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Endophytic fungi of some medicinal plants in Egypt

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ABSTRACT

Thirty endophytic fungal species were isolated from eight healthy medicinal plants grown in their natural habitat in Egypt. These plants were basil, green onion, green pepper, mint, roselle, watercress, white radish and tagetes. Isolates were identified on the basis of morphological characteristics into 14 genera namely *Acremonium, Alternaria, Aspergillus, Cladosporium, Cochliobolus, Epicoccum, Fusarium, Geosmithia, Macrophomina, Paecilomyces, Penicillium, Rhizopus, Scopulariopsis* and *Trichoderma*. Also, a dark septate sterile mycelium isolate belonging to mycelia sterilia group was isolated. These endophytic fungi were evaluated for their biocontrol ability against *Fusarium oxysporum* the causal agent of wheat root rot disease. *Alternaria alternata* and *Cochliobolus lunatus* showed the highest antagonistic activity (71.43 % inhibition value) against the tested *Fusarium oxysporum* followed by *Fusarium oxysporum* and *Cladosporium cladosporioides* with percentages of 68.57 % and 60%, respectively.

INTRODUCTION

Medicinal plants are plants that provide people with medicines to prevent disease, maintain health or cure ailment". The World Health Organization (WHO) defines a medicinal plant as any plant, which in one or more of its organs، contains substances that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical semi-synthesis (Balick*et al.*, 1996). An ethno-biological survey revealed that about 8,000 species of medicinal plants are used as food supplements, medicines, biocides and other phytoch-emicals.

The medicinal plants in Egypt represent a new promising resource as there is a relatively high representation of medicinal species in the native flora, that may induce the accumulation of a high concentration of secondary metabolites and there is a large market demand with higher economic value than other crops. All plant organs such as roots, rhizomes, flowers, leaves, fruits, seeds, as well as oils were used for medicaments in the form of powders, pills, suppositories, creams, pastes and ointments (Haggag, 1997). Endophytes refer to the microorganisms (mostly fungi and bacteria) colonizing the intercellular and intracellular regions of healthy plant tissues at a particular time, whose presence is unobtrusive and asymptomatic (Strobel, 2003and Schulz and Boyle, 2006). Endophytes form a symbiotic relationship with their plant host. It is believed that in many cases the microbes function as the biological defense for the plant against foreign phytopathogens.

The protection mechanism of the endophytes is exerted directly, by releasing metabolites to attack any antagonists or lyse affected cells and indirectly, by either inducing host defense mechanisms or promoting growth (Kloepper and Ryu, 2006).

Antibiotics or hydrolytic enzymes can be released by endophytes to prevent colonization of microbial plant pathogens (Strobel, 2003 and Berg and Hallmann, 2006), or prevent insects (Azevedo *et al.,* 2000) and nematodes (Hallmann *et al.,* 1998) from infecting plants.

Medicinal plants which are known to be used since centuries as an alternative source of medicine, are a valuable source for bioprospecting endophytes. Many studies recently found that endophytic fungi have the ability to protect the host from diseases and limit the damage caused by pathogenic microorganisms (Phongpaichit *et al.,* 2006; Ganley *et al.*, 2008; Sun and Guo, 2012 and Vieira *et al.*, 2012). Moreover, the production of chemical compounds by medicinal plants is believed to be associated with the endophytic fungi.

Wheat (*Triticum aestivum* L. em Thell.) is an important and a strategic cereal crop for the most of world's populations. It is the most staple food of about two billion people (36% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995).

Wheat is mainly cultivated in Egypt, as it is one of world's leading crops and can affect the economy. Diseases are a major cause of yield loss in winter wheat. Wheatcrop is subjected to a number of diseases, which are responsible for reducing its overall production to a greatextent (Haggag, 2013).

Fusarium foot and root rot (FRR) represent major devastating disease of wheat worldwide. Generally, affected wheat plants with root rot disease may be stunted or less vigorous than healthy one. Plants may be yellow, wilted and die prematurely. Root system may be poor with roots and crown tissues discolored and deteriorated (Smiley and Patterson, 1996 and Smiley *et al.,* 2005).

The present study was conducted to investigate the diversity of fungal endophytes of some medicinal plants grown in Egypt and their antagonistic activity against *Fusarium oxsporum* the most common pathogen of root rot of wheat plants.

MATERIALS AND METHODS

Isolation of endophytic fungi

The plant materials

Eight medicinal plants (basil, green onion, green pepper, mint, roselle, watercress, white radish and tagetes) were selected for the present investigation and collected from their natural habitats from El-Qalyubia (El-Qanatir el-Khairiya) and El-Fayoum governorates cultivated fields between April-October, 2012.The plant samples (healthy leaves, stems and roots)were placed in a plastic bag, stored in a refrigerator and then used for isolation of endophytic microorganisms within 72 h after sampling (Kim *et al.*, 2007).

Surface sterilization of the plant materials

The collected plant material was first surface sterilized according to the following method with few modifications. Plant material was first cleaned by washing several times under running tap water to remove soil particles and adhered debris and then left to air-dried. Surface sterilization was performed by sequentially rinsing the plant material with 96% ethanol for 0.5 - 1 minute, then with 2 – 2.5% sodium hypochlorite solution for 1 - 4 minute followed by 96% ethanol for 0.5 minute and finally with sterile distilled water for several times to remove the surface sterilization agents (Oberholzer-Tschütscher 1982; Sieber *et al.*, 1988 and Crous *et al.,* 1995). Finally, plant materials were then dried in between folds of sterile filter papers.

The isolation methods

After surface sterilization, two methods were used for isolation of endophytic microorganism. The first method, after proper drying, the surface-sterilized plant materials were cut into small pieces long 1 cm. (Kim *et al.*, 2007) then three pieces per each plant tissue were placed over the surface of three isolation media (i) Malt yeast extract agar (Schulz *et al.*, 1995) (ii) Potato dextrose agar (Rotem, 1994) and (iii) Tryptic soy Agar (Leavitt *et al.*, 1955). The sterilized tissue pieces were pressed on to the surface of media to ensure the endophytic isolations only from internal tissues of the plant pieces. In the second method, the surface-sterilized plant materials were cut into small pieces then cut longitudinally with a sterile scalpel as the inner tissues were excised and then laid on plates of the isolation media mentioned before (Hung and Annapurna, 2004).

All the plates were analyzed in three replicates, incubated at 28^0C for a about 2 weeks to promote the growth of endophytes and regularly observed. Total colony count was determined and any fungal growth was isolated, purified regularly, transferred onto slants of the isolation medium, accessioned by the plant and plant tissue, and incubated at 28^0C when they appeared then stored at 4^0C till further use.

Species richness

Expresses the total number of each fungal species isolated from each plant.

Relative frequency (%)

Relative frequency (RF) of each isolate was calculated as the number of the isolates of one species divided by the total number of isolates and express as percentage (Photita *et al.,* 2001).

Tissues specifity

Expresses the total number of each fungal genus isolated from each organ tissue of all the medicinal plants.

Identification of endophytic fungi

All endophytic fungi isolated in this study were identified to the genus and species level depending on morphological methods. Identification of the genus *Aspergillus* was carried out using Raperand Fennelli, (1977) reference. Identification of fungal isolates belonging to Hyphomycetes, dematiaceous Hyphomycetes, general Mucorales, the genus *Fusarium* and the genus *Penicillium* was carried out in MYCOLOGICAL CENTER, Faculty of Science, Assiut University (AUMC).

Antagonistic activities of endophytic fungi against *Fusarium oxysporum*

Endophytic fungal isolates were screened for their *in vitro* antagonistic activity against *Fusarium oxysporum* by dual culture technique, which isolated from naturally infected wheat plants grown in Egyptfrom El-Qalyubia (El-Qanatir el-Khairiya) governorate. The endophytic isolates were streaked-inoculated to one side of petri-dishes containing potato dextrose agar medium (PDA) (El-Tarabily *et al.*, 1997; Hassanein, 2010 and Hassanein *et al.*, 2010). The plates were then incubated at 28^0C for 3 days to allow the production and diffusion of metabolites into the agar. *Fusarium oxysporum*(isolated from diseased wheat plants) agar disk from seven days old culture was placed into the opposite side of the inoculated streak plates. Pathogen mycelium disc was also placed on uninoculated potato dextrose agar separately as control. All the plates were incubated at 28°C for 6 days and the plates were examined for inhibition of pathogens growth after 3, 4, 5 and 6 days. The reduction percentage in radial growth of the pathogen was calculated with each endophyte as described by Nouari *et al.* (2013) as follow:

Percentage of inhibition (I %) = A – B / A X 100 Where :

A – Radius of the pathogen in the control plate,

B – Radius of pathogen in the dual culture plate.

RESULTS

The study plants

During the study, endophytic fungi have been isolated from 8 different medicinal plants belonging to 6 families (Table 1).

Table 1: List of medicinal plants studied with their English, latin and family names

Serial no.	English name	Latin name	Family
1	Basil	*Ocimum basilicum*	Lamiaceae
2	Green onion	*Allium wakegi*	Alliaceae
3	Green pepper	*Capsicum annuum*	Solanaeae
4	Mint	*Mentha spicata*	Lamiaceae
5	Roselle	*Hibiscus sabdariffa*	Malvaceae
6	Tagetes	*Tagetes erecta*	Asteraceae
7	Watercress	*Eruca sativa*	Brassicaceae
8	White radish	*Raphanus sativus*	Brassicaceae

Isolation of endophytic fungi

A total of 911 endophytic fungal colony forming units (CFU) were obtained from 8 medicinal plants during the study.

Identification of endophytic fungi from different plants

In the present study, 14 genera were obtained and identified as *Acremonium, Alternaria, Aspergillus, Cladosporium, Cochliobolus, Epicoccum, Fusarium, Geosmithia, Macrophomina, Paecilomyces, Penicillium, Rhizopus, Scopulariopsis* and *Trichoderma* (Table 2).

Table 2: List of endophytic fungal genera speciesand mycelia sterilia isolated from medicinal plants

Genus serial No.	Genus	Species count	Species
1	***Acremonium***	4	*Acremonium atrogriseum* (Panasenko) Gams *Acremonium blochii* (Matruchot) Gams *Acremonium hyalinulum* (Sacc.) W. Gams *Acremonium strictum* Cams
2	***Alternaria***	1	*Alternaria alternate* (Fries) Keissler
3	***Aspergillus***	7	*Aspergillus aculeatus* *Aspergillus glaucus*var. *repens* *Aspergillus parasiticus* *Aspergillus punicus* *Aspergillus sydowii* (Bainier & Sartory) Thom & Church *Aspergillus ustus* *Aspergillus versicolor* (Vuillemin) Tiraboschi
4	***Cladosporium***	1	*Cladosprium cladosporioides* (Fresenius) de Vries
5	***Cochliobolus***	1	*Cochliobolus lunatus* Nelson & Haasis
6	***Epicoccum***	1	*Epicoccum purpurasscens* Ehrenberg
7	***Fusarium***	1	*Fusarium oxysporum* Schlechtendal
8	***Geosmithia***	1	*Geosmithia lavendula* Pitt
9	***Macrophomina***	1	*Macrophomina phaseolina* (Tassi) Goidanch
10	***Paecilomyces***	1	*Paecilomyces lilacinus* (Thom) Samson
11	***Penicillium***	4	*Penicillium cyaneum* Biourge, La Cellule *Penicillium duclauxii* Delacroix *Penicillium oxalicum*Currie & Thom *Penicillium pinophilum* Hedgcok
12	***Rhizopus***	1	*Rhizopus stolonifer* (Ehrenberg) Vuillemin
13	***Scopulariopsis***	1	*Scopulariopsis brumptii* Salvanet – Duval
14	***Trichoderma***	1	*Trichoderma harzianum* Rifai
Total species count		**26 species**	
	Mycelia sterilia isolates		
	Dark septate sterile mycelium	4	One isolate from basil plant One isolate from mint plant One isolate from tagetes plant One isolate from watercress plant
Total mycelia sterile count		**4 isolates**	
Total count		**30**	

Some fungal isolates could not be identified to the genus level. These cultures that not form spores were grouped as mycelia sterilia and divided into different morphological species according to their cultural characteristics. In this study, one morphospecies of mycelia sterilia were recorded and identified as dark septate sterile mycelium isolates.

Thirty endophytic fungal isolates belonging to 14 genera and one mycelia sterilia group (4 isolates) were identified from 8 different medicinal plants.

Among the identified fungi, *Aspergillus* yielded the greatest fungal diversity with 7 different species being isolated from different plants tissues followed by *Acremonium* and *Penicillium* had 4 species for each of them. Mycelia sterilia group was represented by 4 isolates of dark septate sterile mycelium isolates obtained from 4 different plants (basil, mint, tagetes and watercress). One species was isolated and identified from the remaining 11 genera (Table 2).

Species richness of the endophytic fungi in different medicinal plants

The distribution of endophytic fungal species was investigated in the selected medicinal plants and their occurrence in different plant organs tissues also were investigated (Table 3). The diversity and abundance of the species varied according to the plant and the organs tissues tested as shown in Table (3).

Table (3) indicated that, the highest species count was observed with roselle plant (11 species) with the highest in roots and stems tissues (7 and 6 species, respectively) followed by tagetes plant (10 species) in stems, roots and leaves (7, 5 and 5 species, respectively). On the other hand, the same species count was recorded with basil, mint and watercress plants (7 species of each).

Table 3: The distribution and count (CFU) of the endophytic fungal species in different organs tissues of medicinal plants

Plant	Genus	Species	Organ colony count (CFU)			Whole plant colony count (CFU)
			Roots	Stems	Leaves	
Basil	***Acremonium***	*Ac. hyalinulum*	3	30	4	37
	Aspergillus	*A. aculeatus*	1	2	17	20
		A. glaucus var.*repens*	22	18	38	78
	Cladosporium	*Cl. cladosporioides*	-	-	2	2
	Penicillium	*P. duclauxii*	4	3	2	9
	Rhizopus	*R. stolonifera*	-	5	-	5
	Dark septate mycelium	Dark septate mycelium	-	16	13	29
Total count	**6**	**7**	**30**	**74**	**76**	**180**
Green onion	***Acremonium***	*Ac. atrogriseum*	-	12	2	14
	Aspergillus	*A. sydowii*	30	-	10	40
	Scopulariopsis	*Sc. brumptii*	-	5	-	5
Total count	**3**	**3**	**30**	**17**	**12**	**59**
Green pepper	***Scopulariopsis***	*Sc. brumptii*	-	10	10	20
Total count	**1**	**1**	**-**	**10**	**10**	**20**
Mint	***Alternaria***	*Al. alternata*	5	3	-	8
	Aspergillus	*A. aculeatus*	6	2	6	14
		A. parasiticus	34	11	16	61
		A. ustus	4	4	-	8
	Fusarium	*F. oxysporum*	9	-	2	11
	Penicillium	*P. oxalicum*	-	3	1	4
	Dark septate mycelium	Dark septate mycelium	2	3	4	9
Total count	**5**	**7**	**60**	**26**	**29**	**115**

Table 3: Cont.

Plant	Genus	Species	Organ colony count (CFU)			Whole plant colony count (CFU)
			Roots	Stems	Leaves	
Roselle	***Acremonium***	*Ac. blochii*	-	-	1	1
		Ac. strictum	1	15	-	16
	Alternaria	*Al. alternata*	1	-	-	1
	Aspergillus	*A. aculeatus*	8	4	20	32
		A. glaucusvar.repens	27	-	-	27
		A. parasiticus	-	9	18	27
		A. punicus	-	9	-	9
		A. versicolor	40	-	-	40
	Geosmithia	*Ge. lavendula*	-	3	-	3
	Macrophomina	*Ma. Phaseolina*	1	-	-	1
	Paecilomyces	*Pa. lilacinus*	29	3	6	38
Total count	**6**	**11**	**107**	**43**	**45**	**195**
Tagetes	***Alternaria***	*Al. alternata*	-	8	10	18
	Aspergillus	*A. aculeatus*	2	5	1	8
		A. parasiticus	10	12	5	27
	Cladosporium	*Cl. cladosporioides*	-	3	1	4
	Cochliobolus	*Co. lunatus*	3	-	-	3
	Fusarium	*F. oxysporum*	3	-	-	3
	Penicillium	*P. duclauxii*	6	5	-	11
		P. pinophilum	-	1	-	1
	Trichoderma	*T. harzianum*	-	3	-	3
	Dark septate mycelium	Dark septate mycelium	-	-	8	8
Total count	**8**	**10**	**24**	**37**	**25**	**86**
Watercress	***Alternaria***	*Al. alternata*	-	2	2	4
	Aspergillus	*A. aculeatus*	11	2	1	14
		A. parasiticus	13	18	38	69
		A. ustus	-	17	-	17
		A. versicolor	-	6	-	6
	Penicillium	*P. oxalicum*	5	8	-	13
	Dark septate mycelium	Dark septate mycelium	-	2	-	2
Total count	**4**	**7**	**29**	**55**	**41**	**125**
White radish	***Alternaria***	*Al. alternata*	2	3	6	11
	Aspergillus	*A. aculeatus*	6	13	11	30
		A. parasiticus	25	26	25	76
		A. versicolor	-	-	4	4
	Epicoccium	*E. purpurascens*	1	-	-	1
	Penicillium	*P. cyaneum*	9	-	-	9
Total count	**4**	**6**	**43**	**42**	**46**	**131**
Total Count			**323**	**304**	**284**	**911**

Relative frequencies of theendophytic fungi isolated from each plant

The endophytic fungal species isolated from the medicinal plants were classified into 14 genera and one mycelia sterilia group (Table 4).

Aspergillus species had the highest relative frequency values (66.63 %) and considered the most dominant genus isolated followed by *Acremonium*, dark septate sterile mycelium isolates, *Penicillium, Alternaria* and *Paecilomyces* which considered as moderate in their dominance and gave relative frequency values of 7.46 %, 5.27 %, 5.16 %, 4.61 % and 4.17 %, respectively. Finally, *Scopulariopsis* and *Fusarium* species gave the lowest relative frequency values (2.74 % and 1.54 %, respectively).

Infrequently endophytic fungi include 7 genera namely *Cladosporium,* *Cochliobolus, Epicoccum, Geosmithia, Macrophomina, Rhizopus* and *Trichoderma* (Table 4).

All the medicinal plants were found to harbor various endophytic fungi. Among the 8 medicinal plants tagetes, basil and roselle yielded the greatest fungal diversity with 8, 6 and 6, respectively different genera being isolated from different tissues.

The highest number of fungal genera (8) was obtained from tagetes plant as they belong to 7 different genera and one morphospecies of mycelia sterilia. Among these fungal genera, *Aspergillus* was the most frequent (40.70 %) followed by *Alternaria* (20.93 %) and Dark septate sterile mycelium isolate (9.30 %).

The common endophytic fungi had a wide distribution in the medicinal plants with a high species abundance, for example *Aspergillus* were found in all the plants except green pepper which had a relative high occurrence in 7 of these plants (RF= 66.63 %), especially in watercress and white radish (RF= 84.80 % and 83.97 %, respectively).

Table 4: Relative frequencies of the endophytic fungi isolated from medicinal plants

Genus	RF (%)	Medicinal plants								Total genus count
		Basil	Green onion	Green pepper	Mint	Roselle	Tagetes	Water cress	White radish	
Acremonium	Plant count	37	14	-	-	17	-	-	-	68
	RF (%)	**20.56**	**23.73**	**-**	**-**	**8.72**	**-**	**-**	**-**	**7.46**
Alternaria	Plant count	-	-	-	8	1	18	4	11	42
	RF (%)	**-**	**-**	**-**	**6.96**	**0.51**	**20.93**	**3.20**	**8.40**	**4.61**
Aspergillus	Plant count	98	40	-	83	135	35	106	110	607
	RF (%)	**54.44**	**67.80**	**-**	**72.17**	**69.23**	**40.70**	**84.80**	**83.97**	**66.63**
Cladosporium	Plant count	2	-	-	-	-	4	-	-	6
	RF (%)	**1.11**	**-**	**-**	**-**	**-**	**4.65**	**-**	**-**	**0.66**
Cochliobolus	Plant count	-	-	-	-	-	3	-	-	3
	RF (%)	**-**	**-**	**-**	**-**	**-**	**3.49**	**-**	**-**	**0.33**
Epicoccum	Plant count	-	-	-	-	-	-	-	1	1
	RF (%)	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.76**	**0.11**
Fusarium	Plant count	-	-	-	11	-	3	-	-	14
	RF (%)	**-**	**-**	**-**	**9.56**	**-**	**3.49**	**-**	**-**	**1.54**
Geosmithia	Plant count	-	-	-	-	3	-	-	-	3
	RF (%)	**-**	**-**	**-**	**-**	**1.54**	**-**	**-**	**-**	**0.33**
Macrophomina	Plant count	-	-	-	-	1	-	-	-	1
	RF (%)	**-**	**-**	**-**	**-**	**0.51**	**-**	**-**	**-**	**0.11**
Paecilomyces	Plant count	-	-	-	-	38	-	-	-	38
	RF (%)	**-**	**-**	**-**	**-**	**19.49**	**-**	**-**	**-**	**4.17**
Penicillium	Plant count	9	-	-	4	-	12	13	9	47
	RF (%)	**5**	**-**	**-**	**3.48**	**-**	**6.15**	**10.40**	**6.87**	**5.16**
Rhizopus	Plant count	5	-	-	-	-	-	-	-	5
	RF (%)	**2.78**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.55**
Scopulariopsis	Plant count	-	5	20	-	-	-	-	-	25
	RF (%)	**-**	**8.47**	**100**	**-**	**-**	**-**	**-**	**-**	**2.74**
Trichoderma	Plant count	-	-	-	-	-	3	-	-	3
	RF (%)	**-**	**-**	**-**	**-**	**-**	**1.54**	**-**	**-**	**0.33**
Dark septate mycelium	Plant count	29	-	-	9	-	8	2	-	48
	RF (%)	**16.11**	**-**	**-**	**7.83**	**-**	**9.30**	**1.60**	**-**	**5.27**

The genera *Alternaria* and *Penicillium* were isolated from 5 medicinal plants with a major occurrence in tagetes (RF= 20.93 %) and watercress (RF= 10.40 %) respectively, followed by dark septate sterile mycelium isolates and isolated from 4 plants with a major occurrence in basil (RF= 16.11 %). The genus *Acremonium* was obtained from 3 plants with a major occurrence in green onion and basil plants (RF= 23.73 % and 20.56 %, respectively) (Table 4).

Tissues specificity of the endophytic fungi

The occurrence of the endophytic fungi in roots, stems and leaves was investigated for plants under investigation.

Acremonium, Alternaria, Aspergillus, dark septate sterile mycelium isolates, *Paecilomyces* and *Penicillium* were the most frequent fungiand had a special presence in all tissues of these plants (Table 5). *Cladosporium* and *Scopulariopsis* could not found in roots of any of 8 medicinal plants while *Fusarium* could not found in stems of these plants. *Cochliobolus, Epicoccum* and *Macrophomina* colonized only medicinal plants roots while *Geosmithia, Rhizopus* and *Trichoderma* colonized their stems only.

Some endophytic fungi could colonize tissue more than the others. For example, *Aspergillus*, *Paecilomyces* and *Penicillium* were obtained from medicinal plants roots than from stems and leaves while, *Acremonium* species were detected in the stems than in the roots and leaves. Finally, *Alternaria* and dark septate sterile mycelium isolates were found in the leaves than roots and stems.

Table 5: The endophytic fungi in different organs tissues of medicinal plants

Genus	Medicinal plants organs tissues			Total genus count
	Roots	Stems	Leaves	
Acremonium	4	57	7	68
Alternaria	8	16	18	42
Aspergillus	239	158	210	607
Cladosporium	-	3	3	6
Cochliobolus	3	-	-	3
Epicoccum	1	-	-	1
Fusarium	12	-	2	14
Geosmithia	-	3	-	3
Macrophomina	1	-	-	1
Paecilomyces	29	3	6	38
Penicillium	24	20	3	47
Rhizopus	-	5	-	5
Scopulariopsis	-	15	10	25
Trichoderma	-	3	-	3
Dark septate sterile mycelium	2	21	25	48

In vitro* antagonistic activity of the endophytic fungi against *Fusarium oxysporum

The *in vitro* studies showed that among the thirty endophytic fungal species tested, only 7 produced inhibition of radial growth of *Fusarium oxysporum* under the conditions of this study compared to untreated control as shown in Table (6) and Figure (1) and there was a significant difference in the percentage of inhibition of pathogen radial growth by the tested endophytic antagonists.

Table 6: *In vitro* antagonistic activity of the endophytic fungi against *Fusarium oxysporum*

Endophytic fungus	*Fusarium oxysporum*		
	A	B	I (%)
Alternaria alternata	3.5	1	71.43
Aspergillus punicus	3.5	1.6	54.29
Aspergillus versicolor	3.5	2.1	40
Cladosporium cladosporioides	3.5	1.4	60
Cochliobolus lunatus	3.5	1	71.43
Epicoccum. purpurascens	3.5	1.8	48.57
Fusarium oxysporum	3.5	1.1	68.57

Percentage of inhibition I (%) = A – B / A X 100, A – Radius of the pathogen in the control plate, B – Radius of pathogen in the dual culture plate.

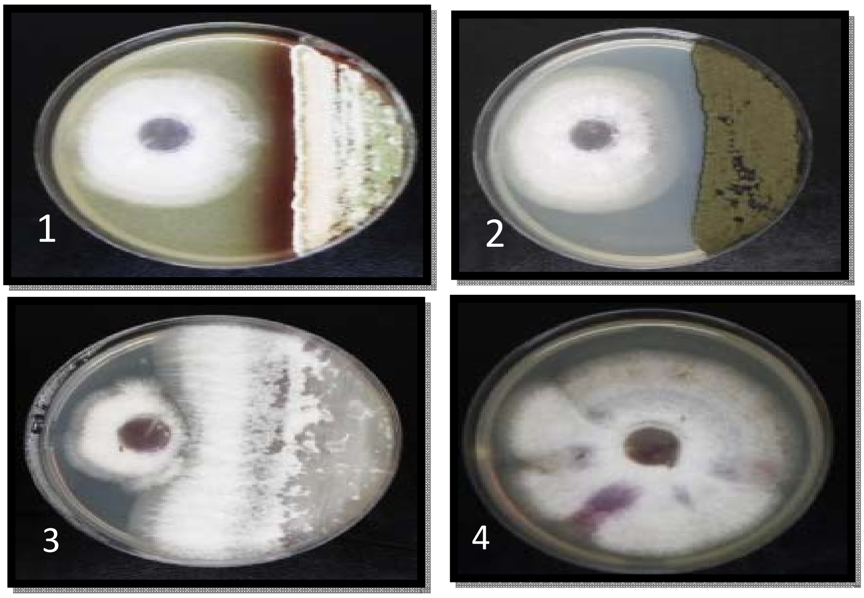

Fig. 1: Antagonistic activity of some active endophytic fungi against *Fusarium oxysporum*.
1. *A. versicolor*, 2.*Cl. cladosporioides*, 3.*F. oxysporum* & 4.*F. oxysporum* (control).

Alternaria alternata and *Cochliobolus lunatus* inhibited the growth of *F. oxysporum* and gave a percentage of inhibition of 71.43 % for both, followed by *Fusarium oxysporum* and *Cladosporium cladosporioides* with percentage of 68.57 % and 60%, respectively. On the other hand, *Aspergillus punicus*, *Epicoccum purpurascens* and *Aspergillus versicolor* gave the lowest percentage of inhibition of *F. oxysporum* (54.28 %, 48.57 % and 40 %, respectively).

DISCUSSION

Plants-associated microorganisms are known to be beneficial, neutral or pathogenic, but others are still poorly understood. Endophytes which are free living organisms able to colonize plant tissues through stomata, wounds or cracks, when a chance is available (Hardoim *et al.,* 2008 and Rodriguez *et al.,* 2009).This relationship is complex and involve many factors. Some studies have shown that endophyte colonization can be stimulated by host plant species, geographic location, seasonality and different tissues of the same plant (Porras-Alfaro and Bayman, 2011).

From our results, it was found that some fungal pathogens are commonly isolated as endophytes and several researches have reported that many of these species can be pathogenic for some hosts and non-pathogenic for others (Malcolm *et al.,* 2013) that depending on environmental conditions and on the host (Sieber, 2007 and Malcolm *et al.,* 2013).

The present study also found that fungal endophytes showed significant differences in their presence, absence and abundance in the study plants. Some plants harbored more endophytic fungi than others (as roselle and tagetes). The endophytic fungi had different relative frequencies in different host plants as *Aspergillus* not only found in most of plantsbut also had the highest relative frequencies within each of them. On the other hand, some other endophytic fungi were detected in only single plant (*Geosmithia* from roselle and *Trichoderma* from tagetes). These results were consistent with the previous reports of Arnold *et al.* (2001) and Bettucci *et al.* (2004).

The endophytic fungi have been found in different tissues of a single plant which is a reflection of tissue specificity (Collado *et al.,* 2001; Frohlich *et al.,* 2000 and Ganley and Newcombe, 2006). The current results showed that there is a difference between various plants tissues and that might be due to their degree of exposure to air, sun, wind, rain, moisture and aeration conditions and availability of nutrients (Andreote *et al.,* 2014).

Although the production of wheat has increased but several diseases have greatly affected its yield and quality. Many *Fusarium* pathogens cause the same essentially symptoms on different crops such as cortical decay of roots, root rot, wilting, yellowing and premature death on the diseased plants (Summerell *et al.,* 2001 and Saremi, 2005). Several *Fusarium* species are dangerous as they known to produce mycotoxins as deoxynivalenol (DON) and nivalenol (NIV) (Bewick *et al.,* 2008) and these toxins can also transport from the roots to the other upper plant parts (Covarelli *et al.,* 2012).

*Fusarium oxysporum*is the most important *Fusarium* species isolated from diseased wheat plants, Mehta (2014) reported that both *F. oxysporum* and *F. solani*are secondary pathogens of wheat plants. Also, Saremi *et al*., (2011) showed that *F. oxysporum* can cause vascular and damping-off diseases on different crops.

Non-pathogenic endophytic microorganisms have a specific interest as biological control agents (Alabouvette *et al.,* 2006 and Berg, 2009). They are well fully adapted to their host plant as they colonize an ecological niche similar to that of plant pathogens (Berg *et al.,* 2005) and can produce secondary metabolites which required for their survival against host defense responses (Strobel and Daisy, 2003 and Brader *et al.,* 2014).

In the present study, all the endophytic fungi isolated from medicinal plants were examined for their antagonistic activity against *Fusarium oxysporum*. Out of these endophytes, only 7 endophytic fungi showed a significant difference in the pathogen radial growth inhibition.

Mycelial growth inhibition of the target pathogen in dual culture method revealed that the highest suppression rate was with a wider inhibition zone. A microbial biological control agent may be showed several mechanisms against plant pathogens during their antagonistic activity. There are about 3 primary mechanisms by which endophytes can promote host resistance to pathogens: by the production of antimicrobial substances toxic to pathogens, by the competition for the same ecological niche and nutrients used by the pathogen and by the production of enzymes that attack the cell wall components of the pathogens, stimulating pathogen cell death (Mandyam and Jumpponen, 2005; Ganley *et al.,* 2008 and Shittu *et al.,* 2009).

Abdel-Sater (2001) showed that the culture filtrates of *Alternaria alternate* and *Cochliobolus lunatus* caused high inhibition of *Pleospora herbarum* that caused spot diseases on onions leaves. Hellwig *et al.* (2002) demonstrated that antimicrobial compounds like altersetin from *Alternaria* sp., showed significant antibacterial activities.

Many studies have indicated that *Fusarium* spp. are the most common species among endophytes from medicinal plants and a potent source of bioactive compounds. Antimicrobial compounds like the penta-ketide (CR3772-2methylbutyraldehyde-substituted-α-pyrone), beauvericin, subglutinol A and B were isolated from *Fusrarium* spp., endophytes of plants *Selaginella pallescens, Cinnamomum kanehirae*, and *Tripterygium wilfordii,* respectively. These compounds showed strong antimicrobial activity against *Candida albicans* and methicillin-resistant *Staphylococcus aureus* (Lee *et al.,* 1995; Sean and Jon, 2000 and Wang *et al.,* 2011).

The antagonistic effect expressed by the *Cladosporium cladosporioides* in dual culture method also reported by Bisht *et al.* (2016) who isolated *Cladosporium cladosporioides* from of *Cupressus torulosa* which were found to be shown strong antagonistic activity against phytopathogens *Macrophomina phaseolina* and *Fusarium solani.*

The dual culture technique reveals that 2 *Aspergillus* species, *Aspergillus punicus* and *Aspergillus versicolor* have an antagonist effect against *Fusarium oxysporum*. Previous reports by Bosah *et al.* (2010) showed that *Aspergillus* species were identified as effective antagonists against the known destructive pathogens to most agricultural crop plants.

The successful in pathogens suppressions in dual culture methods provide useful information on the effective use of these isolates as biological control agents against wheat diseases. Therefore, they may be used as biofertilizer and biocontrol agents for crops production in agricultural environment.

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ARABIC SUMMERY

الفطريات الداخلية في بعض النباتات الطبية فى مصر

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تم عزل ثلاثون نوع من الفطريات الداخلية من ثمانية نباتات طبية سليمة من بيئة نموها الطبيعية في مصر. النباتات المستخدمة هي ريحان، بصل اخضر، فلفل اخضر، نعناع، كركدية، ورد قطيفة، جرجير وفجل ابيض. تم تعريف جميع العزلات تبعا للأسس المورفولوجية الى ١٤ جنس ونوع واحد مورفولوجى من مجموعة الخيوط العقيمة. تم تعريفهم كالاتى هم اكريمونيم، الالترناريا، اسبر جلس، كلادوسبوريم،كوكوبولاس، خيط مقسم عقيم داكن، ابيكوكم، الفيوزاريوم، جوسمثيا، ماكروفومينا، بيكلوميسيس، بنسليوم، ريزوبس، سكوبيلاريوبسسوترايكودرما. وتم دراسة قدرة هذه الفطريات الداخلية في التحكم الحيوي ضد فطر فيوزراريوماوكسيسبورم المسبب لمرض تعفن جذر القمح.

الالترنارياالترناتاوكوكوبولاسلينتس اظهروا اعلى نشاط تثبيط ضد هذا النوع من الفيوزاريوم واعطوا اقوى نسب تثبيط ٧١.٤٣ %، وتوبعت بفيوزاريماوكسيسبورم و كلادوسبوريمكلادوسبوريويدس بنسب ٦٨.٥٧ % و ٦٠ % على التوالي.