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Bio-suppression of Fusarium Wilt Disease in Potato Using Nonpathogenic Potato-associated Fungi

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Abstract

Ten nonpathogenic *Aspergillus* spp. and *Penicillium* spp. isolates, naturally occurring within healthy potato plants and previously selected based on their ability to suppress Fusarium dry rot disease, were evaluated for their *in vitro* antifungal potential against *Fusarium sambucinum*, *F. oxysporum* and *F. graminearum* and their effects against Fusarium wilt severity and on plant growth and production. Tested through the dual culture technique on PDA medium, all isolates tested had significantly decreased *Fusarium* spp. growth relative to the untreated control. Growth inhibition, achieved after 7 days of incubation at 25°C, varied from 32.3 to 42.9% using *Aspergillus* spp. and from 44.1 to 59.6% with *Penicillium* spp. The highest inhibition, by about 55-59%, was noted using isolates E.36.11 (*P. chrysogenum*) and E.39.11 (*Penicillium* sp.). Competition, mycoparasitism, hyphal lysis, early formation of resting structures and mycelial cords, and decreased sporulating ability are the main effects recorded during antagonism exerted toward targeted *Fusarium* species. Fusarium wilt severity, noted 75 days posy-planting, was significantly lowered by 29 to 47% on potato plants treated using 7 out the 10 isolates tested. The highest wilt severity decrease, by 41-47% over the inoculated and untreated control, was achieved using E.13.11 (*A. niger*), E.25.11 (*A. flavus*), E.36.11 (*P. chrysogenum*), and E.29.11 (*P. polonicum*) based treatments. Plant inoculated with *Fusarium* spp. and treated with E.29.11 (*P. polonicum*), E.13.11 (*A. niger*), E.41.11 (*A. terreus*), E.60.11 (*A. flavus*), and E.25.11 (*A. flavus*) showed 36-46% higher aerial part growth. The most interesting improvements of root and tuber fresh weights, achieved using the majority of isolates tested, ranged between 22-40% and 15-21%, respectively. Further investigations are needed to more elucidate the antifungal activity of the extracellular metabolites of the most effective isolates toward *Fusarium* species infecting potato.

Keywords: Associated-fungi; Antifungal potential; Dual culture; *Fusarium* spp.; Plant growth; Wilt severity

Introduction

Potato (*Solanum tuberosum* L.) is an economically important vegetable crop worldwide [1-3]. In Tunisia, potato is threatened by various fungal diseases including vascular wilts. The most common wilt pathogens are *Verticillium dahliae* and to a lesser extent *V. alboatrum* and *V. tricorpus* [4,5]. However, in the last few years, Fusarium wilt of potato has become increasingly widespread in many potato-growing regions and was frequently associated to early dying symptoms leading to 30-50% yield losses and decreased tuber quality [5-8]. *Fusarium* wilt is one of the most important yield limiting diseases in potato production worldwide [9]. In the world, about 15 to 70% of potato fields were reported to be infected with Fusarium wilt causal agents and mainly *F. oxysporum* [10-16].

Wilt pathogens infect their host plants through young roots and then they grow into and up the water-conducting vessels of roots and stems. Infected plants exhibit unilateral leaf yellowing and necrosis at lower leaves, stunting, chlorosis, vascular discoloration, wilt, and eventual death [17,18]. The disease is caused by a complex of *Fusarium* species including *F. eumartii*, *F. avenaceum*, *F. solani*, *F. graminearum*, *F. sambucinum* and mainly *F. oxysporum* [6,7,19-21]. In addition, these wilt agents can interact synergistically with other soilborne wilt pathogens and phytoparasitic nematodes leading to more increased wilt severity and incidence [22]. Moreover, Fusarium wilt is considered as a serious limiting factor to local seed production programs due to the internal tuber infection within vascular tissues without exhibiting apparent external symptoms.

Therefore, disease control is so difficult due to the long lasting in soil of its resting structures, the absence of resistant cultivars and to the limited range of effective fungicides [9,23]. Several other control methods have been also used to suppress potato soilborne diseases including Fusarium wilts such as biocontrol using *Trichoderma* spp. [9,24], soil solarization [19], and green manure based-amendments using folder radish [25]. Recently, an interesting alternative to soilborne disease control that has been developed and gained particular interest is the exploration of plant-associated microorganisms (fungi or bacteria) as biocontrol agents. In fact, these native agents, naturally occurring within plant tissues were reported to play a significant role in their bioprotection against various bioagressors including soilborne fungi. Thus, many research studies have been focused on isolation of plant-associated microorganisms and their release into soil for the improvement of plant health and growth [26-28]. Previous studies have shown that associated fungi may be useful as potential sources of biocontrol agents and bioactive compounds. In fact, their biodiversity together with their capacity to produce bioactive secondary metabolites

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have attracted more attention to study their role in biocontrol of plant bioagressors [29]. Indeed, many endophytic and/or associated microorganisms can synthesize bioactive compounds involved in plant defense against plant pathogens [30-32]. Their antagonistic potential against some soilborne fungi such as *V. dahliae* and *Rhizoctonia solani* has been previously showed [28,33].

In Tunisia, previous Fusarium wilt biocontrol attempts were more focused on fungal agents isolated from soil or compost where biocontrol agents belonging to *Trichoderma*, *Penicillium* and *Aspergillus* genera were used [24]. In a previous study [34], we have demonstrated the ability of 20 nonpathogenic isolates of potato-associated fungi belonging to *Aspergillus, Penicillium, Colletotrichum*, and *Trichoderma*, to suppress Fusarium dry rot disease severity in potato incited by *F. sambucinum* and *F. solani*.

In the present investigation, 10 isolates of potato-associated fungi, previously selected based on their ability to suppress Fusarium dry rot disease by more than 50%, will be evaluated for their antifungal potential against three *Fusarium* species involved in serious plant wilting, their suppressive effect against Fusarium wilt and their impacts on plant growth and production.

Materials and Methods

Potato cultivar

Potato (*Solanum tuberosum* L.) cv. Spunta tubers, the most grown in Tunisia and known to be highly susceptible to vascular wilts were used in all pot experiments. They were previously stored at 6°C for two months and just before being used for the bioassays, they were superficially disinfected using a sodium hypochlorite solution diluted at 10% during 5 min, rinsed with tap water and air dried. They were maintained two weeks at 15-20°C, under 60-80% relative humidity, and natural room light for pre-germination.

Pathogen inoculum

Three *Fusarium* species namely *F. oxysporum, F. graminearum* and *F. sambunicum* were used in the current study. They were originally recovered from potato tubers exhibiting typical symptoms of dry rot disease and/or plants exhibiting Fusarium wilt infection. Their identification and pathogenicity tests were previously demonstrated [35]. They were cultured on Potato Dextrose Agar (PDA) medium amended with 300 mg/L of streptomycin sulphate. Their virulence was maintained through inoculation of wounded and disease-free potato tubers and re-isolation on PDA from rotting tissues.

Plant infection was performed using a mixed inoculum, composed of *F. oxysporum*, *F. sambucinum* and *F. graminearum*, previously shown to be the most aggressive on potato plants (Mejdoub-Trabelsi, unpublished data) [36].

Pathogen inoculum was prepared by scraping off mycelium from 7-day-old cultures, which was suspended into sterile distilled water and shaken for 5 min using an electric blender. The obtained suspension was filtered and equal volumes of conidial suspensions from each *Fusarium* species were mixed and the final concentration of the combined inoculum was adjusted to 10^7 CFU/mL using a Malassez hemocytometer.

Potato-associated fungi used and their culture conditions

Ten isolates of potato-associated fungi belonging to *Aspergillus* and *Penicillium* genera were used in the present study. They were originally isolated from disease-free potato plants removed from several potato-growing fields in Tunisia (Table 1). They were isolated on PDA medium from surface-sterilized stems and tubers. All isolates used were previously subjected to pathogenicity tests on potato tubers and were found to be nonpathogenic. Their identification was based on their macro and micro morphological traits [37,38].

They were selected among 20 isolates, tested in a previous study [34], based on their capacity to suppress Fusarium dry rot disease. They were stored at -20°C in a 20% glycerol solution and were grown on PDA at 25°C for one week before being used in the bioassays.

For plant treatment, conidial suspensions used were prepared as follows. Liquid cultures of each isolate were prepared by transferring five plugs (6 mm in diameter), removed from 7-day-old cultures on PDA, to 150 ml of Potato Dextrose Broth (PDB) and incubated at 25°C for 10 days in a rotary shaker incubator at 120 rpm. The obtained suspension was filtered through double layered cheese cloth and the conidial concentration was adjusted to 10^7 CFU/mL before being used for plant challenge.

Effect of potato-associated fungi against *Fusarium* spp. radial growth

The 10 selected fungal isolates were evaluated for their capacity to inhibit the *in vitro* growth of *F. sambucinum*, *F. oxysporum* and *F. graminearum* using the dual culture technique on PDA medium. The target *Fusarium* species and the isolates tested were cultured in the same Petri plate containing PDA amended with streptomycin (300 mg/L). Agar plugs (6 mm in diameter), taken from 7-day-old cultures of the pathogen or the antagonist, were placed at 2 cm apart from the edge of the Petri plate and equidistant of 5 cm. Control plates were treated with pathogen plugs only. Four replicates were used per individual treatment and the whole experiment was repeated twice. Fungal cultures were maintained at 25°C and the mean diameter of *Fusarium* spp. colonies was noted after 7 days of incubation.

Pathogen growth inhibition (GI) was estimated using Whipps' [38] formula: Growth inhibition % = ((C1-C2) / C1) × 100 where, C1: Mean diameter of pathogen colony in control plates and C2: Mean diameter of pathogen colony in presence of antagonist.

Growth inhibition score (GIS), estimated based on above GI records, was attributed to each isolate tested using an arbitrary 0-4 scale where 0 = pathogen colony overgrowing antagonist, 1=GI comprised

Isolate	Species	Origin	Organ	Cultivar	RFD (%)*
		***Aspergillus* spp.**			
E.41.11	*A. terreus*	Sahline	Stem	Spunta	64.5
E.25.11	*A. flavus*	Chott-Mariem	Tuber	Magda	66.7
E.37.11	*A. flavus*	Sahline	Tuber	Safrane	57.4
E.61.11	*A. nidulans*	Chott-Mariem	Tuber	Spunta	58.7
E.60.11	*A. flavus*	Chott-Mariem	Tuber	Spunta	64.5
E.13.11	*A. niger*	Chott-Mariem	Tuber	Carrera	65.3
		***Penicillium* spp.**			
E.39.11	*Penicillium* sp.	Teboulba	Tuber	Bellini	65.7
E.29.11	*P. polonicum*	Chott-Mariem	Tuber	Evora	56.5
E.44.11	*Penicillium* sp.	Kairouan	Stem	Spunta	59.3
E.36.11	*P. chrysogenum*	Chott-Mariem	Tuber	Spunta	62

*RFD: Ability (in %) to reduce Fusarium dry rot severity based on mean rot penetration as compared to the untreated control noted on potato cv. Spunta tubers inoculated with a mixed inoculum composed of *Fusarium sambucinum* and *F. solani* and treated with the different potato-associated isolates tested [34].

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

between 1 and 25%, 2=GI comprised between 26 and 50%, 3=GI comprised between 51 and 75%, and 4=GI comprised between 76 and 100%.

Hyphal interactions at the confrontation zone between the dual cultured fungi were observed under light microscope and all abnormal morphological alterations in pathogen mycelium, in comparison to the untreated control, were described.

Effects of potato-associated fungi on Fusarium wilt severity and plant growth and production

Seed tubers, showing optimal germination were planted in plastic pots containing a mixture of perlite and peat (1:3 v/v). Two weeks post-planting, pathogen inoculation was performed by watering each plant by 100 ml of a conidial suspension (10^7 CFU/mL) of pathogen inoculum composed of the three *Fusarium* species. Uninoculated control plants were watered using 100 ml of sterile distilled water. Ten days post-inoculation, potato plants were treated through culture substrate drench using 100 ml of the conidial suspensions of the selected associated fungi. Untreated (inoculated and uninoculated) control plants were treated similarly using 100 ml of sterile distilled water. Pots were placed under greenhouse conditions (18-25°C, 14 h light) for 60 days and watered regularly enough to avoid drought stress. Ten plants were used per individual treatment. The whole experiment was repeated twice.

Parameters noted

Fusarium wilt severity was assessed, 75 days post-planting, based on the intensity of foliar damage. Leaf damage index (LDI) was noted using the following arbitrary 0-4 scale where 0 = asymptomatic leaves, 1 = Wilted leaves, 2 = Leaves showing unilateral yellowing, 3 = Leaves showing unilateral necrosis, and 4 = Dead leaves. The effect of the selected fungi on potato plants was also evaluated based on growth and production parameters (aerial part, roots and tuber fresh weights).

Statistical analyses

Statistical analyses of the *in vitro* trial's data were carried out according to a completely randomized factorial design where the antagonistic treatments tested (potato-associated isolates and the untreated control) and the three *Fusarium* species were the two fixed factors. Four replicates were used per individual treatment. The effect of antagonistic treatments tested through the *in vivo* bioassay was analyzed according to a completely randomized design and each individual treatment was replicated ten 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 revealed that mean diameter of *Fusarium* spp. colonies, formed after 7 days of incubation at 25°C, depended significantly (at $P \leq 0.05$) upon *Fusarium* species and antagonistic treatments tested. No significant interaction was recorded between both factors. Indeed, as given in Figure 1, the 10 potato-associated isolates tested had significantly decreased *Fusarium* spp. growth over the untreated control but with a variable degree depending on associated isolates used. Combined data of three *Fusarium* species indicated that the percentage of growth inhibition, over the untreated control, ranged between 32.3 and 42.9% using *Aspergillus* spp. isolates and varied from 44.1 to 59.6% with *Penicillium* spp. The highest inhibition by about 55-59% was recorded using *Penicillium* spp. isolates E.36.11 (*P. chrysogenum*) and E.39.11 (*Penicillium* sp.).

It should be mentioned that *Fusarium* spp. growth was inhibited by more than 42% using 6 out of the 10 potato-associated isolates tested. Furthermore, ranked based on their growth inhibition score (GIS), the majority of *Aspergillus* spp. isolates (excepting E.60.11 when confronted to *F. oxysporum*) and two *Penicillium* spp. isolates (namely E.29.11 and E.44.11) showed similar scores when dual cultured with the three *Fusarium* species whereas E.36.11 and E.39.11 *Penicillium* isolates exhibited 3 as GIS value toward all targeted *Fusarium* species (Table 2). This indicates their highest antifungal potential compared to the other isolates tested and their competitive ability on PDA medium.

In addition, light microscopic studies of hyphal *in vitro* interactions performed at the confrontation zone of *Fusarium* spp. with the potato-associated fungi revealed varied antagonistic effects. Indeed, mycoparasitism, decreased sporulating ability, severe lysis, early formation of chlamydospores, and formation of mycelial cords through anastomosis mechanism were the main effects noted on the treated hyphae as compared to the untreated control ones.

Bio-suppression of Fusarium wilt severity using potato-associated fungi

The efficiency of the potato-associated isolates tested against

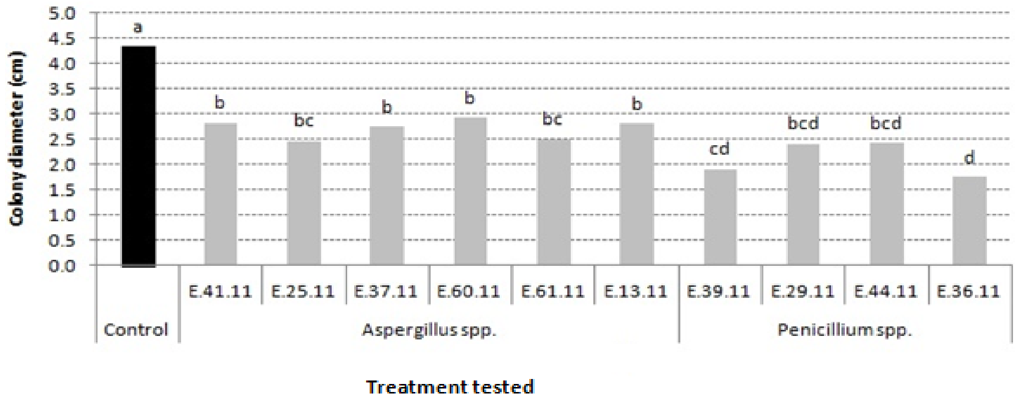

Figure 1: Antifungal potential of 10 isolates of potato-associated fungi recovered from healthy stems and tubers toward *Fusarium* species noted after 7 days of incubation at 25°C as compared to the untreated control (combined data of three *Fusarium* species).

Isolates	*F. sambucinum*	*F. oxysporum*	*F. graminearum*	Average GIS per Isolate tested
***Aspergillus* spp.**				
E.41.11	2	2	2	2
E.25.11	2	2	2	2
E.37.11	2	2	2	2
E.60.11	2	1	2	1.66
E.61.11	2	2	2	2
E.13.11	2	2	2	2
***Penicillium* spp.**				
E.39.11	3	3	3	3
E.29.11	2	2	2	2
E.44.11	2	2	2	2
E.36.11	3	3	3	3

Growth inhibition score (GIS) estimated using an arbitrary 0-4 scale where 0 = pathogen colony overgrowing antagonist, 1 = GI comprised between 1 and 25% and 4 = GI comprised between 76 and 100%.
GI: Growth inhibition of pathogen growth as compared to the untreated control calculated using Whipps' [38] formula.

Table 2: Variation in growth inhibition score (GIS) of the potato-associated fungi tested depending on targeted *Fusarium* species noted after 7 days of incubation at 25°C.

Fusarium wilt incited by *F. sambucinum*, *F. oxysporum* and *F. graminearum* was evaluated based on leaf damage intensity compared to the inoculated and untreated control (Figure 2).

ANOVA analysis revealed that Fusarium wilt severity, noted 75 days post-planting, varied significantly (at $P<0.05$) depending on the antagonistic treatments tested. In fact, plants treated using seven out the ten isolates tested showed significantly lower wilt severity relative to *Fusarium* spp.-inoculated and untreated control ones. Fusarium wilt severity decrease achieved using these seven isolates ranged between 29 and 47% compared to 11-23% recorded using the three remaining ones.

It should be highlighted that Fusarium wilt severity was lowered by more 41-47% using E.13.11 (*A. niger*), E.25.11 (*A. flavus*), E.36.11 (*P. chrysogenum*) and E.29.11 (*P. polonicum*) based treatments. Moreover, potato plants treated with these four isolates exhibited significantly similar disease severity as the uninoculated and untreated control (NIC). The effect of E.36.11 (*P. chrysogenum*) and E.29.11 (*P. polonicum*) on Fusarium wilt severity is illustrated in Figure 3.

Effects of the potato-associated fungi on plant growth and production

The aerial parts fresh weight, noted 75 days post-planting, depended significantly (at $P<0.05$) upon antagonistic treatments tested. This parameter recorded on potato plants treated with 8 out the 10 associated isolates tested (namely E.29.11, E.13.11, E.41.11, E.60.11, E.25.11, E.36.11, E.44.11, and E.39.11) was significantly similar to that noted on the uninoculated and untreated control (NIC) plants. E.29.11 (*P. polonicum*), E.13.11 (*A. niger*), E.41.11 (*A. terreus*), E.60.11 (*A. flavus*), and E.25.11 (*A. flavus*) based treatments led to 36-46% significantly higher aerial parts fresh weight than *Fusarium*-inoculated and untreated control (Figure 4). This parameter was increased by 11-35% using the remaining isolates.

Root fresh weight also varied significantly (at $P<0.05$) depending on antagonistic treatments tested. In fact, plant treatment using 9 out of the 10 potato-associated isolates selected led to 22-40% increase in this parameter relative to the inoculated and untreated control (IC). It should be also indicated that all the potato-associated isolates tested behaved as both controls based on this parameter (Figure 5).

Tuber fresh weight, noted 75 days post-planting, depended significantly (at $P<0.05$) upon antagonistic treatments tested. Tuber yield obtained using 8 out the 10 associated isolates tested was 15-21% higher, even if significantly insignifiant, than that recorded on *Fusarium* spp.-inoculated and untreated control plants (Figure 6).

Discussion

Ten nonpathogenic isolates, previously selected based on their capacity to lower Fusarium dry rot disease incited by *F. sambucinum* and *F. solani* [34], were assessed for their *in vitro* antifungal potential toward *F. sambucinum*, *F. oxysporum* and *F. graminearum* and their suppressive effects against Fusarium wilt severity caused by these species. These fungi, naturally occurring within healthy plants were reported to be more adapted to the ecological niche harboring targeted pathogens and exhibiting, thus, interesting activities in bioprotection of their hosts [39]. They can colonize plant tissues including those of

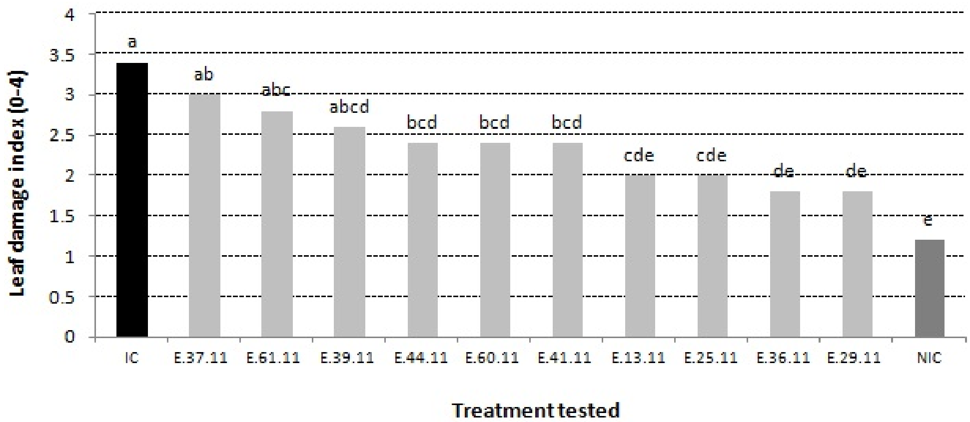

Figure 2: Effect of ten isolates of potato-associated fungi on Fusarium wilt severity noted on potato plants cv. Spunta 75 days post-planting as compared to the controls. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test at $P<0.05$. NIC: Untreated and uninoculated control; IC: Untreated and inoculated control. Inoculation was performed using a mixed inoculum composed of *Fusarium sambucinum*, *F. oxysporum* and *F. graminearum*. E.25.11, E.61.11, E.13.11, and E.60.11: *Aspergillus* spp. isolates recovered from healthy tubers. E.41.11: *A. terreus* recovered from healthy stems. E.36.11, E.37.11, E.29.11, E.39.11: *Penicillium* spp. isolates recovered from healthy tubers; E.44.11: *Penicillium* sp. isolated from healthy stems.

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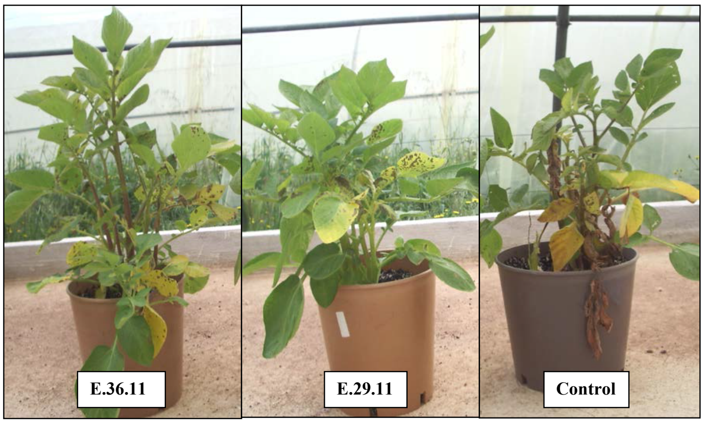

Figure 3: Fusarium wilt severity noted 75 days post-planting on potato plants inoculated with a mixture of *Fusarium oxysporum, F. graminearum* and *F. sambucinum* and treated with some potato-associated isolates as compared to the inoculated and untreated control. E.29.11: *Penicillium polonicum* ; E.36.11: *P. chrysogenum.*

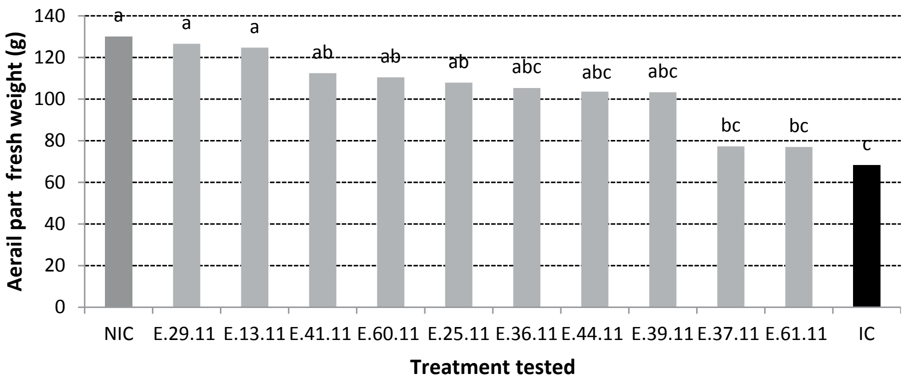

Figure 4: Effect of ten isolates of potato-associated fungi on the aerial part fresh weight noted on potato plants cv. Spunta 75 days post-planting as compared to the controls. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test at *P*<0.05. NIC: Untreated and uninoculated control; IC: Untreated and inoculated control. Inoculation was performed using a mixed inoculum composed of *Fusarium sambucinum*, *F. oxysporum* and *F. graminearum*. E.25.11, E.61.11, E.13.11, and E.60.11: *Aspergillus* spp. isolates recovered from healthy tubers. E.41.11: *A. terreus* recovered from healthy stems. E.36.11, E.37.11, E.29.11, E.39.11: *Penicillium* spp. isolates recovered from healthy tubers; E.44.11: *Penicillium* sp. isolated from healthy stems.

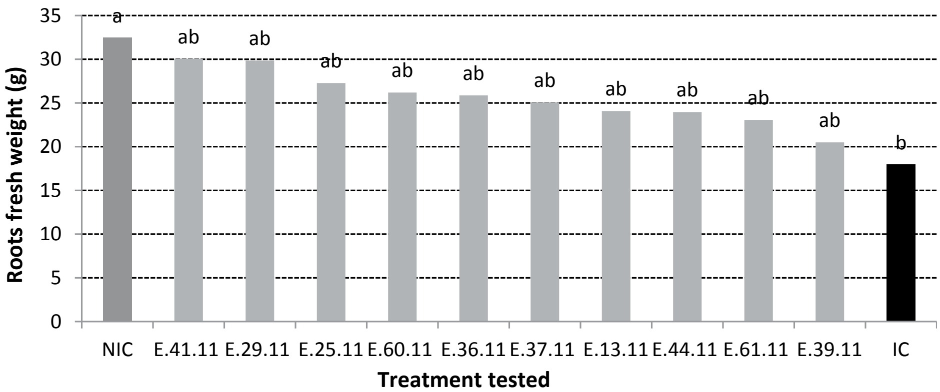

Figure 5: Effect of ten isolates of potato-associated fungi on roots fresh weight noted on potato plants cv. Spunta 75 days post-planting as compared to the controls. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test at *P*<0.05. NIC: Untreated and uninoculated control; IC: Untreated and inoculated control. Inoculation was performed using a mixed inoculum composed of *Fusarium sambucinum*, *F. oxysporum* and *F. graminearum*. E.25.11, E.61.11, E.13.11, and E.60.11: *Aspergillus* spp. isolates recovered from healthy tubers. E.41.11: *A. terreus* recovered from healthy stems. E.36.11, E.37.11, E.29.11, E.39.11: *Penicillium* spp. isolates recovered from healthy tubers; E.44.11: *Penicillium* sp. isolated from healthy stems.

stems, leaves and/or roots without inducing harmful effects and, thus, they are able to protect them from eventual infections [31,40].

Data from the *in vitro* trial showed that the 10 potato-associated isolates tested exhibited antifungal potential toward the three *Fusarium* species tested but with a variable degree depending on antagonists used. In fact, overall inhibition ranged between 32.3 and 42.9% using

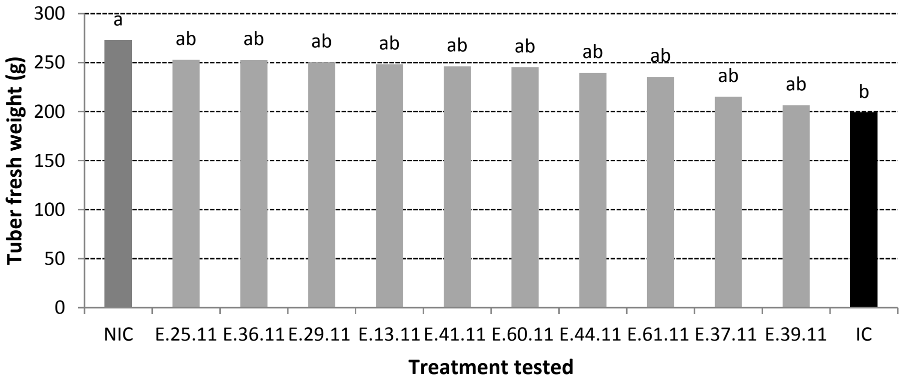

Figure 6: Effect of ten isolates of potato-associated fungi on tuber fresh weight noted on potato plants cv. Spunta 75 days post-planting as compared to the controls. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test at $P<0.05$. NIC: Untreated and uninoculated control; IC: Untreated and inoculated control. Inoculation was performed using a mixed inoculum composed of *Fusarium sambucinum*, *F. oxysporum* and *F. graminearum*. E.25.11, E.61.11, E.13.11, and E.60.11: *Aspergillus* spp. isolates recovered from healthy tubers. E.41.11: *A. terreus* recovered from healthy stems. E.36.11, E.37.11, E.29.11, E.39.11: *Penicillium* spp. isolates recovered from healthy tubers; E.44.11: *Penicillium* sp. isolated from healthy stems.

Aspergillus spp. isolates and between 44.1 and 59.6% using *Penicillium* spp. Thus, this study clearly demonstrated the ability of this group of nonpathogenic fungi to suppress the mycelial growth of targeted *Fusarium* species. These results are in accordance with our previous findings where these isolates were selected among 20 tested for their interesting antifungal potential against *F. sambucinum* and *F. solani* [34]. Several previous studies have indicated that diverse groups of microorganisms naturally colonizing plants may act as biocontrol agents and may be explored as interesting sources for secondary antifungal compounds [32,41-43]. This study is among the few reports on the use of nonpathogenic potato-associated fungi for the biocontrol of pathogenic *Fusarium* species infecting the same host.

In the present study, potato-associated fungi tested had reduced pathogen population and consequently, radial growth through competition for culture medium and more interestingly by inducing various hyphal morphological alterations and disturbance in pathogen growth such as decrease in *Fusarium* spp. sporulating potential, early formation of chlamydospores and mycelial cords through anastomosis mechanism. Moreover, hyphae, non transformed into chlamydospores and/or hyphal cords, showed strong lysis and mycelium vacuolization indicating, thus, the important stress exerted by these bioagents toward targeted pathogens. Similar effects were reported using commonly known biocontrol agents such as *Trichoderma* spp. and/or *Aspergillus* spp., recovered from soil and composts, which were recently explored in the same pathosystem againt Fusarium dry rot pathogens [44,45] and the vascular Fusarium wilt agent i.e. *F. oxysporum* f. sp. *tuberosi* [24]. These effects may be presumably due to the diffusible and/or volatile metabolites released by those fungi during their antagonistic activity [46]. Some previous studies showed a diversity of mechanisms of action, displayed by endogenous agents during antagonism, such as competition, antibiosis, and the synthesis of a wide range of diffusible antifungal metabolites [47]. These results are in accordance with those of Jabnoun-Khiareddine et al. [48] reporting on the ability of endogenous *Penicillium* spp., isolated from healthy *Solanaceous* plants, to suppress *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* the causal agents of Verticillium wilt in Tunisia. In the same way, previous investigations have also explored the possible use of indigenous *Aspergillus* spp. and *Penicillium* spp. associated to date palm composts to control black scurf and stem canker caused by *R. solani* [49].

Data from the *in vivo* trial indicated that for potato plants treated using seven out the ten isolates tested, Fusarium wilt severity incited by a mixed inoculum composed of *F. sambucinum*, *F. oxysporum* and *F. graminearum* was significantly decreased by 29 to 47% over the inoculated and untreated control. Disease severity was lowered by 41-47% using E.13.11 (*A. niger*), E.25.11 (*A. flavus*), E.36.11 (*P. chrysogenum*), and E.29.11 (*P. polonicum*) based treatments. This indicates the interesting bioprotection potential exhibited by the selected agents even though they were applied once and post-pathogen challenge. Their efficacy in suppressing Fusarium wilt disease may be improved through the application of an other reminder treatment and/or their preventive application before pathogen inoculation. In fact, as indicated in previous studies, introduction of antagonists prior to planting into the culture substrate may probably improve their establishment around and within plant subterranean parts; thus, pathogen internal and external progress may be prevented and its subsequent spread decreased [50]. The ability of *Aspergillus* spp. and *Penicillium* spp. to reduce Fusarium wilt disease is in agreement with previous findings such as those of Sharma et al. [51] reporting on the antagonistic potential of *A. versicolor* displayed toward *F. oxysporum* f. sp. *cumini* the causal agent of cumin wilt where disease incidence was lowered by 45.4%. Also, compost-associated *Penicillium* spp. and *Aspergillus* spp. were shown able to suppress potato Fusarium wilt caused by *F. oxysporum* f. sp. *tuberosi* [34]. Similarly, Jabnoun-Khiareddine et al. [52] outlined the bioprotection of tomato against Verticillium wilt based on *in vivo* and *in situ* trials using endogenous *Penicillium* sp. associated to healthy *Solanaceae* plants. Also, Jabnoun-Khiareddine et al. [33] have demonstrated the potential of indigenous *Penicillium* sp. to totally suppress Verticillium wilt of potato when incorporated into the culture substrate 15 days before pathogen challenge. In the same way, Larena et al. [53] have successfully suppressed tomato vascular wilts caused by *V. dahliae* and *F. oxysporum* f. sp. *lycopersici* both under growth chamber and field conditions using *P. oxalicum* based treatments.

Penicillium spp. and *Aspergillus* spp., naturally associated to potato plants and used in the current study, exhibited variable effectiveness in biocontrolling Fusarium wilt and in enhancing growth and production parameters. In fact, results from the *in vivo* trial indicated that plant treatments using E.29.11 (*P. polonicum*), E.13.11 (*A. niger*), E.41.11 (*A. terreus*), E.60.11 (*A. flavus*), and E.25.11 (*A. flavus*) had improved the aerial part growth by 36-46% on *Fusarium* spp. inoculated and treated plants as compared to control. The most interesting improvements of root and tuber fresh weights, achieved using the majority of isolates tested, ranged between 22-40% and 15-21%, respectively. These plant growth promoting effects recorded on infected and biologically treated potato plants are in agreement with previous studies [33,52] recording significant increases in roots and stem fresh weights of tomato plants and in tuber fresh weight of potato achieved using *Penicillium* sp. isolates originally recovered from healthy solanaceous crops (tomato, potato and eggplant). These growth promoting effects displayed by these potato associated fungi may be attributed either to their direct inhibitory effects toward targeted pathogens which was expressed by the recorded decrease in Fusarium wilt severity or to their secondary metabolites probably involved in growth promotion or plant defense response. In this way, *P. oxalicum* was shown able to induce resistance in tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* [54]. Also, Qiu et al. [55] have isolated from *Ginkgo biloba* L. twigs *A. nidulans* and *A. oryzae* which were able to produce phenolic and flavonoid compounds. Production of biologically active secondary metabolites by endogenous microorganisms was also previously mentioned by Verma et al. [56] who have isolated from foliar tissues of a medicinal plant, *Stevia rebaudiana* Bertoni, *A. flavipes* exhibiting interesting suppressive effects against the soilborne fungus *Sclerotinia sclerotiorum*. Moreover, according to Schulz et al. [57], endogenous fungi are known to induce strong antagonistic responses in host plants and these may be sufficient to provide resistance to pathogens that otherwise can invade plants without initiating a strong defense response. This could lead to a cost-effective, environmentally friendly, sustainable, and reproducible yield enhancement of protected crops.

Conclusion

The screening of ten isolates of potato-associated fungi, originally recovered from apparently healthy potato stems and tubers, for their capacity to inhibit the mycelial growth of three *Fusarium* species, to lower Fusarium wilt and to enhance plant growth and production led to the selection of four promising biocontrol agents useful for Fusarium wilt control. Thus, the results from the current study revealed that healthy potato plants may be targeted and explored as potential source of biocontrol agents active against Fusarium wilt in addition to their Fusarium dry rot suppressive effects as demonstrated in our previous study. The most efficient isolates identified as *A. niger*, *A. flavus*, *P. chrysogenum*, and *P. polonicum* will be further studied to elucidate the antifungal activity of their extracellular metabolites against the four *Fusarium* species responsible for these both diseases based on *in vitro* and *in vivo* trials.

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References

1. Wang Q, Zhang E, Li F (2008) Runoff efficiency and the technique of micro-water harvesting with ridges and furrows, for potato production in semi-arid areas. Water Res Manag 22: 1431-1443.
2. Schieber A, Aranda Saldaña MD (2009) Potato peels: A source of nutritionally and pharmacologically interest compounds. Food 3: 23-29.
3. Visser RGF, Bachem CWB, Boer JM, Bryan GJ, Chakrabati SK, et al. (2009) sequencing the potato genome: outline and first results to come from the elucidation of the sequence of the world's third most important food crop. Am J Pot Res 86: 417-429.
4. Jabnoun-Khiareddine H, Daami-Remadi M, El-Mahjoub M (2005) Emergence in Tunisia of new pathotypes of *Verticillium tricorpus* able to attack tomato, aubergine and potato. EPPO Bull 35: 497-503.
5. Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, El-Mahjoub M (2011) Comparative aggressiveness on *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* on potato as measured by their effects on wilt severity, plant growth and subsequent yield loss. Func Plant Sci Biotech 5: 1-8.
6. Daami-Remadi M, El Mahjoub M (2004) Emergence en Tunisie de *Fusarium oxysporum* f. sp. tuberosi agent de flétrissure vasculaire des plants et de pourriture sèche des tubercules de pomme de terre. EPPO Bull 34: 407-411.
7. Ayed F, Daami-Remadi M, Jabnoun-Khiareddine H, El Mahjoub M (2006) Effect of potato cultivars on incidence of *Fusarium oxysporum* f. sp. *tuberosi* and its transmission on progeny tubers. J Agron 5: 400-430
8. Kerkeni A, Daami-Remadi, Khedher MB (2013) In vivo evaluation of compost extracts for the control of the potato Fusarium wilt caused by *Fusarium oxysporum* f. sp. *tuberosi*. Afr J Plant Sci Biotech 7: 36-41.
9. Ommati F, Zaker M, Mohammadi A (2013) Biological control of *Fusarium* wilt of potato (*Fusarium oxysporum* f. sp. *tuberosi*) by Trichoderma isolates under field condition and their effect on yield. J Crop Prot 2: 435-442.
10. Thanassoulopoulos CC, Kitsos GT (1985) Studies on *Fusarium* wilt of potatoes. 1. Plant wilt and tuber infection in naturally infected fields. Potato Res 28: 507-514.
11. Venter SL, Theron DJ, Steyn PJ, Ferreira DI, Eicker A (1992) Relationship between vegetative compatibility and pathogenicity of isolates of *Fusarium oxysporum* f. sp. *tuberosi* from potato. Phytopathology 82: 858-862.
12. Manici LM, Cerato C (1994) Pathogenicity of Fusarium oxysporum f. sp. tuberosi isolates from tubers and potato plants. Potato Res 37: 129-134.
13. Ommati F, Sharifi K (2008) Determination of species and dispersal of potato Fusarium wilt in Semnan province. Page 70. In: Proceedings of the 18th Iranian plant protection congress, 24-27 August 2008, Hamadan, Iran.
14. Saremi H, Amiri ME (2010) Exploration of potato cultivar resistant to the major fungal pathogen on potato wilting disease in Iran. J Food Agric Environ 8: 821-826.
15. Zaheer Z, Shafique S, Shafique S, Mehmoood T (2012) Evaluation of pathogenic potential and genetic characterization of *Fusarium solani*: A cause of *Fusarium* wilt in potato. Afr J Microbiol Res 6: 1762-1765.
16. Gachango E, Kirk W, Schafer R, Wharton P (2012) Evaluation and comparison of biocontrol and conventional fungicides for control of postharvest potato tuber diseases. Biol Control 63: 115-120.
17. Hwang SF, Evans IR (1985) Eumartii wilt of potato in Alberta. Can Plant Dis Surv 65: 57-59.
18. Kucharek T, Jones JP, Hopkins D, Strandberg J (2000) Some diseases of vegetable and agronomic crops caused by Fusarium in Florida. Circular-1025 of Florida Cooperative Extension Service, Institute of Food and Agriculture Science and University of Florida.
19. Triki MA, Priou S, El-Mahjoub M (2001) Effects of soil solarization on soil-borne populations of *Pythium aphanidermatum* and *Fusarium solani* and on the potato crop in Tunisia. Potato Res 44: 271-279.
20. Ayed F (2005) La flétrissure fusarienne de la pomme de terre: comportement variétal et approches de lute chimique et biologique. Mastère en Protection des Plantes et Environnement de l'Institut Supérieur Agronomique de Chott-Mariem, Tunisie pp: 85.
21. Ismail Y, McCormick S, Hijri M (2011) A fungal symbiont of plant-roots modulates mycotoxin gene expression in the pathogen Fusarium sambucinum. PLoS One 6: e17990.
22. Daami-Remadi M, Sayes S, Horrigue-Raouani N, Hlaoua-Ben Hassine W (2009) Effects of *Verticillium dahliae* Kleb., *Fusarium oxysporum* Schlecht. f. sp. *tuberosi* Snyder, Hansen and *Meloidogyne javanica* (Treub.) Chitwood inoculated individually or in combination on potato growth, wilt severity and nematode development. Afr J Microbiol Res 3: 595-604.
23. Ayed F, Daami-Remadi M, Jabnoun-Khiareddine, Hibar K, El Mahjoub M (2006) Evaluation of fungicides for control of *Fusarium* wilt of potato. Plant Pathol J 5: 239-243.
24. Ayed F, Daami-Remadi M, Jabnoun-Khiareddine, El Mahjoub M (2006) Potato vascular Fusarium wilt in Tunisia: Incidence and biocontrol by Trichoderma spp. Plant Pathol J 5: 92-98.
25. Jabnoun-Khiareddine H, Abdallah RAB, Ayed F, Gueddes-Chahed M, Hajlaoui A, et al. (2016) Effect of fodder radish (*Raphanus sativus L.*) green manure on potato wilt, growth and yield parameters. Adv Crop Sci Tech 4: 211.

26. Petrini O, Sieber TN, Toti L, Viret O (1992) Ecology, metabolite production, and substrate utilization in endophytic fungi. Nat Toxins 1: 185-196.

27. Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: endophytism defined. In: Bacon CW, White JF, 2009 editors. Microbial endophytes. New York: Marcel Dekker.

28. Berg G, Zachow C, Lottmann J, Götz M, Costa R, et al. (2005) Impact of plant species and site on rhizosphere-associated fungi antagonistic to Verticillium dahliae kleb. Appl Environ Microbiol 71: 4203-4213.

29. Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19: 792-798.

30. Azevedo JL, Maccheroni JW, Pereira JO, Araújo WL (2000) Endophytic microorganisms: areview on insect control and recent advances on tropical plants. Electr J Biotechnol 3: 40-65.

31. Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol Rev 21: 51-66.

32. Arnold AE, Maynard Z, Gilbert GS (2001) Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol Res 105: 1502-1507.

33. Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, El Mahjoub (2010) Evaluation of several indigenous microorganisms and some bio-fungicides for biocontrol of potato Verticillium wilt. Pest Technol 4: 35-44.

34. Mejdoub-Trabelsi B, Abdallah RAB, Kthiri Z, Hamada W, Daami-Remadi M (2016) Assessment of the antifungal activity of nonpathogenic potato-associated fungi toward *Fusarium* species causing tuber dry rot disease. J Plant Pathol Microbiol (in press).

35. Mejdoub-Trabelsi B, Jabnoun-Khiareddine, Daami-Remadi M (2015) Interactions between four *Fusarium* species in potato tubers and consequences for fungal development and susceptibility assessment of five potato cultivars under different storage temperature. J Plant Pathol Microbiol 6: 293.

36. Petrini O (1986) Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, Heuvel J Van Den (Eds.). Microbiology of the phyllosphere. Cambridge: University Press.

37. Barnett HL, Hunter BB (1998) Illustrated Genera of Imperfect Fungi. 4th ed. APS press.

38. Whipps JM (1987) Effect of media on growth and interactions between a range of soilborne glasshouse pathogens and antagonistic fungi. New Phytol 107: 127-142.

39. Narisawa K, Okhi T, Hashiba T (2000) Suppression of clubroot and Verticillium yellows in Chineese cabbage in the field by the endophytic fungus, *Heteroconium chaetospira*. Plant Pathol 49: 141-146.

40. Kusari S, Singh S, Jayabaskaran C (2014) Biotechnological potential of plant-associated endophytic fungi: hope versus hype. Trends Biotechnol 32: 297-303.

41. Narisawa K, Kawamata H, Currah RS, Hashiba T (2002) Suppression of Verticillium wilt in eggplant by some fungal root endophytes. Eur J Plant Pathol 108: 103-109.

42. Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67: 491-502.

43. Abdel-Motaal FF, Nassar MSM, El- Zayat S, El- Sayed MA, Ichi Ito S (2010) Antifungal activity of endophytic fungi isolated from Egyptian Henbane (*Hyoscyamus muticus L.*). Pak J Bot 42: 2883-2894.

44. Daami-Remadi M, Hibar K, Kihareddine HJ, Ayed F, El Mahjoub M (2006) Effect of two Trichoderma species on severity of potato tuber dry rot caused by Tunisian *Fusarium* complex. Int J Agric Res 5: 877-886.

45. Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, Hibar K, Znaïdi IEA, et al. (2006a) In vitro and in vivo evaluation of individually compost fungi for potato Fusarium dry rot biocontrol. J Biol Sci 6: 572-580.

46. Aydi R, Hassine M, Jabnoun-Khiareddine H, Jannet HB, Daami-Remadi M (2014) Study of the antifungal potential of Aspergillus spp. and their culture filtrates and organic extracts against Fusarium sambucinum. Tunisian J Med Plants Nat Prod 11: 15-29.

47. Backman PA, Sikora RA (2008) Endophytes: An emerging tool for biological control. Biol Control 46: 1-3.

48. Jabnoun-Khiareddine H, Daami-Remadi, Ayed F, El Mahjoub M (2009) Biological control of tomato Verticillium wilt by using indigenous *Trichoderma* spp. Afr J Plant Sci Biotechnol 3: 26-36.

49. El Khaldi R, Daami-Remadi M, Zourgui L, Chérif M (2016) Biological control of stem canker and black scurf on potato by date palm compost and its associated fungi. J Phytopathol 164: 40-51.

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

51. Sharma YK, Lodha SK, Sriram S, Ramanujam B (2015) Comparative efficacy of biological control agents for the management of cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini*. J Spices Arom Crops 24: 18-22.

52. Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, El Mahjoub M (2009) Biocontrol of tomato Verticillium wilt by using indigenous Gliocladium spp. and Penicillium sp. isolates. Dynamic Soil, Dynamic Plant 3: 70-79.

53. Larena I, Sabquillo P, Melgarejo P, De Cal A (2003) Biocontrol of Fusarium and Verticillium wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. J Phytopathol 151: 507-512.

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

55. Qiu M, Xie RS, Shi Y, Zhang H, Chen HM (2010) Isolation and identification of two flavonoid-producing endophytic fungi from *Ginkgo biloba* L. Ann Microbiol 60: 143-150.

56. Verma A, Johri BN, Prakash A (2014) Antagonistic evaluation of bioactive metabolite from endophytic fungus, Aspergillus flavipes KF671231. Journal of Mycology.

57. Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi. A source of novel biologically active secondary metabolites. Mycol Res 106: 996-1004.