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 spp.) 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 post-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 wilt. The most common wilt pathogens are Verticillium dahliae and to a lesser extent V. albostratum 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, stuntting, chlorosis, vascular discoloration, wilt, and eventual death [17,18]. The disease is caused by a complex of Fusarium species including F. cumbartii, 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 bioaggressors 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 biogressors [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. sambucinum 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 through double layered cheese cloth and the mean diameter of pathogen colony in presence of antagonist.

Growth inhibition score (GIS), estimated based on above GI values, was noted after 7 days of incubation.

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 Whipple's [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 between 1-3, 2=GI comprised between 3-6, 3=GI comprised between 6-9, 4=GI comprised between 9-12, and 5=GI comprised between 12-15.

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Origin</th>
<th>Organ</th>
<th>Cultivar</th>
<th>RFD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.41.11</td>
<td>A. terreus</td>
<td>Sahline</td>
<td>Stem</td>
<td>Spunta</td>
<td>64.5</td>
</tr>
<tr>
<td>E.25.11</td>
<td>A. flavus</td>
<td>Chott-Mariem</td>
<td>Tuber</td>
<td>Magda</td>
<td>66.7</td>
</tr>
<tr>
<td>E.37.11</td>
<td>A. flavus</td>
<td>Sahline</td>
<td>Tuber</td>
<td>Safraene</td>
<td>57.4</td>
</tr>
<tr>
<td>E.61.11</td>
<td>A. nidulans</td>
<td>Chott-Mariem</td>
<td>Tuber</td>
<td>Spunta</td>
<td>58.7</td>
</tr>
<tr>
<td>E.60.11</td>
<td>A. flavus</td>
<td>Chott-Mariem</td>
<td>Tuber</td>
<td>Spunta</td>
<td>64.5</td>
</tr>
<tr>
<td>E.13.11</td>
<td>A. niger</td>
<td>Chott-Mariem</td>
<td>Tuber</td>
<td>Carrera</td>
<td>65.3</td>
</tr>
<tr>
<td>E.39.11</td>
<td>Penicillium sp.</td>
<td>Teboulba</td>
<td>Tuber</td>
<td>Bellini</td>
<td>65.7</td>
</tr>
<tr>
<td>E.29.11</td>
<td>P. polonicum</td>
<td>Chott-Mariem</td>
<td>Tuber</td>
<td>Evora</td>
<td>56.5</td>
</tr>
<tr>
<td>E.44.11</td>
<td>Penicillium sp.</td>
<td>Kairouan</td>
<td>Stem</td>
<td>Spunta</td>
<td>59.3</td>
</tr>
<tr>
<td>E.36.11</td>
<td>P. chrysogenum</td>
<td>Chott-Mariem</td>
<td>Tuber</td>
<td>Spunta</td>
<td>62</td>
</tr>
</tbody>
</table>

*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 inoculated with a mixed inoculum composed of Fusarium sambucinum and F. solani and treated with the different potato-associated isolates tested [34].
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 (10⁷ 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 replications 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 ≤ 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

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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).

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Variation in growth inhibition score (GIS) of the potato-associated fungi

Table 2: Calculated using Whipps' [38] formula.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>F. sambucinum</th>
<th>F. oxysporum</th>
<th>F. graminearum</th>
<th>Average GIS per Isolate tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.41.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E.25.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E.37.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E.60.11</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1.66</td>
</tr>
<tr>
<td>E.61.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E.13.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.39.11</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>E.29.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E.44.11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E.36.11</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

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%.

Gl: 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 of 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 of 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 insignificant, 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...
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...
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 against Fusarium dry rot pathogens [44,45] and the vascular Fusarium wilt agent i.e. F. oxysporum f. sp. tuberosi [24]. These effects may be presumable 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-aturum 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. cuminii 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 spp. associated to healthy Solanaceae plants. Also, Jabnoun-Khiareddine et al. [33] have demonstrated the potential of indigenous Penicillium spp. 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 [33,32] 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 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


