SCREENING OF ANTIMICROBIAL AND ANTIOXIDANT SECONDARY METABOLITES FROM ENDOPHYTIC FUNGI ISOLATED FROM WHEAT (*TRITICUM DURUM*)

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Abstract: The emergence of antibiotic-resistant micro-organisms calls for inventive research and development strategies. Inhibition of these pathogenic micro-organisms may be a promising therapeutic approach. The screening of antimicrobial compounds from endophytes is a promising way to meet the increasing threat of drug-resistant strains of human and plant pathogens. In the present study, a total of 20 endophytic fungi and 23 endophytic actinomycetes have been isolated from wheat (*Triticum durum*). Mohamed Ben Bachir variety collected from Bordj Bou Arreridj region (Algeria) during winter 2010. The isolates were screened and evaluated for their antimicrobial and antioxidant activities. Antimicrobial activity was evaluated for crude ethyl acetate extracts using an agar diffusion assay against twelve pathogenic bacteria, yeast, and two phytopathogenic fungi. All extracts showed inhibitory activity on at least one or more pathogenic microorganisms, with an average zone of inhibition varied between 7 mm to 25 mm, and the largest zone was of 25 and 25.3 mm against candida albicans and Escherichia coli respectively. The antioxidant capacity of the extracts was evaluated by β-carotene/linoleic acid assay. Results showed that 60% of these extracts have antioxidant activity, exhibiting 50, 57% to 78, 96% inhibitions. While the inhibitory activity for oxidation of linoleic acid of 40% of them was less than 50%. From the present work it is possible to conclude that these microorganisms could be promising source of bioactive compounds, and warrant further study.

Key words: endophytic microorganisms, antimicrobial activity, antioxidant activity, *Triticum durum*

INTRODUCTION

There is an ever-growing need for new and useful compounds to provide assistance and relief in all aspects of the human condition. Both human pathogens and fungal phytopathogens are prone to develop ''drug'' resistances. The effectiveness of the older types of antibiotics can decrease substantially. In addition, because of safety and environmental problems, many synthetic agricultural agents have been and still are targeted for removal from the market. The removal of such agents creates a need to find alternative ways to control farm pests and pathogens. There is an urgent need to work towards the invention of safer antifungal agents which are expected to be renewable, non-petrochemical, naturally eco-friendly, and easily obtainable (Demain 2000; Liu *et al*. 2001).

Natural products are adapted to a specific function in nature. Thus, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotypes. Endophytic fungi inhabit a biotype that is not well studied (Nithya and Muthumary 2011). The presence of endophytic fungi in plant tissues was discovered more than 75 years ago when Sampson (1935) reported such fungi from *Lolium* grass. The contemporary resurgence of research on endophytic fungi began when Bernstein and Carroll (1977) reported the presence of endophytes in needles of *Pseudotsuga menziesii* (Rajagopal *et al*. 2012).

Endophytes are microorganisms that include bacteria and fungi living within plant tissues without causing any immediate overt negative effects. Endophytes have been found in every plant species examined to date. These microorganisms are recognized as potential sources of novel natural products for exploitation in medicine, agriculture, and industry. More bioactive natural products may be isolated from the microorganisms (Kumar and Sagar 2007). Endophytes are ubiquitous and have a rich biodiversity. It is noteworthy, that of the nearly 300,000 plant species that exist on the earth, each individual plant is the host to one or more endophytes (Strobel and Daisy 2003). In view of the special colonization in certain hosts, it is estimated that there may be as many as 1 million different endophyte species. However, only a handful of them have been described (Andrew and Hirano 1991). This means the opportunity to find new and targeting natural products from interesting endophytic microorganisms, among the myriad of plants in different niches and ecosystems, is great.

Endophytes are the chemical synthesizers inside plants (Owen and Hundley 2004). Many of them are

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capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens. Some of these compounds have been proven useful for novel drug discovery (Guo *et al.* 2008). The possibility that endophytes can biosynthesize associated plant compounds was first comprehended and published by Stierle *et al*. (1993). The publication followed the highly heralded discovery of endophytic *Taxomyces andreanae* that produces the multi-billion dollar anticancer compound Taxol® (generic name: paclitaxel). This compound was isolated from the Pacific yew tree *Taxus brevifolia*. Inspired by this discovery, numerous efforts have been made to identify endophytes as sources of associated natural plant products. Many scientists have become increasingly interested in studying fungal endophytes as potential producers of novel and biologically active compounds. In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic, and anticancer activities have been successfully discovered from the endophytic fungi. These bioactive compounds could be classified as alkaloids, terpenoids, steroids, quinones, lignans, phenols, and lactones (Zhang *et al*. 2006; Xu *et al*. 2008). Endophytes producing Podophyllotoxin (PDT), a well-known aryltetralin lignan with potent anticancer, antiviral, antioxidant, antibacterial, immunostimulation, and anti-rheumatic properties, are obtained from endophytic fungus *Alternaria* sp. isolated from *Sinopodophyllum,* and endophytic fungus *Fusarium oxysporum* obtained from *Sabina recurva* (Gao *et al*. 2007; Kour *et al*. 2008). Endophytes producing Camptothecin (CPT), an antineoplastic agent, are obtained from *Entrophospora infrequens* (Puri *et al*. 2005). Endophytes producing immunosuppressives, for example, subglutinols A and B, are produced by *Fusarium subglutinans* (Lee *et al*. 1995). Pestacin and isopestacin as antioxidant were separated from *Pestalotiopsis microspora* associated with *Terminalia morobensis* (Harper *et al*. 2003).

The most frequently encountered endophytes are fungi (Staniek *et al*. 2008). Endophytic actinobacteria have been also isolated from a variety of healthy plant species ranging from crop plants, such as wheat, rice, potato, carrot, tomato, and citrus. Endophytic actinobacteria are relatively unstudied and are also potential sources of novel natural products for exploitation in medicine, agriculture, and industry (Strobel *et al*. 2004). Endophyte association offers the greatest potential for biocontrol programmes because these fungi are integrated into host systems (Cao *et al*. 2005; Clay 1989). Dewan and Sivasithamparam (1989) reported that a fungal endophyte isolated from wheat provides the host with significant protection from infection by the "take all" fungus. Endophytes enhance plant growth (Igarashi *et al*. 2002), and promote plant establishment under adverse conditions (Hasegawa *et al*. 2006). In Algeria, however, endophytes of crop plants have not been studied. The main aim of the study was to isolate endophytic fungi and actinomycetes from leaves and roots of wheat (*Triticum durum*), and extract bioactive secondary metabolites with the use of solvent, then determine the antibacterial and antioxidant activities.

MATERIALS AND METHODS

Samples collection and isolation of strains

Roots and leaves of wheat (*T. durum*) from the Mohamed Ben Bachir variety were collected from the Bordj Bou Arreridj region (Algeria) during the winter of 2010. Each sample was placed in a separate sterilized bag and brought back to the laboratory. Samples were processed within 24 hours from when they were collected (Tejesvi *et al.* 2007). Samples from leaves and roots were washed under running tap water and cut into several pieces of approx 5 mm diameter. Pieces were surface-sterilized by the immersion sequence of: 96% ethanol for 1 min, sodium hypochlorite (2% available chlorine v/v) for 3 min, 96% ethanol for 30 seconds (Larran *et al.* 2007), and then finally rinsed twice in sterile distilled water. Ten pieces per organ were placed in each Petri dish (a dish contained 2% Potato Dextrose Agar (PDA) supplemented with 250 mg/l chloramphenicol and Starch Casein Agar (SCA) to isolate the fungi and the actinomycetes, respectively. Five replicates (Petri dishes) of each sample organ were made. Dishes were then incubated at 26±2°C (Larran *et al.* 2007; Zin *et al.* 2007). The plates were checked each day after inoculation and any fungi or actinomycetes that appeared were isolated, purified, and then maintained at 4°C on PDA and Nutrient Agar (NA) slopes, respectively, for further identification.

Antagonistic study and identification of endophytes

All the 43 pure isolates of endophytic fungi and acinomycetes were screened for their *in vitro* antagonism against phytopathogenic fungi, *Phytophthora infestans* and *Fusarium oxysporum* f. sp. *albedinis* by dual culture, according to the method described by Orole and Adejumo (2009), and Srividya *et al.* (2012). The percentage reduction in radial growth was calculated for each endophyte as follows:

Percentage of inhibition zones

$$I\,(\%) = \left(\frac{A - B}{A}\right) \times 100$$

where:
A – radius of the pathogen in the control plate,
B – radius of pathogen in the dual culture plate.

Primary screening of all 23 pure isolates of actinomycetes against pathogenic bacteria was done by the perpendicular streak method (Yadav *et al*. 2008). Actinomycete was streaked on the nutrient agar as a straight line and incubated at 27°C. After seven days of incubation, test organisms (*Bacillus* sp., *Salmonella typhi, Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli*, *Pseudomonas aerugenosa* and *Staphylococcus aureus*) were streaked perpendicular to the streak line. After 24 hours of incubation at 37°C, the microbial inhibitions were observed by determining the inhibition distance (in mm) between the endophytic actinomycetes and pathogenic bacteria in dual cultures.

Certain pure cultures of the endophyte fungal isolates that proved strong antagonism against phytopathogenic

fungi were selected. Identification at the genus level was based on the morphology of the fungal culture, the mechanism of spore production, and characteristics of the spores, by following the standard mycological manuals (Pitt and Hocking 1985; Botton *et al*. 1990; Champion 1997). The isolates of actinomycetes were characterized up to genus level according to traditional morphological criteria. The characteristics of colonies on the plate, the distinctive reverse colony color, morphology of substrate and aerial hyphae, morphology and mass color of spores as well as diffusible pigment produced were all taken into consideration (Holt *et al.* 1994; Silva *et al.* 2009; Verma *et al*. 2009).

Fermentation and antimicrobial assay

The strains showing moderate to good activity were selected for secondary screening, which was performed by the agar disc diffusion method. For this reason, Erlenmeyer flasks (250 ml) containing 100 ml of Potato Dextrose Broth (PDB) or Nutrient Broth (NB) were autoclaved at 121°C for 15 min. After this, PDB was inoculated with mycelium plugs from the margins of actively growing cultures on PDA of the seven selected endophytic fungi (A1W, A2W, A6W, A7W from leaves, and A3W, A4W, A5W from roots). Nutrient broth was inoculated with plugs from three selected actinomycetes growing on SCA (A8W, A9W and A10W from roots). The flasks were incubated for 3 weeks on a rotary shaker at 150 rpm and 25°C. The fermentation broths were then filtered through two-folds of cheese cloths. The filtrates were extracted twice with equal volumes of ethyl acetate. The organic solvent extracts were evaporated in a rotary evaporator and then stored at 4°C until used (Kwon *et al.* 2007; Zin *et al.* 2007). The ethyl acetate extracts of endophytic fungi were individually tested against a panel of microorganisms including a total of 15 microbial cultures, 3 gram positive bacteria, (*Bcillus* sp., *S. aureus, E. faecalis*), 9 gram negative bacteria (*S. typhi, E. coli, Serratia marcescens, Enterobacter agglomerans, P. aeruginosa, K. pneumoniae, Stenotrophomonas maltophilia, Citrobacter freundii, Pseudomonas* sp.), 2 phytopathogenic fungi (*F. oxysporum* f.sp. *albedinis, P. infestans*) and a yeast (*Candida albicans*). Test organisms were provided by the Laboratory of Microbiology, Ferhat Abbas University. The dried fungal extracts were dissolved in water and DMSO (9:1) to a final concentration of 1 mg/ml. Antimicrobial tests were then carried out by the agar disc diffusion method using 100 µl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast, and 10^4 spore/ml of fungi spread on NA, Sabouraud Dextrose Agar (SDA) and PDA medium, respectively. The discs (6 mm in diameter) were impregnated with 20 µl of the extracts and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the extracts. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains, 48 h for yeast, and 72 h for filamentous fungi (Baris *et al.* 2006). Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated three times.

Antioxidant activity

In this assay, the antioxidant capacity of the extracts was evaluated by the β-carotene/linoleic acid test according the method described by Dapkevicius *et al.* (1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0,5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade) and 25 µl linoleic acid, and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (30 min 100 ml/min) was added and vigorously shaken. From this reaction mixture, 2,500 µl were dispensed into test tubes, and 350 µl portions of the extracts prepared at 2 g/l concentrations were added. The emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with BHT as the positive control, and with a H_20 and methanol as the negative control. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and H_20 and methanol. The percentage of inhibition of each extract was calculated using the following formula:

$$AA\ \% = \frac{Abs_{test}}{Abs_{BHT}} \times 100$$

where:
– AA% – the percentage of antioxidant activity,
– Abs_{test} – absorbance in the presence of the extract (test),
– Abs_{BHT} – absorbance in the presence of the positive control (BHT).

Data analysis

Statistical analysis was done using SAS/STAT ® 9.2. The results of the antimicrobial activity were analyzed statistically by the two-way ANOVA followed by the Student-Newman-Keuls MULTIP-rank test to compare the average inhibition zones of the extracts. Results of the antioxidant activity analysis was made by the one-way ANOVA followed by the Student-Newman-Keuls MULTIP-rank test to compare the inhibition percentages of endophytes extracts and those of the controls. The results were expressed as mean ±SD, and the measures were repeated three times (n = 3). The difference was considered statistically significant when the p value was ≤ 0,05. Colonization and isolation rates were calculated according to Yuan *et al.* (2010) by the following relations:

Colonization rate = Total number of samples/Total number of samples in that trial

Isolation rate = Total number of isolates yielding in the given trial/Total number of samples in that trial.

RESULTS

Endophytes isolation

After the isolation, a total of 43 isolates were recovered from 200 samples (100 segments of roots and 100 segments of leaves), 20 of them were fungi and 23 actinomycetes. The number of isolates, rate of isolation and of colonization obtained with roots, were higher than those obtained with

leaves. The number of fungal isolates recovered from roots was 12 (60%) with a rate of 0,48% and 32% of isolation and colonization, respectively. Whereas, the number of isolates from leaves was 8 (40%) with an isolation and colonization rate of 0,32% and 24%, respectively. Almost, the same results were obtained with the actinomycetes (Table 1).

Antagonistic study and identification of endophytes

The isolates were tested for anti-pathogen activity by the dual culture. Ten isolates (23%) demonstrated the greatest antagonism activity. The isolates A4W, A8W, A9W and A10W were more active against *P. infestans,* with an inhibition percentage that reached 54.07% as the maximum by the isolate A10W. While isolates A2W, A3W, A5W, A7W, A8W and A9W were more active against *F. oxysporum* f. sp. *albedinis,* with an inhibition percentage that reached to 63,89% as the maximum by the isolate A3W (Table 2). The three isolates of actinomycetes A8W, A9W and A10W, were more effective against most test bacteria, especially *E. coli* (Table 3).

Table 1. Number of isolates, colonization, and isolation rate from different tissues

Endophytes	Organs of the wheat plant					
	leaves			roots		
	number of isolates	isolation rate	colonization rate [%]	number of isolates	isolation rate	colonization rate [%]
Endophytic fungi	8	0.32	24	12	0.48	32
Endophytic actinomycetes	7	0.28	20	16	0.64	48

Table 2. Inhibition percentages of fungal growth by isolated endophytic fungi and actinomycetes with higher activity in the primary screening

Isolates	Inhibition percentage [%]	
	Phytophthora infestans	*Fusarium oxysporum* f. sp. *albedinis*
A1W	8.11	11.11
A2W	5.41	44.44
A3W	21.62	63.89
A4W	45.95	16.67
A5W	29.73	52.78
A6W	5.42	16.67
A7W	00	58.33
A8W	42.96	45.00
A9W	51.85	43.33
A10W	54.07	7.50

Table 3. Antagonistic activity of selected endophytic actinomycetes with high activity against test bacteria in the primary screening

Pathgenic bacteria	Zone of inhibition [mm]		
	A8W stain	A9W strain	A10W strain
Bacillus sp.	4	15	14
Staphylococcus aureus	6	6	7
Enterococcus faecalis	00	13	13
Salmonella typhi	8	8	6
Escherichia coli	22	23	15
Pseudomonas aerugenosa	14	7	13
Klebsiella pneumoniae	8	7	00

Table 4. Identity of selected endophytic fungi and actinomycetes isolated from leaves and roots of *Triticum durum* Desf.

Isolate Code	Taxon	Plant Organ
A 1 W	*Alternaria* sp.	leaves
A 2 W	*Cladosporium* sp.	leaves
A 3 W	*Penicillium* sp. 1	roots
A 4 W	*Penicillium* sp. 2	roots
A 5 W	*Aspergillus* sp.	roots
A 6 W	*Chaetomium* sp.	leaves
A 7 W	*Phoma* sp.	leaves
A 8 W	*Streptomyces* sp. 1	roots
A 9 W	*Streptomyces* sp. 2	roots
A 10 W	*Streptomyces* sp. 3	roots

Table 5. Antimicrobial activity shown by selected endophytic fungi and actinomycetes extracts against tests microorganisms in the secondary screening

Pathogenic Micro-organisms	Inhibition Zones [mm] (mean) ethyl acetate extracts of isolates									
	Alternaria sp.	*Cladosporium* sp.	*Penicillium* sp.	*Penicillium* sp.	*Aspergillus* sp.	*Chaetomium* sp.	*Phoma* sp.	*Streptomyces* sp. 1	*Streptomyces* sp. 2	*Streptomyces* sp. 3
Gram+ Bacteria										
Bacillus sp.	11.7	10.7	13.7	13.0	11.7	12.0	00	12.0	00	16.0
Staphylococcus aureus	12.0	10.7	13.0	10.7	11.7	9.7	00	00	00	00
Enterococcus faecalis	11.7	10.3	12.7	12.0	11.3	11.7	13.0	00	00	00
Gram- Bacteria										
Salmonella typhi	14.3	12.3	14.3	11.3	13.3	11.7	10.0	00	00	11.0
Escherichia coli	18.3	16.0	25.3	19.0	21.7	20.0	15.0	24.5	13.0	15.3
Serratia marcescens	14.3	10.7	12.0	12.0	11.3	10.7	11.0	17.0	13.0	14.0
Enterobacter agglomerans	00	11.0	00	00	00	00	10.0	13.0	10.0	9.0
Pseudomonas aeruginosa	00	00	0.0	00	0.0	0.0	00	00	00	17.0
Klebsiella pneumoniae	12.0	9.7	13.0	12.0	11.7	12.7	14.0	00	00	00
Stenotrophomonas maltophilia	00	12.0	12.3	11.7	13.3	00	10.0	00	00	10.0
Citrobacter freundii	00	00	00	00	00	00	00	00	00	00
Pseudomonas sp.	9.3	9.0	9.0	10.3	11.3	00	00	14.0	00	00
Fungi and yeast										
Candida albicans	20.7	15.0	23.0	20.0	13.0	15.0	14.0	19.0	25.0	16.0
Fusarium oxysporum f .sp. *albidinis*	00	00	19.0	11.0	17.0	10.0	15.0	12.0	15.0	00
Phytophthora infestans	8.0	10.0	14.0	17.0	16.0	10.0	00	16.0	14.0	15.3

(mean): average of three replicates (n = 3)

Table 6. Comparison of average inhibitions of extracts obtained by ethyl acetate and their effect on the growth of test microorganisms

Ethyl acetate extracts	Inhibition zones [mm] Test micro-organisms			
	G^- bacteria	G^+ bacteria	fungi	all micro-organisms
Alternaria sp.	7.593 a	11.778 a	9.556 cd	8.822 abc
Cladosporium sp.	8.963 a	10.556 a	8.333 d	9.156 abc
Penicillium sp. 1	9.556 a	13.111 a	18.667 a	12.089 a
Penicillium sp. 2	8.482 a	11.889 a	16.00 ab	10.667 ab
Aspergillus sp.	9.186 a	11.556 a	15.333 abc	10.889 ab
Chaetomium sp.	6.111 ab	11.111 a	11.667 bcd	8.222 abc
Phoma sp.	7.778 a	4.333 b	9.667 cd	7.467 bc
Streptomyces sp.1	7.611 a	4.000 b	15.667 abc	8.500 abc
Streptomyces sp. 2	4.000 b	00 c	18.000 a	6.000 c
Streptomyces sp. 3	8.481 a	5.333 b	10.444 cb	8.244 abc

Means with the same letter are not significantly different at ($p < 0.05$)

Comparison of microscopic and macroscopic characteristics of the ten selected isolates was done by screening with the identification keys. Thus, we were able to identify these isolates at the genus level. The isolate A5W was found to belong to the *Aspergillus* sp. genus, A1W to the genus *Alternaria* sp., both A3W and A4W to the genus *Penicillium* sp., A2W to the genus *Cladosporium* sp., A6W to the genus *Chaetomium* sp., and A7W to the genus *Phoma* sp. The three isolates A8W, A9W, and A10W were found to belong to the genus *Streptomyces* sp. (Table 4).

Antimicrobial assay

All extracts showed inhibitory activity on at least one or more pathogenic microorganisms. The average zone of inhibition varied between 7 mm to 25 mm. The largest zone was of 25 mm against *E. coli* and *C. albicans* by *Penicillium* sp. 1 and *Streptomyces* sp. 2, respectively. Six isolates were active against all Gram positive: *Bacillus* sp., *S. aureus*, *E. faecalis*, and fungal species *F. oxysporum* f. sp. *albedinis* and *P. infestans*. From the 9 Gram negative species utilized, the extracts were more active against 5 species only, which were: *S. typhimurium*, *E. coli*, *S. marcescens*, *K. pneumoniae* and *S. maltophilia*. The other 4 spe-

cies: *E. agglomerans, P. aeruginosa, C. freundii, Pseudomonas* sp. were not sensitive to most of the extracts (Table 5). The comparison of the mean inhibition zones of endophytes extracts obtained by ethyl acetate and their effect on the growth of different groups of pathogenic microorganisms are presented in table 6. The comparison showed that extracts of *Penicillium* sp. 1, *Penicillium* sp. 2 *and Aspergillus* sp. were the most effective, and exhibited broad spectrum activity on the three different groups of pathogenic microorganisms (fungi, Gram positive and negative bacteria). On other hand, the extracts were more effective on Gram positive bacteria and fungi compared to Gram negative bacteria (Fig. 1).

Antioxidant assay

The crude extracts of fungi and *Streptomycetes* were evaluated for their capacity of antioxidant activity using the *β*-carotene/linoleic acid system oxidation. After 24 hours of incubation, the results showed that some of the sample extracts discouraged linoleic acid oxidation, while others were less active. We recorded that each of the extracts of *Penicillium* sp. 2, and *Aspergillus* sp. had an anti-oxidation activity to linoleic acid, and an inhibition percentage which ranged from 78.961±3.183% to 73.977±1.102%, respectively. These are high percentages compared to Butylated hydroxytoluene (BHT), which was considered to have a 97% inhibition to the oxidation of linoleic acid. While, the isolates *Phoma* sp., *Alternar-*

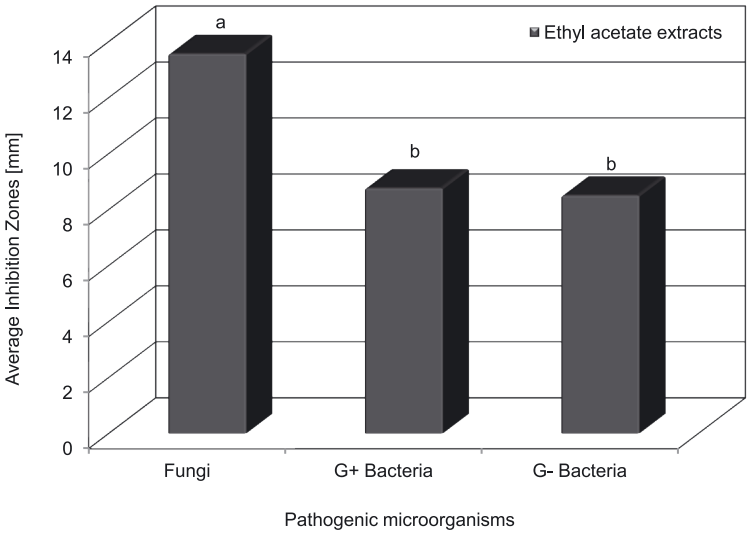

Fig. 1. Comparison of average inhibitions of ethyl acetate extracts on the growth of different groups of pathogenic micro-organisms (fungi, Gram positive and negative bacteria)
Means with the same letter are not significantly different at ($p < 0.05$)

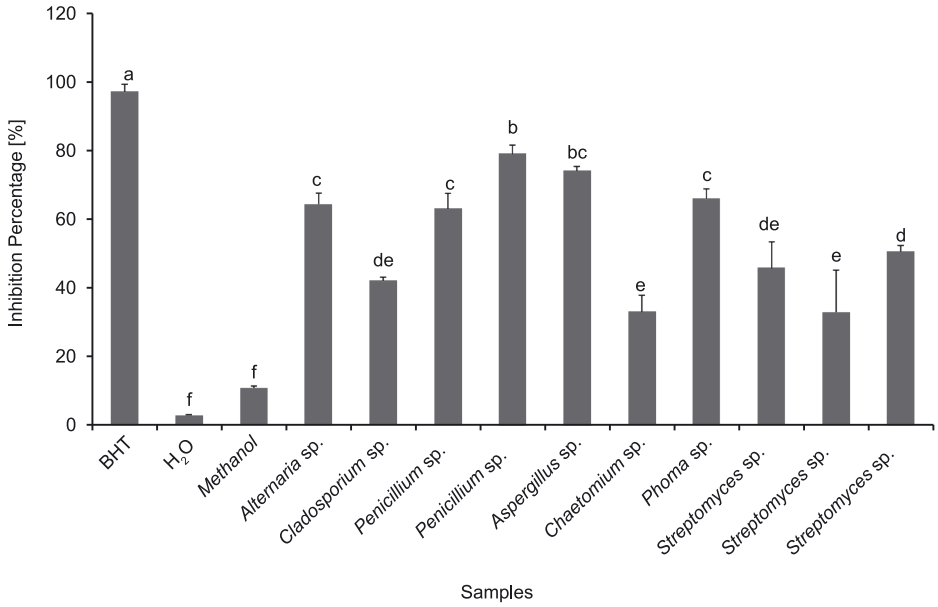

Fig. 2. Antioxidant activity of crude ethyl acetate extracts of endophytes compared to the controls (BHT, MeOH and H_2O) by the test of β-carotene / linoleic acid after 24 hours
Each point represents the mean ±SD (n = 3), each value of the curve followed by the letters shared no significant difference among themselves, at ($p < 0.05$)

ia sp., and *Penicillium* sp. 1 had less inhibition, with inhibition percentages of 65.740±4.486%, 64.218±7.569%, and 63.012±1.734%, respectively. The inhibitory activity of the rest of the isolates: *Cladosporium* sp., *Chaetomium* sp. and three *Streptomycetes* isolates to the oxidation of linoleic acid was less than 50% (Fig. 2).

DISCUSSION

Differences in colonization and isolation rates have been proven in several other studies and are in agreement with our results. The differences were those observed between plants tissues such as roots, leaves, and stems of wheat (*T. aestivum*) (Sieber *et al.* 1988; Larran *et al.* 2007); between roots and leaves of rice (Naik *et al.* 2009; Tian *et al.* 2004) as well as between roots, leaves, stems, and flowers of several species of medicinal plants (Gong and Gou 2009; Lv *et al.* 2010). The difference in endophytes assemblages in the various tissues indicated that some fungal endophytes have an affinity for different tissue types. This affinity might be a reflection of their capacity for utilizing or surviving within a specific substrate (different tissue texture and chemistry) (Huang *et al.* 2008).

Most of the isolated fungal taxa active in this study, belong to the common isolated endophytes and were reported as endophytes in previous studies on crops plants. They were isolated from *T. aestivum* (Larran *et al.* 2002; Larran *et al.* 2007), and from rice *O. sativa* (Tian *et al.* 2004; Naik *et al.* 2009). The most frequently isolated actinomycetes strains from healthy plants were those belonging to the genus *Streptomyces* (Zin *et al.* 2007).

As all taxa of endophytes were from healthy tissues, it appears that either they were non-pathogenenic or the plants had developed a resistance mechanism against the pathogens. This suggests that the isolates recovered here are either avirulent or hypovirulent or are virulent but in a latent phase (Petrini 1991). It is probable that these taxa are not pathogens for their antagonism against phytopathogens. These fungi could be adapted to this host and be antagonists of their pathogens. Depending on their antagonistic capacity, they would be able to displace, reduce, suppress or induce resistance against them (Larran *et al.* 2007). Those active endophytic fungi inside the plants may play an important role in protecting the plant host against pathogenic microorganisms and have an intimate correlation with the development and physiological activity of wheat (Tian *et al.* 2004).

There are many reports about antimicrobial compounds produced by endophytes in cultures that were active against plant and human pathogenic microorganisms. Chareprasert *et al.* (2006) reported an antimicrobial activity exhibited by endophytic fungi isolated from teak and rain trees. These fungi were found to produce some metabolites active against bacteria and yeast. From 67 endophytic fungi isolated from *Q. variabilis*, 19.4% (*Aspergillus* sp., *Penicillium* sp., and *Alternaria* sp.) showed significant antimicrobial activity (Wang *et al.* 2007). In accordance with Lin *et al.* (2007) concerning the study of the medicinal plant *C. acuminate*, 174 endophytic fungi were isolated and from 18 taxa. *Alternaria* (12.6%) was dominant, and three showed antimicrobial activity from 22 *Alternaria* extracts tested. With regard to actinomycetes, Rabah *et al.* (2007) isolated a number of *Streptomyces* with antimicrobial activity. Among them, three isolates (SS15, SS19 and SS7) demonstrated antimicrobial activity against Gram positive and negative bacteria, yeast, and fungi.

Antagonism might be due to the production of biologically active compounds in media (Castillo *et al.* 2002). The reason for the different sensitivity between Gram positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram negative bacteria have an outer polysaccharide membrane which carries the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes. Gram positive should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Pandey *et al.* 2004; Ogundare *et al.* 2006). The results obtained in our study suggest that these endophytes have the potential to be a source for novel bioactive products.

An enormous variety of plants have been studied for new sources of natural antioxidants. Phenolic and flavonoid compounds derived from plants were proved to be potent antioxidants and free radical scavengers. Significant correlations between phenolic compounds and antioxidant properties of medicinal plants were noted (Baghiani *et al.* 2010; Khennouf *et al.* 2010). The same was seen in studies on endophytes. Each of the *Phoma*, *Cladosporium,* and *Chaetomium* fungi were found to have antioxidant activity. This activity was greater among the *Chaetomium* fungus accompanied by a greater proportion in the total phenolic content compared with other active isolates. The same was with the endophytic fungus *A. alternata* (Fernandes *et al.* 2009). Furthermore, ethyl acetate is often used as an extraction solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols (Scholz and Rimpler 1989). On the other hand, Conde *et al.* (2008) have reported that ethyl acetate allowed the highest phenolic content and the selective removal of nonphenolic compounds. Therefore, it could be that the antioxidant activity of ethyl acetate extracts of the endophytes isolated from wheat was caused by the presence of phenolic compounds in the extracts.

The present results may lead to the conclusion that endophytes are considered to be a potential source for novel bioactive products (Strobel 2003). The data presented in this study demonstrated that extracts of endophytic fungus and *Streptomycetes* isolated from wheat, have antimicrobial and antioxidant activities, especially *Penicillium* sp. 1, *Penicillium* sp. 2, and *Aspergillus* sp. Thus, endophytic fungi and actinomycetes play an important role in the search for natural compounds. Endophytic fungi and actinomycetes might also represent an alternative source for the production of therapeutic agents and bioactive metabolites that are not easily obtained by chemical synthesis, and which have a high activity against pathogenic microorganisms. However, this work will serve as a prelude to more comprehensive studies on the chemistry and biology of the bioactive natural products produced by these endophytes. Further examination can be done to learn if endophytes may have the potential to serve as a biological control or as new pharmacological agents.

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