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Full Length Research Paper

Prevalence of extended spectrum β-lactamases in multidrug resistant strains of Gram-negative bacteria

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Extended spectrum beta-lactamases (ESBLs) are enzymes that are now becoming major sources of resistance to β-lactamase antibiotics more especially in enteric bacteria such as *Klebsiella pneumoniae*, *P. aeruginosa* and *Escherichia coli*. Increase in the frequency of the β-lactam resistance in bacteria has become a serious threat for treating bacterial infections caused by enterobacteriaceae. This study aims to determine the prevalence of ESBLs producers among enteric Gram negative bacteria isolated from patients in OAUTHC, Ile-Ife, Osun State. The subject used in this study involved patients presenting with evidence of hospital infections. The collected sputum specimens were processed for the isolation and identification of Gram negative bacilli. Resistance of the isolates to different antibiotics tested was determined using the standard disc diffusion method of Kirby-Bauer. Phenotypic and confirmatory test of the isolates were done for ESBL production using double disc synergy test (DDST) on Mueller Hinton agar according to CLSI 2013 guidelines. Results reveal that the highest occurrence of bacteria was recovered in *Klebsiella* species (35%), followed by *Enterobacter* sp. (13%), *P. aeruginosa* (10%), *Escherichia coli* and *Salmonella* sp. (7%) while the least occurs in *Proteus* sp. All the isolates were 100% resistance to class piperacillin, 76.6% to beta-lactamase inhibitor and 52% resistance to cephalosporins while they were susceptible to carbapenems, nitrofurans, and fluoroquinolones. The prevalence of ESBLs producing isolates was 51.1% while non-ESBL producing strains were 48.8%. Therefore, it can be concluded that extended spectrum beta lactamases are gradually increasing in Nigeria with co-resistance to some other classes of antibiotics which is very alarming. There is a limited number of drugs sensitivity for these bacteria and the drug of choice is imipenem, ciprofloxacin and ofloxacin.

Key words: Multidrug resistance, extended spectrum beta-lactamases (ESBLs), Gram negative bacteria.

INTRODUCTION

Extended spectrum β-lactamases (ESBLs) are beta-lactamases enzymes that are capable of conferring bacteria resistance to the penicillins, first, second and third generation cephalosporins, and aztreonam and those that do not confer resistance to the cephamycins or carbapenems groups of antibiotics. They confer resistance by hydrolysis of these antibiotics which are inhibited by β-lactamase inhibitors such as clavulanic

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acid, tazobactam or sulbactam, and they are encoded by genes that can be exchanged between bacteria (Paterson and Bonomo, 2005; Shaikh et al., 2015). The first ESBL was identified by Knothe et al. (1983) in a nosocomial *Klebsiella pneumoniae* strain isolated in Germany in 1983 since then over 500 variants of the clavulanic acid-inhibited form (TEM, CTX-M, SHV, OXA) have been described worldwide (Kiratisin et al., 2008). They are most prevalent in *Klebsiella* sp and their epidemiology reflects a mixture of mutations, plasmid transfer and or clonal spread (Livermore and Woodford, 2006). The most common ESBL phenotypes arise from point mutations in the *bla*TEM *,bla*SHV*,* or *bla*CTX genes resulting in alteration of the primary amino acid sequence of the enzyme (Bradford, 2001; Gniadkowski, 2001; Paterson and Bonomo, 2005; Paterson, 2006). In the past years there has been an increase in the incident of extended spectrum beta lactamase infections and those related to ESBL infections.

This study has been carried out to examine the occurrence of ESBLs among Gram negative isolates from patients with respiratory tract infections.

METHODS

The study was conducted on in-patients and out-patients attending Obafemi Awolowo University Teaching Hospital Complex (OAUTHC). Ethical approval was obtained from the Hospital Ethical and Research Committee of the institution. Sputum samples were collected aseptically from subjects between the period of January, 2015 and February, 2016. Sample size was determined using Cochran's formula of $N= Z^2 pq/d^2$ (Bartlett et al., 2001). In all, a total of 471 enteric bacteria isolates (Gram-negative) were collected altogether from both inpatients and outpatients. The morphological and conventional methods of the bacteria strains were done and confirmation of the bacteria was determined by biochemical test and with the use of API 20E kit. (API Bio Merieux, Germany). The resistance of isolates to different antibiotics was done using the method of Kirby-Bauer standard disc diffusion method.

Extended spectrum beta-lactamase production from the isolates were screened by using disc diffusion of cefotaxime (CTX) and ceftazidime (CAZ) placed on Muller Hinton agar plate seeded with the isolates according to the CLSI recommendations.

Phenotypic confirmatory test for ESBL positive strains were determined by double disc synergy test (DDST) for all the ESBL producing isolates using CLSI (2015) guidelines. Bacterial colony was re-suspended in nutrient broth to 0.5 McFarland standards and was inoculated directly on a Mueller-Hinton agar plates. Single discs containing ceftazidime with and cefotaxime was placed center to center, 25 mm apart to the amoxycillin/clavulanic acid (20/10 µg) disc on a lawn culture of the isolate on Mueller-Hinton agar plate. The plates were then incubated at 37°C for 18 - 24 h. An enhanced cleared zone of inhibition towards the amoxycillin/clavulanic acid (20/10 µg) disc showed positive ESBL production (Iroha et al., 2009).

RESULTS

Four hundred and seventy-one enteric Gram-negative bacteria which consist of thirty-two different species were recovered from patients with respiratory tract infection. They are *K. pneumoniae*, *Citrobacter freundii, Escherichia coli, Proteus mirabilis, Aeromonas salmonicida, Serratia liquefaciens* and *Salmonella arizonae*. Out of four hundred and seventy-one (471) isolates, 241 (51%) isolates produced extended spectrum beta-lactamase, while 230 (49%) were not extended spectrum beta-lactamase producers (Table 1). The ESBL producers are 51% while non-ESBL producing strain are 48.8% (Table 2). Most of the ESBL-producers were multiple drug resistant to piperacillin, augmentin, ceftazidime, cefoxtazime, cefuroxime, cefixime and beta lactam inhibitor, 95.6 and 96.4% of isolates were found sensitive to imipenem and ofloxacin respectively while other isolates showed resistance to piperacillin (100%) (Table 3). The multiple antibiotic resistance (MAR) phenotypes of bacterial isolates recovered from patients diagnosed with LRTI in Ile-Ife showed diversities of MAR patterns which occurred among the isolates (Table 4).

DISCUSSION

The antibiotic resistance among the respiratory pathogens is a major barrier that might interfere with an effective treatment. The emergence of multidrug resistant strains poses a major threat to the patients globally. Among the isolates, various mechanisms of antibiotic resistance have been attributed, among which production of beta-lactamases is a leading cause of resistance. Extensive use of broad spectrum antibiotics has further increased the multidrug resistance. Carbapenem-hydrolyzing beta-lactamases of Ambler class B (metalloenzymes), Ambler class D (oxacillinases) and extended-spectrum beta-lactamases (ESBLs) of Ambler class A are sources of multidrug resistance in Gram-negative isolates (Noyal et al., 2009).

In this study, two hundred and forty-one (51.1%) bacterial isolates were extended spectrum beta-lactamases producers. Sherchan et al. (2012) reported 20% ESBL production among the Gram-negative isolates which is in contrast to this present study. Statistically, there were no significant differences ($P>0.05$) among the ESBL producers and non-ESBL producers. The emergence of these ESBLs in Gram-negative bacilli is becoming a therapeutic challenge. Carbapenem group of antibiotics play a vital role in the management of Gram-negative infection, because of their broad spectrum activity and stability to hydrolysis by most of the beta-lactamase including ESBLs (Sherchan et al., 2012).

Another finding in this study was that the isolates showed low level of susceptibility to third generation cephalosporins such as cefotaxime, ceftriazone, and ceftazidime, that is, they were highly resistant to third generation cephalosporins. This may be due to the fact that many of the organisms are ESBL producers which are evident from these findings as they showed ESBL

Table 1. Percentage distribution of bacterial pathogens isolated.

Bacteria pathogens	Number of occurrence	Percentage
Klebsiella sp.	166	35.2
Enterobacter sp.	53	11.3
Proteus vulgaris	2	0.4
Pseudomonas aeruginosa	47	5.0
Escherichia coli	33	7.0
Providencia stuartii	4	0.8
Shigella sp.	28	5.9
Aeromonas sp.	29	6.1
Citrobacter sp.	37	7.8
Salmonella sp.	32	6.7
Serratia sp.	20	4.2
Yersinia sp.	10	2.3
Total number of isolates	**471**	**100**

Table 2. Frequency of ESBL producing bacteria isolates.

Bacterial isolates	No of isolates	ESBL positive (%)	ESBL negative (%)
Enterobacter sp.	63	18 (28.6)	45 (71.4)
Klebsiella sp.	166	102 (61.4)	64 (38.6)
Proteus sp .	2	2 (100)	0
P. aeruginosa	35	16 (34.0)	31 (66.0)
Escherichia coli	33	22 (66.7)	11 (33.3)
Serratia sp.	20	14 (70)	6 (30)
Aeromonas sp.	29	13 (44.8)	16 (55.2)
Citrobacter sp.	37	23 (62.2)	14 (37.8)
Salmonella sp.	32	13 (40.6)	19 (59.4)
Providencia sp.	4	0	4 (100)
Yersinia sp.	10	4 (40)	6 (60)
Shigella sp.	28	14 (50)	14 (50)
Total	**471**	**241 (51.1)**	**230 (48.8)**

Table 3. Antibiotic resistance among isolates cultured from sputum samples of patients diagnosed with RTI.

Classes of antibiotics tested	Specific antibiotics	Number of isolates resistant	% Resistance
Penicillins	Piperacillin	471	100
Cephalosporins (Cephems)	Cefoxtazime	208	44.1
	Cefoxitin	128	27.1
	Cefuroxime	176	38.0
	Cefixime	239	50.7
	Ceftazidime	178	37.7
	Augmentin	293	62.2
Beta-Lactamase Inhibitor	Amoxicillin-clavulanic acid	361	76.6
Carbapenems	Imipenem	21	4.4
Nitrofurans	Nitrofurantoin	75	15.9
Aminoglycosides	Gentamicin	58	12.3
Fluoroquinolones	Ciprofloxacin	20	4.24
	Ofloxacin	17	3.60

Table 4. Multiple antibiotics resistance profile of the isolates.

Multiple antibiotics resistance profile of the isolates	No of isolates	Overall (%)
Aug, Prl	25	25 (6.28)
Amc, Aug, Prl	233	
Amc, Gen, Prl	9	
Aug, Ofl, Prl	2	260 (65.3)
Aug, Nit, Prl	8	
Aug, Ipm, Prl	4	
Aug, Gen, Prl	4	
Amc, Aug, Gen, Prl	22	
Amc, Aug, Ipm, Prl	3	
Amc, Aug, Ofl, Prl	3	
Amc, Gen, Nit, Prl	1	86 (21.6)
Aug, Ipm, Nit, Prl	3	
Aug, Gen, Ofl, Prl	6	
Amc, Aug, Nit, Prl	48	
Aug, Gen, Nit, Ofl, Prl	1	
Amc, Aug, Ipm, Nit, Prl	12	
Amc, Aug, Gen, Nit, Prl	3	22 (5.5)
Amc, Aug, Gen, Ofl, Prl	5	
Amc, Aug, Ofl, Nit, Prl	1	
Amc, Aug, Gen, Nit, Ofl, Prl	1	
Amc, Aug, Ipm, Ofl, Nit, Prl	1	3 (0.75)
Amc, Aug, Ofl, Gen, Ipm, Prl	1	
Amc, Aug, Gen, Ipm, Nit, Ofl, Prl	1	1 (0.25)
Total number of MAR pattern = 23	**398**	**84.5**

production at a very high rate, especially in K*lebsiella* spp, *E. coli* and *P. aeruginosa*. Other reason may be that the third generation cephalosporins antibiotics have been misused for a long period by individuals, so that over this time pathogens have developed and become resistance. The misuse of broad spectrum antibiotics, insufficient hygiene, immunosuppression, and a prolonged stay in the hospital are some other major aetiological factors that enhance the chances of multi drug resistant infections (Manjunath et al., 2011). Studies from Indian and Nigeria reported high level of resistance (87 - 89%) and (84.8 - 96%) respectively to cephalosporins (Egbebi and Famurewa, 2011; Gupta et al., 2012). The decreased susceptibility of third generation cephalosporins could also be due to production of ESBL and AmpC beta-lactamases. In the present study, 23.4% isolates were sensitive to amoxicillin-clavulanic acid. Such low level of sensitivity has also been reported in other Indian studies stating 49.3% to as low as 9% (Gupta et al., 2012; Namratha et al., 2015).

Conclusion

The prevalence and incidence of respiratory infections has been on the increase side, which are caused by beta lactam resistant organisms and due to the production of different enzymes has increased recently. Detection of strains producing ESBL is of paramount importance in both hospital acquired and community acquired isolates. The close monitoring of this pathogens in laboratories is required to minimize the spread of these bacteria and help to select appropriate antibiotics to combat them. The detection of extended spectrum beta lactamase producing isolates in the study area are multidrug resistance; thus, there is a need for constant and careful surveillance for multidrug-resistant bacteria in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Investigation on biosuppression of Fusarium crown and root rot of tomato (*Solanum lycopersicum* L.) and growth promotion using fungi naturally associated to *Solanum linnaeanum* L.

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Fusarium crown and root rot (FCRR) is a serious tomato disease in Tunisia which is difficult to control due to its soilborne nature and to the luck of genetic resistance. In the current study, native *Solanum linnaeanum* was explored as potential source of effective fungal agents for disease biocontrol. Eight fungal isolates, recovered from *S. linnaeanum* plants growing in the Tunisian Centre-East and shown able to colonize roots, crowns and stems of tomato (*Solanum lycopersicum* L.) seedlings, were tested for their ability to inhibit *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), the causal agent of this disease, and to promote plant growth. Tomato seedlings inoculated or not with FORL and treated using tested fungal isolates, exhibited significant increments in their growth parameters. Tested as conidial suspensions or cell-free culture filtrates, I74 and I92 isolates were the most active leading to 92.8% decrease in FCRR severity and 89.3 to 95.2% lowered vascular browning extent as compared to FORL-inoculated and untreated controls. These two isolates were microscopically and macroscopically described and identified using rDNA sequencing gene as being *Penicillium crustosum* I74 (MF188258) and *Fusarium proliferatum* I92 (MF188256). Pathogen mycelial growth was inhibited by 29.4 to 78.1% using their conidial suspensions and by 67.5 to 82% with their cell-free culture filtrates. *P. crustosum* I74 and *F. proliferatum* I92 showed chitinolytic, proteolytic and amylase activities. Only I92 isolate exhibited a lipolytic activity. Our study clearly demonstrated that I74 and I92 isolates were promising candidates for suppressing FCRR severity and promoting tomato growth. Further investigations are required for elucidating their mechanisms of action involved in disease suppression and plant growth promotion.

Key words: Antifungal activity, associated fungi, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Solanum linnaeanum*, tomato growth.

INTRODUCTION

Fusarium crown and root rot (FCRR) is one of the most damaging tomato diseases worldwide infecting more severely roots and crowns (Rowe and Farley, 1977). The causal agent is a soilborne fungus named *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) (Jarvis and Shoemaker, 1978). Infection process leads to subsequent development of crown cankers, root rots, vascular discoloration, and severe yellowing and wilting of leaves. Severe disease outbreaks may lead to quick plant dieback and induce serious crop and yield losses (Hibar et al., 2006; Ozbay and Newman 2004; Can et al., 2004). This pathogen is difficult to suppress in soil due to its airborne dissemination to neighboring plants and to its long survival in soils as chlamydospores even in absence of host plants (Rowe and Farley, 1977). The limited effectiveness of chemical fungicides and the lack of resistance in the most commercially grown tomato cultivars led to increased focus in the search for other effective alternatives such as biological control. This control method is now increasingly considered as a key alternative for sustainable agriculture (Berg et al., 2017; Zheng et al., 2017). Different microbial agents were found to be efficient in controlling FORL such as non pathogenic *Fusarium oxysporum* (Alabouvette and Olivain, 2002), *Trichoderma harzianum* (Ozbay et al., 2004; Hibar et al., 2005), binucleate *Rhizoctonia solani* (Muslim et al., 2003) and *Fusarium equiseti* (Horinouchi et al., 2008). A significant decrease, by 50 to 73% in FORL radial growth, was achieved using some biofungicides and natural greenhouse conditions, Hibar et al. (2006) succeeded in decreasing disease FCRR incidence to 5.5% using biofungicide based *T. harzianun* strain T22.

In the last decades, plant-associated endophytic fungi were widely explored as effective antagonists and environmentally friendly tools for biocontrol of plant diseases (Staniek et al., 2008). These agents are able to grow within plant tissues for at least part of their life cycle without inducing any harmful effects to their hosts (Bacon and White, 2000). They are able to protect their associated host plants against various bio-aggressors and abiotic stresses (Backman and Sikora, 2008). In fact, such plant protection may be achieved by activation of its defense mechanisms (Kavroulakis et al., 2007) or by the inhibition of the pathogens, hence reducing the severity of incited diseases (Kuldeau and Bacon, 2008). These effects may be accomplished by various bioactive secondary metabolites including auxins (Vadassery et al., 2008) and indole derivatives (Strobel et al., 2004). and Sikora, 1995). In fact, the endophytic isolate of *F. oxysporum* strain Fo47, applied as root treatment, had significantly suppressed Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* (Aimé et al., 2013). Fakhro et al. (2010) noted 30% decrease in Verticillium wilt on tomato plants colonized by *Piriformospora indica*. *Penicillium* species EU0013 significantly decreased Fusarium wilt incidence (Alam et al., 2010) and *F. equiseti* GF191 successfully controlled FCRR disease by the secretion of antifungal compounds (Horinouchi et al., 2007). Endophytic *Fusarium solani* significantly limited root infection by FORL and subsequent disease development (Kavroulakis et al., 2007).

Moreover, some beneficial plant-associated endophytes could promote plant growth by increasing its nutrient uptake and/or by enhancing its tolerance to environmental stresses (Kuldeau and Bacon, 2008). Several investigations dealing with fungal endophytes have evidenced their plant growth-promoting potential (PGP) and biocontrol potency (Mahmoud and Narisawa, 2013; Bogner et al., 2016) due to their capacity to release growth hormones, abscisic acid (You et al., 2012) and plant-growth regulatory substances (Wiyakrutta et al., 2004).

Previous studies demonstrated that wild Solanaceae plants may be explored for isolation of biocontrol agents and extraction of biologically active compounds (Bhuvaneswari et al., 2013; Aydi Ben Abdallah et al., 2016). In this regard, Veira et al. (2012) demonstrated the biodiversity of fungal agents recovered from *Solanum cernuum* Vell and their strong antifungal potential. The endophytic fungus *Zygo Rhizopus* species isolated from *Solanum nigrum* displayed antibacterial activity (Sunkar and Nachiyar, 2011). Endophytic *Aspergillus ustus* isolated from *Solanum tuberosum* promoted growth and induced resistance against different pathogens in *Arabidopsis thaliana* (Marina et al., 2011).

Solanum linnaeanum L. (syn. *S. sodomaeum*) is a wild solanaceous species native to southern Africa and a common weed in Northern Africa and Southern Europe (Ono et al., 2006). This species is rich in alkaloids, steroids and saponins and glycoalkaloids (Elabbara, 2014) but not previously explored as potential source of isolation of potent endophytic fungi that may be used as biocontrol agents.

The present study aimed to isolate *S. linnaeanum* endophytes, evaluate their ability to suppress FCRR severity, to enhance tomato growth and to inhibit FORL *in vitro* growth.

To the best of the authors' knowledge, this is the first report on potential use of fungi naturally associated to *S. linnaeanum* for suppression of this disease and for the enhancement of tomato growth.

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MATERIALS AND METHODS

Aiming to search for potent biological control agents active against the tomato pathogen *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), the approach consists of the isolation of endophytic fungi from *S. linnaeanum* and to assess their capacity to colonize tomato seedlings. Selected endophytic fungi will be further investigated, using their conidial suspensions and cell-free culture filtrates, for their capacity to suppress disease and to enhance tomato growth.

Pathogen isolation and inoculum preparation

F. oxysporum f. sp. *radicis-lycopersici* (FORL) isolate used in the current work was originally recovered from tomato plants presenting characteristic symptoms of FCRR disease expressed as plant wilting, vascular discoloration, and severe crown and root rots. Pathogen isolate was gratefully provided by the Laboratory of Phytopathology of the Regional Research Centre on Horticulture and Organic Agriculture at Chott-Mariem, Sousse, Tunisia.

Before being used for antifungal bioassays, FORL isolate was grown at 25°C for 5 days on Potato Dextrose Agar (PDA) medium amended with streptomycin sulphate (300 mg/L).

For mass-production of inoculum, a mycelial plug (5 mm in diameter) of FORL, removed from 5-days-old cultures, was grown in Potato Dextrose Broth (PDB) and incubated for 5 to 7 days under continuous shaking at 150 rpm. The obtained conidial suspension extracted from liquid culture by filtration through sterile Whatman No. 1 filter paper to remove mycelium and the obtained conidial suspension was adjusted to 10^7 conidia/mL using a hemocytometer (Hibar el al., 2006; Mutawila et al., 2016).

Plant material preparation and growth conditions

Tomato cv. Rio Grande seeds were surface sterilized by immersion into 70% (v/v) ethanol for 2 min, then in 0.2% (v/v) sodium hypochlorite (NaOCl) for 3 min (Akaladious, 2015). They were rinsed several times with sterile distilled water (SDW) and sown in alveolus plates (7 × 7 cm) containing sterilized peat ^{TM} (Floragard VertriebsGmbH für gartenbau, Oldenburg). Seedlings were cultured under controlled conditions (24 to 26°C, 12-h photoperiod and 70% relative humidity) for about 28 days and watered regularly to avoid water stress. Seedlings at the two-true-leaf growth stage were used for all *in vivo* trials.

Wild plant material and isolation of associated fungi

Fresh and healthy *S. linnaeanum* leaves, stems, fruits and flowers were collected from Tunisian littoral, Monastir (latitude 35°42'32.4"N, longitude E10°49'19.9") in November 2013. Fresh materials were thoroughly washed under running tap water to eliminate any adhering soil particles.

Under aseptic conditions, five leaf, stem, fruit and flower samples were surface sterilized according to Kjer et al. (2009) protocol. Samples were immersed in 70% (v/v) ethanol for 1 min, then in 10% (v/v) sodium hypochlorite for 5 min, again in a 70% (v/v) ethanol for 30 s, and finally rinsed three times in SDW (3 min each). Sterility checks were performed for each sample to verify the efficiency of the disinfecting process. For these tests, 0.1 mL from the last rinse water was spread on solid PDA medium previously poured in Petri plates. Cultures were incubated 6 days and regularly checked for the presence of growing fungal colonies. Absence of such colonies is an indicator of the efficiency of the disinfecting process (Pimental et al., 2006). The surface-disinfected plant tissues were blotted dry on sterilized filter papers. They were transversely sectioned into pieces of 1 cm in length using a sterile razor blade, which were placed in Petri plates containing PDA. Ten pieces were plated out in each plate and three plates were used per each sample. Plates were incubated at 25°C and examined daily for any fungal growth emerging from the plated fragments. Once growing fungal colonies are observed, they were individually transferred to new PDA plates and incubated at 25°C. The collected fungal cultures were purified using the single-spore isolation technique and stored at 4°C or in 20% glycerol (v/v) at -20°C or in -20% until future use.

Morphology of developing pure colonies was examined and characterized and spores produced by each fungal isolate were observed microscopically to determine the taxonomic status of each isolate under magnification and used in the identification of the isolated endophytes. Fungal isolates recovered from *S. linnaeanum* species were divided into 13 different morphotypes. One isolate from each morphotype was selected for the screening of the endophytic colonization ability.

Preparation of conidial suspensions

Conidia of fungal isolates associated to *S. linnaeanum* were harvested from growing colonies and suspended in 100 mL PDB. Cultures were incubated at 25°C for 12 days under continuous shaking at 150 rpm (Xiao et al., 2013). Liquid cultures were filtered through Whatman No. 1 filter paper and the obtained conidial suspension was adjusted to 10^6 conidia/mL (Harman, 2004).

Preparation of cell-free culture filtrates

Fungal isolates were grown in PDB medium and incubated for 15 days at 28°C under continuous shaking at 150 rpm (Sharma et al., 2016). Obtained liquid cultures were filtered through Whatman No. 1 filter paper and filtrates were first centrifuged thrice for 10 min at 10,000 rpm then further sterilized by filtration through a 0.22 µm pore size filter (Zhang et al., 2014) before use.

Test of endophytic colonization ability

Collected fungal isolates were screened for their endophytic behavior and ability to colonize tomato tissues. In fact, for each individual treatment (each tested isolate), a group of five tomato roots cv. Rio Grande seedlings (at two-true-leaf stage) were dipped for 30 min into 25 mL of isolate conidial suspension (10^6 conidia/mL) (Bhat et al., 2003). Control seedlings were dipped in equal volume of SDW. Tomato seedlings were transferred to individual pots (12.5 × 14.5 cm) filled with commercialized peat and cultured at 20 to 25°C, with 70 to 85% relative humidity and a 12 h photoperiod during 60 days. To check their ability to colonize tomato tissues, tested fungal isolates were recovered from tomato roots, crowns and stems according to Hallmann et al. (2006) procedure. Plates were maintained at 25°C and examined daily for any growing fungal colonies. Colonies exhibiting similar morphological traits as the wild-type ones were selected and considered as endophytes.

The colonization frequency (F) was calculated according to Kumareson and Suryanarayanan (1998) formula as follows:

F (in %) = Number of segments colonized by the test fungus/Total number of segments plated × 100.

The percent of fungal colonization per target organ was arsine transformed before performing statistical analysis.

Assessment of FCRR suppression ability

Fungal colonies exhibiting macro-morphological diversity and re-

isolated onto PDA medium with a frequency exceeding 20% were picked separately onto PDA. Conidial suspensions and cell-free culture filtrates of eight fungal isolates were screened for their ability to suppress FCRR disease on tomato cv. Rio Grande under greenhouse conditions.

Tomato seedlings were transplanted into individual pots (12.5 × 14.5 cm) containing commercialized peat. The tested biological treatments were applied to seedlings as culture substrate drench with 20 mL of a conidial suspension (10^6 conidia/mL) or a cell-free supernatant prepared as detailed earlier. Inoculation was performed one week post-treatment as substrate drench with 20 mL of FORL conidial suspension (10^7 conidia/mL) (Horinouchi et al., 2007). Uninoculated control (negative control or NC) seedlings were watered with SDW only. Positive control (IC) plants were challenged with the same volume of FORL conidial suspension and watered with SDW.

All plants were cultured in a greenhouse at 20 to 25°C, with 70 to 85% relative humidity and a 12 h photoperiod. Five replicates of one seedling each were used for each individual treatment. The whole experiment was repeated two times. At 60 days post-inoculation with FORL (DPI), the parameters noted were disease severity, root length, shoot height, roots and shoot fresh weights and FORL re-isolation frequency (percentage of pathogen isolation from roots, collars and stems) on PDA.

FCRR severity was evaluated based on the above and below ground damage and on the vascular browning extent (from collar). Disease damage was assessed based on a 0 to 3 rating scale, where: 0= no symptoms and 3= dead seedlings (Vakalounakis and Fragkiadakis, 1999).

The frequency of FORL re-isolation from roots, collars and stems was calculated using the following formula (Moretti et al., 2008):

$$IR (\%) = r/R \times 100$$

where r = number of fragments showing pathogen growing colonies and R = total number of fragments plated on PDA medium.

Assessment of growth-promoting ability

Eight selected endophytic fungal isolates were screened *in vivo* for their ability to improve tomato growth using their conidial suspensions or their cell-free culture filtrates.

Biological treatments were performed by dipping roots of a group of five tomato cv. Rio Grande seedlings (at two-true-leaf growth stage) for 30 min into fungal conidial suspensions and another group into cell-free filtrates (Bhat et al., 2003; Saraf et al., 2017). Seedlings were transferred to individual pots (12.5 × 14.5 cm) containing commercialized peat. Control seedlings were similarly challenged using SDW. All seedlings (treated and controls) were grown under greenhouse conditions and regularly watered with tap water to avoid water stress. All treatments replicated five times and the whole experiment was repeated twice. At 60 days post-treatment, parameters noted were root length, shoot height and fresh weight of roots and shoots.

Assessment of the *in vitro* antifungal activity

Eight endophytic isolates were evaluated for their capacity to inhibit the *in vitro* growth of FORL using the dual culture technique. Two agar plugs (6 mm in diameter) one colonized by the pathogen (removed from a 5-days-old culture at 25°C) and a second by the test fungus (removed from a 7-days-old culture at 25°C) were deposed equidistantly 2 cm apart on PDA medium supplemented with streptomycin sulfate (300 mg/L) (Dennis, 1971). Three replicates of one plate each were considered for each individual treatment and the whole experiment was repeated twice. Control plates were inoculated by only one FORL plug. Cultures were incubated at 25°C for 5 to 6 days. Mean diameter (cm) of FORL colony was recorded when pathogen reached the center of control plates. Growth inhibition percentage of FORL was calculated according to the following (Kaewchai, 2010) formula:

$$\text{Growth inhibition } (\%) = [(dc - dt)/ dc] \times 100$$

where dc = mean colony diameter in control plates; dt = mean colony diameter in treated plates.

Assessment of the *in vitro* antifungal activity of cell-free culture filtrates

Five fungal isolates were chosen based on their ability to suppress FCRR disease severity by more than 50% over control and to reduce FORL mycelial growth by more than 60%. The selected isolates were grown on PDB medium. Cultures were incubated under continuous shaking at 150 rpm at 25°C for 30 days (Xiao et al., 2013).

A 2 mL-sample of each tested culture filtrate was centrifuged thrice at 10,000 rpm for 10 min. Collected supernatant fluids were sterilized by filtration through a 0.22 µm pore size filter. Control treatment was the PDB filtrate. Filtrates were added at the concentration of 10% (v/v) aseptically to Petri plates containing molten PDA medium amended with streptomycin sulfate (300 mg/mL) (w/v). After medium solidification, three 6 mm agar plugs colonized by FORL were placed equidistantly in each Petri plate. Three replicate plates for each tested treatment were used and all the experiment was repeated twice. Cultures were incubated at 25°C for 5 days. The diameter of pathogen colony (in treated and control plates) was measured and the pathogen growth inhibition rate was calculated as described earlier.

Identification of the best antagonistic and plant growth promoting fungal isolates

The genomic DNA extraction of the four selected fungal isolates was performed using the DNA Mini Kit (Analytik Jena, Biometra) according to manufacturer instructions. For each test fungus, the ITS region, the widely used for general fungal identification (White et al., 1990), was amplified by polymerase chain reaction (PCR) using both universal fungal primers: ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The PCR reaction was performed in a total reaction volume of 25 µl containing 5 µl of buffer (5×), 2.5 µl of dNTP (2 mM), 1.5 µl of $MgCl_2$ (25 mM), 0.25 µl Taq polymerase (5 U/µl), 2.5 µl of each primer (6 µM), 5.75 µl of ultra-pure water and 5 µl of genomic DNA templates (10 ng).

The amplification program, performed in an OpticonII (Biorad) Thermal Cycle, included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min. Amplification was terminated by a final extension step of 7 min at 72°C. The obtained PCR products were electrophoresed in agarose gel 1% (w/v) stained with ethidium bromide, and visualized under UV light. Gene sequencing was carried out in a private laboratory (Biotools, Tunisia). ITS sequences were analyzed with Basic Local Alignment Search Tool (BLAST) through GenBank (http://www. blast.ncbi.nlm. nih.gov/).

Enzymatic activity displayed by the best antagonistic and plant growth promoting isolates

The most effective fungi (I74 and I92 isolates) in suppressing FCRR

Table 1. Fungal isolates from *Solanum linnaeanum* leaves, stems, flowers and fruits on PDA medium and their relative isolation frequency.

Identification	Leaf		Stem		Flower		Fruit		F Total (%)
	N	F (%)	N	F (%)	N	F (%)	N	F (%)	
Fusarium	4	3.33	5	4.17	3	2.5	2	1.67	11.7
Alternaria	3	2.50	2	1.67	3	2.5	4	3.33	10.0
Penicillium	4	3.33	7	5.83	4	3.33	3	2.50	15.0
Aspergillus niger	4	3.33	4	3.33	1	0.83	1	0.83	8.3
Aspergillus flavus	2	1.67	3	2.50	3	2.5	1	0.83	7.5
Aspergillus nidulans	1	0.83	2	1.67	1	0.83	0	0.00	3.3
Trichoderma	1	0.83	2	1.67	3	2.5	2	1.67	6.7
N Total	19	-	25	-	18	-	13	-	-
F Total	25.33	-	33.33	-	24	-	17.33	-	100

N: Number of isolates; F: isolation frequency (%).

disease were screened for their ability to produce extracellular enzymes (namely amylases, lipases, proteases, and chitinases) using qualitative techniques as described subsequently. All assays were carried out in triplicates.

Amylase activity

Amylase activity was tested by growing fungal isolates on Glucose Yeast Extract Peptone Agar (GYEP) medium amended with 0.2 g starch. After incubation at 25°C for 4 days, plates were flooded with 1% iodine in 2% potassium iodide and the formation of white zones around colonies, induced by the digestion of starch added to medium, indicated a positive reaction (Sunitha et al., 2013).

Lipolytic activity

For lipase activity, fungal isolates were grown on Peptone Agar (PA) medium amended with sterilized tween 20 diluted at 1% v/v. Plates were incubated at 25°C for 3 to 7 days. The presence of a visible precipitate around the colony, due to the formation of calcium salts of the lauric acid released by the enzyme, indicated a positive lipase activity (Sunitha et al., 2013).

Proteolytic activity

For protease activity, 10-day-old grown fungal agar plugs (3 mm in diameter) were spot inoculated on Casein Starch Agar with 1% skimmed milk and incubated at 25°C for 96 h. After incubation, the formation of clear halos around fungal colonies indicated a positive proteolytic activity (Alecrim et al., 2017).

Chitinolytic activity

Chitinase activity was tested by inoculating fungal plugs on chitin-based medium (Sharaf et al., 2012). Cultures were maintained at 25 ± 2°C for 10 days. Isolates displaying chitinolytic activity grew on the medium (Okay et al., 2008).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0. Each experiment was repeated twice. Data were analyzed according to a completely randomized design. Means were separated using LSD or Duncan Multiple Range tests (at $p < 0.05$).

RESULTS

Endophytic fungi isolation frequency and diversity

Data given in Table 1 revealed that a total of 75 fungal isolates were recovered from *S. linnaeanum* leaves, stems, flowers and fruits. There was a difference in the isolation frequency of isolates depending on plant parts explored. In fact, 19 isolates (25.3% of the total collected) were originated from leaves, 25 (33.3%) from stems, 18 (24%) from flowers and 13 (17.3%) from fruits. Interestingly, a macroscopic variability was noticed between the 75 collected fungal isolates. They were affiliated to 5 genera, namely *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Trichoderma* based on their macro- and micro-morphological traits.

It should be highlighted that *Aspergillus* was the mostly isolated genus (19.1%). The isolation frequency of *Penicillium, Fusarium*, *Alternaria*, and *Trichoderma* were 15, 11.6, 10, and 6.7%, respectively (Table 1).

Endophytic colonization ability

Based on the colony characteristics and morphology, the 75 fungal isolates recovered from *S. linnaeanum* species were divided into 13 different morphotypes. One isolate from each morphotype was selected for endophytic colonization screening. Results revealed that all treated plants remained healthy until the end of the experiment. The thirteen isolates tested were found to be non-pathogenic and were selected for further screenings.

ANOVA analysis revealed that tomato colonization

Table 2. Re-isolation frequency (%) of endophytic fungal isolates from tomato cv. Rio Grande roots, crowns and stems noted 60 days post-inoculation.

Isolate	Roots	Crowns	Stems
NC	20^{e}	13.33^{e}	10.0^{d}
I71	66.67^{b}	63.33^{b}	53.33^{b}
I72	16.67^{e}	10^{e}	6.67^{d}
I74	83.33^{a}	73.33^{a}	66.67^{a}
I75	56.67^{c}	50^{c}	50^{b}
I78	13.33^{e}	6.67^{e}	6.67^{d}
I81	10^{e}	6.67^{e}	3.33^{d}
I83	66.67^{b}	63.33^{b}	53.33^{b}
I84	10^{e}	6.67^{e}	6.67^{d}
I85	56.67^{c}	50^{c}	50^{b}
I87	13.33^{e}	10^{e}	13.33^{cd}
I90	36.67^{d}	26.67^{d}	23.33^{c}
I92	73.33^{b}	68.75^{b}	56.67^{b}
I93	33.33^{d}	27.02^{d}	23.33^{c}

NC: Untreated control; I71, I75: isolates from flowers; I74, I92: isolates from leaves; I83, I90: isolates from stems; and I85, I93: isolates from fruits.

frequency, noted 60 days post-treatment, depended significantly (at $p < 0.05$) upon fungal treatments tested. Data shown in Table 2 showed that colonization frequency ranged between 10 and 83.3% from roots, between 10 and 73.3% from crowns, and between 3.3 and 66.6% from stems. The highest colonization frequencies from roots, crowns and stems (83.3, 73.3 and 66.6%, respectively) were noted on plants treated with I74 isolate. I71, I83 and I92 isolates had successfully colonized tomato plants where their respective colonization frequencies were estimated at 66.6 to 73.3, 63.3 to 68.7 and 53.3 to 56.6%, from roots, crowns and stems. The lowest colonization ability was expressed by I72, I78, I81, I84 and I87 isolates where the frequency noted varied from 3.3 to 16.6%.

Fungal isolates inoculated to tomato seedlings, successfully re-isolated onto PDA medium with a frequency exceeding 20% and showing similar traits as the wild type ones were classified as endophytes. Thus, 8 isolates out of the 13 tested and fulfilling the earlier mentioned conditions (namely I71, I74, I75, I83, I85, I90, I92 and I93) were selected for the *in vivo* screening of their antifungal activity against FORL and their plant growth-promoting effects.

Effect of endophytic fungal isolates on FCRR severity

Suppressive potential of conidial suspensions

ANOVA analysis revealed that FCRR severity, based on above- and below-ground damage and noted on tomato plants 60 days post-inoculation with FORL, varied significantly (at $p < 0.05$) depending on biological treatments. Data given in Figure 1A (a) showed that six out of the eight isolates tested had significantly decreased in disease severity by 50 to 92.8% relative to pathogen-inoculated and untreated control. I74- and I92-based treatments were found to be the most effective in suppressing FCRR severity by 92.8% on tomato plants challenged with FORL as compared to control. Moreover, I71, I75, I83, and I85 isolates exhibited significantly similar ability to decrease FCRR severity, by 50 to 64.2% as compared to control and by 40% relative to hymexazol-treated control (or FC).

Also, as shown in Figure 1A (b), the vascular discoloration extent (from collar) was lowered by 21.3 to 90.2% as compared to infected control following treatments using conidial suspensions of tested isolates. Similarly, I74- and I92-based treatments were found to be the most efficient in suppressing the vascular discoloration extent by 89.8% versus control. Also, interestingly, I71, I83 and I85 isolates had lowered this parameter by 51.4 to 59.2% relative to FORL-inoculated and untreated control and by 31.8% compared to hymexazol. Re-isolation frequency of FORL onto PDA medium from roots, crowns and stems of treated tomato plants varied depending on tested biological treatments. Data given in Figure 1A (c) showed a reduction in FORL re-isolation frequency by 23.3 to 56.6, 10 to 70 and 41 to 79.3% from roots, crowns and stems, respectively, as compared to FORL-inoculated and untreated control (96.6 to 100%).

Suppressive potential of cell-free culture filtrates

The suppressive potential of cell-free culture filtrates,

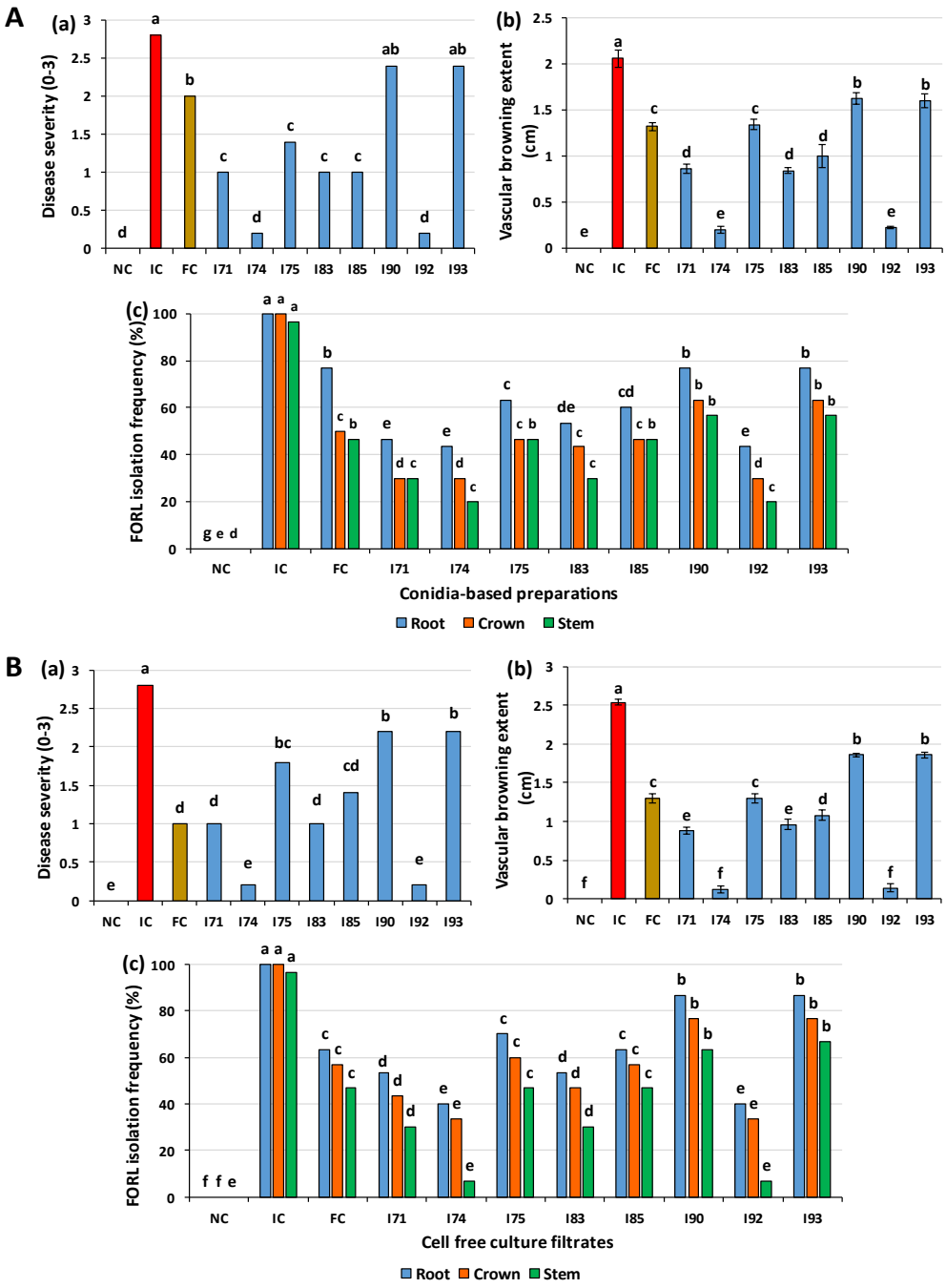

Figure 1. Effects of endophytic fungal isolates recovered from *Solanum linnaeanum* (A) and their cell-free culture filtrates (B) on Fusarium Crown and Root Rot severity and pathogen re-isolation frequency, as compared to controls, noted 60 days post-inoculation. NC: Negative control: Uninoculated and untreated. IC: Positive control: Inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and untreated. FC: Inoculated with FORL and treated with hymexazol-based fungicide; I71, I75: Isolates from flowers; I74, I92: Isolates from leaves; I83, I90: Isolates from stems; and I85, I93: Isolates from fruits. FORL isolation was performed on PDA medium and the frequency was noted after 60 days of incubation at 25°C. Bars sharing the same letter are not significantly different according to Duncan Multiple Range test at $p < 0.05$.

noted 60 days post-inoculation with FORL, varied significantly (at $p < 0.05$) depending on tested isolates. Results presented in Figure 1B (a) showed a significant (at $p < 0.05$) decrease in FCRR severity, based on leaf and root damage intensity, ranging between 21.4 and 92.8% compared to FORL-inoculated and untreated

control. Interestingly, cell-free filtrates of I74 and I92 were found to be the most efficient treatments by suppressing FCRR symptoms, by 92.8% relative to control, more efficiently than the reference fungicide (hymexazol) (64.2%). Data shown in Figure 1B (b) revealed that FCRR severity, as estimated based on the vascular discoloration extent, was significantly (at $p < 0.05$) reduced by 26.7 to 95.2% compared to FORL-inoculated and untreated control. Cell-free culture filtrates from I74 and I92 isolates were found to be the most effective in reducing this parameter by 94.4 to 95.2%. Treatments with I71, I83 and I85 filtrates were more efficient than hymexazol where the decrease in the vascular browning extent ranged between 57.4 and 68.3%, as compared to control. Pathogen re-isolation frequency onto PDA medium from treated tomato plants also varied depending on tested cell-free filtrates. Figure 1B (c), showed 13 to 60, 23.3 to 66.6, and 34.4 to 82.7% decrease in FORL re-isolation frequency from tomato roots, crown and stems, respectively, compared to control (96.6 to 100%), following treatments with filtrates of tested isolates.

Growth-promoting effect of endophytic fungal isolates on FORL-inoculated tomato plants

Plant growth-promoting ability of conidial suspensions

ANOVA analysis revealed a significant variation (at $p < 0.05$) in all tomato growth parameters (root length, plant height, root and aerial part fresh weights), noted 60 days post-inoculation, depending on biological treatments tested. In fact, as shown in Figure 2A (a), a significant enhancement in tomato root length, by 29.8 to 90.4% as compared to FORL-inoculated and treated control, was noted depending on treatments tested. Plant treatment using I74 conidial suspensions led to the highest increase (by 90.4% over control) in root length. Interestingly, I71, I83 and I92 isolates significantly improved this parameter by 48.8 to 50% over hymexazol-treated control.

Results given in Figure 2A (b) showed a variable ability to increase the root fresh weight depending on biological treatments tested. The highest increment (of about 88 to 91%) was recorded on plants treated with I74 and I92 conidial suspensions. Treatments with I71 and I83 conidial suspensions led to 36.5 to 40.2% increase in this parameter over hymexazol-treated control.

Data graphed in Figure 2A (c) showed that all tested biological treatments significantly improved shoot height by 34.7 to 90.3% versus FORL-inoculated and untreated control and by 6.7 to 50.7% over pathogen-free controls. The highest shoot height increments (90.3 and 85.5%) were recorded on plants treated with I74 and I71 conidial suspensions, respectively.

Figure 2A (d) illustrates the significant (at $p < 0.05$) increments in the shoot fresh weight noted using all tested biological treatments as compared to FORL-inoculated (31.4 to 91%) or to pathogen-free and untreated control plants (8.6 to 58%). The highest improvement of shoot fresh weight (by 91%) was recorded on plants treated with I74 conidial suspension. It should also be highlighted that all fungal treatments tested improved shoot weight by 0.4 to 35.2% relative to fungicide-treated control.

Plant growth-promoting ability of the cell-free culture filtrates

Growth parameters (root length, shoot height, roots and shoot fresh weights), noted on tomato seedlings 60 days post-inoculation with FORL, varied significantly depending on tested biological treatments.

All fungal cell-free culture filtrates tested significantly (at $p < 0.05$) improved root length of FORL-inoculated and treated tomato seedlings by 33.6 to 92.9% as compared to the untreated control and by 14.39 to 65.15% over pathogen-free ones (Figure 2B (a)). The highest increment in this parameter (92.9%) was induced by I74 filtrate. All tested treatments induced significant improvement of root extent by 2.7 to 48.2% relative to FORL-inoculated and fungicide-treated control (FC).

Data given in Figure 2B (b) showed that all tested cell-free culture filtrates significantly (at $p < 0.05$) improved root fresh weight over controls. Increments of this parameter, compared to FORL-inoculated control, ranged between 35.3 and 90.7% and the highest one was recorded on tomato plants treated with I74 filtrate. All tested filtrates, except those from I90 and I93 isolates, increased root fresh weight by 24.7 to 45.8% over FORL-inoculated and hymexazol-treated control and over pathogen-free control.

Results presented in Figure 2B (c) revealed that all tested filtrates significantly (at $p < 0.05$) improved by 29.4 to 92.6% the height of tomato shoots, as compared to FORL-inoculated and untreated control. I74 filtrate was found to be the most effective treatment leading to in 92.64% increase in shoot height. Moreover, an improvement by 86.7% was achieved using I92 filtrate as compared to pathogen inoculated and untreated control. All tested filtrates had significantly increased this parameter by 0.5 to 49.7% and by 6 to 57.8% over FORL-inoculated and fungicide-treated control (FC) and versus pathogen-free control, respectively.

Data given in Figure 2B (d) showed that all cell-freefiltrates tested significantly (at $p < 0.05$) increased shoot fresh weight as compared to pathogen-inoculated and untreated control. The highest improvement (94.8%) was achieved using I74 filtrate. More interestingly, all tested filtrates, except those from I90 and I93 isolates, even promoted shoot fresh weight in treated tomato plants by 6.6 to 43.3 and 10.7 to 49% as compared to FORL-inoculated and hymexazol-treated control and to pathogen-free control, respectively.

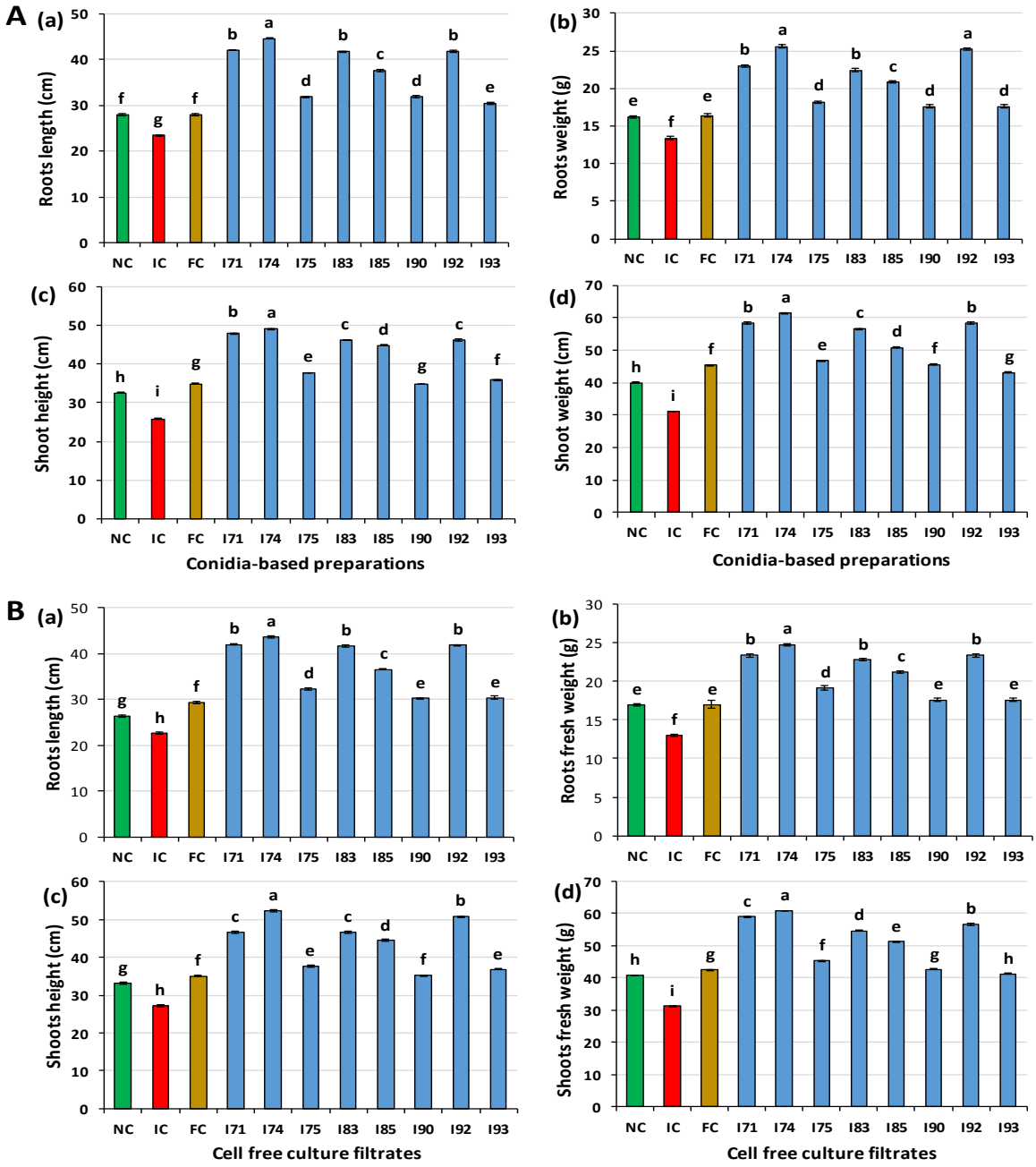

Figure 2. Effects of conidia-based preparations (A) and cell-free culture filtrates (B) from endophytic fungal isolates recovered from *Solanum linnaeanum* on tomato growth parameters noted 60 days post-inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici* as compared to controls. NC: Negative control: Uninoculated and untreated control. IC: Positive control: Inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and untreated. FC: Inoculated with FORL and treated with hymexazol-based fungicide; I71, I75: Isolates from flowers; I74, I92: Isolates from leaves; I83, I90: Isolates from stems; and I85, I93: Isolates from fruits. Bars sharing the same letter are not significantly different according to Duncan Multiple Range test at $p < 0.05$.

Tomato growth-promoting ability of endophytic fungal isolates on pathogen-free plants

The eight fungal isolates tested did not induce any disease symptoms when inoculated to tomato plants which remained healthy till 60 days post-inoculation. As they were found to be non pathogenic, their conidial suspensions and their cell-free culture filtrates were further screened for their ability to promote growth of pathogen-free tomato plants.

Growth-promoting effects of fungal conidial suspensions

ANOVA analysis revealed that plant growth parameters (root length, root fresh weight, shoot height, and shoot fresh weight), noted 60 days post-treatments, varied significantly (at $p < 0.05$) depending on tested biological treatments. Data given in Figure 3A (a) revealed that the maximum improvement in tomato root length, estimated at 85.2 to 91.8% over pathogen-free and untreated control, was achieved following treatments using I71, I74 and I92 conidial suspensions. As measured based on root fresh weight, treatments with I74 and I92-conidial suspensions led to the highest increase in this parameter (83.8%) (Figure 3A (b)). Results graphed in Figure 3A (c) showed that all conidial suspensions tested had significantly enhanced shoot height as compared to pathogen-free and untreated control. The greatest increase was achieved using I74 conidial suspension. Furthermore, treatments based on I71, I83 and I92 conidia had significantly similar effect on this parameter where the recorded promotion varied between 64 and 67.5%. Data given in Figure 3A (d) revealed that shoot weight increase achieved following biological treatments ranged between 40 and 84% as compared to untreated control where I74- and I92-based treatments were the most effective leading to 82.6 to 84% increase in this parameter. Interestingly, I71 and I83 conidialsuspensions had significantly improved shoot weight by 69.3% over control.

Growth-promoting effects of cell-free culture filtrates

ANOVA analysis revealed a significant (at $p < 0.05$) variation in tomato growth parameters (root length, root fresh weight, shoot height, and shoot fresh weight), noted 60 days post-treatments, depending on cell-free filtrates tested. As shown in Figure 3B (a), a significant increase in root length, by 18 to 76.27% over control, was induced by all tested filtrates. The highest enhancement of root length, by 72.6 to 76.2% as compared to pathogen-free control, was achieved using I74 and I92 filtrates. As estimated based on root fresh weight, I74 and I92 filtrates induced the highest increase in this parameter by 87.8% followed by those from I71 and I83 isolates (Figure 3B (b)). The least increase (by 17.7% over control) in root fresh weight was induced by treatments with I90 and I93 filtrates. Results presented in Figure 3B (c) showed that all fungal treatments tested had significantly (at $p < 0.05$) increased shoot height by 20.6 to 61.9% relative to the untreated control. The highest enhancement, by 59.3 to 61.9% versus control, was induced by I74 and I92 filtrates. Interestingly, I71 and I83 filtrates had significantly improved this growth parameter by about 46.4 to 47%. Data provided in Figure 3B (d) revealed that all filtrates tested had significantly (at $p < 0.05$) enhanced shoot fresh weight by 13.7 to 85.3% in treated plants as compared to the untreated control ones. Treatments with I74 and I92 filtrates were found to be the most effective in enhancing this parameter by 80.7 to 85.3% over control. Importantly, I71 and I83 filtrates had also improved shoot growth by about 60.5 to 61.4%.

***In vitro* antifungal activity of endophytic fungal isolates toward FORL**

ANOVA analysis revealed a significant (at $p < 0.05$) decrease in FORL colony diameter, noted after 5 days of incubation at 25°C, depending on biological treatments tested as compared to the untreated control. As shown in Figure 4(a), the reduction in FORL mycelial growth varied from 29.4 to 78.1% depending on treatments. The highest inhibition, of about 77.2 to 78.1% versus control, was achieved using I74 and I92 isolates (Figure 5A). I71-, I83- and I85-based treatments had suppressed FORL radial growth by 60 to 62.3%.

***In vitro* antifungal activity of cell-free culture filtrates toward FORL**

Five selected fungal isolates were screened for their *in vitro* antifungal activity against FORL using their cell-free culture filtrates. They were chosen based on their ability to suppress FCRR disease severity by more than 50% over control and to reduce FORL mycelial growth by more than 60%.

ANOVA analysis revealed a significant (at $p < 0.05$) variation in FORL mycelial growth depending on cell- free filtrates tested. In fact, Figure 4(b) showed that the highest decrease in FORL mycelial growth, by about 81.2% versus control, recorded using I74 and I92 filtrates. Thus, the most effective antifungal metabolites against FORL were found to be those from I74 and I92 isolates (Figure 5B).

Characterization of the two best antagonistic and plant growth-promoting fungal isolates

Morphological characterization

Colonies of the most bioactive fungal isolates (namely I74 and I92) were morphologically characterized based on colony appearances, mycelial textures and pigmentations on PDA medium at 25°C. Macroscopically, colonies of I74 isolate showed a rapid growth (about 6 to 7 mm/d), the surface texture is velvety to powdery. Colonies are initially white becoming green to blue green after 3 to 4 days of incubation. The plate reverse color is white to yellowish. As for micro, its morphological traits, hyphae are septate and hyaline. Conidiophores are simple or branched. Phialides are grouped in brush-like

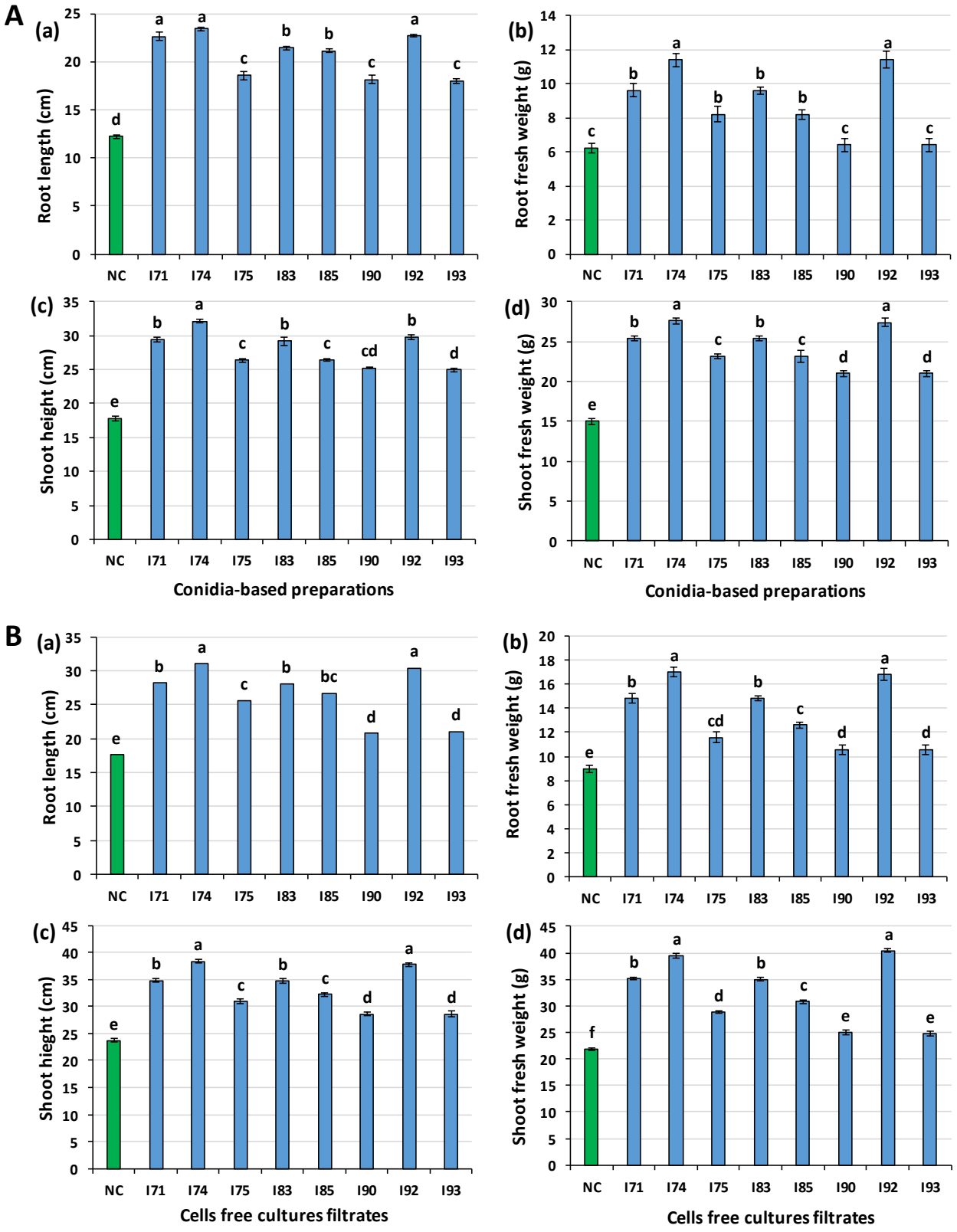

Figure 3. Comparative plant growth-promoting ability of conidial suspensions (A) and cell-free culture filtrates (B) of endophytic fungal isolates recovered from *Solanum linnaeanum* noted on tomato cv. Rio Grande plants 60 days post-treatment. NC: Untreated control; I71, I75: Isolates from flowers; I74, I92: Isolates from leaves; I83, I90: Isolates from stems; and I85, I93: Isolates from fruits. Bars sharing the same letter are not significantly different according to Duncan Multiple Range test at $p < 0.05$.

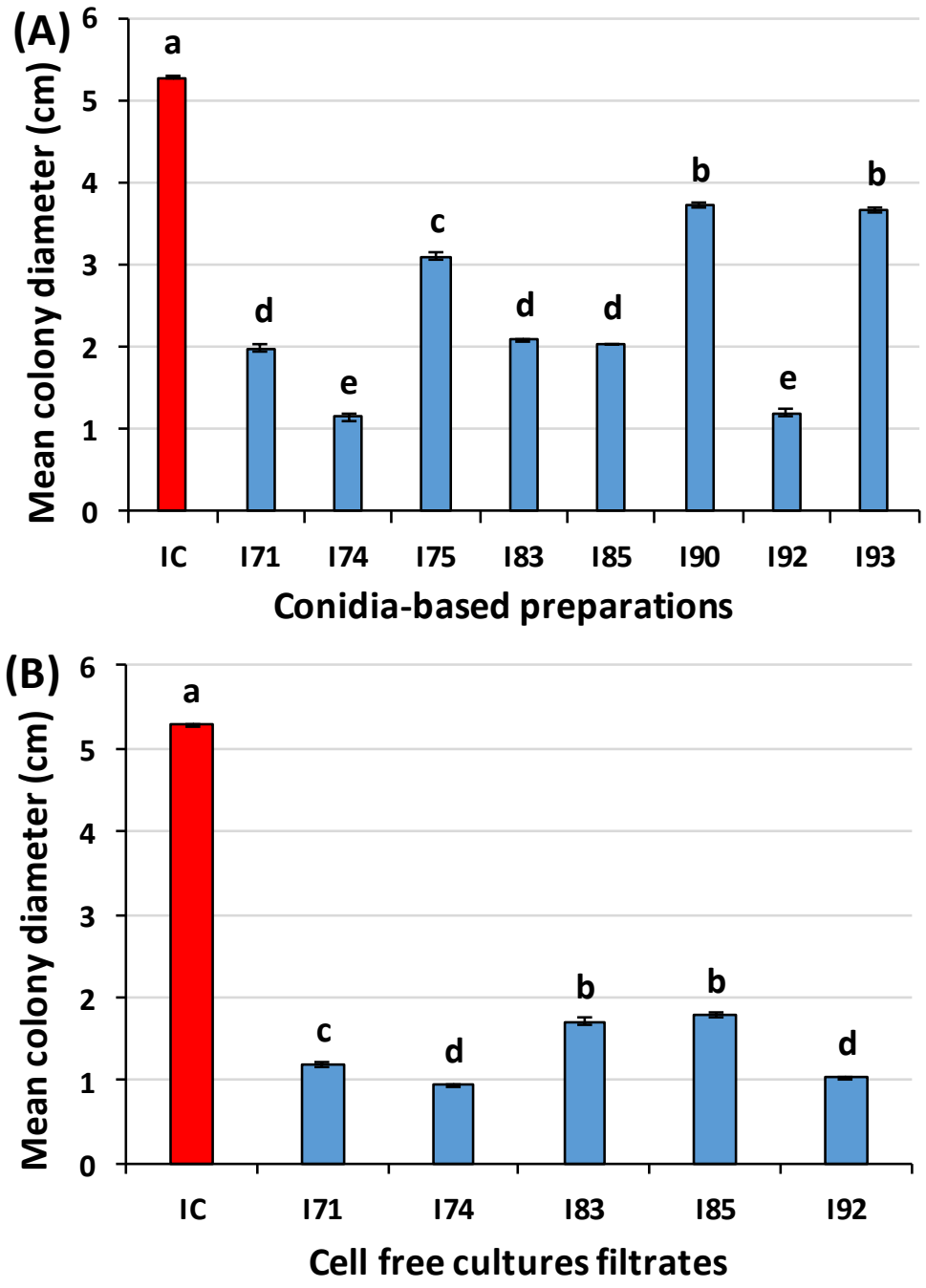

Figure 4. Antifungal activity of endophytic fungal isolates recovered from *Solanum linnaeanum* (A) and their cell-free culture filtrates (B) toward *Fusarium oxysporum* f. sp. *radicis-lycopersici* noted after 5 days of incubation at 25°C compared to control. IC: Untreated control; I71, I75: Isolates from flowers; I74, I92: Isolates from leaves; I83, I90: Isolates from stems; and I85, I93: Isolates from fruits. Bars sharing the same letter are not significantly different according to Duncan Multiple Range test at $p < 0.05$.

clusters (penicilli) at the ends of the conidiophores. Conidia are unicellular, round to ovoid, hyaline or pigmented, rough walled or smooth, in chains. Dimension of conidia is of about 3.5 to 4.2 µm (Figure 6).

PDA culture of I92 isolate showed abundant aerial mycelia that are initially white in color and later change to violet-purple. Monophialides and polyphialides produce conidia in chains. The macroconidia have 1 to 3 septa, a slightly curved apical cell and a scarcely developed base cell. The size of macroconidia was of about 17.3 to

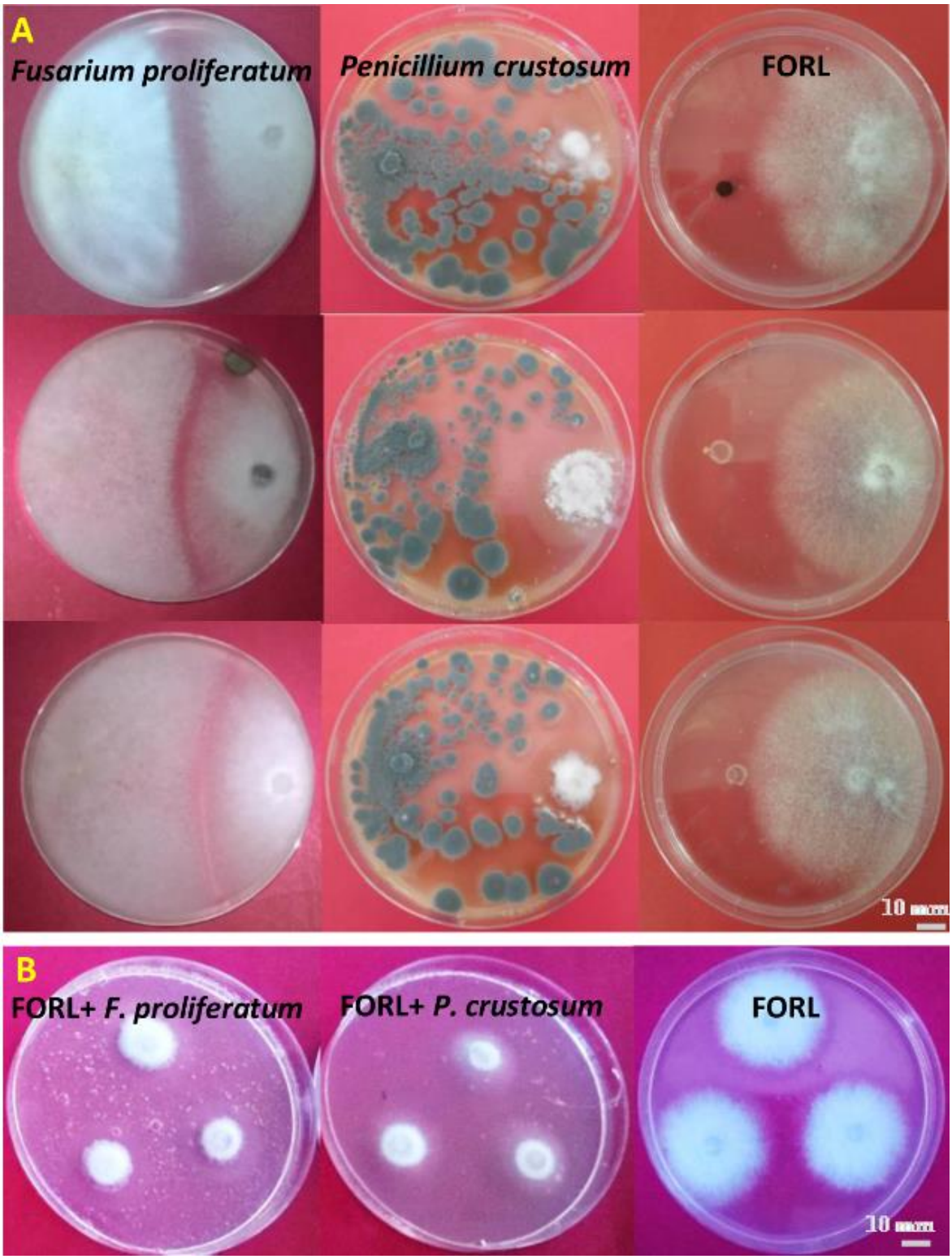

Figure 5. Inhibition of *Fusarium oxysporum* f. sp. *radicis-lycopersici* mycelial growth when dual cultured with some endophytic fungal isolates recovered from *Solanum linnaeanum* (A) or grown on PDA amended with 1 mL of their cell-free culture filtrates (B) noted after 5 days of incubation at 25°C.

38.1 × 1.9 to 3.1 µm. The microconidia are oval in form, with a flat base and no septa (Figure 6). The size of

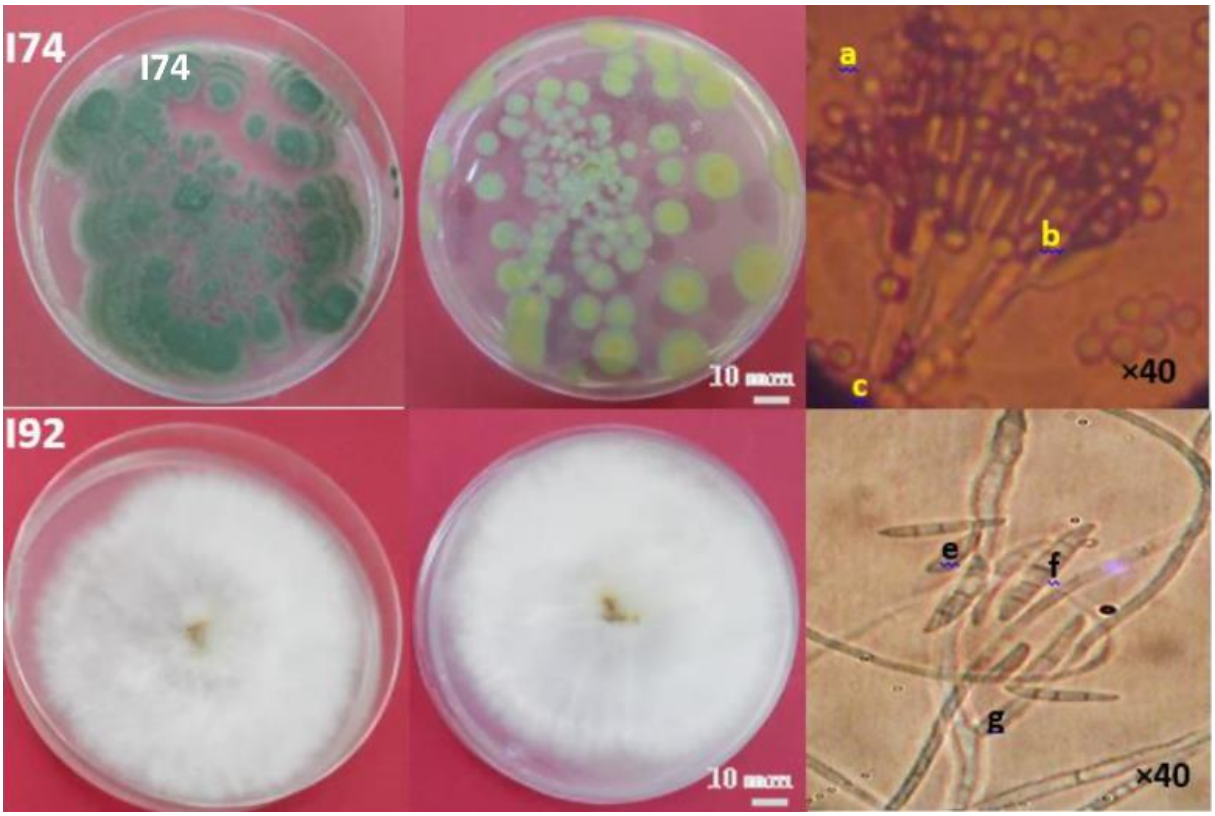

Figure 6. Macroscopic and microscopic features of the most bioactive isolates (I74 and I92) recovered from *Solanum linnaeanum* and grown on PDA medium for 7 days at 25°C. a: Conida; b: Phalides; c: Conidiophore e: Microconidia; f: Macroconidia g: Monophialide.

microconidia was of about 2.4 to 11.9 × 1.2 to 3.8 µm.

Molecular identification

The electrophoresis of PCR products of genomic DNA samples on 1.0% (w/v) agarose gel using a 100 bp size marker as a reference, showed bands of 600 bp for each fungus. Blast analysis of sequenced rDNA gene homology and the phylogenetic analysis based on neighbor-joining (NJ) method with 1000 bootstrap sampling revealed that the isolate I74 belonged to the genus *Penicillium* with 100% of similarity with *Penicillium crustosum* (MF188258) (Table 3 and Figure 7A). Blast analysis of sequenced rDNA gene homology and the phylogenetic analysis based on neighbor-joining (NJ) method with 1000 bootstrap sampling revealed that the isolate I92 belonged to the genus *Fusarium* with 100% of similarity with *Fusarium proliferatum* (MF188256) (Table 3 and Figure 7B). The nucleotide sequences used of representative isolates were obtained from Genbank database under the following accession numbers: (A) KP216913 (*P. crustosum* isolate S5-Z-3-14), KP216901 (*P. crustosum* isolate S4-Z-3-20), KU527788 (*Penicillium commune* isolate MC-11-L), KT876718 (*Penicillium expansum* isolate A1-4), KP857656 (*Penicillium* spp. isolate AQG11), AY425983 (*Penicillium griseoroseum* isolate VIC), KX243323 (*Penicillium griseofulvum* isolate 2159A), AF527057 (*Penicillium farinosum*), KX243331 (*Penicillium solitum* isolate RS1), (B) KM013437 (*F. proliferatum* isolate SWUKJ1.1120), JQ846048 (*Gibberella intermedia* isolate 5439), KX065004 (*Fusarium fujikuroi* isolate Zbf-S13), KT351610 (*Fusarium* spp. isolate T11), KC817122 (*Fusarium chlamydosporum* isolate UFSM-F8), JF499677 (*Gibberella moniliformis* isolate FM24), JN646039 (*Fusarium subglutinans* isolate PK2), HQ451889 (*F. oxysporum* isolate FOCCB-2), KX262965 (*Fusarium verticillioides* isolate BPS180), and for the fungal isolates tested: (I74) and (I92). The tree topology was constructed using ClustalX (1.81).

Hydrolytic enzyme activities

Both isolates I74 and I92 were able to produce protease, amylase, and chitinase. However, only isolate I92 was able to produce lipase enzyme (Table 4).

DISCUSSION

Biological control of FCRR disease in tomato has been

Table 3. Identification of the two most bioactive endophytic isolates (I74 and I92) by DNA sequencing genes.

Isolate	Accession number	Most related species	Sequence homology (%)
I74	MF188258	S5-Z-3-14, *Penicillium crustosum;* S4-Z-3-20, *P. crustosum*	100
I92	MF188256	SWUKJ1.1120, *Fusarium proliferatum*	100

I74 and I92: Fungal isolates recovered from surface-sterilized *Solanum linnaeanum* leaves.

Table 4. Enzymatic activity displayed by both endophytic fungi (I74 and I92) recovered from *Solanum linnaeanum* leaves.

Isolate	Amylase	Lipase	Protease	Chitinase
I74	+	-	+	+
I92	+	+	+	+

+: Presence of enzymatic activity; -: Absence of enzymatic activity.

extensively accomplished using several fungal agents (Alabouvette and Olivain, 2002; Hibar et al., 2006; Horinouchi et al., 2008). However, the exploration of endophytic fungi as biocontrol agents against this disease is rarely considered (Kavroulakis et al., 2007). Interestingly, some wild *Solanum* species have been explored as potential sources of bioactive molecules and biocontrol agents (Khan et al., 2015; Aydi Ben Abdallah et al., 2016). In the present study, a collection of fungal isolates naturally associated with *S. linnaeanum* was screened for its ability to suppress FCRR and to promote tomato growth when applied as conidial suspensions or cell-free culture filtrates.

A total of 75 fungal isolates were recovered from *S. linnaeanum* leaves, stems, flowers and fruits. The frequency of isolates collected varied depending on host organs targeted for isolation. Stems harbored 33.3% of recovered isolates. Also, previous studies have demonstrated that colonization rate of endophytic fungi is more prevalent in stems than in the other organs (Li et al., 2012; Zheng et al., 2013) as stems are persistent whereas the other organs are deciduous (Li et al., 2012). In contrast, Kharwar et al. (2011) found that endophytic fungi associated with *Mansoa alliacea* were more prevalent in leaves (72.2%) which could be explained by the wide surface of leaves that facilitates the penetration of fungi and also since leaves are more rich in cellulose (Navralitova et al., 2017).

According to their macroscopic and microscopic traits, fungal isolates recovered from *S. linnaeanum* were affiliated to five genera, namely *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Trichoderma*. *Aspergillus* was found to be the most dominant genus with a relative isolation frequency of 19.1% followed by *Penicillium* (15%). *Aspergillus* and *Penicillium* associated to *Solanum*-species were reported in previous studies (El-Hawary el al., 2017; Jena and Tayung, 2013). In fact, *A. flavus* (El-Hawary et al., 2016), *Aspergillus* spp. (SNFSt), *Aspergillus* spp. (SNFL) (El-Hawary el al., 2017) and *Penicillium funiculosum* (Khan et al., 2013) were obtained from *S. nigrum*. For instance, *P. crustosum* was isolated from *Juniperus procera* (Gherbawy and Elhariry, 2014). *Penicillium purpuogenum*, *P. lanosum* and *P. oxalicum* were isolated from *S. rubrum* leaves, stems and fruits (Jena and Tayung, 2013). *P. crustosum* was also recovered from various sources such as *Coffea arabica* seeds, berry and crown (Vega et al., 2010), *Persea americana* roots (Hakizimana et al., 2011), *Quercus robur* branches (Nicoletti et al., 2013) and *Capsicum annum* plants (Paul et al., 2012). On the other hand, *F. proliferatum* was naturally associated to *Dysoxylum binectariferum* (Kumara et al., 2012), *Jatropha curcas* (Kumar and Kaushik, 2013), leaf and root from *Brassica napus* (Zhang et al., 2014).

Fungal isolates associated to *S. linnaeanum* were evaluated for their capacity to control FCRR disease under greenhouse conditions using their conidial suspensions or cell-free culture filtrates. Results from the current study clearly demonstrated that I74 and I92 isolates exhibited the highest disease-suppressive effects. These isolates were identified based on rDNA sequencing as *P. crustosum* (I74) and *F. proliferatum* (I92). They were also found to be the most efficient in enhancing growth of tomato plants inoculated with FORL. This indicates that wild *S. linnaeanum* species could be an effective source of isolation of effective fungi, able to colonize and to protect cultivated tomato plants against FCRR disease. In fact, many fungal endophytes are shown capable to produce a variety of extracellular metabolites responsible for the protection of their host plants from their associated pathogens (Meng et al., 2011; Zhang et al., 2014). Other previous studies demonstrated the ability of endophytic *Fusarium* species (such as *F. solani* and *F. equiseti*), isolated from healthy tomato root tissues, to colonize root tissues of cultivated tomato seedlings and to protect them from FORL

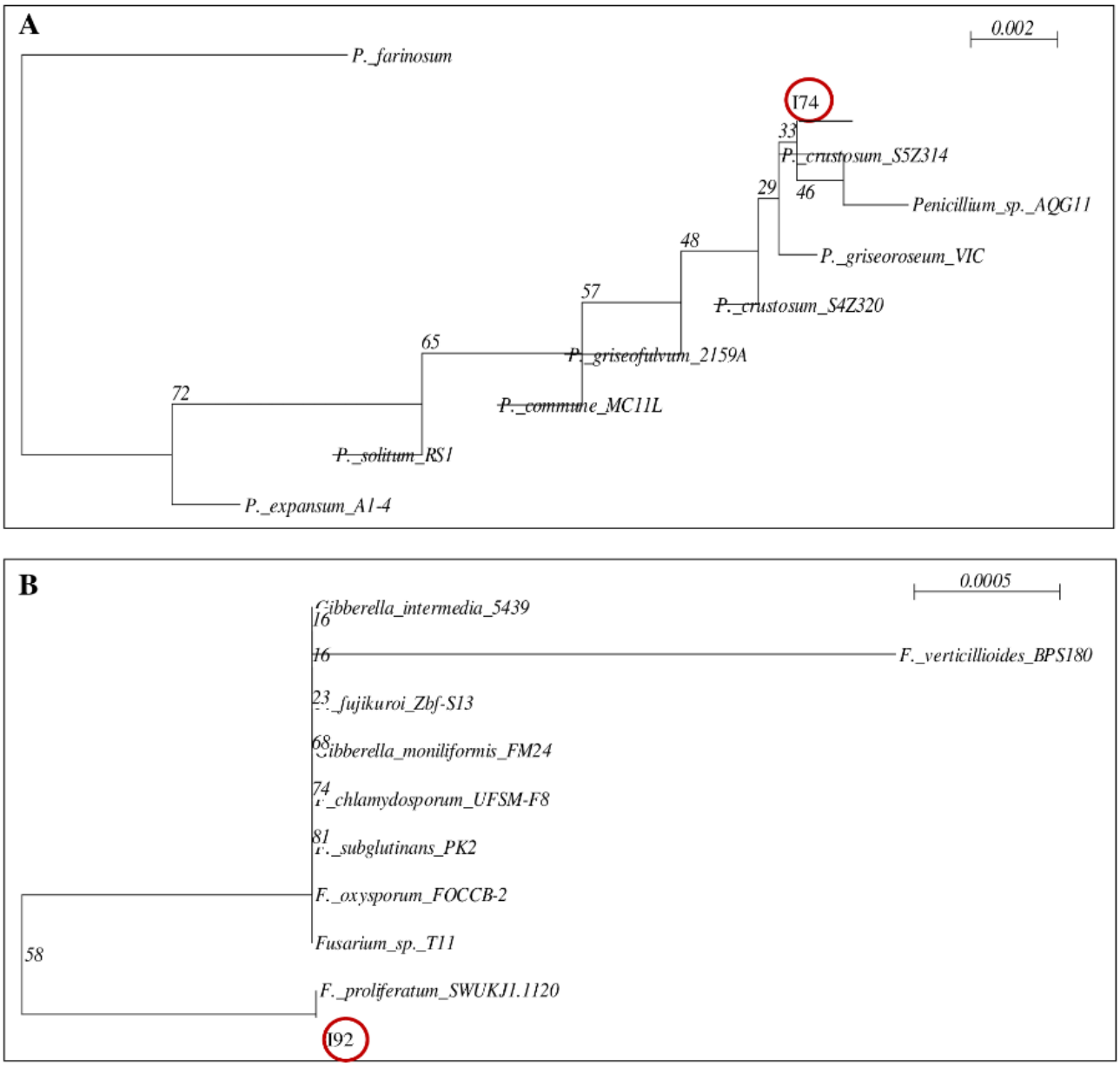

Figure 7. Neighbor-joining phylogenetic tree of rDNA ITS sequences of the most active endophytic isolates I74 **(A)** and I92 **(B)** recovered from *Solanum linnaeanum* and their closest phylogenetic relatives.

infections (Horinouchi et al., 2007; Kavroulakis et al., 2007). Moreover *F. proliferatum* culture filtrates have significantly reduced lesion diameter on detached leaves of *B. napus* caused by *Sclerotinia sclerotiorum* (Zhang et al., 2014). A significant decrease in Verticillium wilt severity was noted on tomato plants treated by *P. indica,* a root-associated endophytic fungus (Fakhro et al., 2010).

In the present study, the ability of conidial suspension preparations and cell-free culture filtrates from the tested endophytic fungi to improve root and shoot growth of treated tomato seedlings compared to pathogen-free ones. I74 (*P. crustosum*) and I92 (*F. proliferatum*) conidial preparations were found to be the most effective in improving root and shoot growth over the untreated control. In addition, cell-free filtrates from these isolates led to the highest enhancement of tomato growth over control. This growth promotion can be achieved directly through the antagonistic activity of endophytic agents against pathogenic fungi or indirectly through an activation of plant defense. These findings are in accordance with previous works reporting on the ability of these two species to improve plant growth (Schulz and Boyle, 2005), through the enhancement of nutrients

uptake (phosphorus, sulfur, calcium, magnesium, and potassium), and their ability to produce diverse bioactive metabolites and enzymes including phytohormones and jasmonic (Lorenzo et al., 2004), abscisic (Shinozaki and Yamaguchi-Shinozaki, 2007), and salicylic acids (Raskin, 1992). In a previous study, the endophytic fungi *Phoma glomerata* LWL2 and *Penicillium* spp. LWL3 promoted the growth of rice seedlings by optimizing the uptake and the assimilation of nutrients (Waqas et al., 2012). Also, endophytic *P. commune* (Choi et al., 2005) and *P. funiculosum* (Khan et al., 2011) are able to produce gibberellins (Lee et al., 1998). Gibberellins were also found in supernatants of endophytic *Aspergillus caespitosus* and *Phoma* spp. (Khan et al., 2014) and *P. citrinum* (Khan et al., 2008). It should be highlighted that this funding is the first report showing the growth-promoting ability of the most bioactive isolates *P. crustosum* (I74) and *F. proliferatum* (I92) recovered from *S. linnaeanum.*

Tested using the dual culture method, conidial suspensions from the tested endophytic fungi exhibited a significant antifungal activity toward FORL. In fact, the highest inhibition of pathogen radial growth was achieved using I74 (*P. crustosum*) and I92 (*F. proliferatum*) isolates. Also, these isolates were found to be potential protease-, chitinase-, and amylase-producing agents. Thus, this interesting antifungal potential could be justified to the capacity of these fungal isolates to inhibit FORL growth via the synthesis of extracellular cell wall-degrading enzymes such as chitinases, proteases and amylases. In fact, based on previous studies, endophytic fungi can produce extracellular hydrolases as a resistance mechanism against pathogenic invasion. Such enzymes include pectinases, cellulases, lipases, and laccase (Prabavathy and Valli Nachiyar, 2012). Extracellular metabolites present in cell-free culture filtrate of the endophytic fungi, tested at 10% (v/v) in this study, were found to be effective in suppressing FORL *in vitro* growth. Interestingly, filtrates of I74 (*P. crustosum*) and I92 (*F. proliferatum*) induced the highest decrease (by 80.4 to 82%) in pathogen mycelial. Similarly, previous report showed that *P. crustosum* exhibited a significant antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans* and *F. solani* (Gherbawy and Elhariry, 2014). Interestingly, *Penicillium* species are well reported to produce antifungal metabolites. In fact, fungitoxic metabolites produced by *P. crustosum* were shown to be effective to inhibit the mycelial growth of *F. solani* (Nicoletti et al., 2004). Additionally, bioactive metabolite produced by an endophytic *F. oxysporum* strain 162 significantly reduced the mycelial growth of *Phytophthora cactorum*, *Pythium ultimum* and *R. solani* (Hallmann and Sikora, 1995). In a previous work, an antifungal compound, hypocrellin B, was produced by the endophytic *Penicillium chrysogenum* recovered from *Fagonia cretica* and showed to be involved in its antifungal activity displayed against *Microbotryum violaceum* and *Trichophyton rubrum* (Meng et al., 2011).

Conclusion

Endophytic fungi are potentially interesting sources of bio-based products useful in sustainable agriculture. To the best of our knowledge, *S. linnaeanum* was firstly reported in the current study as a potential source of isolation of endophytic fungi with antifungal potential against FORL. The present study led to the selection of two potent biocontrol agents shown to be efficient for FCRR control and for the improvement of tomato growth. According to rDNA gene sequencing, the most bioactive endophytic fungi were identified as *P. crustosum* (I74), and *F. proliferatum* (I92). Interesting enzymatic activities (chitinase, protease, lipase and amylase) were demonstrated for these two selected isolates and seemed to be responsible for their antifungal potential against FORL. The study suggests that wild solanaceous species are interesting source of isolation of promising endophytic fungal isolates with FCRR suppression and biofertilizing abilities. Further chemical and molecular studies are required to identify the bioactive compounds involved in pathogen suppression and growth promotion.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of maize and peanut crops on Ivory Coast northern soil biological activities and their response to arbuscular mycorrhizal fungi inoculation

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The overuse of chemical fertilizers in agriculture remains an environmental concern, especially in sub-Saharan areas of Africa where soil degradation lead to low crop yield. Crop inoculation with beneficial microorganisms appears a good alternative to reduce chemical substances and improve yield. In this regard, studies on soil biological activities and inoculation experiments on maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) crops were conducted. The aim of this work was to evaluate the effect of these crops on soil microbial activities and to assess their response to inoculation with two fungi (*Glomus aggregatum* and *Glomus etunicatum*) alone or in combination. Rhizospheric and non rhizospheric soils were collected in peanut and maize fields at Takali in northern Côte d'Ivoire. Soil enzymes activities, total microbial biomass, AMF spore and rhizobia densities were determined in these soils. Then, mycorrhizal inoculation experiment of these plant species was conducted in a greenhouse located at Nangui Abrogoua University, Abidjan, Côte d'Ivoire. After three months, plant growth and yield, mycorrhizal and nodulation parameters were measured. Results showed that maize has significantly improved enzymes activities, spore density and total microbial biomass of soil. Effect of peanut was only significant on chitinase. Moreover, soil rhizobia density was reduced under this crop effect. Maize significantly improved these parameters more than peanut. Inoculation results showed a significant enhancement of the height and shoot dry weight of maize with single inoculation with *G. aggregatum* or *G. etunicatum* even if a low mycorrhization rate was observed. However, in peanut, the mixed inoculants (*G. aggregatum* + *G. etunicatum*) significantly increased the pod weight and nodulation parameters. Results showed the importance of plant cover in the improvement of soil biological quality including enzymes activities and microbial community and suggested that the effect of mycorrhizal inoculation is influenced by many factors as plant species, AMF and soil environment characteristics.

Key words: *A. hypogaea*, *Z. mays*, soil enzyme activities, microbial biomass, soil microorganisms' density, mycorrhizal inoculation.

INTRODUCTION

For developing countries, the economy of Côte d'Ivoire is based primarily on agriculture and more than 50% of population lives mainly from this activity, which confirms its importance for food security (Kouakou et al., 2010). Maize and peanut are two foods crops that are sources of income for local population, which the main growing area is Korhogo in northern country. Maize *(Zea mays* L.) is a cereal, belonging to grass family (Kellogg, 2001), cultivated for its carbohydrate-rich seeds. It is the third most cultivated cereal in the world after rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.).

Indeed, world food security depends on ample supply of these three major cereals (Ferrar, 1995). It's used in both human and animal feed. Peanut (*Arachis hypogaea* L.) is a legume grown for its high protein and oil content. Its fatty and amino acid composition and its taste and flavor are important features attributed (Asibuo et al., 2008). It is considered to be one of the most important oilseed crops worldwide. This plant is also cultivated in rotation to improve the nitrogen content of the soil and thus contributes to its fertility.

However, in northern Côte d'Ivoire, crop yields remain low and environmental concerns caused by, the expensive chemical fertilizers (Dobermann and Cassman, 2004) support researchers for new sustainable strategies to promote soil fertility and thereby, improve crop production. In this context, exploitation of soil microbial communities such as arbuscular mycorrhizal fungi (AMF), to improve food quantity and quality (Barea, 2015), has been considered as safe, inexpensive and environmentally friendly.

AMF establish a mutual relationship with plant roots which benefit from water and nutrients that fungus collects in soil; in turn it feeds on carbon allocated by plant (Parniske, 2008). They contribute to the mineral nutrition of plants even during drought and other environmental stress (Martínez-García and Pugnaire, 2009; Martínez-García, 2010). Several studies have demonstrated the stimulatory effect of these symbiontes on growth parameters in grasses and legumes (Smith et al., 1998) and in decomposition of organic matters. Positive effects of AMF and phosphorus application were observed on the growth and phosphatase activities of peanut (Doley and Jite, 2012).

In the same way, Mustafa et al. (2010) found beneficial effect of inoculation with *Glomus mossae* on growth parameters of maize. Others stimulatory effects of these fungi in ecosystems by, governing a large number of crucial soil processes including soil nutrient biochemical cycling, decomposition of litter and the establishment of soil living components were also demonstrated (Chen et al., 2015). These beneficial effects are due mainly to enzymatic complex such as phosphatase, which participates to organic phosphorus decomposition and improves soil phosphorus concentration that is, an important index to assess soil phosphorus bioavailability (Panettieri et al., 2014).

Bacteria and fungi are the main source of enzymes in the soil which constitute the largest fraction of soil organisms in terms of biomass and number and the main factor influencing litter decomposition in soil (Berg and McClaugherty, 2014). Their activities are particularly relevant at the root–soil interface microhabitats known as the rhizosphere, where microorganisms are stimulated by carbon substrates provided by plant rhizodeposits (Hirsch et al., 2013). Compared to bulk soil, the rhizosphere soil is characterized by higher concentrations of nutrients and labile organic C (Duineveld et al., 2001). Therefore, controlling the components in agricultural soils is a crucial feature of the biological components of these soils, as a vital aspect of sustainable crop farming system (Malherbe and Marais, 2015).

However, these beneficial actions of AMF are difficult to generalize because plant response to mycorrhization depends on several parameters such as AMF species, plant and the environmental conditions (Rodríguez-Echeverria et al., 2016). Moreover, studies on soil enzymes activities are concentrated on temperate areas; and very few data are available in tropical environment in the literature (Acosta-Martinez et al., 2007). So far, no studies have been carried out on the inoculation of maize and peanut with AMF strains and on enzymes activities under crop influence, in Côte d'Ivoire, are more accurately in Korhogo area. The aim of this study is not only to assess the impact of peanut and maize crops on soil enzymes activities and total microbial biomass, but also to evaluate the effect of two arbuscular mycorrhizal fungi (*Glomus aggregatum* and *G. etunicatum)* on growth parameters and yield of these crops.

MATERIALS AND METHODS

Soil sampling

Soils used for the biological activities were collected in October 2016, in peanut and maize monoculture fields in Takali (9°25 N; 5°35 W) located in Korhogo in northern Côte d'Ivoire. For each crop, a composite sample of rhizospheric soil under maize or peanut was collected by removing five plants and shaking the soil surrounding of the roots. Composite soil used as control was also

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collected on bulk without any vegetation.

These soils were used to study biological activities beneathmaize and peanut. For a greenhouse experiment, another soil collection was done at ten points of the plot, to obtain a representative composite soil sample of the targeted plot. Physical and chemical parameters of this composite soil were determined at the Centre de Recherche en Océanographie (CRO) of Abidjan, Côte d'Ivoire. The pH (water) was measured in the supernatant of a soil / distilled water mixture in a ratio of 1:2.5.

Organic and mineral matters content were determined according to Moreno et al. (2001). Contents of total nitrogen (N) and phosphorus (P) were quantified according to Bremner (1960) and Sherrell and Saunders (1966), respectively by atomic absorption spectrometer after digestion with concentrated sulfuric acid. Potassium (K) was analyzed by means of argon plasma ionization source mass spectrometer (ICP-MS) according to Rao and Talluri (2007) method.

Soil biological activities

AMF spore density in rhizospheric and non rhizospheric soils was determined by, counting after extraction through moistening sieving method as described by Gerdeman and Nicholson (1963). The number of rhizobia was evaluated by the most probable number plant infection (MPN) method of Brockwell (1980) and siratro (*Macroptilium atropurpureum*) was used as trap plant. It consists of dilute soils used to inoculate siratro seedlings with soil suspension.

Four enzymes activities: acid phosphatase, β-glucosidase, fluorescein diacetate (FDA) and chitinase were assayed in rhizospheric and non rhizospheric soils collected in fields of peanut and maize. For each enzyme activity, three replicates were carried out. Acid phosphatase activity was measured according to Tabatabai and Bremner (1969) method. Soils were incubated with modified universal buffer (pH 6.5) and *p*-nitro-phenyl-phosphate substrate at 37°C under stirring during 1 h. Reaction was stopped with $CaCl_2$ (0.5 M) and NaOH (0.5 M) and the supernatant was taken and assayed in spectrophotometer at 400 nm.

β-glucosidase was evaluated according to Hayano (1973), the substrate used was *p*-nitro-phenyl-β-glucopyranoside. Soils were also incubated with substrate and citrate phosphate buffer (pH 5.8) during 2 h at 37°C under stirring. After incubation, the reaction was stopped with Na_2CO_3 (0.2 %), mixed and the optic density was read also at 400 nm. FDA activity was determined as described by Adam and Duncan (2001) in presence of potassium phosphate buffer (pH 7.6) and fluorescein diacetate substrate. After 1 h of incubation at 30°C under stirring, reaction was stopped by addition with 1 mL of pure acetone, mixed and centrifuged. Then, 1 mL of supernatant was taken and assayed at 490 nm.

For chitinase activity, soils were incubated with acetate buffer (pH 5.5) and substrate *p*-nitro-phenyl-β-glucosaminide during 1 h under stirring and stopped by addition of $CaCl_2$ (0.5 M) and NaOH (0.5 M) (Parham and Deng, 2002). Results were reported in µg of product released per hour and gram of dry soil following µg /h/g.

Soil total microbial biomass was also determined according to the fumigation-extraction method as described by Amato and Ladd (1988). Two extractions to KCl were done at T0 (extraction before fumigation) and T10 (extraction after fumigation). For T0 extraction, soils were incubated in the presence of KCl under stirring and after decantation; the supernatant was taken and stored at -20°C for colorimetric analysis. Before T10 extraction, fumigation is previously done. It is intended to kill all living microorganisms in the soil and targeted to incubate soils in presence of chloroform vapors at obscurity. After 10 days of incubation, the extraction to KCl was done as in the case of samples T0. Total biomasses in carbon (C), ammonium (NH_4^+) and nitrate (NO_3^-) were measured in these samples.

Fungal inoculum preparation

Fungal inoculum *G. aggregatum* and *G. etunicatum* were supplied by the Laboratoire Commun de Microbiologie IRD/ISRA/UCAD of Dakar, Senegal. *G. aggregatum*(Schenck and Smith emend. Koske; DAOM 227 128) was isolated from Djignaki (Senegal) and *G. etunicatum* from Dijon (France). These strains were chosen for their performance in an efficiency test on many plants (Kruger et al., 2012). They were isolated and multiplied on sterile soil, poor in phosphorus with maize as trap plant under greenhouse conditions.

Three months after cultivation, roots were harvested and mycorrhizal inoculums were prepared as described by Plenchette et al. (1989). Each inoculum of fungi consists of sand, spores, hyphae and mycorrhizal root fragments. It contained an average of 40 spores per gram of soil and roots fragments with 80% of colonization (Guissou et al., 1998). The mixed inoculum was obtained by a mixture of equivalent quantities of the two fungi and contained approximately the same spore's number and infective propagules of each fungal species.

Greenhouse inoculation test

The inoculation experiment was conducted in a greenhouse (5°23 N to 4°0 W) located at the University of Nangui Abrogoua (UNA) in Abidjan, Côte d'Ivoire during three months (from October to December 2016). The average temperature and humidity were 31.2°C and 38.80%, respectively during the day and 26°C and 62.5% at night.

Seeds of maize CNRA-GMPR-18 and peanut CNRA-ara 8-20 varieties were provided by the *Centre National de Recherche Agronomique* of Abidjan. These two varieties have a short cycle of 90 days. Peanut seeds were surface-scarified in 70% calcium hypochlorite solution ($CaCl_2O_2$) for 8 min and then rinsed several times with sterile water (Gottardi and Nagl, 1998). The maize seeds were surface-sterilized in 20% bleach for 10 min and rinsed several times with sterile water, allowed to soak for 30 min (Hite et al., 1999). They were then pre-germinated in Petri dishes containing 0.9% agar and incubated for 72 h at 28°C in the dark (covered with aluminum foil) in an oven.

The pre-germinated seedlings were transferred into plastic bags containing about 1 kg of non-sterile soil moistened slightly with tap water. For each plant species (maize or peanut), four inoculation treatments were applied: inoculation with *G. aggregatum* (Ga), *G. etunicatum* (Ge), mixed inoculum (Ga+Ge) and control without inoculum. Also, for each treatment, 9 replicates were performed in a completely randomized block. The inoculation was done at sowing with 20 g of fungal inoculum. Mixed treatment consists in inoculating seedlings with 10 g of Ga and 10 g of Ge. Plants were watered every day to maintain soil water content close to field capacity during 3 months.

The height of plants was measured every two weeks during experimentation and after three months of cultivation, they were harvested. Shoot, root and total weights of peanut and maize plants were obtained after drying at 70°C for 48 h. Before drying the peanut roots, the fresh nodules and pods of each plant were detached, counted and weighed separately. Frequency and intensity of mycorrhization of peanut and maize roots were determined according to Phillips and Hayman (1970) method. For that, roots were previously rinsed with tap water and placed in tubes containing 10% KOH. The tubes were then boiled in a water bath at 90°C for 60 min.

This step makes it possible to empty the cytoplasm content of

Table 1. Soil enzymes activities beneath maize and peanut crops.

Plants species	β-Glucosidase (µg p-Np/h/g)		Chitinase (µg p-Np/h/g)		Phosphatase (µg p-Np/h/g)		FDA (µg fda/h/g)	
	RS	NRS	RS	NRS	RS	NRS	RS	NRS
Maize	51.09±7.43^{aB}	40.65±0.78^{a}	3.90±0.76^{bB}	1.60±0.21^{a}	214.35±19.13^{bB}	134.76±18.38^{a}	99.47±17.33^{aA}	73.57±12.93^{a}
Peanut	32.27±7.47^{aA}	31.17±5.90^{a}	1.37±0.21^{bA}	0.82±0.21^{a}	129.53±34.38^{aA}	117.23±13.35^{a}	94.86±17.04^{aA}	67.86±9.52^{a}

RS: rhizospheric soil; NRS: non rhizospheric soil. For each enzyme activity and plant species, in column, values following with the same minuscule letter for RS and NRS or with the same majuscule letter for RS are not significantly different according to student test t ($p<0.05$).

Table 2. Soil physical and chemical characteristics.

Parameter	Values
pH	5.25
Organic matters (%)	4.23
Mineral matters (%)	2.53
Total nitrogen (mg/kg)	1.01
Total phosphorus (mg/kg)	4.57
Potassium (mg/kg)	244.27

the cells and to facilitate the coloration. The roots were rinsed abundantly with tap water to remove KOH, and then stained with 0.05% trypan blue which is brought to water bath at 80°C for 30 min. For each sample, root fragments of about 1 cm were mounted between slide and cover slide crushed in 20% glycerol and observed under a microscope. Estimation of root colonization by AMF was carried out using the method of Trouvelot et al. (1986) according to a rating system based on 6 classes. Mycorrhizal frequency (F %) and intensity (I %) were measured as follows:

F % = (number of mycorrhizal fragments / total number of fragments observed) × 100

Where (F %) is the frequency of mycorrhization reflecting the importance and the percentage of fragments of infected roots, with n as the total number of root fragments observed.

I% = (95n5 + 70n4 + 30n3 + 5n2 + n1) / total number of fragments observed

Where (I%) is the intensity of the cortex colonization expressing the portion of the cortex colonized with respect to the entire root system, with n5, n4,…, n1 as the number of fragments, respectively, denoted as 5, 4,…, 1.

Data analysis

The data obtained were analyzed using the XLSTAT 2010 software. The means values of different parameters were compared by the ANOVA according to the Student Newman Keuls test ($p<0.05$) for the inoculation test. Percentage data of root mycorrhizal colonization were arcsine transformed prior to analysis. Analyses were performed separately for each plant species.

For soil biological activities, data were performed with test t of Student for two independent samples. The comparison was done between rhizospheric and non rhizospheric soils for each parameter and plant species.

The aim of this test was to evaluate the impact of crop of maize or peanut on the parameters studied. Another comparison was assayed between the two crops in order to know the crop which has improved most of these parameters.

RESULTS

Crop effect on soil enzymes activities

Soil enzymes activities are more important in rhizospheric (RS) than in non rhizospheric soils (NRS) for all activities measured (Table 1). Indeed, chitinase activities were significantly improved both in beneath peanut and maize crops, respectively by 67.07 and 143.75% compared to non rhizospheric soils.

Acid phosphatase was significantly increased in RS with maize by 59.06% but not with peanut. However, there was no significant difference between RS and NRS regarding FDA and β-glucosidase. Compared to peanut, maize crop significantly improved β-glucosidase, chitinase and phosphatase activities, respectively by 58.32, 184.67 and 65.89% except FDA. Results on soil physical and chemical characteristics are given in Table 2.

Impact of plant crops on total microbial biomasses, and AMF spores and rhizobial densities

Results presented in Table 3 showed that similarly to enzyme activities, maize crop (RS) significantly improved total microbial biomasses C, NH_4^+ and NO_3^-

Table 3. Soil total microbial biomass beneath maize and peanut crops.

Parameter	Biomass C(µg C/g)		NH_4^+ (µgN-NH_4^+/g)		NO_3^- (µgN-NO_3^-/g)	
	RS	NRS	RS	NRS	RS	NRS
Maize	23.50±0.50^{bB}	11.50±0.50^{a}	1.40±0.20^{bB}	0.77±0.12^{a}	7.77±1.15^{bB}	3.10±0.46^{a}
Peanut	14.00±2.00^{aA}	17.33±1.61^{a}	0.90±0.01^{aA}	0.93±0.15^{a}	3.93±0.64^{aA}	4.50±0.26^{a}

RS: rhizospheric soil; NRS: non rhizospheric soil. For each parameter and plant species, in column, values following with the same minuscule letter for RS and NRS or with the same majuscule letter for RS are not significantly different according to student t test ($p<0.05$).

Table 4. Densities of AMF spores and rhizobia in soil beneath maize and peanut crops.

Number	Spores/50g of soil		Rhizobia/g of soil	
	RS	NRS	RS	NRS
Maize	1180±67.02^{bB}	917.67±143.49^{a}	ND	ND
Peanut	1036±47.03^{aA}	871.33±69.24^{a}	8.1 10^{3a}	1.08 10^{4a}

RS: rhizospheric soil; NRS: non rhizospheric soil. For each density and plant species, in column, values following with the same minuscule letter for RS and NRS or with the same majuscule letter for RS are not significantly different according to student test t ($p<0.05$).

Table 5. Plant growth parameters of maize and peanut inoculated with arbuscular mycorrhizal fungi.

Plant species	Treatment	Plant growth				Pods yield	
		Height (cm)	SDW (g)	RDW (g)	TDW (g)	Number	Weight (g)
Peanut	Ga	34.06±6.24^{a}	3.16±0.63^{a}	0.46±0.12^{a}	3.62±0.73^{a}	1.44±0.53^{a}	2.1±0.52^{a}
	Ge	37.26± 5.04^{ab}	3.91±1.11^{a}	0.67±0.22^{b}	4.58±1.26^{a}	2.11±0.67^{a}	3.78±1.06^{b}
	Ge+Ga	42.80±5.95^{b}	4.29±0.97^{a}	0.70±0.12^{b}	4.99±1.03^{a}	2.22±0.88^{a}	4.0±1.28^{b}
	Control	38.33±2.86^{ab}	4.00±0.94^{a}	0.71±0.14^{b}	4.72±1.05^{a}	1.44±0.52^{a}	2.15±0.61^{a}
Maize	Ga	66.7±8.14^{b}	2.45±0.70^{ab}	1.05±0.35^{a}	3.50±1.03^{a}	-	-
	Ge	65.61±6.7^{b}	2.59±0.48^{b}	1.44±0.30^{a}	4.03±0.75^{a}	-	-
	Ge+Ga	61.26±3.45^{ab}	2.24±0.22^{ab}	0.92±0.19^{a}	3.16±0.35^{a}	-	-
	Control	59.56±5.03^{a}	1.91±0.48^{a}	0.86±0.32^{a}	2.77±0.74^{a}	-	-

For each plant in column, Values following by same letters are not significantly different according to student Newman-keuls test ($p<0.05$). Ga: Glomus aggregatum; Ge: Glomus etunicatum.

two times, compared to non rhizospheric soils (NRS). In contrary, peanut negatively affect these parameters, but any significant difference was observed.

Same trends were also observed with AMF spore density which was significantly improved under maize crop influence by 28.68%, compared to non rhizospheric soil and by 13.90% compared to peanut soils (Table 4). Peanut crop also increased AMF spore density by 18.94% compared to NRS.

However, surprisingly rhizobial density was decreased from 1.08 10^4 to 8.1 10^3 Rhizobia/g (25% discount) by peanut crop even if there was no significant difference.

Effect of mycorrhizal inoculation on crop growth and yield

Results showed that pods weight of peanut was significantly increased by 86.05% in mixed inoculums (Ga + Ge) compared to the non-inoculated control (Table 5). The pods weight was nearly two times higher in plants inoculated by Ge alone and the mixed (Ga + Ge). However, plant height, shoot, root and total dry weight and pods number were not significantly improved in comparison with control. The peanut root dry matter of plants inoculated with Ga was lower than non-inoculated control.

With maize plants, significant increase was obtained when plants were inoculated alone with Ge (10.16%) or Ga (12%) (Table 5). The cocktail of the two inocula had no significant effect on maize plants. Maize shoot dry weight (SDW) was improved by fungal inoculation with a significant effect in plants inoculated with Ge (35.60%) in comparison to the control. In contrast, root dry weight (RDW) and total dry weight (TDW) were not significantly improved by AMF inoculation.

Table 6. Nodulation and mycorrhization parameters.

Species	Treatment	Nodulation		Mycorrhization	
		Nod. Number	Nod. Weight (mg)	Frequency (%)	Intensity (%)
Peanut	Ga	60.00±8.41[a]	143.00±8.92[a]	7.22±2.54[a]	0.13±0.06[a]
	Ge	70.11±12.1[b]	166.50±9.97[b]	8.89±2.55[a]	0.16±0.02[ab]
	Ge+Ga	78.78±6.94[b]	186±12.54[b]	10±2.89[a]	0.24±0.03[b]
	control	72.33±7.47[b]	171.00±11.84[b]	9.44±2.54[a]	0.19±0.06[ab]
Maize	Ga	-	-	11.84±2.38[a]	0.19±0.05[a]
	Ge	-	-	15.41±2.22[a]	0.27±0.08[a]
	Ge+Ga	-	-	10.49±3.08[a]	0.16±0.03[a]
	Control	-	-	10.49±3.08[a]	0.16±0.03[a]

For each plant species, values followed by the same letter in the columns are not statistically different according to student Newmann-Keuls test ($p<0.05$). Mycorhizal values were prior transformed in arcsine before data analysis.

Effect of inoculation on microbial symbiosis

Results of nodulation and mycorrhization parameters are presented in Table 6. Peanut nodulation parameters (nodules number and weight) were not significantly improved by AMF inoculation. Surprisingly, inoculation with Ga significantly decreased the number and fresh weight of nodules compared to the non-inoculated control.

No positive effect of AMF inoculation on mycorrhizal parameters of maize and peanut roots was observed in comparison to non-inoculated control. Moreover, treatment Ga significantly decreased the mycorrhizal intensity of peanut roots compared to that of roots inoculated with the mixed treatment (Ge+Ga).

DISCUSSION

Plants improve soil biological activities

Enzyme activities were higher in rhizospheric soil beneath maize and peanut compared to non rhizospheric soils. That suggests the importance of plant cover in the soil microbial activities. The rhizosphere is a narrow region of the soil that is directly influenced by root secretions and associated microbial activity, and sustains dense populations of root-associated and free-living microorganisms (Cheng et al., 2014). Thus, biological activity in topsoil and litter layer is not only governed by abiotic factors such as pH, humidity, temperature but also by biotic factors including interaction with microbial biomass (Zhou et al., 2016).

In fact, soil enzymes activities are provided mainly from microorganisms such as soil bacteria and fungi, widely distributed which involved in degradation of organic matter in field soils (Tedersoo et al., 2014; Wardle and Lindahl, 2014). However, maize crop has significantly improved these activities compared to peanut crop, except FDA. These results indicated the importance of incorporating plants of the grass family in a crop system and its influence on soil density microorganisms and their biological activity. Indeed according to Natywa and Selwet (2011), maize produces significant quantities of root exudates secretions that may include amino acids, hydrocarbons, vitamins, organic acids, and enzymes. These substances stimulate the growth and development of microorganisms in the rhizosphere of this plant.

In this study, phosphatase activities were the highest (214.35±19.13 µg p-Np/h/g of dry soil), that can be due to their ability to persist in soils for long periods by binding to soil organic matter and clays (Matus, 2014). The high content of phosphatase in soils may also be due to the poor availability of this element in soils therefore to a high P demand of plants and microorganisms living in soil. In fact, soil physical and chemical analysis revealed that our soils were poor in P with an average value of 4.57 mg/kg of dry soil. These results demonstrate preferentially the development of a mechanism in response to a mineral deficit such as P to produce phosphatase when soil P resources are limited. Our investigations showed also that phosphatase content was significantly higher in maize soil than soil beneath peanut, which may be due to high density of AMF spores in maize soil and a greater secretion of this enzyme by maize roots. In contrary, Maseko and Dakora (2013) have reported that legumes secrete more phosphatase enzymes than cereal which might be explained by a higher requirement of P by legumes in the symbiotic nitrogen fixation process. The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties (Kidd et al., 2016).

β-glucosidase plays an important role in the degradation of glucose polymer and regulates the supply in glucose; an important carbon energy source for growth and activity of soil microorganisms (Merino et al., 2016). It was significantly higher in rhizospheric soil

under maize compared to that of peanut. These results can be explained also by the high number of AMF spores beneath maize crop compared to that of peanut. In fact, β-glucosidase is derived predominantly from heterotroph fungi (Turner et al., 2002) which require a lot of amount of carbon providing from glucose for the establishment of mycorrhiza (Böhme and Böhme**,** 2006). N-acetyl-β-glucosaminidase or chitinase catalyzes the hydrolysis of chitin, a linear polymer of β-1,4-N-acetylglucosamine units which is abundance next to cellulose.

Chitinase was significantly higher in rhizospheric soils of peanut and maize compared to non rhizospheric soils. This may be attributed to the fact that, the oxidative functional activity of microbial communities in the rhizosphere is higher than that of non rhizospheric soil (Yang et al., 2013). This heightened chitinase activity may be due to the higher carbon resources in the rhizosphere soil, which is considered as the driving force for microbial activity and density as reported by Yang et al. (2013).

Fluorescein diacetate is hydrolyzed by a number of different enzymes, such as proteases, lipases and esterase. This activity was higher in rhizospheric soils of peanut and maize than in non rhizospheric soils but no significant effect was observed. That may be due to the fact that, FDA is hydrolyzed by a non-specific group of enzymes which are widely present in soils (Adam and Duncan, 2001).

In our study, microbial biomasses in carbon, ammonium, nitrate and AMF spores density were significantly higher in soil beneath maize compared to peanut rhizospheric soils. These observations suggest that maize rhizosphere herbages many microorganisms than that of peanut. Indeed, soil microbial biomass is the weight in term of C and N of all living microorganisms in soil and it's recognized as a sensitive indicator of environmental change (Li and Chen, 2004). The occurrence of microorganisms depends on the presence of allopathic compounds secreted by roots as well as mutual interactions between different groups of microorganisms in the soil (Bowles, 2014). Plant-induced differences in microbial communities due to the rhizosphere effect are well established (Hamdan and Kavazanjian, 2016). Thus, plant cover and consequently soil moisture can be an important driver of a soil microbial community (Lange et al., 2014). However, a decrease of these biomasses (C, NH_4^+, NO_3^-) and rhizobia density was found in rhizospheric soil of peanut compared to non rhizospheric soil. This may be due to the fact that rhizobia infect root legume and develop into the roots of their host. These observations may be due to the rhizobia sensitivity to cropping system and to soil acidity (Hungria and Vargas, 2000), whose pH was 5.25 and root exudates secrete acid also. According to Landon (1991), the optimum soil pH for legume plants is between 6.5 to 7.0. Moreover, Moir and Moot (2010) have shown a low persistence of legume species in soils of low pH (pH <5.8).

Plant response to mycorrhizal inoculation

Height and shoot dry weight (SDW) of maize plants were significantly improved by the single inoculation (Ga or Ge). These results are in agreement with other studies which showed that inoculation with AMF improved the growth parameters of plants (Ndoye et al., 2013; Diatta et al., 2014; Sánchez-Roque et al., 2016). This increase of SDW and height of maize plants results in a good mineral nutrition, which can be due to the introduced AM fungi. The lowest values recorded following inoculation with the mixed treatment (Ge+Ga) compared to single treatments with Ga and Ge were found in maize in all parameters. These results corroborated with those of Baxter and Dighton (2001) who suggest that co-inoculation with endomycorrhizal fungal strains does not necessarily improve plant development parameters. The low rate of mycorrhization of AMF species observed can be attributed to antagonism between fungal strains, competition for nutrients such as carbohydrates and environmental conditions of the trial such as reduced growing substrate (nursery condition), temperature, soil pH, moisture content or phosphorus availability of the soil (Dalpé, 1997).

Although contradictory in peanut, the best values were observed by the co-inoculation treatment (Ga+Ge) in comparison to treatment alone Ga or Ge. Moreover, a significant effect was observed in pods weight with the mixed inoculum (Ga+Ge) and treatment Ge, which may be due to the introduced AMF (Gill and Singh, 2002). That can be explained by the synergy between the two strains of fungi and bacteria such as rhizobia. In fact, peanut is a legume which associates with rhizobia and co-inoculation with fungi can promote the synergy between the three symbiontes (native rhizobia, *G. etunicatum* and *G. aggregatum*). Some rhizosphere bacteria function in synergy with mycorrhizae, thus promoting their growth and protection while others might interfere negatively (Barea et al., 2002). This assertion might justify the fact that the mixed inoculum was more effective in peanut and not in maize. However no significant effect was observed on height, SDW, mycorrhization (frequency and intensity) and nodulation parameters (number and weight of nodules) of peanut. These results are consistent with those of Morte and Honrubia (2002) who found that the height and dry weight of *Phoenix canariens* inoculated with *Glomus deserticola* and *G. intraradices* were not different from those of uninoculated control. Sgrott et al. (2012) also found in their study that AMF had no significant effect on plant height but increased total biomass. In addition, inoculation with Ga decreased nodulation parameters

and root dry weight of peanut in comparison to control plants. This result on peanut inoculation with Ga is in disagreement with those obtained by Leye et al. (2015) on the inoculation with AMF on sesame where they found satisfactory results. These contradictory results confirm once again the variability of plant's response to inoculation as a function of the fungal species. It has been shown that plant response to microbial inoculation depends not only on the inoculum strain, host plant and environmental conditions, but also on the compatibility between these factors (Azcon et al., 1991). The significant decrease observed in root dry weight (RDW) and nodulation parameters of peanut plant inoculated mainly with Ga could be due to a diversion of carbohydrates substances by the introduced AMF (Waceke et al., 2001). Also, these results might be explained by non-efficiency of this AMF or to competitiveness with native soil microorganisms (Graham, 2008). Indeed, inoculation is beneficial only if the strains used are more competitive than the existing strains in the soil (Bâ et al., 1996). In fact, in a study of the symbiosis Glycine-Glomus-rhizobium, it was showed the antagonist effects between endomycorrhizal colonization and the nodulation which may be due to competition for carbohydrates. Thus, they act as parasites which exploit soil resources and reduce host growth (Lau et al., 2012). The failure of inoculation with AMF on non-sterile soil was also observed by Plenchette et al. (2000) and it's due in partly to high energetic costs of this symbiosis. In fact, in mycorrhizal symbiosis AMF can consume up to 20% of C produced by their host plant to supply substrate indispensable to their growth (Bago et al., 2000). This negative response to endomycorrhizal inoculation of peanut with Ga would also be related to the origin (Senegal) of this strain. Some authors have reported that the introduction of non-indigenous strains may be a barrier to successful inoculation (Chi et al., 2013). The use of indigenous strains of AMF can improve these parameters in this case.

Conclusion

Results suggest that maize significantly improved soil enzymes activities, total microbial biomass and AMF spores density. However, no significant effect was observed with peanut crop on these parameters apart from chitinase activity. Moreover, this crop has decreased soil rhizobia density and total microbial biomass.

Single treatment with Ge or Ga significantly increased the growth parameters of maize. However in peanut, yield parameters were significantly improved by AMF cocktail. These results highlight not only the importance of plant cover in the rehabilitation of soil bio-functioning but also the variability of plant response to microbial inoculation and the potential beneficial effects of these fungi on few growth parameters of plants.

The inoculums can be used to improve the yields of these two crops. However, it is important to select indigenous AM fungi from crop substratum in order to reduce competitiveness effect for a better response of the plant to mycorrhizal inoculation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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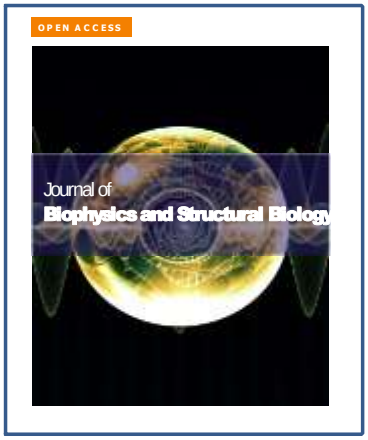

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