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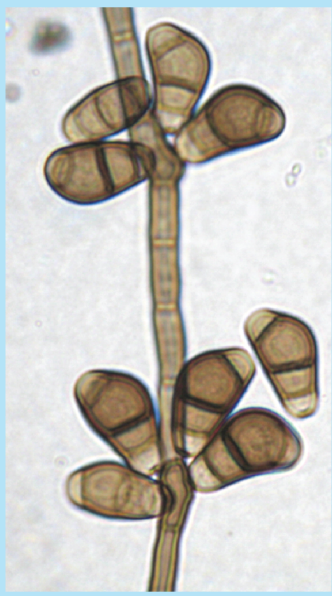
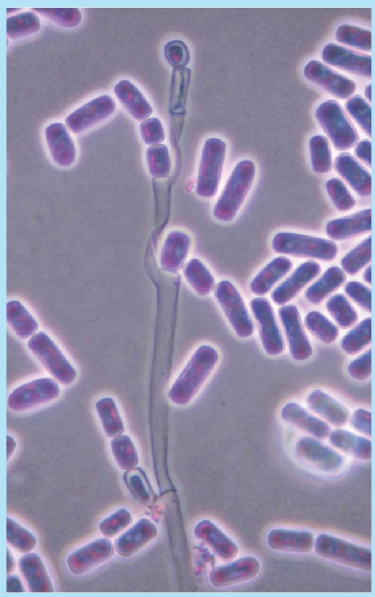

Volume 12 : 2021

ISSN (Print) : 2090 - 7583

ISSN (Online) : 1047 - 2357

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http://www.aun.edu.eg/aumc/journal/index.php

Journal of Basic & Applied Mycology (Egypt) 12 (2021): 27-41 **p-ISSN 2090-7583, e-ISSN 2357-1047**
 http://www.aun.edu.eg/aumc/Journal/index.php

Endophytic mycobiota and enzymatic capacity in wild and cultivated plants in New Valley, Egypt: A comparative analysis

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Received 18/5/2021, Accepted 25/5/2021

Abstract: The ongoing research resulted in the isolation and identification of 38 endophytic mycobiota species from 20 genera associated with four wild and four cultivated plants collected from El-Kharga, New Valley Governorate, Egypt. The most prominent genera were *Aspergillus*, *Alternaria*, *Fusarium*, *Exserohilum*, and *Monascus*, accounting for 36.24 %, 11.13 %, 10.95 %, 7.35 %, and 4.25 % of total fungi, respectively. *Aspergillus* was found in 100 % of wild plants and 75 % of cultivated plants, *Alternaria* (50 % and 25 %), *Fusarium* (25 % and 75 %), *Exserohilum* (50 % and 25 %), and *Monascus* (50 % and 25 %). Wild plants had a higher overall CFU count (260/200 segments), number of genera (14) and species (26) than cultivated plants (187 CFUs/200 segments, 10 genera and 16 species). *A. terreus* and *A. flavus* were the most common on wild plant species, while *F. oxysporum* and *F. solani* were the most common on cultivated plant species. Wild plant-isolated fungi showed increased growth ability in endoglucanase, exoglucanase, and pectinase enzymes with powerful strains *A. flavus* AY-72 from *Prosopis farcta* produced acumulative output of 242.81 IU/ml/min of pectinase, dark sterile mycelia AY-133 from *Trifolium sativum* producing 14.6 IU/ml/min of endoglucanase and *F. solani* AY-126 from *Ammi majus* producing 65.5 IU/ml/min of exoglucanase. The current results stated that wild plants have more efficient enzyme-generating strain sources than cultivated plants, with higher enzyme activity.

Kew words: Endophytic fungi, pectinase, cellulase, submerged fermentation, wild, cultivated, plants.

Introduction

Endophytic microbes are the depopulated tissues of plants wherein they pass any or all of their life cycle without introducing disease symptoms in their host (Petrini 1991 and Abdel-Sater *et al*. 2021). Host tissues, including leaves, branches, bark, bones, vegetables, flowers and seeds, may be dominated by fungal endophytes (Rodriguez *et al*. 2009). In this symbiotic partnership, fungal endophytes typically provide protection and nutrients from the host, while the host plant may benefit from a range of qualities including the defense against natural enemies such as parasites and herbivores (Schardl *et al.* 2004 and Singh *et al.* 2011); Fostering plant production (Hamayun *et al.* 2010); and growing plant exposure to abiotic stress factors such as salinity and toxicity of heavy metals in soil (Khan *et al.* 2014).

Some wild or cultivated plants are considered to harbor endophytic fungi, which are essential sources of numerous bioactive secondary metabolites and enzymes that are useful in several industries (Zou *et al.* 2000, Strobel *et al.* 2004, Krishnamurthy *et al.* 2008). Endophytic fungi are largely unexplored sources of metabolites that are of interest to the pharmaceutical and agricultural industries. A single Endophyte may generate many bioactive metabolites. As a result, more focus has been given to the role of endophytes in the processing of various natural products with greater bioactivity (Prabavathy and Valli 2012). In addition to other enzymes, pectinases and cellulases are among the most essential enzymes produced by endophytic fungi as responsible for resistance towards infective attack and of acquiring nutrients from the host. These enzymes have various industrial applications, and are of considerable significance. There are growing

attempts, from diverse environments, to identify and understand endophytic fungi.

Consequently, the purposes of the present research were to analyze and compare the biodiversity of endophytic fungi in both wild and cultivated plants in New Valley Governorate, Egypt. Also, to evaluate their capacities to generate hydrolyzing enzymes in their environment, and to address the main question of this study, is there any disparity in the capacity of fungi to produce bioactive enzymes derived from various sources?

Materials and Methods

1. Sampling area

New Valley Governorate is situated in the Western Desert and bordered by the Eastern Governorates of Minya, Assiut, Sohag, Qena, and Aswan, from the West Egyptian frontier with Libya and from the North are the Governorates of Matrouh, and the Coastal Oasis of 6th October City and from the South Egyptian boundary with Sudan. It occupies 440098 km^2, about 44 % of Egypt's total area and about 66 % of Egypt's southern area.

2. Sampling

During April 2018, leaves and roots from eight healthy and mature plants, of which four were of wild species and four cultivated crops, were collected from the El-Kharga Oasis, New Valley Governorate. Ten replicates were harvested in sterile polyethylene bags from each of the wild (*Ammi majus*, *Cressa cretica*, *Polypogen monspeliensis*, *Prosopis farcta*) and cultivated (*Allium cepa*, *Trifolium alexandrinum*, *Trifolium sativum* and *Zea mays*) plants (Figure 1). The samples gathered were then delivered immediately to the laboratory to isolate the endophytic fungi.

3. Identification of the collected plant species

Plant species collected in the current investigation (Figure1) have been defined according to morphological and taxonomic characteristics for each plant species at the Assiut University Herbarium, Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt, with the assistance of curators of the Assiut University Herbarium.

4. Sample preparation and surface sterilization

Prior surface sterilization, every collected sample's leaves and roots were vigorously washed with tap water to extract the dust and then with distilled water and cut into 5-cm pieces. The following immersion series was used for surface sterilization: 5% sodium hypochlorite for 3 min, 70% ethanol for 1 min, and washing with sterile purified water 3 times each for 1 min. 1 cm from both ends of each segment was cut off in aseptic conditions to create a 3-cm section (Al-Bedak *et al.* 2020a).

5. Isolation of endophytic fungi

Segments of each sample (5 segments) were plated on Petri-dishes (5 each sample) containing 1 % glucose-Cz (Ismail *et al.* 2017) with the following composition (g/l): Glucose, 10; Na_2NO_3, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01; $CuSO_4$, 0.005; Rose Bengal, 0.05; chloramphenicol, 0.25; agar, 15 and the final pH 7.3. The plates were incubated for 7-21 d at 25°C. Counts of CFUs of each fungal isolate were calculated per ٥٠ segments in every sample. Pure cultures of the fungal isolates have been preserved on PDA slants at 4°C (Smith and Onions 1994) and on cotton balls as defined by Al-Bedak *et al.* (2019). The number of accessions to the Assiut University Mycological Center (AUMC) culture collection was provided for fungal species with the highest enzymatic activity.

6. Screening of pectinase and endoglucanase production on solid medium

The potential of pectinase and cellulases (endoglucanase and exoglucanase) production was detected on sucrose-free Czapek's agar medium, supplemented with pectin (from citrus peel) and carboxymethyl cellulose (CMC) as the primary source of carbon, respectively. Fifty µl of spore suspension from a 7-d-colony of growing fungal strain was applied individually to each 5-mm-diameter well on the agar plate (Moubasher *et al.* 2016). The plates were then incubated at 30°C for 48 h. The transparent areas that developed around the wells were more apparent as 0.25 % aqueous iodine solution saturated the plates. The diameters of the clear areas (in mm) were determined against the brown color of the enzyme-indicating test media.

7. Enzyme production in submerged fermentation

For the production of endoglucanase, exoglucanase and pectinase, both positive fungal strains was developed individually in conical flasks of 250-ml Erlenmeyer each containing the broth medium of 50 ml sucrose-free Czapek's supplemented by 1 % pectin or 1 % CMC as the sole source of carbon. The flasks were then individually inoculated with a 1 ml spore suspension containing $1x10^7$ spore/ml of 7-d-old cultures of the strains tested. Then, the inoculated flasks were incubated for 7 d at 30°C in 150 rpm shaking environment.

8. Enzyme extraction

After the incubation period, the contents of the flasks were filtered individually via filter papers (Whatman No.1), and the filtrate was then centrifuged at 10000 xg for 10 min at 4°C. The transparent supernatants have been used as a source of the enzyme cellulase or pectinase.

9. Enzymes assay and protein estimation

Endoglucanase, exoglucanase and pectinase production was determined by mixing 0.1 ml filtered crude enzyme with 0.9 ml of 1 % carboxymethyl cellulose (CMC), microcrystalline cellulose (MCC), and pectin respectively (prepared in 50 mM Na-citrate buffer, pH 5.0). The reaction mixture was incubated at 50°C for 20 min (Bailey *et al.* 1992) and the process was stopped by introducing 2 ml of 3, 5-dinitrosalicylic acid (DNS) (Miller 1959) and boiling in a water bath for 10 min. After cooling, the color absorbance was measured at 540 nm using UV-Visible spectrophotometer (T80+). The amount of reducing sugar liberated was quantified using standard curves of glucose. One unit of the enzyme is defined as the amount of enzyme that liberates 1 µmol of the reducing glucose equivalent per minute under the standard assay conditions (Ghose and Bisaria 1987). The enzyme activity was calculated according to the following formula (Moubasher *et al.* 2016, Al-Bedak *et al.* 2020b).

$$\textbf{Enzyme activity} = \textbf{Absorbance x DF x } \left(\frac{1}{x}\right)\left(\frac{1}{y}\right)\left(\frac{1}{t}\right)\left(\frac{1}{slope}\right)$$

Where: DF = the dilution factor for enzyme; x = the volume of enzyme used; y = the volume of hydrolysate used for assay of reducing sugars; t = the time of hydrolysis; slope is determined from a standard curve. Soluble protein was estimated by Folin Lowry's method using bovine serum albumin as standard (Lowry *et al.* 1951).

Results

1. Biodiversity of endophytic fungi on the tested plants

A total of 38 species belonging to 20 genera of endophytic mycobiota have been isolated from both wild and cultivated plants collected from El-Kharga Oasis, New Valley Governorate, on one occasion during April 2018. The wild plants had the higher CFUs (260), the number of genera (14) and species (26) than the cultivated plants, which produced 187 CFUs, 10 genera and 17 species. Nineteen species of total fungi recovered were recorded from wild plants

only, while 12 fungal species were isolated from cultivated plants only (Table 1).

Aspergillus (represented by 8 species) was the most widespread genus in wild plants (144 CFUs) over the cultivated plants (18 CFUs). It accounted for 55.4% and 36.24% of fungi in wild plants and gross fungi. *Aspergillus terreus* was the most prevalent species with 15.66 % of the total fungi, although only one plant was reported, followed by *A. flavus* containing 11.9 % of the fungi in wild plants and 6.9 % of the total fungi, collected from the leaves and roots of 2 out of 4 wild plants respectively (Table 1).

Alternaria (4 species) fell second to *Aspergillus*, containing 9.2 % of wild plant fungi and 5.4 % of overall fungi. *A. turkisafria* by 4.2%, led by *A. alternata* with 1.9 %, then *A. perangusta* and *A. tenuissima* containing 1.5 % of the overall sum of fungi in wild plants. *Microascus* (2 species) was the *Alternaria* runner, representing 7.3 % of wild plant fungi. It was the most widespread genus isolated from all the wild plants studied. The most prevalent species of *Microascus* is *M. cinereus* accompanied by *M. terreus*, which constitutes 5.4% and 1.9% respectively of the gross fungi of wild plants. It is worth noting that *Prosopis farcta* is the richest endophytic fungi plant amongst the four wild plants studied. *Exserohilum*, described by two species, fell next to *Microascus*, representing 6.9 % of the total fungi in wild plants with *E. rostratum* was reported from the roots of 2 wild plants and composed of 3.35 % of the total fungi followed by *E. gedarefense* (2 plants) representing 4 % of the total fungi respectively (Table 1).

Alternaria perangusta, *A. turkisafria*, *Arthrenium euphorbiae*, *Aspergillus acidus*, *A. eucalypticola*, *Scopulariopsis fimicola*, *Setosphaeria khartoumensis*, *Stachybotrys zhanymuensis* were recorded in this study for the first time in Egypt to the best of our knowledge.

2. Biodiversity of endophytic fungi in cultivated plants

Fusarium (represented by 4 species) was the most widespread genus in cultivated plants, accounting for 18 % of the fungi in cultivated plants and 10.5 % of the total fungi, followed by *Alternaria* and *Aspergillus*. It has been retrieved from 3 plants out of 4 cultivated plants. *F. oxysporum* was the most prevalent species with 14.4 % of the total fungi in cultivated plants, but only one plant was reported, followed by *F. solani*, which produced 6.95 % of the fungi in cultivated plants and recovered from the roots of 2 cultivated plants.

Alternaria and *Aspergillus* (3 species each) were runners in *Fusarium*, representing 11.8 % and 9.6 % of the gross fungi in cultivated plants respectively. *Alternaria tenuissima* is the most widespread species of *Alternaria* in cultivated plants, contributing 6.95 % of the total fungi followed by *A. alternata* and *A. gaisen* containing 2.7 % and 2.1 % of the gross cultivated fungi. The most dominant species of *Aspergillus* is *A. flavus* was accompanied by *A. nidulans* composed of 4.8 % and 3.2 % , respectively, then *A. tubingensis* 1.6 % of overall fungi in cultivated plants. *Alternaria gaisen* and *Exserohilum gedarefense* are recorded in the present study for the first time in Egypt.

3. Preliminary screening of endophytic fungi for pectinases and cellulases activity

Sixty-two isolates (out of 88)were capable of expressing pectinase enzymes, of which 7 isolates were strong producers, 26 were moderate and 29 were low, Whereas, 58 were capable of producing cellulase, of which 21 were strong producers, 16 were moderate and 21 were low (Table 2).

4. Submerged production of endoglucanase, exoglucanase and pectinase

The most active 28 fungal isolates were quantitatively assessed for endoglucanase, exoglucanase, and pectinase synthesis in submerged fermentation using the sucrose-free Cz broth medium modified with 1 % pectin or CMC or avicel as the sole carbon source. Enzymes derived from wild plants had higher levels of endoglucanase, exoglucanase, and pectinase production (14.12 IU/ml/min, 65.5 IU/ml/min, and 242.81 IU/ml/min, respectively) than fungal species isolated from cultivated plants (13.78 IU/ml/min, 40.156 IU/ml/min, and 218.64 IU/ml/min, respectively (Table 3).

Aspergillus flavus AY-72 produced a cumulative output of 242.81 IU/ml/min for pectinase. The best performance for Dark sterile mycelia AY-133 was 14.6 IU/ml/min and *F. solani* AY-126 was 65.5 IU/ml/min for endoglucanase and exoglucanase, respectively (Table 3).

The final distinction between wild and cultivated plants can be outlined in table (4). In this sense, wild plants have been the best sources of fungi and their extracellular enzymes, as well as the enzymatic activities. In comparison, wild plants were superior to cultivated plants in the recording of fungal species that appeared here for the first time in Egypt (8 species) over the cultivated plants (2 species). 60.4 % and 72.9 % of fungi from wild plants had the ability to synthesize cellulase and pectinase enzymes, respectively, while 75.0 % and 70.0 % of fungi from cultivated plants could produce the cellulase and pectinase, respectively (Table 4).

Discussion

The present study has shown that endophytic fungal lineages have been collected from all plant species studied and that certain plants have the same fungal genera and species, which means that endophytic fungi may be the same in plants belonging to different families. Altogether, 38 species related to 20 genera were recovered from the leaves and roots of all tested plants. The high incidence genera were represented by *Aspergillus*, *Fusarium* and *Alternaria*. *Aspergillus* was the most common genus recovered from 7 plants with *A. terreus* being the most prevalent species followed by *A. flavus*.

These current results agreed, to some extent, with the findings of Raviraja (2005) who studied the endophytic fungi in five Brazilian medicinal plants and found that *Aspergillus* and *Penicillium* were isolated in high frequencies. In this sense, previous studies that were carried out on wild plants, the genera *Fusarium*, *Aspergillus*, *Nigrospora*, *Stachybotrys*, *Rhizoctonia* and *Macrophomina* were obtained (Khan *et al.* 2007, Gherbawy and Gashgari 2014 and Carbungco *et al.* 2015).

Endophytic fungi produce enzymes such as amylases, cellulase, lipases and proteases, as part of their mechanism to overcome the defense of the host against microbial invasion and to obtain nutrients for their development. In addition, these enzymes are essential for endophytic fungi to colonize in the plant tissue. The array of enzymes produced differs between fungi and often depends on the host and their ecological factors. In the current study, 88 fungal isolates were screened for their abilities to produce pectinase and cellulase enzymes. The results obtained revealed that 70.45 % of the total isolates tested could produce pectinase enzyme and 67.0 % could produce cellulase enzyme. In this sense, Sunitha et al. (2013) observed that 62.0% and 32.0% of their endophytic isolates tested were positive for pectinase and cellulase, respectively, but their fungi tested were isolated from plants separate from ours. In another analysis of cellulolytic activity in salt

marsh fungi, 100 % of the isolates studied demonstrated cellulase production (Gessner 1980), whereas 66.0 % of the fungi isolated from the wild plant *Brucea javanica* could produce cellulase enzyme (Choi *et al.* 2005).

Cellulases have been widely used in agricultural, biofuel, detergent, fermentation, food, paper pulp, and textile industries (Kuhad *et al.* 2011). Screening of the isolates for cellulase activity was attempted with a view of endophytes penetrating the plant tissue through the lignocellulosic wall with the help of the hydrolytic enzymes, cellulases being predominant among them (Carroll and Petrini 1983). In addition, it was reported that some endophytes might behave as latent saprophytes, and when the host dies, they use these enzymes for tissue degradation to obtain nutrients (De Aldana *et al.* 2013).

The current results demonstrated that 71.59 % of total isolates could hydrolyze pectin in SmF, of which *Aspergillus*, *Alternaria*, and *Fusarium* isolates were the higher producers. *Aspergillus flavus* AY-72 being the potent giving rise to 242.481 IU/ml/min. In this regard, many fungal species have been reported to degrade pectin substrate and produce pectinases enzymes either in submerged or solid-state fermentation such as *Aspergillus awamori* (Botella *et al.* 2007 and Dasari 2020), *A. niger* (Bai *et al.* 2004 and El Enshasy *et al.* 2018), *A. flavus*, (Abdel-Sater *et al.* 2021), *A. terreus* (Martínez- Trujillo *et al.* 2011 and Abdel-Sater *et al.* 2021), *Penicillium chrysogenum* (Banu et al. 2010 and de MB Silva *et al.* 2020), *Fusarium moniliforme* (Niture and Pant 2004 & 2007 and Rajathi *et al.* 2020), *F. oxysporum* (Di Pietro and Roncero1996 and Dong et al. 2020), *Alternaria alternata* (Isshiki et al. 2001, Anand *et al.* 2008 and Faten and Abeer 2013), *Cladosporium cladosporioides* (Bastos *et al.* 2013 and Poveda *et al.* 2018) and *Trichoderma reesei* (Olsson et al. 2003 and Beier *et al.* 2020). However, the results reported by Shubha and Srinivas (2017) and Choi *et al.* (2005) showed that pectinase production was absent in all the endophytic fungi of *Brucea javanica*.

Conclusion

This study compares mycobiota isolated from wild and cultivated plant species. It can be deduced that wild plant species is a viable source of fungal species with high enzyme activity and a promising propensity for enzyme synthesis. This research will also help to focus attention on wild plants, which are a potential source of species capable of producing compounds of high economic value.

Compliance with ethical standards

The manuscript is original. No part of the manuscript has been published before nor is any part of it under consideration for publication at another journal.

Conflict of interest

The authors declare that they have no conflict of interest. All authors fully agree for submission of the manuscript.

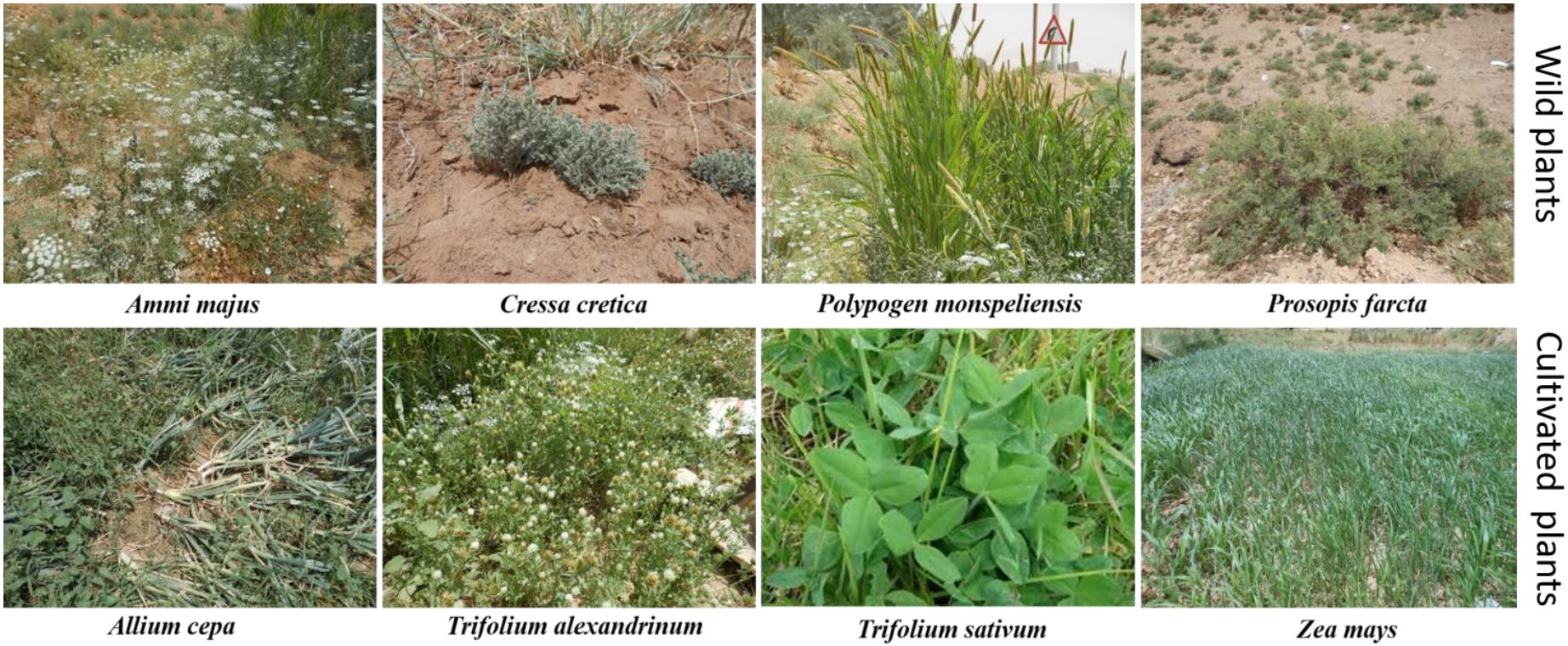

Figure 1: Some healthy and mature wild (upper) and cultivated (lower) plant species collected from El-Kharga Oasis, New Valley Governorate during this study.

Table 1: CFUs (calculated to the total CFUs of each fungus per 25 segments of leaves (L) or roots (R) of each plant sample), Gross total CFUs and % gross total CFUs of fungi isolated from 4 wild and 4 cultivated plants collected from El-Kharga Oasis, New Valley Governorate on 1 % glucose-Cz at 25 °C during April 2018.

Fungi	Wild plants								Cultivated plants									
	Cressa cretica		***Ammi majus***		***Polypogen monspeliensis***		***Prosopis farcta***		***Trifolium alexandrinum***		***Zea mays***		***Allium cepa***		***Trifolium sativum***			
	L	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**CFU**	**%CFU**
Acrophialophora fusispora							14										14	3.13
Alternaria			**19**		**5**										**22**		**49**	**10.95**
A. alternata					5										5		10	2.24
A. gaisen															4		4	0.89
A. perangusta			4														4	0.89
A. tenuissima			4												13		17	3.8
A. turkisafria			11														11	2.46
Arthrenium euphorbiae							3										3	0.67
Aspergillus	**33**	**37**	**8**	**3**	**24**	**26**	**13**			**5**	**10**		**3**				**162**	**36.24**
A. acidus							3										3	0.67
A. eucalypticola						3											3	0.67
A. flavus			8	3	7	13	**3**			5	4						43	9.6
A. neoniger							3										3	0.67
A. nidulans											6						6	1.34
A. niger					14												14	3.13
A. parasiticus					3	10	4										17	3.8
A. terreus	33	37															70	15.66
A. tubingensis													3				3	0.67
Chaetomium globosum												3					3	0.67
Exserohilum				**1**	**3**	**14**						**15**					**33**	**7.35**
E. rostratum				1		14											15	3.35
E. gedarefense					3							15					18	4.0
Fusarium							**3**			**27**				**9**		**11**	**50**	**11.13**
F. circinatum							3										3	0.67
F. dimerum														4			4	0.89
F. oxysporum										27							27	6.0
F. solani														5		8	13	2.9
F. subglutinans																3	3	0.67
Graphium penicillioides															3		3	0.67

Fungi	**Wild plants**								**Cultivated plants**									
	Cressa cretica		***Ammi majus***		***Polypogen monspeliensis***		***Prosopis farcta***		***Trifolium alexandrinum***		***Zea mays***		***Allium cepa***		***Trifolium sativum***			
	L	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**CFU**	**%CFU**
Macrophomina phaseolina				11						8							19	4.25
Microascus	**3**		**5**	**5**	**3**		**3**										**19**	**4.25**
M. cinereus	3		5		3		3										14	3.13
M. terreus				5													5	1.12
Nigrospora oryzae							3										3	0.67
Penicillium griseoroseum					3												3	0.67
Phoma pomorum																3	3	0.67
Rhizoctonia solani									10	5							15	3.35
Scopulariopsis fimicola							3										3	0.67
Setosphaeria khartoumensis					3												3	0.67
Stachybotrys zhanymuensis							3										3	0.67
Stemphylium botryosum															8		8	1.8
Torula sp.							3										3	0.67
Dark mycelia sterilia				3			2				2				3		10	2.24
White mycelia sterilia							1										1	0.22
Yeast spp.											8	6	20	3		3	40	8.95
CFUs	**36**	**37**	**32**	**23**	**41**	**40**	**51**	**0**	**10**	**45**	**20**	**24**	**23**	**12**	**36**	**17**	**447**	
Total CFUs	**73**		**55**		**81**		**51**		**55**		**44**		**35**		**53**			
Total genera (20)	3		7		8		10		5		3		2		5			
Total species (38)	3		9		12		13		5		4		3		8			
	CFUs = 260 (15 genera & 26 species)								**CFUs = 187 (10 genera & 17 species)**									

Table 2: Preliminary screening of pectinases and cellulases production by endophytic fungi recovered from leaves and roots of some wild and cultivated plants collected from El-Kharga Oasis, the New Valley Governorate, Egypt, during April 2018.

Fungal species	No. of isolates tested	Preliminary screening							
		Pectinases				Cellulases			
		Positive	L	M	H	Positive	L	M	H
Acrophialophora fusispora	1	1		1		1		1	
Alternaria									
A. alternata	2	1	1			2	1	1	
A. gaisen	1	1	1						
A. perangusta	1	1		1					
A. tenuissima	2	2	2			1		1	
A. turkisafria	1					1		1	
Arthrenium euphorbiae	1					1		1	
Aspergillus									
A. acidus	1								
A. eucalypticola	1								
A. flavus	11	6	4	1	1	5	2	1	2
A. neoniger	1								
A. nidulans	1	1		1		1		1	
A. niger	1	1		1					
A. parasiticus	3	1	1			2	2		
A. terreus	6	6		5	1	5	3	1	1
A. tubingensis	1								
Chaetomium globosum	1	1		1		1	1		
Exserohilum									
E. rostratum	2	1	1						
E. gedarefense	2	2	1	1		2		1	1
Fusarium									
F. circinatum	1	1		1		1	1		
F. dimerum	1	1			1	1		1	
F. oxysporum	1	1	1			1	1		
F. solani	3	2	1	1		3	1		2
F. subglutinans	1					1		1	
Graphium penicillioides	1	1		1		1		1	
Macrophomina phaseolina	4	3	2		1				
Microascus									
M. cinereus	5	5	2	3		5		3	2
M. terreus	2	2		2		1		1	
Nigrospora oryzae	1	1	1			1	1		
Penicillium griseoroseum	1	1		1					
Phoma pomorum	1								
Rhizoctonia solani	4	3		3		3	2		1
Scopulariopsis fimicola	1	1			1	1			1
Setosphaeria khartoumensis	1	1	1			1			1
Stachybotrys zhanymuensis	1								
Stemphylium botryosum	3	3	1	2		3	2		1

Fungal species	**No. of isolates tested**	**Preliminary screening**							
		Pectinases				**Cellulases**			
		Positive	**L**	**M**	**H**	**Positive**	**L**	**M**	**H**
Torula sp.	1					1			1
Dark sterile mycelia	4	3	3			2			2
White sterile mycelia	1					1			1
Yeast spp.	10	8	6		2	9	4		5
Total isolates	**88**	**62**	**29**	**26**	**7**	**58**	**21**	**16**	**21**
No. of genera	**20**	**15**	**9**	**11**	**4**	**16**	**7**	**8**	**10**
No. of species	**38**	**28**	**14**	**16**	**5**	**27**	**11**	**14**	**12**

Table 3: Comparison between fungi from wild and cultivated plants in terms of their production of endoglucanase, exoglucanase and pectinase in SmF.

Fungal species	**Isolate no.**	**Wild plant species**			**Cultivated plant species**		
		Endoglucanase IU/ml/min	**Exoglucanase** IU/ml/min	**Pectinase** IU/ml/min	**Endoglucanase** IU/ml/min	**Exoglucanase** IU/ml/min	**Pectinase** IU/ml/min
Aspergillus flavus	AY-148	8.96	46.379	-	-	-	-
A. flavus	AY-151	-	-	203.56	-	-	-
A. flavus	AY-281	-	-	175.91	-	-	-
A. flavus	**AY-72**	1.64	-	**242.81**	-	-	-
A. terreus	AY-2	-	-	177.43	-	-	-
A. terreus	AY-87	10.45	29.253	-	-	-	-
Exserohilum gedarefense	AY-136	-	-	-	12.64	26.593	
Fusarium dimerum	AY-95	-	-	-	-	-	185.30
F. solani	AY-96	-	-	-	8.69	26.593	-
F. solani	AY-126	-	**65.5**	-	6.31	34.731	-
Microascus cinereus	AY-146	8.91	30.9	-	-	-	-
M. cinereus	**AY-147**	**14.12**	26.6	-	-	-	-
Macrophomina phaseolina	AY-5	-	-	234.07	-	-	-
Rhizoctonia solani	**AY-62**	-	-	-	13.78	30.875	-
Scopulariopsis fimicola	AY-249	3.9	35.396	230.62	-	-	-
Setosphaeria khartoumensis	AY-168	3.38	49.198	-	-	-	-
Stemphylium botryosum	AY-127	-	-	-	3.66	30.449	-
Torula sp.	AY-248	8.77	29.598	-	-	-	-
Yeast sp.	AY-138	-	-	-	9.03	31.247	-
Yeast sp.	**AY-229**	-	-	-	-	-	**218.64**
Yeast sp.	AY-232	-	-	-	9.0	**40.1**	-
Yeast sp.	AY-237	-	-	-	11.99	31.274	-
Yeast sp.	AY-239	-	-	-	2.5	32.338	201.12
Yeast sp.	AY-242	-	-	-	9.3	26.6	-
Dark sterile mycelia	**AY-133**				**14.6**		
Dark sterile mycelia	AY-149	0.11	26.593	-	-	-	-
Dark sterile mycelia	AY-233	-	-	-	4.6	30.183	-
White sterile mycelia	AY-247	3.98	39.491	-	-	-	-

Table 4: Comparison between wild and cultivated plants in terms of their biodiversity, and pre-screening and production of endoglucanase, exoglucanase and pectinase enzymes.

Comparison parameter	**Wild plants**	**Cultivated plants**
Total CFUs	260	187
Total genera	14	10
Total species	26	16
Most common genera	*Aspergillus*, *Alternaria*, *Microascus*	*Fusarium*, *Alternaria*, *Aspergillus*
Most common species	*A. terreus*, *A. flavus*	*F. oxysporum*, *F. solani*
No. of new-recorded species	8 out of 26 (30.77 %)	2 out of 16 (12.5 %)
Positive cellulase-producers	29 out of 48 (60.4 %)	30 out of 40 (75 %)
Positive pectinase-producers	35 out of 48 (72.9 %)	28 out of 40 (70 %)
Endoglucanase-potent strains	*M. cinereus* AY-147 14.1 IU/ml/min	Dark sterile mycelia AY-133 **14.6 IU/ml/min**
Exoglucanase-potent strains	*F. solani* AY-126 **65.5 IU/ml/min**	Yeast sp. AY-232 40.1 IU/ml/min
Potent strains for pectinase	*A. flavus* AY-72 **242.81 IU/ml/min**	Yeast sp. AY-229 218.64 IU/ml/min

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