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Antifungal Potential of Extracellular Metabolites from *Penicillium* spp. and *Aspergillus* spp. Naturally Associated to Potato against *Fusarium* species Causing Tuber Dry Rot

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Abstract

Culture filtrates and chloroform extracts of *Aspergillus* and *Penicillium* species isolated from disease-free potato tubers were screened for their ability to suppress four Fusarium species responsible for potato dry rot in Tunisia. Percent inhibition of *Fusarium* spp. mycelial growth, based on the sealed plate method, ranged between 4 and 53%. Cell-free culture filtrates of test fungi displayed interesting antifungal potential toward *Fusarium* spp. The highest inhibitions, up to 50%, were achieved using filtrates of *A. flavus* and *P. chrysogenum*. All chloroform extracts of the tested isolates had inhibited *Fusarium* spp. growth where those from *P. chrysogenum* and *A. flavus* had decreased their radial growth by 76% compared to their relative controls. Tested as tuber treatment prior inoculation, the rot lesion diameter was decreased by 37.61 and 38.58% using *P. polonicum* and of *A. niger* cell-free culture filtrates, respectively. The most effective chloroform extracts in suppressing rot penetration were those from *A. flavus* and *A. niger* leading to 46.25 and 50.62% decrease in this parameter, compared to control, respectively.

Keywords: *Aspergillus* spp.; Chloroform extracts; Culture filtrates; *Fusarium* spp.; *Penicillium* spp.; Potato dry rot

Introduction

Fusarium dry rot is a damaging postharvest potato (*Solanum tuberosum* L.) disease. It is a fungal disease that represents a serious threat to tubers under cold and traditional storage conditions. Tuber losses ranged between 6 and 25% and more than 60% of infected tubers were reported in some cases [1]. Various Fusarium species are involved in disease development [2,3]. *F. solani*, *F. sambucinum*, *F. oxysporum* and *F. graminearum* are the most prominent in Tunisia [4-6]. Disease symptoms develop mainly on wounded tubers as shallow small brown lesions. The periderm eventually sinks and may wrinkle in concentric rings as the underlying dead tissue desiccates [1]. Causal agents of dry rot are both soil- and tuber-borne pathogens [7]. *Fusarium* spp. survives for many years as chlamydospores in soil or as colonizers of living plants or crop debris. Thus, the soil constitutes a source of inoculum [8].

Chemical control of disease using benzimidazoles fungicides has led to the development of thiabendazole-resistant strains in many potato-producing countries including Tunisia [9,10]. Moreover, commercially grown potato cultivars were found to be susceptible to highly susceptible Fusarium species complex [11,12].

An interesting approach for the management of this post-harvest disease has gained an increasing attention. It consists of using non-pathogenic agents, naturally associated to plants, as biocontrol agents [13]. These microorganisms are able to colonize healthy plant tissues without inducing any visible harmful effects [14,15]. They are involved in plant protection as they are capable to produce various compounds active in inducing plant defense and disease suppression [16-18]. Naturally plant-associated fungi and bacteria are used as biological agents against many fungal plant pathogens particularly *Fusarium* spp. [19-21].

Additionally, various reports have indicated that naturally based compounds and microbial biocontrol agents, generally considered as environmentally safe, could constitute interesting components of integrated dry rot management strategies [8]. Furthermore, a great number of metabolites such as alkaloids, peptides, steroids, terpenoids, phenols, quinines, and flavonoids, with antimicrobial activities, are produced by endogenous agents [22,23]. Thus, many plant-associated microorganisms (bacteria and fungi) can protect their host plants through the synthesis of biologically active compounds [24].

Among the fungal agents used, atoxigenic *Penicillium* and *Aspergillus* species were widely explored as promising biocontrol agents due to their ability to produce several antibiotic metabolites [25-28]. Various *Aspergillus* species recovered from solarized soils and/or composts (*A. niger*, *A. flavus*, *A. terreus*, *A. nidulans*) have successfully suppressed potato postharvest diseases such as Pythium leak incited by *Pythium ultimum* tuber dry rot induced by *F. sambucinum* and pink rot caused by *Phytophthora* sp. [29-32]. Several studies have also pointed out the usefulness of bioactive metabolites from naturally plant-associated fungi. In fact, according to Frisvad and Larsen the genus *Aspergillus* is capable to release various compounds including bioactive peptides and enzymes [33,34]. Furthermore, *P. chrysogenum* exhibited an inhibitory effect towards *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *vasinfectum* [35].

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Applied as conidial suspensions, cell-free culture supernatants and organic extracts, non-pathogenic fungi displayed interesting antifungal activity against some plant pathogenic fungi [36]. In fact, Hassine et al. demonstrated the ability of chloroform extract of *Penicillium* sp. to suppress grey mold, early blight and anthracnose of tomato fruits caused by *Botrytis cinerea*, *Alternaria solani* and *Colletotrichum coccodes*, respectively [37]. Aydi Ben Abdallah et al. have previously demonstrated the antifungal activity of cell-free culture filtrates and various organic extracts from non-pathogenic *A. niger*, *A. terreus* and *A. flavus*isolates towards *P. ultimum* [38].

In our recent studies, potato-associated fungi belonging to the genera *Aspergillus* (*A. niger*, *A. flavus*, *A. terreus* and *A. nidulans*) and *Penicillium* (*P. polonicum* and *P. chrysogenum*) had efficiently suppressed rot penetration, caused by a mixed infection with *F. sambucinum* and *F. solani* and Fusarium wilt induced by a Fusarium species complex composed of *F. graminearum*, *F. sambucinum* and *F. oxysporum* [21,39]. Several other studies have reported the possible biocontrol of Fusarium dry rot using *Penicillium* spp. and *Aspergillus* spp. [27,31,32]. It is interesting to seek for new bioactive compounds exhibiting antifungal activities from plant-associated fungi [39,40]. A mass of bioactive natural products isolated from nonpathogenic fungi have been reported in recent few years, among them antimicrobial active substances including alkaloids, peptides, steroids, terpenoids, quinines, flavonoids, aliphatic compounds and phenols [22,41].

Searching for potentially bioactive metabolites from naturally plant-associated fungi is a promising approach of controlling plant diseases based on eco-friendly alternatives.

In the present study, potato-associated and non-pathogenic *Aspergillus* spp. and *Penicillium* spp. will be screened for their ability to produce extracellular metabolite(s) active against *Fusarium* spp. through the investigation of the effects of their cell-free culture filtrates and their organic extracts toward target Fusarium species.

Materials and Methods

Potato tubers

Visibly disease-free and undamaged potato (*Solanum tuberosum* L.) cv. Spunta tubers were used in the present work. This cultivar is the most grown in Tunisia and susceptible to major potato postharvest diseases including Fusarium dry rot [5]. Before use, tubers were previously stored at 6°C for two months. Twenty-four hours before, they were gently washed in running tap water to remove adhering soil and allowed to dry under room conditions. They were disinfected by immersing into 10% sodium hypochlorite solution for 5 min, rinsed thrice with sterile distilled water (SDW) and air dried.

Fusarium species

F. oxysporum, *F. graminearum*, *F. solani* and *F. sambucinum* used in this work were originally recovered from potato tubers exhibiting dry rot symptoms. They were cultured on Potato Dextrose Agar (PDA) medium supplemented with 300 mg L^{-1} (w/v) of streptomycin sulphate. Their virulence was preserved by bimonthly inoculation of freshly wounded and healthy tubers and re-isolation on PDA.

Isolation and culture of the potato-associated fungi

The potato-associated fungi used in the present work were originally recovered from visibly disease-free tubers collected from various potato-growing fields in Tunisian Centre-East (Chott-Mariem, N35°56'20.451"; E10°33'32.028"). They were previously selected for their effectiveness against *Fusarium* spp. in a previous work [21]. Four isolates, namely E.13.11 (*Aspergillus niger*; KU305732), E.25.11 (*A. flavus*; KU305733), E.36.11 (*Penicillium chrysogenum*; KU305735) and E.29.11 (*P. polonicum*; KU305734), molecularly identified, were used in the present work. Stock cultures were maintained 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.

Test of the antifungal potential of volatile compounds from the potato-associated fungi

The antifungal potential of *Aspergillus* and *Penicillium* spp. against *Fusarium* spp. was evaluated based on the sealed plate method. This technique consists in culturing the antagonist and the test pathogen into separate half Petri plates poured with PDA medium amended with streptomycin sulfate (300 mg L^{-1}) (w/v). An agar plug of test antagonist (6 mm in diameter), cut from an actively growing colony, was placed in the centre of the bottom plate half whereas the pathogen agar plug was deposited in the centre of the top plate half. Both plates were wrapped with parafilm layers to prevent loss of volatile compounds [32]. Thus, the four target pathogens were exposed to the influence of volatile substances released by *Aspergillus* spp. and *Penicillium* spp.

For this confrontation method, control plates were plated by pathogen plugs only. The diameter of *Fusarium* spp. colonies (submitted or not to volatiles from tested antagonists) was noted after 7 days of incubation at 25°C. The mycelial growth inhibition rate was calculated based on the following formula:

$$I\,\% = [(C2-C1)/C2] \times 100$$

where C2: Mean colony diameter (two perpendicular measurements) of target pathogen in control plates and C1: Mean colony diameter of target pathogen confronted to antagonist. Three replicate plates were used for each individual treatment and the whole experiment was repeated at least twice.

Antifungal potential of cell-free culture filtrates of potato-associated fungi

Each tested antagonistic agent was cultured at room temperature (25-27°C) in Potato Dextrose Broth (PDB) and subjected to continuous stirring at 150 rpm for 30 days [33,37]. After incubation, mycelial pellets were separated from the supernatant by filtration through Whatman No.1 filter papers and the collected filtrate was further centrifuged thrice for 10 min at 10,000 rpm. Supernatant fluids were collected and sterilized by filtration through a 0.22 µm pore size filter.

The antifungal potential of cell-free culture filtrates of *Aspergillus* spp. and *Penicillium* spp. against *Fusarium* spp. was assessed according to Vibha method [36]. Filtrates were aseptically injected at the concentration 20% (v/v) into Petri plates containing molten 10 mL PDA medium cooled at 45°C and amended with streptomycin sulfate (300 mg L^{-1}) (w/v). After solidification of the mixture, three agar plugs of the target pathogen (6 mm in diameter) were placed equidistantly in each Petri plate. In control plates, PDB medium filtrate was added at the same proportion to PDA.

The effect of tested treatments on pathogen growth (in treated and untreated control plates) was measured as described above. Each individual treatment was replicated three times and the whole experiment was repeated twice.

Antifungal potential of chloroform extracts from potato-associated fungi

The extracellular metabolites released by *Aspergillus* spp. and *Penicillium* spp., grown in PDB medium for 30 days, were extracted with chloroform using a separating funnel. Ten milliliters of cell-free culture filtrate of each test antagonist were poured in a separating funnel. Then, 10 mL of chloroform were added carefully. The funnel was reversed several times by degassing from time to time. The mixture was allowed to settle for few minutes with the cap open. The organic phase i.e. the lower phase was collected. The aqueous phase was replaced in the funnel and the extraction was repeated two other times by adding 10 ml of chloroform for each step. The solvent was evaporated in a rotary evaporator at 90°C with a slight rotation of 150 rpm.

Extract of the organic phase was dissolved in 2 mL of methanol. A volume of 500 µL of each type of extract was injected into Petri plates containing molten 10 ml PDA medium amended with streptomycin sulfate (300 mg L^{-1}) (w/v). After medium solidification, three pathogen agars plugs (6 mm diameter), removed from an actively growing colony, were arranged in an equidistant manner in each plate. Filtrate from un-amended PDB medium was used as a control. Fungal cultures were maintained at 25°C for 7 days. The effect of tested extracts on pathogen growth was measured as previously described.

Effect of cell-free culture filtrates and chloroform extract from *Aspergillus* spp. and *Penicillium* spp. on potato dry rot severity

Preparation of pathogen inoculum: Pathogenic inoculum was composed by two species, i.e., *F. sambucinum* and *F. solani*, previously demonstrated as the most aggressive Fusarium species complex involved in the severest dry rot decays in Tunisia, was used for tuber inoculation. Inoculum preparation was initiated by scraping off mycelium from 7 day old cultures and homogenizing it with SDW for 5 min in a blender [12]. The obtained suspension wasfiltered through double layered cheese cloth and the conidial suspension was adjusted to 10^7conidia mL^{-1} using a Malassez haemocytometer. Equal volumes of *F. solani* and *F. sambucinum* conidial suspensions were combined and used for tuber inoculation.

Preparation of fungal secondary metabolites: Cell-free culture filtrates and chloroform extracts of the four potato-associated fungi (*A. niger, A. flavus, P. polonicum* and *P. chrysogenum*) were prepared as previously described and were screened for their ability to suppress potato dry rot disease jointly incited by *F. sambucinum* and *F. solani*.

Tuber treatment and inoculation: Prior to inoculation, each potato tuber was wounded (6 mm in diameter and in depth) at two sites along a line joining the two ends using a sterile cork borer. The occasioned wounds were immediately treated by injecting 100 µL of cell-free culture filtrates or chloroform extracts of the four potato-associated fungi. After 24 h, tubers were inoculated with 100 µL of pathogen inoculum. The positive control tubers were inoculated with pathogens but treated with SDW only. The negative control tubers were challenged with an un-colonized agar plug and treated similarly with SDW. All tubers were incubated at 25°C for 21 days in plastic bags.

Evaluation of Fusarium dry rot severity: The mean diameter of dry rot lesions was measured at the end of the incubation period. Then, tubers were longitudinally sectioned through along the two wounds and the maximum depth (p) and width (l) of the diseased necrotic tissue were measured. Rot penetration (P) was calculated using the following formula [42]:

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

Statistical Analyses

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0 and mean comparisons were performed using the Duncan's Multiple Range test (at $P \leq 0.05$). Statistical analyses were carried out for *Fusarium* spp. colony diameters following a completely randomized factorial design where treatments (potato-associated isolates and the untreated control), the four Fusarium species were the two fixed factors. For the *in vitro* tests of the antifungal activity of cell-free culture filtrates and chloroform extracts, the experiment was performed according to a completely randomized design where treatments (filtrates or chloroform extracts and controls) and the four Fusarium species were the two fixed factors. Each individual treatment was replicated three times. For tuber bioassays, the experiment was performed according to a completely randomized design where the treatments tested (filtrates or extracts and controls) were the sole fixed factor and each individual treatments was replicated six times.

Results

Effect of potato-associated fungi on *Fusarium* spp. mycelial growth

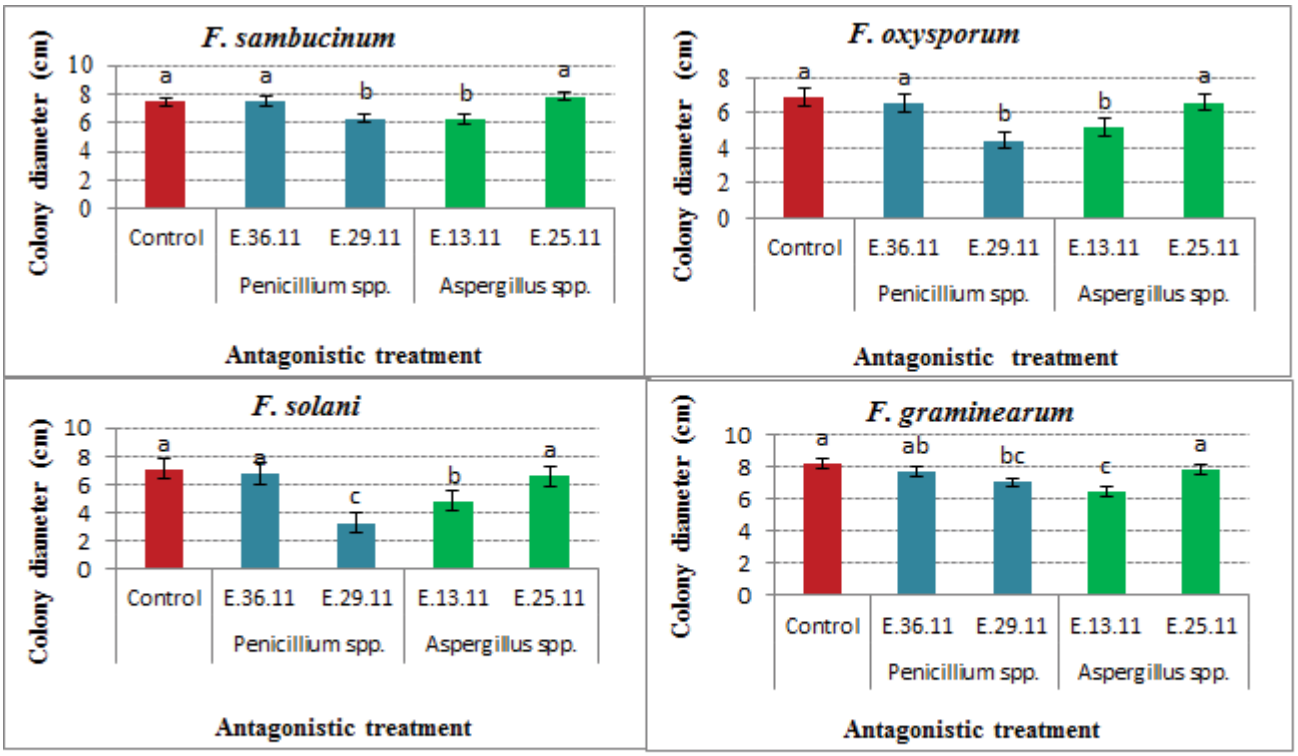
Analysis of variance showed that the average colony diameter of *Fusarium* spp., recorded after 7 days of incubation at 25°C, varied significantly ($P<0.01$) depending on Fusarium species, antagonistic treatments and their interactions.

The antifungal activity of volatile compounds from the test potato-associated fungi varied depending on target Fusarium species. In fact, *F. sambucinum,* growth inhibition varied between 7 and 43% where volatiles released by E.29.11 (*P. polonicum*) and E.13.11 (*A. niger*) displayed the highest antifungal activity. *F. oxysporum* growth inhibition was reduced by 4 to 36% where the highest reduction was attributed to E.13.11 and E.29.11 volatile metabolites. *F. solani* growth was inhibited by 7 to 53%, compared to control, where volatiles from *P. polonicum* and *A. niger* were found to be the most active. *F. graminearum* radial growth was lowered by 4 to 24% depending on isolates tested but the highest antifungal potential was displayed by *P. polonicum* and *A. niger* volatile compounds. However, the two isolates, E.25.11 (*A. flavus*) and E.36.11 (*P. chrysogenum*), were found to be less active at distance against all *Fusarium* species (Figures 1 and 2).

Results given in Figure 2 indicated the antifungal effect displayed by E.29.11 (*P. polonicum*) depending on target *Fusarium* species, which was expressed by 19, 53, 14 and 36% decrease in *F. sambucinum*, *F. solani*, *F. graminearum* and *F. oxysporum* growth, respectively over their respective controls. It should be highlighted that, whatever the antagonistic isolates tested, the mycelial growth inhibition of all Fusarium species varied from 4 to 53% in the sealed plate method (Figure 3).

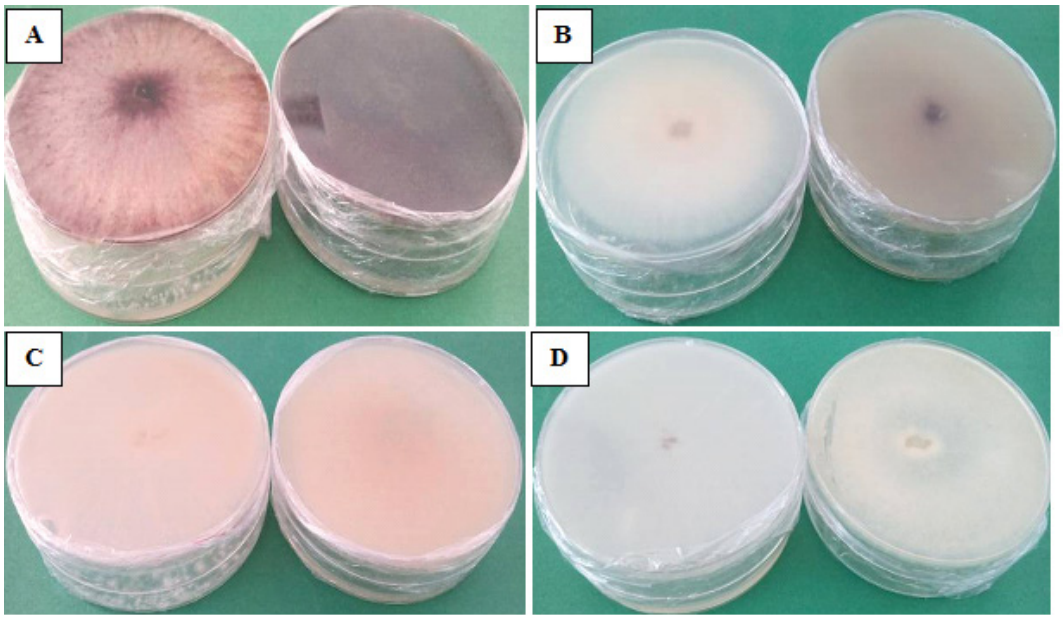
Antifungal potential of cell-free culture filtrates of potato-associated fungi toward *Fusarium* spp.

Analysis of variance, performed for the average colony diameter of *Fusarium* spp. colonies, revealed a highly significant ($P \leq 0.01$) variation depending on target Fusarium species and cell-free culture filtrates from the potato-associated fungi tested. In fact, Figure 3 showed that the tested cell-free culture filtrates exhibited a highly significant inhibitory effect ($P \leq 0.01$) against *Fusarium* spp. where growth inhibition of



E.36.11: *Penicillium chrysogenum*; E.13.11: *Aspergillus niger*; E.25.11: *A. flavus*; E.29.11: *P. polonicum;* Control: PDB culture medium. Bars affected by the same letter are not significantly different according to Duncan's multiple range tests at $P \leq 0.01$

Figure 1: Effect of potato-associated fungi on *Fusarium* spp. mycelial growth using sealed plate method, noted after 7 days of incubation at 25°C.



A: *F. graminearum*; B: *F. oxysporum*; C: *F. sambucinum*; D: *F. solani*. Control is the Petri plate situed at the right of each picture

Figure 2: Effect of volatile metabolites produced by*Penicillium polonicum* (E.29.11) on the mycelial growth of *Fusarium* spp., noted after 7 days of incubation at 25°C, compared to the untreated controls.

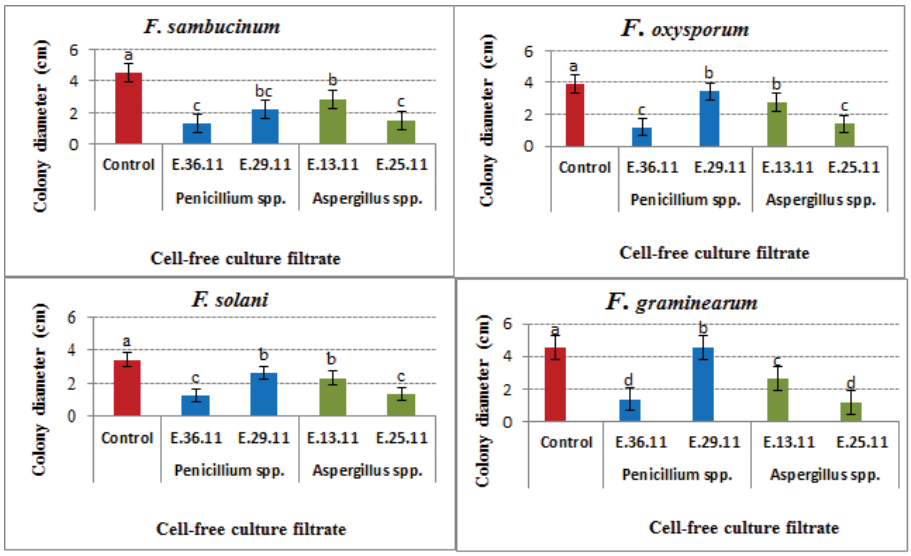
target Fusarium species varied between 36 and 70% for *F. sambucinum*, 11 and 69% for *F. oxysporum*, 23 and 63% for *F. solani* and from 41 to 73% against *F. graminearum*.

It should be highlighted that the use of the cell-free culture filtrates of E.25.11 (*A. flavus*) and E.36.11 (*P. chrysogenum*) has led to more than 50% inhibition of *Fusarium* spp. growth compared to the untreated

control. In fact, the filtrates of these two isolates decreased the radial growth of *F. sambucinum*, *F. solani*, *F. oxysporum* and *F. graminearum* by 51-70, 60-63, 53-69 and 69-73%, respectively, relative to their respective untreated controls (Figure 4).

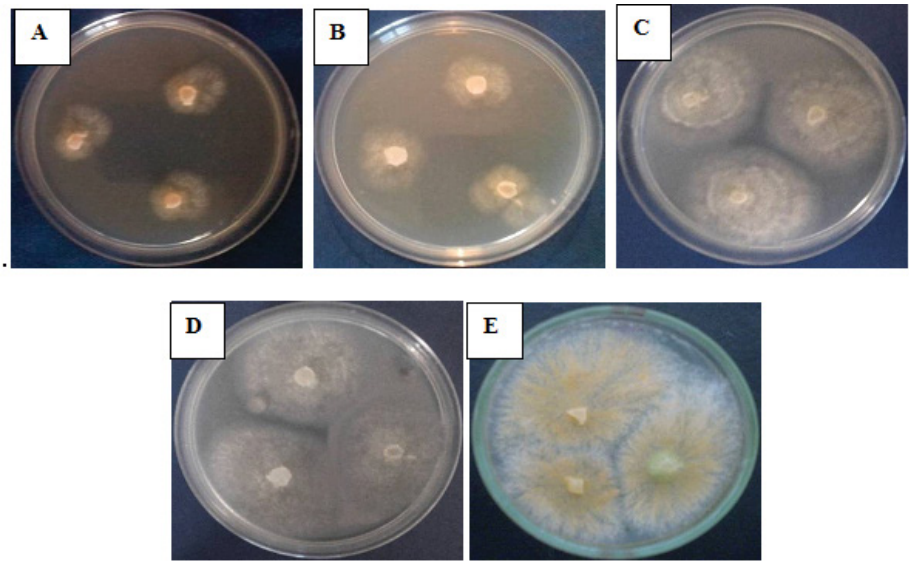
Antifungal potential of chloroform extracts from potato-associated fungi toward *Fusarium* spp.

A highly significant (at $P \leq 0.01$) variation in the average diameter of *Fusarium* spp. colonies was observed depending on tested chloroform extracts from *Aspergillus* spp. (E.13.11 and E.25.11) and *Penicillium* spp. (E.29.11 and E.36.11). All chloroform extracts had inhibited *Fusarium* spp. growth relative to their respective controls. The percentages of growth inhibition varied depending on extracts tested from 52 to 76% for *F. sambucinum*, from 39 to 74% for *F. oxysporum*, from 47 to 72% for *F. solani* and from 40 to 79% for *F. graminearum*. Chloroform extracts from E.36.11 (*P. chrysogenum*) and E.25.11 (*A. flavus*) isolates displayed, in the majority of cases, the highest antifungal potential with 76% decrease in *Fusarium* spp. colony diameters relative to controls (Figure 5).



E.36.11: Cell-free culture filtrate from *Penicillium chrysogenum*; E.13.11: Cell-free culture filtrate from *Aspergillus niger*, E.25.11: Cell-free culture filtrate from *A. flavus*, E.29.11: Cell-free culture filtrate from *P. polonicum*. Control: PDB culture medium. The filtrates were added at the concentration of 20% (v/v). Bars affected by the same letter are not significantly different according to Duncan's multiple range tests at $P \leq 0.01$

Figure 3: Effect of potato-associated fungi cell-free culture filtrates on *Fusarium* spp. mycelial growth, noted after 7 days of incubation at 25°C.



A: Cell-free culture filtrate from E.36.11 (*Penicillium chrysogenum*); B: Cell-free culture filtrate from E.25.11 (*Aspergillus flavus*); C: Cell-free culture filtrate from E.29.11 (*P. polonicum*); D E.13.11: Cell-free culture filtrate from *A. niger*;E:Control Filtrate of Potato Dextrose Broth (PDB). The filtrates were added at the concentration of 20% (v/v)

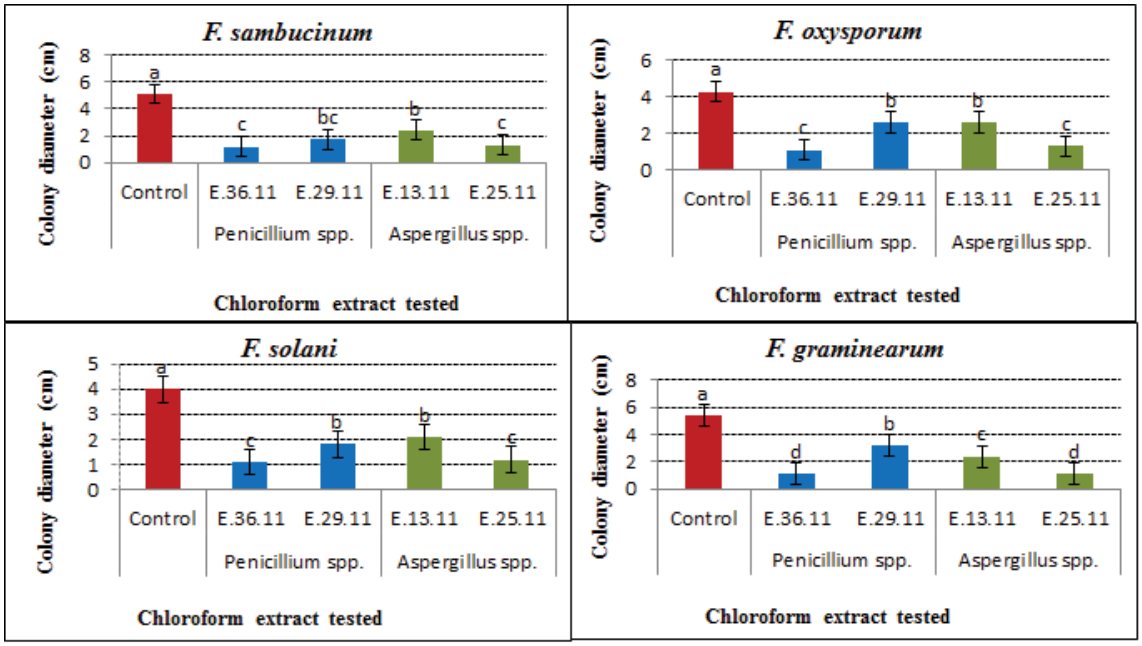
Figure 4: Effect of the cell-free culture filtrates of potato-associated fungi on the mycelial growth of *Fusarium sambucinum* observed after 7 days of incubation at 25°C.

Effect of cell-free culture filtrates and chloroform extracts from potato-associated fungi on Fusarium dry rot severity

Fusarium dry rot severity noted on potato tubers, as measured by the lesion rot diameter and mean pathogen penetration, varied significantly (at $P \leq 0.01$) depending on tested cell-free culture filtrates. In fact, rot lesion diameter, noted 21 days post-treatment, was reduced by 22 to 38%, relative to *Fusarium* spp.-inoculated and untreated control, depending on filtrates tested (Figure 6). The highest decrease in this parameter, by about 37 and 38% versus control, was noted following tuber treatments with cell-free culture filtrates of E.29.11 (*P. polonicum*) and E.13.11 (*A. niger*), respectively.

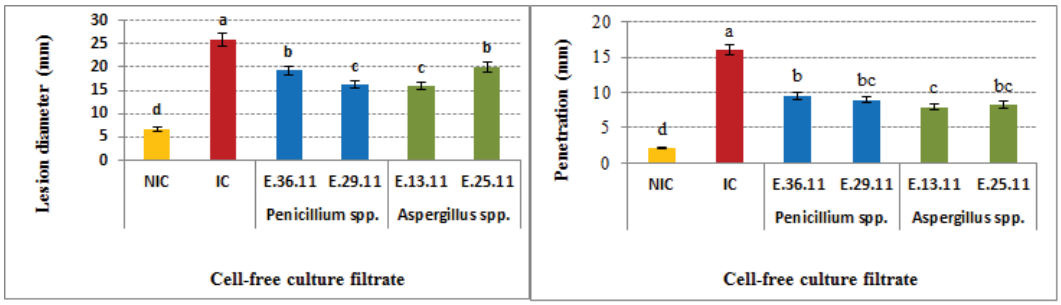
The four tested culture filtrates had also decreased average rot penetration induced by *Fusarium* spp. by 40 to 50% relative to pathogen-inoculated and untreated control. Cell-free filtrates from E.13.11 (*A. niger*), E.29.11 (*P. polonicum*) and E.25.11 (*A. flavus*) were the most efficient in suppressing rot penetration by 50.62%.

In the case of organic extracts bioassay, analysis of variance showed that the lesion diameter and the average rot penetration varied significantly (at $P \leq 0.01$) depending on treatments tested. In fact, the first disease severity parameter was lowered by 23.22 to 39.68% and the second one was reduced by 41.25 to 50.62%, compared to control, using chloroform extracts of the four potato-associated fungi tested (Figure 7). Extracts from E.29.11 of *P. polonicum* and E.13.11 of *A. niger* showed the highest efficacy in reducing dry rot lesion diameter to 39%. The most effective extracts in decreasing rot penetration were those of



E.36.11: Chloroform extract from *Penicillium chrysogenum*; E.13.11: Chloroform extract from *Aspergillus niger*; E.25.11: Chloroform extract from *A. flavus*; E.29.11: Chloroform extract from *P. polonicum*;Control: PDB culture medium. Chloroform extracts were added at 5% (v/v). Bars affected by the same letter are not significantly different according to Duncan's multiple range tests at $P \leq 0.01$

Figure 5: Effect of chloroform extracts from potato-associated fungi on *Fusarium* spp. mycelial growth noted after 7 days of incubation at 25°C.



Inoculation was performed using a mixed inoculum composed of *Fusarium sambucinum*and*F. solani.* E.36.11: Cell-free culture filtrate from *Penicillium chrysogenum*; E.13.11: Cell-free culture filtrate from *Aspergillus niger*; E.25.11: Cell-free culture filtrate from *A. flavus*; E.29.11: Cell-free culture filtrate from *P. polonicum*;NIC: Uninoculated and untreated control; IC: Inoculated and untreated control. Bars affected by the same letter are not significantly different according to Duncan's multiple range tests at $P \leq 0.01$

Figure 6: Effect of cell-free culture filtrates of the four potato-associated fungi tested on potatoFusarium dry rot severity, as estimated by rot lesion dimaeter (A) and penetration (B), noted after 21 days of incubation at 25°C as compared to controls.

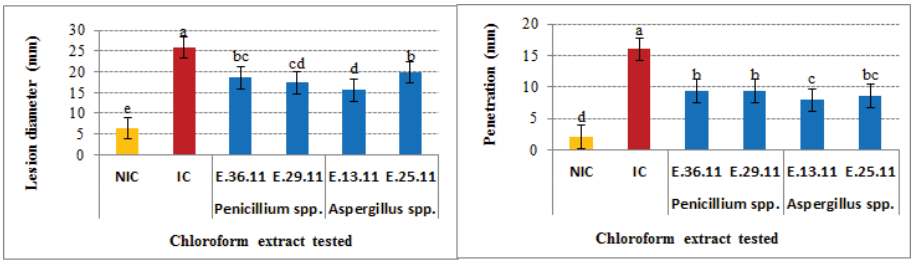


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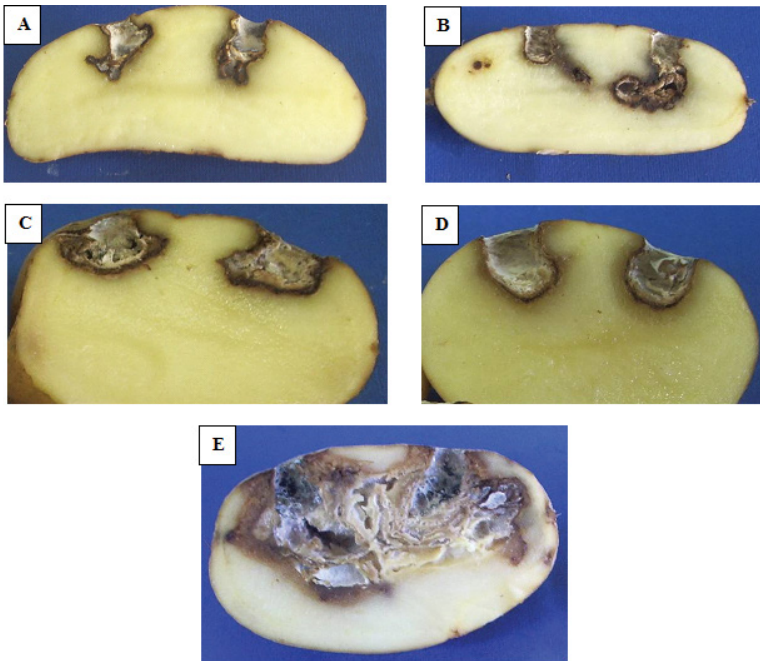
Figure 7: Effect of chloroform extracts from four potato-sassociated fungi tested on Fusarium dry rot severity, as estimated by rot lesion dimaeter (A) and penetration (B), noted after 21 days of incubation at 25°C.



A: Chloroform extract from E.13.11 (*Aspergillus niger*); B: Chloroform extract from E.25.11 (*A. flavus*); C: Chloroform extract from E.29.11 (*Penicillium polonicum*); D: Chloroform extract from E.3.11 (*P. chrysogenum*; E: Inoculated and untreated control

Figure 8: Severity of potato Fusarium dry rot recorded after 21 days of incubation at 25°C of tubers inoculated with *F. sambucinum* and *F. solani* and treated with chloroform extracts of four potato-associated fungi as compared to the inoculated and untreated control.

E.25.11 of *A. flavus* and E.13.11 of *A. niger* where this parameter was lowered by 46.25 and 50.62%, respectively (Figure 8). Results shown in Figure 8 illustrated the variable efficiency of organic extracts from the tested potato-associated fungi against dry rot severity as compared to *Fusarium* spp. inoculated and disease-free untreated controls.

Discussion

The plant-associated fungi can grow within plant tissues, aboveground as well as belowground, without inducing any disease symptoms [8,23]. Their species diversity and their capability to produce various biologically active metabolites were behind the wide number of investigations on endogenous fungi. Their promising biological activity leads to their direct or indirect use as biocontrol agents against various plant diseases [39]. The main objective of the present study was to demonstrate the ability of non-pathogenic associated fungi, occurring ubiquitously within potato tubers, to release extracellular antifungal metabolites for their eventual use for Fusarium dry rot control. In this study, the cell-free culture filtrates and the chloroform extracts of four potato-associated *Penicillium* spp. and *Aspergillus* spp. isolates, selected from previous works using their conidial suspensions, were screened for their potential to suppress Fusarium dry rot in potato based *in vitro* and *in vivo* bioassays [21,38].

Volatile metabolites from *A. niger* (E.13.11) and *P. polonicum* (E.29.11) were found to be the most effective in limiting *Fusarium* spp. *in vitro* growth. In the same sense, nine fungal endogenous isolated from *Musa* spp., grass weeds, *Mimosa pudica* and *Allamanda* spp., tissues were shown able to suppress Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4) as demonstrated

based on their ability to produce volatile inhibitory metabolites [43]. This finding is in agreement with previous works recording similar mycelial growth inhibitions using *A. niger*, *A. terreus* and *A. flavus* to control *Pythium ultimum*. The inhibitory potential of the most effective isolate CH1 (*A. niger*) was about 19.94% by direct contact as compared to 11.24% recorded at distance [30]. The diverse functions of fungal Volatile Organic Compounds (VOC) can be developed for use in biotechnological applications for biocontrol and mycofumigation. Morath et al. [44] reported on the agricultural interest of fungal VOCs in the control fungal pests.

Our interest to find a new natural fungitoxic compounds led us to examine culture filtrates of these potato-associated fungi *in vitro* against the mycelial growth of *F. sambucinum*, *F. solani*, *F. oxysporum* and *F. graminearum*. Among the tested cell-free culture filtrates, those of E.25.11 (*A. flavus*) and E.36.11 (*P. chrysogenum*) were found to be highly effective growth inhibitors against all tested fungi by inducing more than 50% inhibition of *Fusarium* spp. growth compared to the untreated control. Fungi of the genus *Penicillium* are well known as potential producers of antibacterial than antifungal substances although in the last decades, numerous authors have reported activity of secondary metabolites from *Penicillium* spp. against fungal plant pathogens. Florjanczyk et al. [45] demonstrated that extracellular fractions from *P. chrysogenum* had inhibited the growth of *Cryphonectria parasitica*, the causative agent of blight on American chestnut (*Castanea dentata*). The current findings suggest that the antifungal activity of the cell-free culture filtrates from 30 days old *Aspergillus* spp. and *Penicillium* spp. against *Fusarium* spp. may be due to their biologically active and stable secondary metabolites. Similarly, Wang et al. [46] found metabolites with antifungal activity in an endogenous fungus *Penicillium* sp. associated to *Hopea hainanensis*. Culture filtrates from *P. canescens* and *P. janczewskii* isolates showed inhibitory activities against *Rhizoctonia solani* [47]. In the same way, Rouissi et al. [48] demonstrated the fungicidal effects of secondary metabolites produced by a *P. expansum* (R82) strain active toward *Botrytis cinerea*, *Colletotrichum acutatum* and *Monilinia laxa*. Concerning culture filtrates of *Aspergillus* spp., they showed important antifungal potential against *Fusarium* spp. especially the isolate of *A. flavus*. In the same way, Vibha demonstrated the inhibitory effects of metabolites from most ubiquitous *Aspergillus* spp. (*A. ochraceus*, *A. niger*, *A. fumigatus*, *A. flavus* and *A. terreus*) against *R. solani* [36].

The four cell-free culture supernatants were subjected to an extraction with chloroform to confirm the antifungal potential of their extracellular metabolites. Interestingly, results from the current study clearly demonstrated that the chloroform extracts of these two fungal isolates, namely E.36.11 (*P. chrysogenum*) and E.25.11 (*A. flavus*), exhibited the highest disease inhibitory effect of *Fusarium* spp. The production of bioactive secondary metabolites by *Penicillium* species is well documented. In the same sense, Hassine et al. [37] reported that the chloroform extracts of two isolates of *Penicillium* sp. and two isolates of *Gliocladium* spp. isolated from soil and compost displayed antifungal activity *in vitro* and *in vivo* against *B. cinerea*, *A. solani* and *Colletotrichum coccodes* associated to tomato fruit rots. Also, Kaiserer et al. [49] have highlighted that the protein PAF (*Penicillium*-derived antifungal protein) secreted by *P. chrysogenum* strain Q176 was found to be active against phytopathogenic *Aspergillus* spp., *B. cinerea*, *Fusarium* spp., etc. Accordingly, Yang et al. [50] also demonstrated that the substances produced by *P. oxalicum* strain PY-1 were effective against several plant-pathogenic fungi. Bioactive components are chemical compounds often referred to as secondary metabolites. Goyal et al. [51] specified that fungi are believed to be the future microbial cell factories for the production of food grade pigments and enzymes. Mohammed et al. [52] have focused their studies on the secondary metabolites produced by *A. flavus* and identified thirty one bioactive compounds in its methanolic extract such as aromatic rings, alkenes, aliphatic fluoro compounds, aromatic nitro compounds, ammonium ions and organic nitrate. In accordance, Verma et al. [53] demonstrated that the ethyl acetate extract of *A. flavipes*, associated to the medicinal plant *Stevia rebaudiana,* displayed antifungal activity toward the soilborne fungus *Scletotinia sclerotiorum*. Total inhibition of *Pythium ultimum* growth was induced by extracellular metabolites of *A. niger*, *A. terreus* and *A. flavus* recovered from soil and compost teas [38].

In our previous investigations, potato-associated fungi (*Penicillium* spp. and *Aspergillus* spp.) were reported to display antagonistic potential against Fusarium dry rot caused by a mixture infection with *F. sambucinum* and *F. solani* on potato tubers and towards Fusarium wilt induced by a Fusarium species complex composed of *F. graminearum*, *F. oxysporum* and *F. sambucinum* [21,38]. In the present investigation, their secondary metabolites were further evaluated for their antagonistic potential against *F. solani* combined to *F. sambucinum*, the most aggressive agents of dry rot in potato tubers. Few studies on antifungal metabolites extracted from *Penicillium* spp. and *Aspergillus* spp. tested *in vivo* are available and to the best of our knowledge, this is the first report on the potential of these potato-associated fungi, through their secondary metabolites, against postharvest pathogens especially *Fusarium* spp. In this study, culture filtrates and chloroform extracts of *Aspergillus* spp. and *Penicillium* spp., screened for their potential to control Fusarium dry rot and were shown able to exhibit disease-suppressive effects, by reducing dry rot severity in comparison to pathogen-inoculated and untreated control. Other studies reported the inhibitory effects of secondary metabolites of antagonistic fungi against *Fusarium* spp. and other plant pathogens both *in vitro* and *in vivo*. In fact, similar results to those used in this study have previously demonstrated the biocontrol efficacy of *Aspergillus* spp., recovered from soil and compost, against infection by *F. sambucinum* in potato tubers [31]. However, there is no report on the use of *Aspergillus* spp., isolated from healthy potato tubers, to control Fusarium dry rot disease on this plant.

This study presents the first evidence that endogenous *Aspergillus* species (both *A. niger* and *A. flavus*) can reduce disease severity caused by the most aggressive Fusarium species. Interestingly, results from the current study clearly demonstrated that these two non-pathogenic *Aspergillus* species, were potent biocontrol agents and source of bioactive metabolites. In the same sense, Jabnoun-Khiareddine et al. [19] highlighted the potential of endogenous fungi to reduce plant severity disease when they reported the ecological interest of *Trichoderma* spp., associated to healthy tomato, eggplant and potato plants, for an efficient control of tomato Verticillium wilt caused by *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus*. In the same way, assessed in a 4 year field study, non-aflatoxigenic, indigenous *A. flavus* isolates, K49 and CT3, were reported to have potential use for biocontrol through reducing aflatoxin contamination of corn [54]. Fusarium dry rot control using extracellular metabolites from *A. flavus* proved its capability to produce hydrolytic enzymes. In fact, naturally plant-associated fungi produce extracellular hydrolyases as a resistance mechanism to overcome attack by the host against pathogenic invasion. Such enzymes including pectinases, esterases, cellulases and lipases, proteinase, α-1,4-glucan lyase and phosphatases [40]. Shemshura et al. [55] reported that the antagonism of *A. candidus* against two plant-parasitic nematodes is primarily due to the production of carboxylic acids such as citric acid. Recently, Sreevidya et al. [56] found that *P. citrinum* VFI-51 isolated

from five different herbal vermicomposts and chickpea rhizosphere soil samples in India have inhibited *Macrophomina phaseolina* in both dual culture as well as secondary metabolite production assays where *P. citrinum* VFI-51efficiency was expressed by 85% decrease in charcoal rot severity on roots compared to positive control. When this promising isolate was selected for purification of its active secondary metabolites, the purified compound matched with the structure of citrinin. It should be noted that production of citrinin was reported by *Aspergillus* spp. and many *Penicillium* species, including *P. citrinum* [57]. In addition, since we did not specifically extract and quantify the amount of each of the compounds produced by the endogenous and their influence on *Fusarium* spp., we can only conclude that the presence of these extracellular metabolites may have a subsequent impact on the growth of Fusarium species. Their potency as inhibitors cannot be gauged accurately, although percentages of inhibition comprised between 40 and 50% offered some preliminary understanding. The control of dry rot disease in potato tubers by *P. polonicum* could also be due to its capability to produce hydrolytic enzymes. In fact, Sreevidya et al. [58] showed that *P. citrinum* VFI-51 was able to produce siderophore, indole acetic acid (IAA), hydrocyanic acid (HCN), lipase, protease and β-1,3 glucanase. Siderophores help plants not only to acquire iron but also helps in disease suppression.

This study clearly demonstrated that the cell-free culture filtrates and the chloroform extracts of potato-associated fungi (*Aspergillus* and *Penicillium* species) are potential sources of fungitoxic chemicals and their importance in controlling different plant pathogens associated with Fusarium dry rot disease.

Conclusion

This study revealed that non-pathogenic potato-associated fungi *Penicillium* and *Aspergillus* spp., with antifungal potential against *Fusarium* spp., were found to be potent source of extracellular metabolites. Thus, it can be concluded that naturally occurring potato-associated fungi and their extracellular metabolites can suppress *in vitro* and *in vivo* growth *Fusarium* species infecting potato tubers. Further chemical studies are needed to more elucidate the efficiency of the secondary metabolites of these four antagonists and to identify major compounds present in their chloroform extracts.

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References

1. Stevenson WR, Loria R, Franc GD, Weingartner DP (2001) Compendium of Potato Diseases, 2nd ed. The American Phytopathological Society, St. Paul.
2. Peters JC, Lees AK, Cullen DW, Sullivan L, Stroud GP, et al. (2008) Characterization of *Fusarium* spp. Responsible for causing dry rot of potato in great Britain. Plant Pathol 57: 262-271.
3. Gachango E, Hanson LE, Rojas A, Hao JJ, Kirk WW(2012) *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis 96: 1767-1774.
4. Chérif M, Omri N, Hajlaoui MR, Mhamdi M, Boubaker A (2001) Effet de quelques fongicides sur un agent de pourriture sèche des tubercules de pomme de terre *Fusarium roseum* var. *sambucinum* et sur Trichoderma antagonistes. Annales de l'INRAT 74: 131-149.
5. Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, El Mahjoub M (2006a) Effect of temperatures on aggressivity of tunisian Fusarium species causing potato (*Solanum tuberosum* L.) tuber dry rot. J Agron 5: 350-355.
6. Mejdoub-Trabelsi B, Jabnoun-Khiareddine H, Daami-Remadi M (2012) Effect of Fusarium species and temperature of storage on the susceptibility ranking of potato cultivars to tuber dry rot. Pest Technol 6: 41-46.
7. Choiseul J, Allen L, Carnegie SF(2007) Fungi causing dry tuber rots of seed potatoes in storage in Scotland. Potato Res 49: 241-253.
8. Bojanowski A, Avis TJ, Pelletier S, Tweddell RJ (2013) Management of potato dry rot. Postharvest Biol Technol 84: 99-109.
9. Daami-Remadi M, El Mahjoub M (2006) Présence en Tunisie d'isolats de *Fusarium sambucinum* résistants aux benzimidazoles: Développement *in vitro* et agressivité sur tubercules de pomme de terre. Biotechnologie, Agronomie, Société et Environnement 10: 7-16.
10. Ocamb CM, Hamm PB, Johnson DA (2007) Benzimidazole resistance of Fusarium species recovered from potatoes with dry rot from storages located in Columbia Basin of Oregon and Washington. Amer J Potato Res 84: 169-177.
11. Esfahani MN (2011) Comparative pathogenicity of Fusarium species on some of the potato cultivars. Plant Breed Seed Sci 64: 45-53.
12. Mejdoub-Trabelsi B, Jabnoun-Khiareddine H, 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 Microb 6: 293.
13. Berg G, Grubes M, Schloter M, Smallas K(2014) Unraveling the plant microbiome: Looking back and future perspectives. Front Microbiol 5: 148.
14. Petrini O, Sieber TN, Toti L, Viret O (1992) Ecology, metabolite production and substrate utilization in Endophytic fungi. Nat Toxins 1: 185-196.
15. Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: Endophytism defined. In Microbial endophytes. New York: Marcel Dekker.
16. Azevedo JL, Maccheroni JW, Pereira JO, Araújo WL (2000) Endophytic microorganisms: A review on insect control and recent advances on tropical plants. Electro J Biotech 3: 40-65.
17. 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.
18. Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: Progress, challenges and frontiers. Fungal Biol Rev 21: 51-66.
19. 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.
20. Aydi Ben Abdallah R, Mokni-Tlili S, Nefzi A, Jabnoun-Khiareddine H, Daami-Remadi M (2016) Biocontrol of Fusarium wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Nicotiana glauca* organs. Biol Control 97: 80-88.
21. Mejdoub-Trabelsi B, Aydi Ben Abdallah R, Kthiri Z, Hamada W, et al. (2016a) Assessment of the antifungal activity of non-pathogenic potato-associated fungi toward Fusarium species causing tuber dry rot disease. J Plant Pathol Microbiol 7: 343.
22. Yu F, Han T, Wu JZ, Huan BK, Qin LP (2009) Antifungal secondary metabolites from endophytic *Verticillium* sp. Biochem Syst Ecol 37: 162-165.
23. Kusari S, Hertwek C, Spiteller M (2012) Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chem Biol 19: 792-798.
24. Arnold, AE, Maynard Z, Gilbert GS (2001) Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol Res 105: 150-1507.
25. 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. In: Advances in Verticillium: Research and Disease Management, APS Press.
26. Posoda F, Vega F (2005) Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). Mycologia 97: 1195-1200.
27. Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, Hibar K, Znaïdi IEA, et al. (2006b) *In vitro* and *in vivo* evaluation of individually compost fungi for potato Fusarium dry rot biocontrol. J Biol Sci 6: 572-580.
28. Bhattacharyya PN, Jha. DK (2011) Optimization of cultural conditions affecting growth and improved bioactive metabolite production by a subsurface *Aspergillus* strain TSF 146. Intern J Appl Biol Pharm Tech 2: 133-143.

29. Daami-Remadi M, Dkhili I, Jabnoun-Khiareddine H, El Mahjoub M (2012) Biological control of potato leak with antagonistic fungi isolated from compost teas and solarized and non-solarized soil. Pest Technol 6: 32-40.

30. Aydi Ben A, Hassine M, Jabnoun-Khiareddine H, Ben Jannet H, Daami-Remadi M (2013) Valorisation des *Aspergillus* spp. comme agents de lutte biologique contre Pythium et optimisation de leur pouvoir antagoniste *in vitro* et *in vivo*. J Med Plant Nat Prod 9: 70-82.

31. AydiR, Hassine M, Jabnoun-Khiareddine H, Ben Jannet H, Daami-Remadi M (2014a) Study of the antifungal potential of *Aspergillus* spp. and their culture filtrates and organic extracts against *Fusarium sambucinum*. J Med Plant Nat Prod 11: 15-29.

32. Aydi Ben Abdallah R, Mejdoub-Trabelsi B, Jabnoun-Khiareddine H, Daami-Remadi M (2015) Soil-borne and compost-borne *Aspergillus* species for biologically controlling post-harvest diseases of potatoes incited by *Fusarium sambucinum* and *Phytophthora erythroseptica.* J Plant Pathol Microb 6: 313.

33. Dennis C, Webster J (1971) Antagonistic properties of species- groups of Trichoderma II. Production of volatile antibiotics. Trans Brit Mycol Soc 57: 41- 48.

34. Frisvad JC, Larsen TO (2015) Chemodiversityin the genus *Aspergillus*. Appl Microbiol Biotechnol 99: 7859-7877.

35. Dong H, Cohen Y (2002) Induced resistance in cotton seedlings against Fusarium wilt by dried biomass of *Penicillium chrysogenum* and its water extract. Phytoparasitica 30: 77-87.

36. Vibha M (2010) Effect of fungal metabolites and amendments on mycelium growth of *Rhizoctonia solani*. J Plant Protec Res 50: 93-97.

37. Hassine M, Aydi R, Jabnoun-Khiareddine H, Ben Jannet H, Daami-Remadi M (2013) Activité antifongique *in vitro* et *in vivo* des extraits organiques de *Penicillium* sp. et de *Gliocladium* spp. contre trois agents de pourriture des fruits de tomate. Microbiologie et Hygiène Alimentaire 25: 57-66.

38. Aydi Ben Abdallah R, Hassine M, Jabnoun-Khiareddine H, Haouala R, Daami-Remadi M (2014b) Antifungal activity of culture filtrates and organic extracts of *Aspergillus* spp. against *Pythium ultimum*. J Plant Protec 9: 17-30.

39. Mejdoub-Trabelsi B, Aydi Ben Abdallah R, Ammar N, Kthiri, Z, Hamada W, et al. (2016b) Bio-suppression of Fusarium wilt disease in potato using nonpathogenic potato-associated fungi. J Plant Pathol Microb 7: 347.

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

41. Tan RX, Zou WX (2001) Endophytes: A rich source of functional metabolites. Natural product reports 18: 448-459.

42. Lapwood DH, Read PJ, Spokes J (1984) Methods for assessing the susceptibilityof potato tubers of different cultivars to rotting by *Erwinia carotovora* subsp. *atroseptica* and *carotovora*. Plant Pathol 33: 13-20.

43. Ting ASY, Mah SW, Tee CS (2010) Identification of volatile metabolites from fungal endophytes with biocontrol potential towards *Fusarium oxysporum* f. sp. *cubense* race 4. Am J Agricult Biotech Sci 5: 177-182.

44. Morath SU, Hung R, Bennett JW (2012) Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. Fungal Biol Rev26: 73-83.

45. Florjanczyk A, Barnes R, Kenney A, Horzempa J (2016) Soluble material secreted from *Penicillium chrysogenum* isolate exhibits antifungal activity against *Cryphonectria parasitica* - the causative agent of the American Chestnut Blight. J Plant Pathol Microbiol 7: 348.

46. 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.

47. Nicoletti R, Raimo F, Carella A (2003) Searching *Penicillium* isolate antagonistic to *Rhizoctonia solani* in suppressive soils in Salento. J Plant Pathol 85: 295.

48. Rouissi W, Ugolini L, Martini C, Lazzeri L, Mari M (2013) Control of postharvest fungal pathogens by antifungal compounds from *Penicillium expansum*. J Food Protect76: 1879-1886.

49. Kaiserer L, Oberparleiter C, Weiler-Gorz R, Burgstaller W, Leiter E, Marx F (2003) Characterization of the *Penicillium chrysogenum* antifungal protein PAF. Arch Microbiol 180: 204-210.

50. Yang L, Xie J, Jiang D, Fu Y, Li G, Lin F (2008) Antifungal substances produced by *Penicillium oxalicum* strain PY-1 potential antibiotics against plant pathogenic fungi. W J Microbiol Biotech 24: 909-991.

51. Goyal S, Ramawat, KG, Mérillon JM (2016) Different shades of fungal metabolites: An overview. Fungal Metabolites, pp: 1-29.

52. Mohammed GJ, Kadhim MJ, Hussein HM (2016) Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. Int J Pharmacogn Phytochem Res 8: 889-905.

53. VermaA, Johri BN, Prakash A (2014) Antagonistic evaluation of bioactive metabolite from endophytic fungus, *Aspergillus flavipes* KF671231. J Mycology.

54. Abbas HK, Zablotowicz RM, Bruns HA, Abel CA (2006) Biocontrol of aflatoxin in corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. Biocont Sci Technol 16: 437-449.

55. Shemshura ON, BekmakhanovaNE, Mazunina MN, Meyer SLF, Rice CP, et al. (2016) Isolation and identification of nematode-antagonistic compounds from the fungus *Aspergillus candidus*. FEMS Microbiol Lett 363: 1-9.

56. Sreevidya M, Gopalakrishnan S (2016) *Penicillium citrinum* VFI-51 as biocontrol agent to control charcoal rot of sorghum (*Sorghum bicolor* L.). Afr J Microbiol Res 10: 669-674.

57. Pitt JJ (2002) Biology and ecology of toxigenic *Penicillium* species. Adv Exp Med Biol 504: 29-41.

58. Sreevidya M, Gopalakrishnan S, Melø TM, Simic N, Bruheim N, et al. (2015) Biological control of *Botrytis cinerea* and plant growth-promotion potential by *Penicillium citrinum* in chickpea (*Cicer arietinum* L.). Biocontrol Sci Tech 25: 739-755.