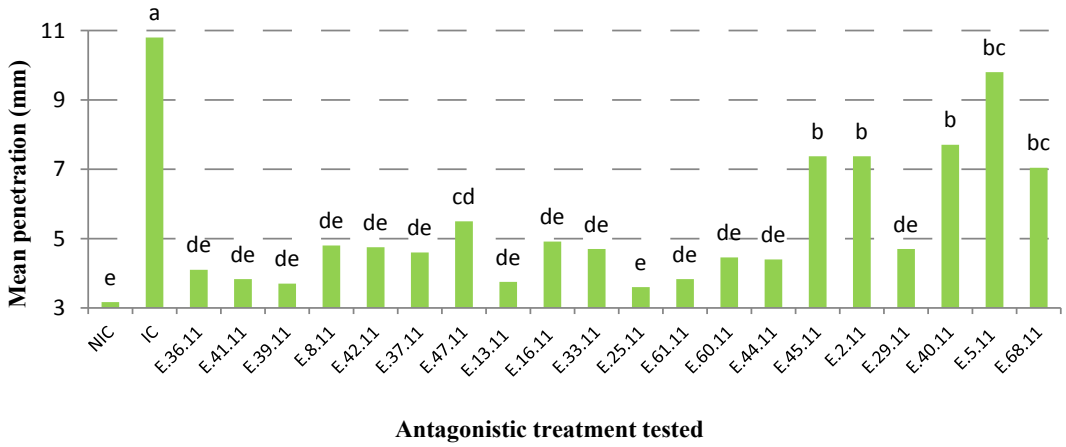

Figure 3: Effect of tuber treatment, 24 h prior inoculation with *Fusarium* spp., with 20 isolates of potato-associated fungi on Fusarium dry rot penetration noted after 21 days of incubation at 25°C as compared to the controls. Bars affected by the same letter are not significantly different according to Duncan's multiple range test at *P* = 0.05. IC: Untreated and inoculated control; NIC: Untreated and uninoculated control. Inoculation was performed using mixed inoculum composed of *F. sambucinum* and *F. solani*. *Penicillium* spp: E.36.11, E.29.11, E.37.11, E.16.11, and E.44.11 were isolated from tubers; E.39.11 was isolated from roots and E.45.11, E.40.11 were isolated from stems. *Aspergillus* spp.: E.41.11, E.42.11, E.47.11, E.13.11, E.33.11, E.25.11, E.61.11, E.60.11, E.2.11, E.5.11, E.68.11 were isolated from tubers and E.8.11 was isolated from stems.

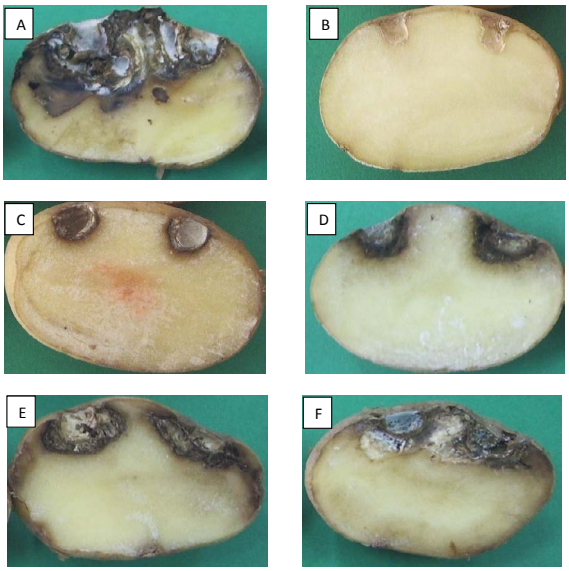

Figure 4: Effect of tuber treatment using some isolates of potato-associated fungi on dry rot severity noted on potato tubers after 21 days of incubation at 25°C as compared to the untreated controls. A: Inoculated with mixed inoculum composed of *Fusarium solani* and *F. sambucinum* and untreated; B: Uninoculated with both pathogens and untreated; C: Inoculated with *Fusarium* spp. and treated with E.25.11 (*Aspergillus flavus*); D: Inoculated with both pathogens and treated with E.13.11 (*A. niger*); E: Inoculated with *Fusarium* spp. and treated with E.61.11 (*A. nidulans*); F: Inoculated with *Fusarium* spp. and treated with E.39.11 (*Penicillium* sp.).

associated fungi has been reported to be an interesting source for secondary antifungal metabolites [25,27,33-35]. Furthermore, the antagonistic potential of the potato-associated bacteria toward pathogenic soilborne fungi was also demonstrated by several authors [21,36,37].

The associated fungi belong to very diverse polyphyletic group of microorganisms; they can thrive asymptomatically within plant tissues, aboveground as well as belowground, including those of stems, leaves and/or roots [26,38]. Their biological diversity coupled with their capability to biosynthesize bioactive secondary metabolites has provided the impetus for a number of investigations on endophytic fungi. This leads to their direct or indirect use as biocontrol agents against numerous diseases [39]. The originality of the present study resides in the indigenous nature of the bioagents tested and in the multiple criteria used for elucidation of their suppressive effects against Fusarium dry rot.

In this study, various potato-associated fungi were tested *in vitro* and *in vivo* for their antagonistic potential toward dry rot agents. An assessment scheme was developed to evaluate their effectiveness by using the dual culture technique and tuber bioassay. All the potato-associated isolates tested had inhibited the mycelial growth of *Fusarium* spp. *in vitro*. Their antagonistic activity was verified not only by their competitive potential on culture medium but also the various damages caused on pathogen mycelium. In fact, previous studies

have shown the involvement of a diversity of mechanisms of action during antagonism including competition, antibiosis, and production of extracellular lytic enzymes such as chitinases and β-1,3-glucanases [40]. In the present study, the majority of potato-associated fungi tested exhibited antifungal potential toward *Fusarium* spp. This finding is in accordance with previous studies reporting on suppression of pathogenic fungi using fungal isolates recovered from the same host as is the case of *Trichoderma koningii*, *Alternaria alternata*, *Phoma* sp., and *Acremonium strictum* were isolated from maize roots which are shown able to parasitize *F. oxysporum, F. pallidoroseum*, *F. verticillioides*, and *Cladosporium herbarum* [41]. Moreover, Jabnoun-Khiareddine et al. [42] signaled the ecological interest of the endogenous *Trichoderma* spp., isolated from several apparently healthy *Solanaceous* plants such as tomato, eggplant and notably potato in the control of tomato Verticillium wilt caused by *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus*.

Colletotrichum, *Alternaria*, *Trichoderma*, *Aspergillus* and *Penicillium*, tested for their antagonistic potential toward *Fusarium* spp., showed several mechanisms of action during antagonism. In fact, they lowered pathogen radial growth and caused strong alterations in *Fusarium* spp. mycelium. Hyphal damage, noted via light microscopic studies, was expressed by a strong lysis and a premature formation of the resting structures (i.e. chlamydospores). In addition and in response to the biotic stress exerted notably by *Aspergillus* and *Penicillium* species, pathogen formed mycelial cords through anastomosis mechanism. Similar effect was also reported for the same pathosystem in the case of Fusarium dry rot [18] and Fusarium wilt diseases [43]. Previous investigations have also explored the possibility of using associated fungi like *Penicillium* and *Aspergillus* spp. to control the take-all disease of wheat [44-45]. In the same way, Wakelin et al. [34] also demonstrated the potential of *P. radicum* to inhibit the growth of *G. graminis* var. *tritici*, *R. solani*, *Pythium irregular* and *Phytophthora cinnamomi in vitro*.

The *in vivo* evaluation of the selected potato-associated fungi tested against a mixed inoculum composed of *F. solani* and *F. sambucinum* revealed that some isolates screened have shown relative consistency both *in vitro* and *in vivo* in reducing dry rot severity. In this study, the potato-associated isolates tested were applied as tuber treatment prior to pathogen challenge and they have probably colonized successfully the site of inoculation leading to the recorded inhibition of dry rot development. Disease-suppressive effects of endophytes towards fungal pathogens have been extensively demonstrated in other pathosystems [41,46]. The current results revealed the suppressive effect of potato-associated against Fusarium dry rot. In accordance, previous Tunisian studies have shown the effectiveness of *Aspergillus* and *Penicillium* genera, originally recovered from compost extracts and solarized soils [18,47], in controlling *Pythium* spp. [48], *Fusarium* spp., and *Phytophthora erythroseptica* the causal agents of watery rot, dry rot and pink rot, respectively [20]. Compost-associated *Penicillium* spp., *Aspergillus* spp., and *Talaromyces assiutensis* were also shown able to suppress black scurf and stem canker potato disease caused by *R. solani* [49]. However, to our knowledge, this is the first report on the use of non pathogenic potato-associated fungi for the biocontrol of pathogenic *Fusarium* species. Thus, these isolates, occurring ubiquitously within potato plants could be used as promising candidates for Fusarium dry rot biocontrol and may be other potato diseases such as Fusarium wilt and tuber blemishing diseases.

Conclusion

To conclude, this is the first report focused on the assessment of the antifungal potential of the potato-associated fungi isolated from healthy potato organs (tubers, roots and aerial parts). These results provide a basis for new and innovative concepts in the biological control of Fusarium dry rot disease. Based on the above presented preliminary results, it can be concluded that naturally occurring potato-associated fungi can suppress *in vitro* and *in vivo Fusarium* species infecting their host plant (potato) as clearly demonstrated above under artificial conditions. Thereafter, and based on their suppressive effects against Fusarium dry rot severity, the first 10 most effective potato-associated fungi will be further screened of their suppressive effects against Fusarium wilt disease on potato plants. Also, additional testing is still needed to confirm their efficacy under various field conditions and to identify the chemical composition of their bioactive compounds.

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