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Molecular Characterization and Fungicidal Activity of some Isolated Endophytic Fungi from some Wild Plants in Egypt

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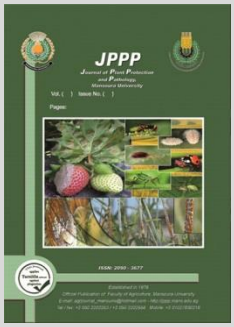

ABSTRACT

Endophytic fungi have several beneficial properties like the production of various bioactive compounds and antimicrobial impacts against many plant pathogens. So, they are qualified to be good biological control agents. The present study aimed to detect endophytic fungal isolates from eight wild plants in Egypt, identify these isolated fungal strains, determine the colonization frequency %, the most prevalent fungal strains and study the antimicrobial activity of these dominant isolates. Fifteen endophytic fungal strain was isolated from the selected wild plant species and morphologically identified. The most four dominant fungal strains were selected and confirmed their identification based on the internal transcribed spacer (ITS) region sequence by using the polymerase chain reaction (PCR) with the primer pairs ITS1/ITS4. The results revealed that among the selected plant species, *Calotropis procera* had the highest endophytic fungi diversity (relative frequency 25%). The most dominant isolates were molecularly identified as *Aspergillus niger*, and *Beauveria bassiana* with dominance percentage 15.25% followed by *A. terreus* (13.5%) then *Trichoderma harzianum* (11.86%). The antagonistic activity of these four isolates against some plant pathogenic fungi using both dual culture technique and the ethyl extracts of these isolates at different concentration (100, 200, 300 ppm) were examined. The results proved the efficiency of almost all of these isolates in repressing the pathogen growth except endophytic *B. bassiana*, the effect was not clear. This study revealed that wild plants are a good source of many endophytic fungal strains that may be exploited in different fields like the biological control field.

Keywords: Antifungal Activity; Endophytes; Ethyl Acetate Extracts; plant pathogens

INTRODUCTION

In the current years, the control of plant disease has faced great challenges. The use of chemical fungicides is an effective method but by its continuous applications leads to the appearance of fungicide -resistance strains of plant pathogenic organisms due to its adaptability and the breakdown of plant resistance, negative effects on the environment and human health especially, if residues persist in the soil or migrate off-site and enter waterways. For all these previous reasons, there is a great demand to find an alternative method to eliminate these pathogens. (Rotolo *et al*. 2018).

Recently, Biological control is an effective and essential method for fungal disease management. Among the biocontrol agents, the utilization of endophytes which are highly widespread and diverse microorganisms (bacteria, fungi and unicellular eukaryotes) that spend whole or a part of their life cycle within intact tissues of the host without inducing pathogenic symptoms (Borges *et al*. 2009). The most prevalent microbes present as endophytes in plants are fungi and bacteria, but the highly isolated are fungi (Staniek *et al*. 2008). Fungal endophytes display a range of symbiotic relationships with their hosts, it can be mutualistic in which benefits pervade both partners (Card *et al*. 2016). Some endophytes may have a mutualistic relation for one plant species only, but not for another (Hardoim *et al*. 2015).

These types of microorganisms have shown special troth in food safety, plant growth, crop protection, phytoremediation and ecological balance (Sudha *et al*. 2016; Murphy *et al*. 2018) and many are known to stimulate the host resistance against abiotic and biotic stress (Gill *et al*. 2016). Endophytic fungi are currently considered as a rich source of new bioactive natural products with antimicrobial, insecticidal, and anticancer activities (Strobel and Daisy 2003; Yu *et al*. 2010; Kharwar *et al*. 2011; Han *et al*. 2013). The production of such compounds by an endophyte is not indiscriminate but may be related to its ecological niche (Haque *et al*. 2005)

Determination of antimicrobial action against phytopathogenic organisms can be used as an easy and efficient examination method to several fungal endophyte strains which can act as bioactive agents (Geris *et al*. 2003; Zhang *et al*. 2012; Kusari *et al*. 2013; Qadri *et al*. 2014). Currently, a great number of fungal endophytes isolated from numerous hosts have been characterized by its highly antimicrobial activities because it contains many bioactive compounds like terpenoids, alkaloids, phenylpropanoids, aliphatic compounds, polyketides and peptides (Mousa and Raizada 2013). There are 230 metabolites produced by plant-associated microbial strains, including many fungal endophytes, as reported by Gunatilaka (2006). Hence, This study is carried out to evaluate the diversity of endophytic fungi colonizing some wild plants in Egypt, and investigate its molecular characterization and antimicrobial activity.

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MATERIALS AND METHODS

Plant sample collection

This study was conducted on eight healthy and mature wild plants selected from the Delta region, Egypt from Jan to Mar 2019. The wild plants used as sources of endophytic fungi were listed in Table 1.After plant selection, disease-free parts of the plant, that is, stem, root, and leaves, were excised with a sterile scalpel and put in sterile plastic bags at 4 °C until transported to the laboratory on the same day for the isolation of the endophytic fungi. The taxonomic identification and laboratory procedures were carried out at the Plant Taxonomists and Microbial Molecular Biology Laboratories in Botany Department, Faculty of Science, Mansoura University.

Table 1 . List of wild plants used as sources of endophytic fungi.

No.	Wild Plant	Family
1	*Tamarix nilotica*	Tamaricaceae
2	*Calotropis procera*	Apocynaceae
3	*Typha domingensis*	Typhaceae
4	*Ammi majus*	Apiaceae
5	*Anagallis arvensis*	Primulaceae
6	*Convolvulus arvensis*	Convolvulaceae
7	*Solanum nigrum*	Solanaceae
8	*Rumex vesicarius*	Polygonaceae

Isolation and identification of endophytic fungi:

The endophytic fungi were isolated using a modified method described by Hallmann *et al*. (2006). The plant samples were thoroughly washed in running tap water for 10 min followed by distilled water to remove dust and debris and then air-dried. Leaves and small branches of each plant sample were individually cut into small fragments using a sterile scalpel into 1 cm long segments after this immersion in 75% ethanol for 1 min, 5% sodium hypochlorite solution for 3 min, 75% ethanol for 30 s and finally swilled with sterile distilled water for 3 to 5 s and dried by sterilized filter papers under aseptic condition. About 6–8 sterilized segments of each plant species were inoculated on potato dextrose agar (PDA) supplemented with chloramphenicol (50 µg/mL, Merck) and streptomycin sulfate (250 µg/mL, Sigma) to suppress bacterial growth and incubated at 28 °C until the outgrowth of endophytic fungi appeared. Pure cultures were inoculated on PDA plates free of antibiotics and incubated for 14 d at 28 °C. After this incubation period, these pure cultures were stored under 20% glycerol at -70 °C used as a stock culture for further studies.

The isolates of endophytic fungi were identified by the morphology of the fungal culture, including colony and medium color, colony characters, spore characters, mycelium characters and reproductive characteristics by following the standard mycological manuals according to Domsch *et al*. (1980). The colonizing frequency (CF%) of each endophytic fungus and the percentage of the dominant endophytic fungi percentage was calculated (Petrini and Fisher 1988; Kumar and Hyde 2004). The most dominant fungal isolates were selected for molecular identification and further experimental investigation.

$$CF\,\% = \left(\frac{\text{Number of segments colonized by single endophytes}}{\text{Total number of segments analyzed}}\right) \times 100$$

Molecular identification of endophytic fungi:

The four dominant fungal isolates were screened for molecular identification. The DNA was prepared according to Sharma *et al*. (2008). Fungal isolate was grown in 15mL of potato dextrose broth medium (PDB) at 25 ºC with continuous shacking at 250 rpm for 7 d. The cell pellet was collected by centrifugation at 13,000 rpm for 4 min and washed by distilled water. About 0.05 g of fungal mycelia were scraped and homogenized in liquid nitrogen using sterile mortar and pestle. The powdered mycelium was suspended in one ml of extraction buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 2% CTAB), shacked gently and incubated for 10 min at 65 ºC then centrifuged at 12,000 rpm for 5 min to remove the remained of cell lysate and add equal volume of chloroform isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 5 min. Transfer upper aqueous phase in clean Eppendorff then add two volumes of ice-cold isopropanol and incubated at -20 ºC for 10 min to precipitate DNA. After this one µL of RNase (20mg/mL) was added and incubated at 37 ºC for about one h. DNA pellet was collected by centrifugation at 13,000 rpm for 15 min and dissolve the pellet in the suitable volume of sdH_2O then left for an hour before being analyzed by gel agarose electrophoresis. DNA was fractionated in agarose gel (0.8%) by electrophoresis for 1h at 100 V in TAE buffer (40 mM Tris; 2 mM EDTA; 20 mM glacial acetic acid, pH 8), stained with ethidium bromide, visualized under UV illumination and photographed. The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified by PCR with the primer ITS1/ITS4 (5/ TCCGTAGGGTGAACCTGCGG /3) / (5/ TCCTCCGCTTATTGATATGC /3) (White *et al*. 1990).

The PCR was performed in 50 µL reaction volume which contained PCR buffer (10 mM tris- HCl PH 8.3, 50 mM KCl, 2 mM $MgCl_2$), 250 µM each of dGTP, dATP, dCTP and dTTP, 2 units of Taq DNA polymerase,100 pmol of each primer and DNA template. Components were overlaid with a drop of mineral and DNA amplification started with denaturing the template DNA at 94 ºC for 5 min followed by 35 cycles. Each cycle consisted of denaturation at 94 ºC for 1 min, annealing for at 50 ºC for 1 min and extension at 72 ºC for 1 min, and a final extension step at 72 ºC for 7 min. The PCR product was analyzed by agarose gel electrophoresis. The PCR products were purified by Quick gel extraction and PCR purification combo kit. Using an automated DNA sequencer (ABI PRISM 3700), the PCR products were sequenced then the Sequences were submitted to GenBank on the NCBI (http://www. ncbi.nlm.nih.gov). The resulting Sequences in this work were compared with the GenBank database using the BLAST software on the NCBI (http://www. ncbi.nlm. nih.gov/ BLAST/).

Antifungal activity

Assay for antifungal activity using dual culture plate technique.

The antifungal activity of the four most common endophytic fungal isolates was tested by dual culture plate technique (Katoch and Pull 2017) against three plant pathogenic fungi (*Alternaria solani, Fuzarium solani* and *Rhizochtonia solani*) which obtained from Plant-Pathogen Research Institute, Agriculture Research Center, Giza, Egypt.

Endophytes and all the test pathogens were grown on PDA plates for 7 d at 25 ºC. Two mm. diameter plugs of newly grown endophyte and test pathogens were taken with

the help of cork borer. Endophytic and pathogenic fungi were aseptically placed 30 mm away from each other on the opposite sides on PDA medium and incubated at 25 ºC for 7 d. Simultaneously the disc of an endophyte and each test pathogen were placed separately on the PDA plate which served as control. All the inoculated plates were allowed to grow. Three replicates were used for each pathogen. After seven days the inhibition zone between endophytic and pathogenic fungi was determined by observed the growth of endophytic and pathogenic fungi on the plates.

Assay for antimicrobial activity using ethyl acetate extracts of isolated endophytic fungi

For Preparation of ethyl acetate extracts, Fungal conidia (30 mL, 1×10^6 conidia/mL) of four fungal isolates were inoculated on 300 ml of potato dextrose broth (PDB) medium and incubated for the production of the seed inoculum at 25 °C for 7d. The seed inoculums were inoculated again in about 4 L of PDB and incubated at 25 °C for 21 d after incubation period the metabolites were extracted by the method of Chen *et al*. (2018) and Abdullah, (2019) using ethyl acetate (EthOAc) from the cell-free culture supernatant.

Aliquots of the fungal culture were mixed with a half volume of ethyl acetate (1: 0.5) and mixed vigorously then the mixture was put in separating funnel and leave them two hours. The organic phase from the above extraction mixture was collected and concentrated by using a rotary evaporator at 45 ºC under reduced pressure (RE-52A, Shanghai YaRong Biochemical Instrument Factory, Shanghai, China) then the crude fraction extract stored at -20 ºC until use.

The EthOAc extracts of each fungal isolates were weighed and dissolved in 25 mL dimethyl sulfoxide (DMSO) as a stock solution. The concentration of the stock solution was calculated by this formula ((wt. (g) /25) × 10^6) to obtain part per million (ppm) concentration. Three concentrations from each extract were prepared by dilution in sterilized distilled H_2O to use in antimicrobial activity experiment.

Freshly prepared fungal spores suspension of plant pathogenic fungal strains was inoculated in the center of plates with PDA medium using a micro applicator to spread and cover the whole plate then incubated at 25 °C for 7 d. Mycelial discs (1 cm) were removed and cultured on PDA medium having the prepared concentration of ethyl acetate extract of isolated endophytic fungal strains and incubated at 25 ºC for 7 d. Simultaneously, PDA plates with DMSO were inoculated by pathogenic fungi served as control. There were 5 Petri dishes of each treatment as replicates. After 7 d of incubation, the colony diameter of the pathogen was measured and recorded in each treatment and the percentage of growth inhibition (GI %) of the plant pathogenic fungi was calculated using the following formula (Ghildiyal and Pandey 2008).

$$GI\ \% = 1 - \left(\frac{Diameter\ of\ pathogen\ colony\ in\ treatment}{Diameter\ of\ pathogen\ colony\ in\ control}\right) \times 100$$

RESULTS AND DISCUSSION

Results

Isolation and Identification of endophytic fungal strain

Wild plants were selected and screened for the presence of endophytic fungi. Most isolates were recorded during the 14th d of incubation. Different endophytic taxa exhibited various relative frequencies on diverse plants (Fig. 1). *Calotropis procera* had the highest endophytic diversity (relative frequency 25%), however, *Solanum nigrum* had the lowest endophytic diversity (relative frequency 5%). Fifty nine isolates belonging to 15 species and nine genera were obtained from stem and root samples and identified morphologically as shown in Table 2. Among these isolates, the most prevalent species were *Aspergillus niger*, and *Beauveria bassiana* with dominance percentage of 15.25% followed by *Aspergillus terreus* (13.5%) then *Trichoderma harzianum*,11.86% while *Curvularia lunata*, *Drechslera bicolor* and *Penicillium expansum* recorded the lowest dominance 1.7%.

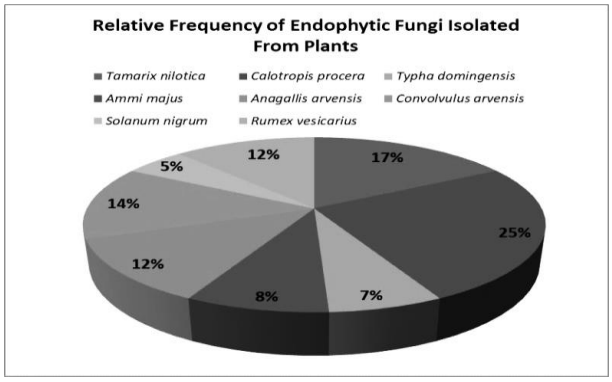

Fig. 1. Relative frequency of endophytic fungi isolated from wild plants.

Table 2. Frequency of endophytic fungi isolated from eight wild plants.

Endophytic fungi	Number of isolates	CF %	Dominant fungi %
1.*Alternaria alternata*	3	25	5.10
2.*Aspergillus niger*	9	75	15.25
3.*Aspergillus terreus*	8	62.5	13.50
4.*Aspergillus terricola*	2	12.5	3.38
5.*Beauveria bassiana*	9	50	15.25
6.*Cladosporium cladosporioides*	2	12.5	3.38
7.*Curvularia lunata*	1	25	1.70
8.*Drechslera bicolor*	1	12.5	1.70
9.*Fusarium oxysporum*	3	37.5	5.08
10. *Fusarium solani*	2	25	3.38
11. *Penicillium citrinum*	2	25	3.38
12. *Penicillium chrysogenum*	4	62.5	6.77
13. *Penicillium expansum*	1	37.5	1.70
14. *Trichoderma harzianum*	7	50	11.86
15. *Trichoderma viride*	5	37.5	8.47
Total number of isolates	59		

CF = Colonization Frequency

In molecular identification of the different endophytic fungi, varieties of techniques were used depending on the gene and protein contents of each strain. Each one of the used techniques has its own advantages, but the added value of their combination turned out to be of great validity in characterization. Molecular identification, of which only four dominant fungal isolates were successfully amplified using primers ITS1 and ITS4 and the obtained DNA homology sequence compared to the data contained in GenBank using the BLAST program on NCBI. BLAST searches revealed their identities as members of three different genera (Fig. 2) which belongs to the phylum Ascomycota. Therefore, BLAST and phylogenetic analyses

of other genomic regions should be combined with those of the ITS region to improve the accuracy of identification.

Isolates no. 80787 and 31139 were related to fungal endophytes for ITS sequences that belonged to *A. niger* and *A. terreus*, respectively Fig. 2(a, b). Isolates no 175879 was *B. bassiana* and Isolates no 39527 was closely related to *Trichoderm harzianum* (Fig. 2c, d). The ITS sequences obtained through this study were deposited in the NCBI GenBank by Accession no MT645496, MT645497, MT645498 and MT645500 for *A. niger, B. bassiana, A. terreus* and *T. harzianum*, respectively, for future reference.

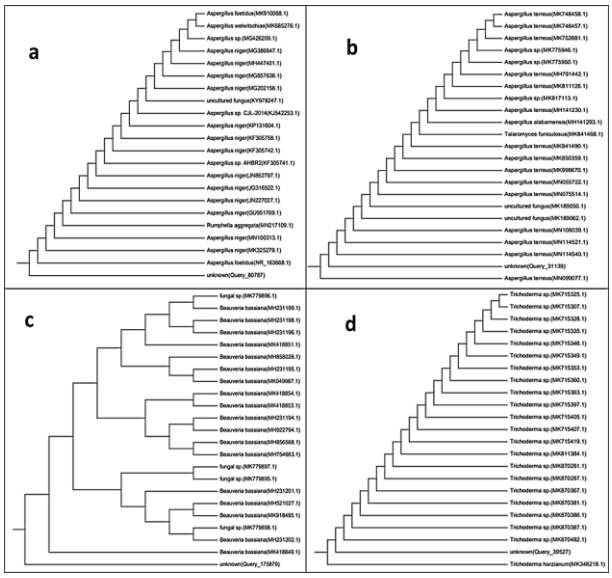

Fig. 2. Phylogenetic tree based on ITS sequences of endophytic fungal isolates and fungal ITS sequences from the GenBank. A: *Aspergillus niger*. B: *Aspergillus terreus*. C: *Beauveria bassiana*. D: *Trichoderma harzianum*.

Antagonistic activity of isolated fungal strains using dual culture technique

The antagonistic activity of the most common four isolated endophytic fungi against three strains of phytopathogenic fungi (*Fusarium solani*, *Rhizoctonia solani* and *Alternaria solani*) was examined *in vitro* by dual culture plate technique and as shown in Table 3 and Fig. 3. All of the isolated endophytes exhibited a negative effect on the microbial growth of *F. solani* and *R. solani* whoever in the case of pathogenic *A. solani* the effect of *B. bassiana* was not distinguished compared with other three endophytic strains.

Table 3. The antagonistic activity of tested endophytic fungal strains against some phytopathogenic fungi using dual culture technique.

endophytic fungal isolates	The sensitivity of plant pathogenic fungi to beneficial fungal strains on PDA plates		
	Rhizoctonia solani	***Alternaria solani***	***Fusarium solani***
Aspergillus niger	+	+	+
Aspergillus terreus	+	+	+
Trichoderma harzianum	+	+	+
Beauveria bassiana	+	-	-

(+) There is antagonism (-) : There isn't antagonism

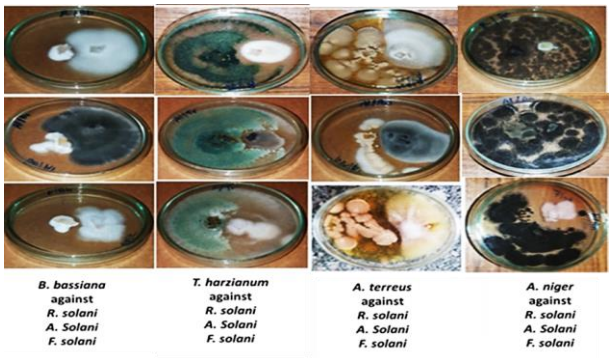

Fig. 3. Antagonistic activity of isolated fungal strains against some plant pathogenic fungi on PDA plates

Inhibitory effect of the ethyl acetate extract of isolated fungal strains against some plant pathogenic fungi

Ethyl acetate extract was prepared from these fungi separately and the yield of metabolites was estimated as shown in Table 4. *Aspergillus niger* was at the forefront in the production of metabolites followed by *A. terreus* then *T. harzianum* and *B. bassiana* which recorded the least amount of metabolites. The antifungal activity of the prepared ethyl acetate extracts of the endophytic fungi at different concentrations (100, 200, 300 ppm) against the test phytopathogenic fungi (*F. solani*, *R. solani* and *A. solani*) were evaluated and the results (Table 4; Fig. 4) show that almost all extracts exhibited antimicrobial activity against the test organisms except in case of *B. bassiana* extract at concentration 100 ppm where no antimicrobial activity recorded while at concentrations 200 ppm and 300 ppm the antimicrobial activity was the lowest. On the other hand, the ethyl acetate extracts of *A. niger*, *T. harzianum* and *A. terreus* gave a high antimicrobial activity against the test pathogenic fungi which increased by increasing its concentrations.

Table 4. Yield of metabolites derived from ethyl acetate extracts of endophytic fungal strains and its antagonistic activity against some phytopathogenic fungi.

Ethyl acetate extracts of isolated fungal strains	Yield of metabolites (mg)/ 4L culture broth medium	Conc. (ppm)	Growth inhibition% (GI %)		
			Fusarium solani	***Rhizoctonia solani***	***Alternaria solani***
Aspergillus niger	821	100	48	61	53
		200	60	75	68
		300	74	91	81
Aspergillus terreus	596	100	21	29	23
		200	32	38	34
		300	45	50	48
Trichoderma harzianum	298	100	45	51	49
		200	53	60	58
		300	63	71	67
Beauveria bassiana	257	100	0	0	0
		200	21	16	0
		300	30	20	17

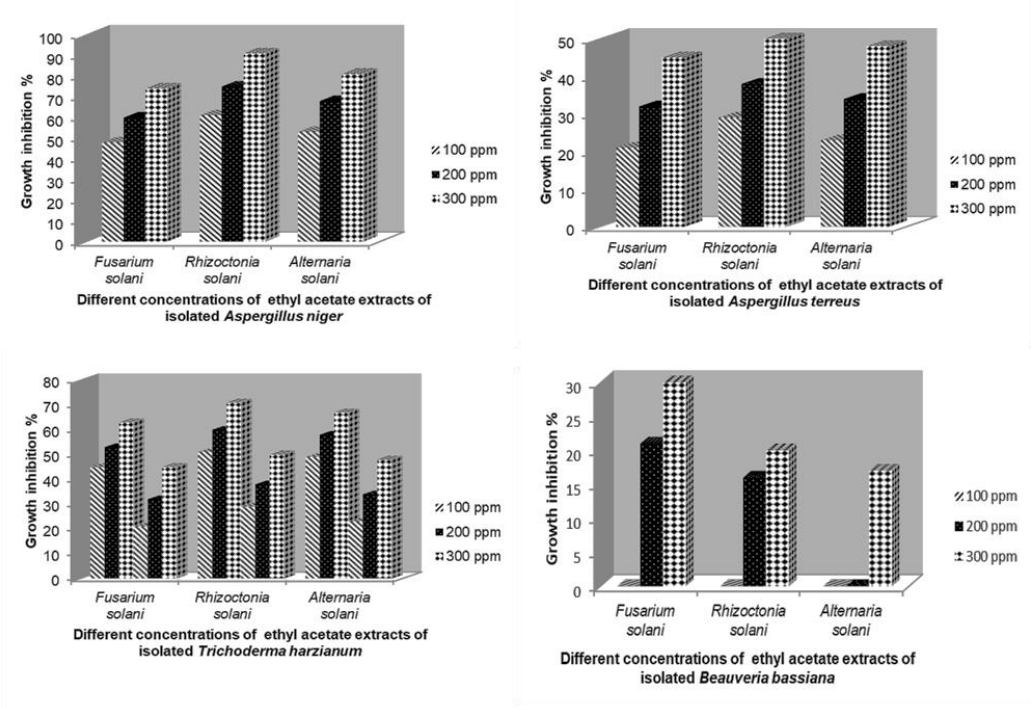

Fig. 4. Effect of different concentrations of ethyl acetate extracts of isolated endophytic fungi.

Discussion

Endophytes reside asymptomatically inside healthy plant tissues to obtain food and shelter and at the same time, the previous studies proved its benefits for the host plants such as the production of multiple effective metabolites which increase host fitness and resistance against some physiological stresses and several types of plant pathogens (Gond *et al*. 2012). The presence of endophytes in many plant species has been well affirmed. In the current study, eight wild plants were checked for the existence of fungal endophytes and almost all of the isolates were registered in the first two weeks of incubation. This finding is in correspondence with results obtained by El-Maghraby *et al*. (2013) for the rate of isolation of endophytic fungi from three different leguminous plants. Among the tested host plants, *Calotropis procera* recorded the highest endophytic diversity and the most common fungal species were *A. niger*, and *B. bassiana* followed by *A. terreus* then *T. harzianum*. These finding is in agreement with Aharwal *et al*. (2014) who isolated 12 different endophytic fungal species from *C. procera* in India where *Aspergillus* spp. were the most prevalent. Also, Gashgari *et al*. (2016) found similar results by screening endophytes colonized some medicinal plants in Saudi Arabia. Moreover, among the fungi isolated from *Hyoscyamus muticus* L. in Eygpt, the endophytic fungi belonging to *Aspergillus* spp. were the most common (Abdel-Motaal *et al*. 2010). Some of the recorded fungal strain in the current survey famed by its phytopathogenic ability and this isn't wondered because the balance between pathogenicity and endophytism of the fungal strain in the different plant hosts determine whether it is an endophyte in one plant or as a pathogen in another (Tan and Zou 2001). The same fungal strains have been isolated as endophytes from different host plants: *Melia azedarach* (Geris *et al*. 2003); *Thymus decussatus* (Selim *et al*. 2011); *Ipomoea carnea* (Tayung *et al*. 2012) ; *Solanum mauritianum* (Pelo *et al*. 2020) .

Recent studies have successfully employed molecular techniques for the identification of endophytic fungi (Morakotkarn *et al*. 2007). As a reinforcement of phenotypic identification in this current study, the four most dominant fungal isolates were selected for molecular identification based on ITS sequences and the results show a full consistency between the molecular identification and the morphological identification. Previous studies indicated that different *Aspergillus* spp have been isolated as endophytes from different plants such as *Ziziphus* sp., *Cynodon dactylon, Pinus thunbergii*, *Spergularia marina* and *Ensete ventericosum* (El-Nagerabi *et al*. 2013; Kim *et al*. 2014; Chauhan *et al*. 2019).

Recently, it is known that some of the host plants depend on their mutualistic relationship with endophytic fungi in protection against pathogens (Tan and Zou 2001). In our study, the negative effect of the four endophytic fungal strain on the growth of tested pytopathogenic fungi was obvious by using dual culture technique with an exception in the case of endophytic *Beauveria bassiana* with pathogenic *Alternaria solani*. The antifungal activity of endophytic *A. niger, A. terreus* and *Trichoderma* spp against some phytopathogenic fungi have been reported in many studies (Gherbawy and Gashgari 2014; Gherbawy and Elhariry 2016; Talapatra *et al*. 2017). To the best of our knowledge, the antifungal activity of Endophytic *Beauveria bassiana* has not been widely reported but it recorded a significant level of antagonism against some phytopathogenic fungi such as *Botrytis cinerea*, *Fusarium oxysporum* and *Rhizoctonia solani* (Shternshis *et al*. 2014; Barra-Bucarei *et al*. 2020). Several previous studies reported that endophytic fungi produce metabolites with antimicrobial action (Porras-Alfaro and Bayman 2011; Katoch *et al*. 2017). These results enhanced the effective role of endophytic fungi in the protection of their host plants from fungal pathogens.

Previous studies reported that extraction with ethyl acetate was the most powerful process for obtaining secondary endophytic fungal metabolites (Garcia *et al*. 2012; Chen *et al*. 2018) and recorded the highest antimicrobial activity in comparison to other different solvents (Malhadas *et al*. 2017). So that, crude ethyl acetate extracts of the selected endopytic fungal isolates were prepared with different concentrations and examined for its antifungal activity against tested phytopathogenic fungi. As obvious from the present study, *A. niger* recorded the highest amount of metabolites followed by *A. terreus* then *T. harzianum* and *B. bassiana*. Different *Aspergillus* species were known by the production of a wide range of secondary metabolites which have been used as antimicrobial agents (Losada *et al*. 2009). By examination of the antifungal potency of the prepared ethyl acetate extract (at 100, 200 and 300conc.), the extract of *A. niger* at all concentration was the most effective in growth inhibition of phytopathogenic fungal strains followed by *T. harzianum* then *A. terreus* this may be due to the highest amount of metabolites produced by *A, niger*. The effect of *Aspergillus* spp. isolates against the tested phytopathogenic fungi have been proven by different previous studies but their carcinogenic properties limit their exploitation in the biological control field (Elazab 2019). Nowadays, most *Trichoderma* strains famed with the ability to produce diverse types of volatiles and non-volatiles toxic metabolites such as harzianic acid and mycotoxins which induce antimicrobial activities and ethyl acetate is the best solvent for extracting secondary metabolites from *Trichoderma* spp. isolates (Leylaie and Zafari 2018). The ethyl extract from *T. harzianum* was more effective in controlling the growth of many plant pathogens as *Scleromitrula shiraiana* (Chen *et al*. 2018). Although the total amount of metabolites produced by *T. harzianum* was lower than that of *A. terreus* in this study, the antifungal effect of the ethyl extract of *T. harzianum* was higher than its corresponding of *A. terreus.* This may be suggested the various types of metabolites produced by *T. harzianum* and its high efficiency as an antimicrobial agent. On the other hand, the lowest antimicrobial effect of ethyl acetate extract of *B. bassiana* may be due to smaller amounts of its active compounds as reported by Tolulope *et al*. (2015) who attributed the noticeable variations in antagonistic effect by fungal extracts to the amount and concentration of the active compounds and this is also confirmed by Joel and Bhimba (2013) who declared that the active ingredients in the fungal extract and the solubility of the active compounds in the solvent used (ethyl acetate) may cause the antimicrobial effect.

CONCLUSIONS

Finally, this is may be the first study in which some endophytic fungal strains were isolated from eight wild plants in Egypt. Molecular characterization including sequencing and phylogenetic analysis were successful for identifying the most common endophytic fungal isolates. Almost of all of these strains had a potential antimicrobial activity against some phytopathogenic fungi. The results obtained in this study suggested that wild plants in Egypt may harbor diverse strains of endophytic fungi which can be exploited for different purposes such as biocontrol agent and a source of many different active metabolites and this need further future research.

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التوصيف الجزيئي والفاعلية الابادية للفطريات لبعض الفطريات النباتية الداخلية المعزولة من بعض النباتات البرية في مصر

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تمتلك الفطريات النباتية الداخلية العديد من الخصائص المفيدة مثل إنتاج العديد من المركبات النشطة بيولوجيًا وتأثيرات مضادات الميكروبات ضد العديد من مسببات الأمراض النباتية. لذلك ، فانها مؤهلة لتكون عوامل مكافحة بيولوجية جيدة. هدفت الدراسة الحالية إلى الكشف عن العزلات الفطرية النباتية الداخلية في ثمانية نباتات برية في مصر و توصيف هذه السلالات الفطرية المعزولة و وتحديد النسبة المئوية لتكرار الاستعمار و والسلالات الفطرية الأكثر انتشارًا وأخيرا دراسة النشاط المضاد للميكروبات لهذه العزلات السائدة. تم عزل خمسة عشر سلالة فطرية من النباتات البرية المختارة وتم توصيفها مظهريا . تم اختيار السلالات الفطرية الأربعة السائدة وتأكيد توصيفها جزيئيا بناءً على تسلسل منطقة ITS علي DNA باستخدام تفاعل البلمرة المتسلسل (PCR) مع زوج البادئات / ITS1 ITS4. أظهرت النتائج أنه من بين الأنواع النباتية المختارة ، كان *Calotropis procera* يحتوي على أعلى تنوع في الفطريات النباتية الداخلية بنسبة ٢٥ %. تم التوصيف الجزيئي للعزلات الأكثر انتشارا وكانت *Aspergillus niger* و *Beauveria bassiana* بنسبة ١٥,٢٥٪ يليها *A. Terreus* بنسبة ١٣,٥٪ ثم *Trichoderma harzianum* بنسبة ١١,٨٦٪. تم اختبار النشاط المضاد لهذه العزلات الأربع ضد بعض الفطريات الممرضة للنبات باستخدام كل من تقنية الزراعة المزدوجة ومستخلصات الإيثيل لهذه العزلات وذلك بتركيزات مختلفة (١٠٠ ، ٢٠٠ ، ٣٠٠ جزء في المليون). أثبتت النتائج كفاءة كل هذه العزلات في تثبيطها لنمو المسببات المرضية للنبات و لم يكن التأثير واضحاً في حالة فطر *B. bassiana*. كشفت هذه الدراسة أن النباتات البرية مصدر جيد للعديد من السلالات الفطرية النباتية الداخلية التي يمكن استغلالها في مجالات مختلفة مثل مجال المكافحة البيولوجية.