Studies in Fungi 6(1): 78–91 (2021) www.studiesinfungi.org ISSN 2465-4973
Article

Doi 10.5943/sif/6/1/4

Endophytic mycobiota of wild medicinal plants from New Valley Governorate, Egypt and quantitative assessment of their cell wall degrading enzymes

Abdel-Sater MA[1], Abdel-Latif AMA[2], Abdel-Wahab DA[2] and Al-Bedak OA[3*]

[1]*Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71511, Egypt*
[2]*Department of Botany, Faculty of Science, New Valley University, El-Kharga, 72511, Egypt*
[3]*Assiut University Mycological Centre, Assiut University, Assiut 71511, Egypt*

Abdel-Sater MA, Ayat MA Abdel-Latif, Dalia A Abdel-Wahab, Al-Bedak OA 2021 – Endophytic mycobiota in some wild medicinal plants at New Valley Governorate, Egypt, and their cell wall degrading enzymes, Studies in Fungi 6(1), 78–91, Doi 10.5943/sif/6/1/4

Abstract

The present study isolated and identified 32 species of endophytic mycobiota belonging to 18 genera associated with 8 wild medicinal plants collected from El-Kharga Oasis, New Valley Governorate, Egypt. *Fusarium* was the most common genus followed by *Alternaria* and *Aspergillus*. *Convolvulus arvensis* was the plant with the highest number of endophytes over the other plant species, while *Moringa oleifera* reported the lowest number of endophytes. In addition, the entomopathogenic fungus *Beauveria bassiana*; was recorded for the first time from leaves of *Portulaca oleracea*. One hundred and twenty-three isolates representing 32 species were screened for their abilities to produce pectinase, carboxy methyl cellulase (CMCase) and avicellase enzymes on sucrose free-Cz supplemented, individually with 1% pectin or 1% CMC or 1% avicel as a sole carbon source, respectively. Ninety-four isolates produced pectinase while 66 isolates produced cellulases. The quantitative assays of the three enzymes for high-producers were performed in submerged fermentation using sucrose-free Cz broth. *Aspergillus* was the superior in the production of the three enzymes with the potent strains were *A. terreus* AUMC 14287 for CMCase (22.0 IU/ml/min) and avicellase (47.868 IU/ml/min) and *A. terreus* AUMC 14278 for pectinase (225.43 IU/ml/min).

Kew words – cellulose – endophytic fungi – medicinal plants – pectinase – submerged fermentation

Introduction

Endophytic microorganisms colonize in plant tissues in which they spend part or all their life cycle without causing disease symptoms in the host (Petrini 1991). Fungal endophytes may inhabit in different organs of the host including leaves, stems, bark, roots, fruits, flowers and seeds (Rodriguez et al. 2009). Generally, in this symbiotic relationship fungal endophytes receive shelter and nutrients from the host, while the host plant may benefit from an array of attributes which include protection against natural enemies such as pathogens and herbivores (Schardl et al. 2004, Singh et al. 2011), plant growth promotion (Hamayun et al. 2010) and increasing the resistance of plants to abiotic stresses such as salinity and heavy metal toxicity in soil (Khan et al. 2014). Some medicinal plants are known for harboring endophytic fungi, which are important sources of various

Submitted 12 April 2020, Accepted 26 November 2020, Published 1 February 2021
Corresponding Author: Osama A. Al-Bedak – e-mail – osamaalbedak@gmail.com

bioactive secondary metabolites and enzymes valuable for the pharmaceutical industry (Zou et al. 2000, Strobel et al. 2004, Krishnamurthy et al. 2008).

Endophytic fungi are relatively unexplored producers of metabolites useful in pharmaceutical and agricultural industries. A single endophyte can produce several bioactive metabolites. As a result, the role of endophytes in the production of various natural products with greater bioactivity have received increased attention (Prabavathy & Valli 2012). Pectinases and cellulases, besides other enzymes, are the most important enzymes produced by endophytic fungi as a resistance mechanism against pathogenic invasion and to obtain nutrition from the host. These enzymes have various industrial applications, thus of major interest. Increasing efforts are being taken to characterize and identify endophytic fungi from medicinal plants. Therefore, the present work was designed to study the biodiversity of endophytic fungi in some wild medicinal plants from the New Valley Governorate, Egypt, and to evaluate their ability to produce extracellular pectinases and cellulases.

Materials and methods

Sampling area

The New Valley Governorate is located at the Western Desert of Egypt. It encompasses 440,098 km^2, which is approximately 44% of the total area of Egypt and 66% of the area of Western Sahara. It is demarcated by the Governorates of Minya, Assiut, Sohag, Qena and Aswan from the east, by Libya and the Governorates of Matrouh and the Marine Oasis of the 6th of October City from the West and by Sudan from the South. The New Valley includes four large Oases namely El-Kharga (the sampling sites), El-Dakhla, El-Bahariya and El-Farafra, and the capital is El-Kharga (Fig. 1).

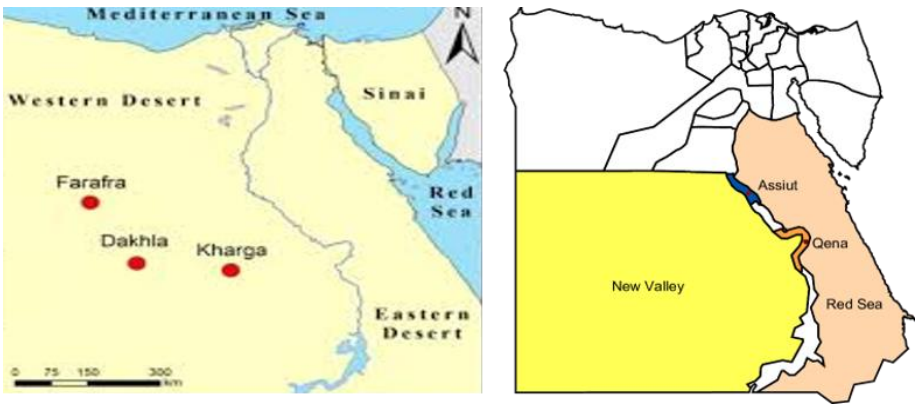

Fig. 1 – Location of the New Valley Governorate showing study site.

Sample collection and identification of plant species

Healthy and mature plant leaves and roots of eight wild medicinal plants were collected from El-Kharga Oasis, New Valley Governorate once during April 2018. Ten replicates from each of *Alhagi graecorum*, *Anagallis arvensis*, *Calotropis procera*, *Chenopodium ambrosioides*, *Convolvulus arvensis*, *Moringa oleifera*, *Portulaca oleracea*, and *Ricinus communis* plants were collected in sterile polyethylene bags and promptly brought to the laboratory for isolation of fungi. The plant species collected in the current investigation were identified according to morphological features and taxonomical characters at the Assiut University Herbarium, Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt (Fig. 2).

Sample preparation and surface sterilization

Prior to surface sterilization, leaves and roots of each sample were thoroughly washed with tap water to remove the dust followed by distilled water. The samples were then cut into 5-cm

segments. The samples were surface sterilized using the following sequence; 5% sodium hypochlorite for 3 min, 70% ethanol for 1 min, and washing with sterile distilled water 3 times each for 1 min. In aseptic conditions, both ends of each segment (1 cm) was cut off to produce a 3-cm segments (Al-Bedak et al. 2020).

Isolation of endophytic fungi

Segments of each sample were plated on Petri-dishes containing 1% glucose-Cz 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 (Ismail et al. 2017). The plates were incubated for 7-21 days at 25°C. Counts of CFUs of each fungal isolate were calculated per 25 segments in every sample. The obtained fungi were identified morphologically to the species level at the Assiut University Mycological Centre according to their macroscopic and microscopic characteristics. Pure cultures of the fungal strains were preserved for further investigations on PDA slants, as well as on cotton balls (Al-Bedak et al. 2019) at 4°C in the culture collection of the Assiut University Mycological Centre.

Phenotypic identification of fungi

The obtained fungi in this study were identified morphologically to the species level at the Assiut University Mycological Centre according to their macroscopic and microscopic characteristics. The following references were used for the identification of fungal genera and species (purely morphologically, based on macroscopic and microscopic features): Booth (1971), Ellis (1976), Pitt (1979), Domsch et al. (2007), Moubasher (1993), de Hoog et al. (2000), Samson et al. (2004), Leslie & Summerell (2006), Simmons (2007) and Al-Bedak et al. (2020).

Screening of pectinase and endoglucanase production on solid medium

Production of pectinase and endoglucanase was detected on sucrose-free Czapek's agar medium amended with pectin (from citrus peel) and CMC as a sole carbon source, respectively. 50 µl of spore suspension from 7-day-old culture of each fungal strain was individually added to each 5-mm diameter well on the agar plate (Moubasher et al. 2016). The inoculated plates were incubated for 2 days at 30ºC. The clear zones formed around the wells were more visible when the plates were flooded with 0.25% (w/v) aqueous iodine solution. The diameters of the clear zones were measured (in mm) against the brown color of the test medium indicating enzyme production.

Production of pectinases and cellulases under submerged fermentation

All positive fungal strains were grown, individually in 250-ml Erlenmeyer conical flasks each containing 50 ml sucrose-free Czapek's broth medium supplemented with 1% pectin or 1% CMC as sole carbon source. The flasks were then inoculated individually with 1 ml spore suspension containing 1 x 10^7 spore/ml of 7-day-old culture of the tested strains. The inoculated flasks were then incubated at 30ºC in shaking condition of 150 rpm for 7 days.

Enzyme extraction

After incubation period, the flasks contents were individually filtered through filter papers (Whatman No. 1) and the filtrate was then centrifuged at 10000 xg for 10 min at 4ºC. The clear supernatants were used as a source for CMCase or pectinase enzyme.

Pectinase assay

The enzyme production was determined by mixing 0.9 ml of 1% pectin (prepared in 50 mM Na-citrate buffer, pH 5.0) with 0.1 ml of filtered crude enzyme, and the mixture was incubated at 50°C for 15 min in a water bath (Bailey et al. 1992). The reaction was stopped by the addition of 2 ml of 3,5-dinitrosalicylic acid (DNS) and the contents were boiled in water bath for 10 min (Miller 1959). After cooling, absorbance was measured at 540 nm using Cary 60 UV-Vis spectrophotometer. The amount of reducing sugar liberated was quantified using calibration curve

of glucose. One unit of pectinase is defined as the amount of enzyme that liberates 1 µmol of glucose equivalents per minute under the standard assay conditions.

Cellulases (CMCase and avicellase) assay

The cellulases activity was determined by mixing 0.9 ml of 1% CMC or 1% avicel (prepared in 50 mM Na-citrate buffer, pH 5.0) with 0.1 ml of filtered crude enzyme, and the mixture was incubated at 50°C for 15 min in a water bath (Bailey et al. 1992). The reaction was stopped by the addition of 2 ml of 3,5-dinitrosalicylic acid (DNS) and the contents were boiled in water bath for 10 min (Miller 1959). After cooling, absorbance of the developed color was measured at 540 nm using Cary 60 UV-Vis spectrophotometer. The amount of reducing sugar liberated was quantified using calibration curve of glucose. One unit of CMCase or avicellase is defined as the amount of enzyme that liberates 1 µmol of glucose equivalents per minute under the standard assay conditions. Glucose concentration was calculated using the calibration curve.

$$\textbf{Glucose concentration} = \frac{\text{Absorbance}}{\text{slope } (=1.0472)} \text{ mg/ml (= g/L)}$$

$$\textbf{Enzyme concentration} = \frac{\text{Glucose concentration (g/L)}}{0.00018} \text{ IU/L}$$

The enzyme activity (pectinase or CMCase or avicellase) was calculated according to the following formula (Moubasher et al. 2016)

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

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

Results

Biodiversity of endophytic fungi

A total of 32 species related to 18 genera of endophytic mycobiota were recovered on 1% glucose-Cz at 25°C from healthy and mature plant leaves and roots of eight wild medicinal plants, collected from El-Kharga Oasis, the New Valley Governorate. The high incidence in genera were recorded in *Alternaria*, *Aspergillus* and *Fusarium*. *Fusarium* (represented by 2 species) was the most common and encountered total CFU constituting 37.0% of total fungi. It was recovered from 7 plants out of 8. *F. oxysporum* was the most prevalent species encountering 23.1% of total fungi, however it was recorded from 3 plants only, followed by *F. solani* giving rise to 13.9% of total fungi and it was the most frequent recovered from 6 plants. *Alternaria* (7 species) came next to *Fusarium* and it was comprised 24.0% of total fungi with *A. alternata* being the most common *Alternaria* species recorded from 4 plants and was comprised 10.33% of total fungi followed by *A. tenuissima* (from 4 plants) comprising 7.42% of total fungi. *Aspergillus* (5 species in addition to 2 unknown species) was the runner of *Alternaria* comprising 17.5% of total fungi. It was the most frequent genus isolated from all the studied plants. The most prevalent *Aspergillus* species were *A. terreus* followed by *A. flavus* constituting 7.42% and 5.84% of total fungi respectively (Table 1).

Aspergillus parasiticus, *Macrophomina phaseolina* were found in 4 plant species, *A. longipes* and *Stemphylium botryosum* in 3 plants, *A. citri*, *A. fumigatus*, and *C. spicifera* in 2 plants while *Acremonium rutilum*, *Beauveria bassiana*, *Chaetomium senegalense*, *Cladosporium exile*, *Clonostachys rosea*, *C. solani*, *Pseudoallescheria boydii*, *Rhizoctonia solani*, *Rhizopus microsporus*, *Scopulariopsis fimicola*, *Stemphylium botryosum* and *Verticillium fungicola* were

recorded each in one plant species. *Convolvulus arvensis* was the richest plant with endophytes containing 14 species belonged to 8 genera and recording the highest CFUs of 79 per 25 segments over the remaining plant species, while *Moringa oleifera* was the poorest in endophytes with 5 species belonging to 2 genera and the lowest CFUs of 13 per 25 segments. It is worth mentioning that *Beauveria bassiana*; the known entomopathogenic fungus was recorded for the first time from leaves of *Portulaca oleracea* as an endophyte (Table 1).

Preliminary screening of endophytic fungi for pectinases and cellulases production

One-hundred and twenty fungal isolates representing 31 species related to 17 genera of endophytic fungi were screened for their abilities to produce pectinase and endoglucanase on sucrose free-Cz supplemented with 1% pectin or 1% CMC as a sole carbon source, respectively. Ninety-four isolates could produce pectinase enzyme, of which 18 were high producers, 25 moderate and 51 low. 66 isolates could produce cellulase, of which 13 were high producers, 16 moderate and 37 low (Appendix 1).

Submerged production of pectinases and cellulases (CMCase and avicellase)

The quantitative assay of pectinase, CMCase and avicellase for high-producing isolates were performed in submerged fermentation using sucrose-free Cz broth medium amended with 1% pectin or CMC or avicel as the sole carbon source. Of these, 17 isolates could produce pectinase enzyme with a relative activity ranged from 147.84 IU/ml/min to 225.43 IU/ml/min while 14 could produce CMCase (1.84 IU/ml/min – 22.0 IU/ml/min) and avicellase (26.0 IU/ml/min – 47.87 IU/ml/min). Six isolates were found to have the abilities to produce the three enzymes, of which *Aspergillus* was the superior with the potent strains were *A. terreus* AUMC 14278 for pectinase activity giving 225.43 IU/ml/min and *A. terreus* AUMC 14287 for CMCase producing 22.0 IU/ml/min and avicellase recording 47.868 IU/ml/min (Tables 2-4).

Discussion

In the current study, endophytic mycobiota in healthy and mature leaves and roots of eight wild medicinal plants were isolated on 1% glucose-Cz at 25°C from sample collected once in April 2018. This study is considered as the first in the New Valley Governorate, Egypt for evaluation of endophytic fungi from these medicinal plants. There is a growing body of literatures that recognize the importance of endophytic fungi across a number of disciplines in recent years as biological sources of a wide range of valuable compounds including plant growth regulatory, antibacterial, antifungal, antiviral, insecticidal substances to enhance the growth and competitiveness of the host in nature (Anwar et al. 2007, Kaur & Kalia 2012, Khairnar et al. 2012, Al-Snafi 2015, Muhammad et al. 2015, Syed et al. 2016, Khan Marwat et al. 2017).

The current results revealed that endophytic fungal assemblages were obtained from all plant species examined and some plants were occupying by the same fungal genera and species, indicating that endophytic fungi can be the same in plants belonging to different families. Altogether, 32 species related to 18 genera were recovered from the leaves and roots of all tested plants.

The high occurrence genera where described by *Fusarium*, *Aspergillus* and *Alternaria*. *Fusarium* was the most widespread genus retrieved from 7 plants. *F. oxysporum* is the most dominant led by *F. solani*. Such latest observations have, to some degree, been compatible with the reports of Raviraja (2005) who researched endophytic fungi in five Brazilian medicinal plants and found that *Aspergillus* and *Penicillium* were isolated at high frequencies, however, *Fusarium oxysporum* was reported at low levels from leaves of two plants tested. Previous studies on plants of the same size as ours have previously been conducted with the genera *Fusarium*, *Aspergillus*, *Nigrospora*, *Stachybotrys*, *Rhizoctonia* and *Macrophomina* from Moringa leaves (Carbungco et al. 2015). Almost similar results were obtained in other studies on *Calotropis procera* in Karachi (Khan et al. 2007) or in Saudi Arabia (Gherbawy & Gashgari 2014).

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 (Patil et al. 2015). In addition, these enzymes are essential for endophytic fungi to colonize in the plant tissue (Sunitha et al. 2013). The array of enzymes produced differs between fungi and often depends on the host and their ecological factors (Sunitha et al. 2013). In the current study, 120 fungal isolates were screened for their ability to produce pectinase and cellulase. The results obtained revealed that 78.0% of the total isolates tested could produce pectinase enzyme and 55.0% could produce cellulase enzyme.

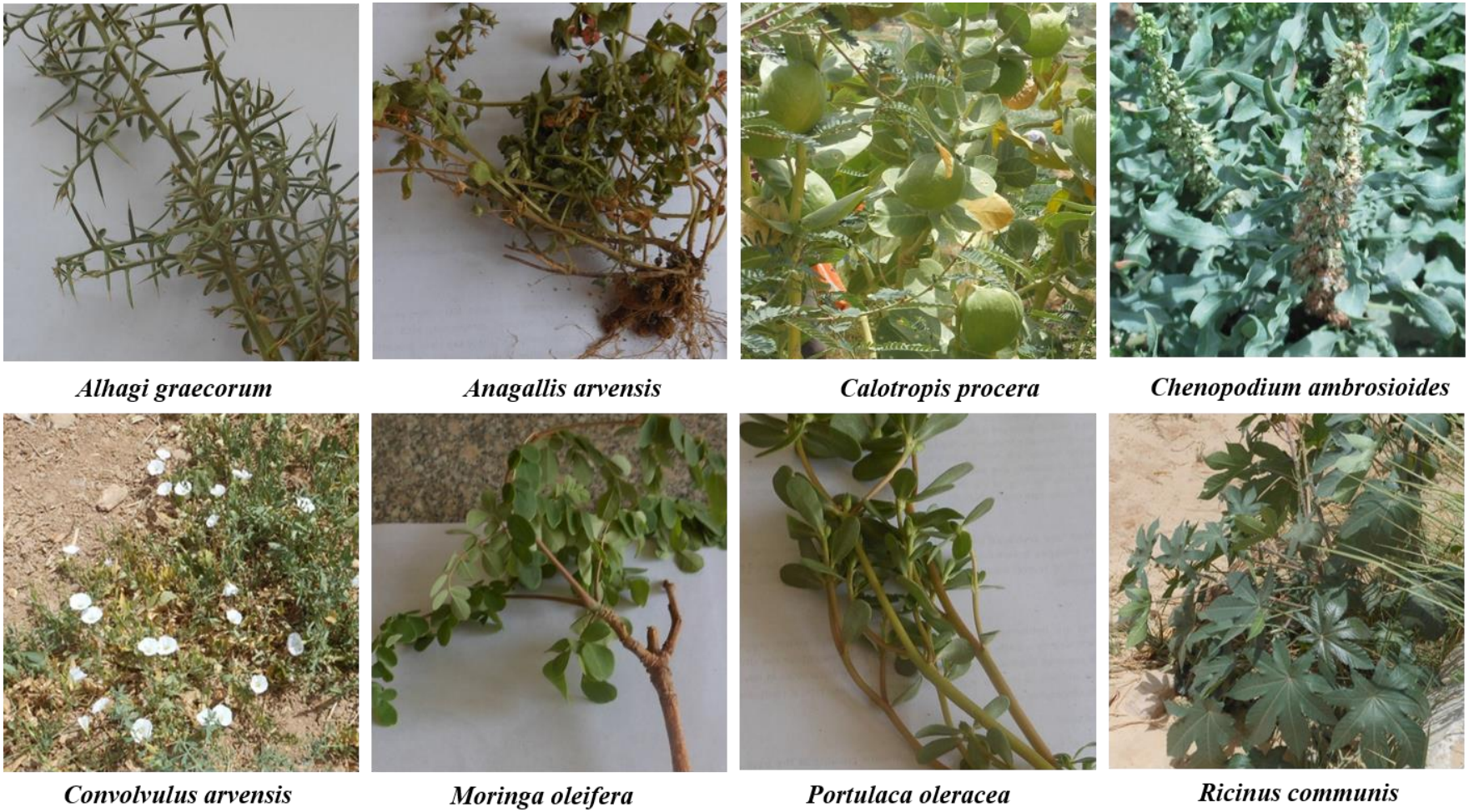

Fig. 2 – Wild medicinal plant species collected from El-Kharga Oasis, the New Valley Governorate, Egypt, during April 2018.

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 8 wild medicinal plants collected from El-Kharga Oasis, New Valley Governorate on 1 % glucose-Cz at 25°C during April 2018.

Fungal genera & species	**Plant species**																**Gross total**	
	Alhagi graecorum		***Convolvulus arvensis***		***Chenopodium ambrosioides***		***Calotropis procera***		***Ricinus communis***		***Angallis arvensis***		***Moringa oleifera***		***Portulaca oleracea***			
	L	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**CFU**	**%CFU**
Acremonium			**4**														**4**	0.89
A. rutilum			1														1	0.22
A. sclerotigenum			3														3	0.67
Alternaria				**1**	**34**		**36**			**1**	**20**		**15**				**107**	**24**
A. alternata				1	21		20				4						46	10.33
A. brassicicola										1							1	0.22
A. chlamydospora													8				8	1.79
A. citri							1				7						8	1.79
A. citri macularis							4										4	0.89
A. longipes					3		3						1				7	1.57
A. tenuissima					10		8				9		6				33	7.42
Aspergillus	**1**	**10**	**3**	**2**	**1**	**1**	**20**	**10**	**21**	**2**	**2**		**2**		**1**	**2**	**78**	**17.5**
A. creber			2														2	0.44
A. flavus		1		1		1			19	2	1					1	26	5.84
A. fumigatus					1				1								2	0.44
A. keveii													1				1	0.22
A. parasiticus				1				8			1				1	1	12	2.70
A. terreus	1	9	1				20		1				1				33	7.42
A. tubingensis								2									2	0.44
Beauveria bassiana															1		1	0.22
Chaetomium senegalense								1									1	0.22
Cladosporium exile									1								1	0.22
Clonostachys solani			1														1	0.22
Curvularia spicifera		19		1													20	4.49
Fusarium		**7**	**33**	**37**		**14**		**12**		**14**	**22**	**6**				**20**	**165**	**37**
F. oxysporum		6	33	36							22	6					103	23.1
F. solani		1		1		14		12		14						20	62	13.9
Macrophomina phaseolina				1		4				1		1					7	1.57
Penicillium olsonii									21								21	4.72
Pseudoallescheria boydii										2							2	0.44
Rhizoctonia solani											1	2					3	0.66

Table 1 Continued.

Fungal genera & species	Plant species																Gross total	
	Alhagi graecorum		*Convolvulus arvensis*		*Chenopodium ambrosioides*		*Calotropis procera*		*Ricinus communis*		*Angallis arvensis*		*Moringa oleifera*		*Portulaca oleracea*			
	L	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**		
Rhizopus microspores																1	1	0.22
Sarocladium kiliense				2													2	0.44
Scopulariopsis fimicola					1												1	0.22
Stemphylium botryosum							6		1		6						13	2.92
Verticillium fungicola			2														2	0.44
Yeast spp.		1				1			1	1	1		1		8	1	15	3.37
CFUs	**1**	**37**	**43**	**44**	**36**	**20**	**62**	**23**	**45**	**21**	**52**	**9**	**18**	**0**	**10**	**24**	**445**	**100**
No. of genera	1	4	5	6	3	4	3	3	5	6	6	3	3	0	2	4		
No. of species	1	6	7	8	5	4	7	4	7	6	9	3	6	0	2	5		
Total CFUs	38		87		56		85		66		61		18		34		**445**	
Total genera (18)	4		9		6		5		10		7		3		5			
Total species (32)	6		14		9		11		12		10		6		6			

The quantitative assay of the three enzymes for high-producers were performed in submerged fermentation using sucrose-free Cz broth. *Aspergillus* was superior in the production of the three enzymes with the potent strains were *A. terreus* AUMC 14287 for CMCase and avicellase, and *A. terreus* AUMC 14278 for pectinase. Almost similar results were reported by (Sunitha et al. 2013) who found that 62.0% and 32.0% of their tested endophytic isolates were positive for pectinase and cellulase respectively, however, their tested fungi were isolated from plants differ from ours. In another study of cellulase activity of fungi inhabiting salt marshes, 100% of the tested isolates showed cellulolytic activity (Gessner 1980), while 66.0 % of fungi isolated from *Brucea javanica* could produce cellulase enzyme (Choi et al. 2005). The main endophytic fungi work in literature involves screening for secondary metabolites of antimicrobial and antioxidant activity. Not many explored the possibility of endophytic fungi as industrially essential biotechnological reservoirs of enzymes.

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 & 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). Studies also estimated that microbial pectinase accounts for 25% of global food and industrial enzymes revenues and is increasingly growing in the market (Oumer 2017). In addition, enzymes are a well-established global industry that is expected to hit USD 6.3 billion in 2021 (Oumer & Abate 2018).

The current results revealed that 78.3% of total isolates could hydrolyze pectin in submerged fermentation, of which 77.14% were *Aspergillus* isolates, 76.9% *Alternaria*, and 86.95% *Fusarium* showed positive results. The present findings were in concurrence with those of Sunitha et al. (2013)

who reported that 62% of their tested fungi were pectinase producers, and better than results obtained by Shubha & Srinivas (2017) who found that 30% of their tested fungi had the pectinolytic activity. However, Choi et al. (2005) have reported that pectinase production was absent in all the endophytic fungi of *Brucea javanica*.

Aspergillus species was superior in pectinase activity with *A. terreus* being the potent strain giving rise to 163.244 IU/ml which is more than the result of pectinase production (106.7 IU/ml) produced by *Aspergillus* sp. Gm (KC et al. 2020) and much more the outcome of pectinase production (1.524 IU/ml) stated by (Sopalun & Iamtham 2020) from endophytic fungi isolated from Thai Orchids.

The production of plant cell-wall digestive enzymes is now a focus of current research. Many such researches have been done into the production of cellulase and pectinase due to the huge number of application scenarios of these enzymes (Jalis et al. 2014, Edor et al. 2018, Ismail et al. 2018, Li et al. 2020, Xue et al. 2020).

Table 2 Pectinase production and activity of some endophytic fungi.

Fungal species	**AUMC no.**	**Pectinase**		
		Glucose g/l	**Production IU/ml**	**Activity IU/ml/min**
Aspergillus flavus	14274	19.27	107.063	147.8
A. flavus	14289	27.82	154.555	213.4
A. fumigatus	14283	27.44	152.438	210.5
A. terreus*	**14278**	**29.4**	**163.244**	**225.4**
A. terreus	14287	26.34	146.326	202.0
A. terreus	14293	26.0	144.814	200.0
A. terreus	14279	24.14	134.103	185.2
Cladosporium exile	14294	23.91	132.846	183.4
Curvularia spicifera	14276	23.6	131.016	180.9
C. spicifera	14273	29.0	161.255	222.7
Fusarium solani	14277	23.3	129.472	178.8
F. solani	14292	21.6	119.954	165.6
Macrophomina phaseolina	14272	23.253	129.185	178.4
M. phaseolina	14275	23.0	127.854	176.5
Penicillium olsonii	14295	23.73	131.843	182.0
Yeast sp.	14289	24.85	138.066	190.7
Yeast sp.	14281	22.9	127.275	175.8

* The highest producer showed in **bold**

Table 3 Endoglucanase (CMCase) production and activity of some endophytic fungi.

Fungal species	**AUMC no.**	**Endoglucanase (CMCase)**		
		Glucose g/l	**Production IU/ml**	**Activity IU/ml/min**
Aspergillus flavus	14274	0.41	2.282	3.15
A. fumigatus	14283	0.3	1.684	2.32
A. terreus	14278	1.9	10.623	14.7
A. terreus*	**14287**	**2.874**	**15.966**	**22.0**
A. terreus	14280	2.68	14.895	20.6

Table 3 Continued.

Fungal species	AUMC no.	Endoglucanase (CMCase)		
		Glucose g/l	Production IU/ml	Activity IU/ml/min
A. terreus	14282	1.47	8.164	11.3
A. terreus	14284	1.9	10.579	14.6
A. terreus	14285	1.5	8.328	11.5
A. terreus	14288	1.0	5.575	7.7
Clonostachys rosea	14291	0.24	1.332	1.84
Curvularia spicifera	14276	1.3	7.167	9.9
C. spicifera	14273	1.39	7.711	10.65
Fusarium oxysporum	14290	0.757	4.206	5.8
F. solani	14286	0.7	3.910	5.4

* The highest producer showed in **bold**

Table 4 Avicellase production and activity of some endophytic fungi.

Fungal species	AUMC no.	Avicellase		
		Glucose g/l	Production IU/ml	Activity IU/ml/min
Aspergillus flavus	14274	4.213	23.405.5	35.55
A. fumigatus	14283	4.232	23.512	35.71
A. terreus	14278	4.049	22.496	34.17
A. terreus*	**14287**	**5.672**	**31.51**	**47.87**
A. terreus	14280	4.418	24.51	37.23
A. terreus	14282	3.706	20.588	31.3
A. terreus	14284	3.671	20.395	30.98
A. terreus	14285	3.876	21.53	32.71
A. terreus	14288	4.907	27.258	41.41
Clonostachys rosea	14291	3.466	19.258	29.25
Curvularia spicifera	14276	3.592	19.958	30.32
C. spicifera	14273	3.782	21.008	31.91
Fusarium oxysporum	14290	3.085	17.139	26.0
F. solani	14286	3.709	20605	31.3

* The highest producer showed in **bold**

Conclusion

The current research investigates the ecology of endophytic fungi in wild medicinal plants in the New Valley Governorate, Egypt and determines their ability to produce hydrolyzing enzymes. The study managed to retrieve a total of 120 fungal isolates from just eight plants, indicating their widespread distribution. The study also confirmed the ability of these fungal isolates to produce pectinase and cellulase. The potent strains of *Aspergillus* was the superior in enzymes production with *A. terreus* AUMC 14287 for CMCase and avicellase, and *A. terreus* AUMC 14278 for pectinase. The study further highlights the promising ability to produce extracellular enzymes by endophytic fungi, thus showing the importance of further analysis to resolve key issues in this area.

Conflict of interests

The authors have not declared any conflict of interests.

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Appendix 1 Preliminary screening of pectinases and cellulases production by endophytic fungi recovered from leaves and roots of eight wild medicinal plants collected from El-Kharga Oasis, the New Valley Governorate, Egypt, during April 2018.

Fungal species	**Number of isolates tested**	**Preliminary screening**							
		Pectinases				**Cellulases**			
		Positive	**L**	**M**	**H**	**Positive**	**L**	**M**	**H**
Acremonium	**2**	**2**	**1**	**1**		**2**	**1**		**1**
Acremonium rutilum	1	1	1			1	1		
Acremonium sclerotigenum	1	1		1		1			1
Alternaria	**26**	**20**	**14**	**6**		**6**	**4**	**2**	
A. alternata	8	7	5	2		2	2		
A. brassicicola	1	1		1					
A. chlamydospora	1	1		1		1	1		
A. citri	3	2	2						
A. citri macularis	2					2		2	
A. longipes	3	3	1	2		1	1		
A. tenuissima	8	6	6						
Aspergillus	**35**	**27**	**11**	**9**	**7**	**21**	**6**	**7**	**8**
A. flavus	9	7	3	2	2	4	1	2	1
A. fumigatus	2	1			1	2		1	1
A. parasiticus	6	3	2	1		2		2	
A. terreus	15	14	5	5	4	12	4	2	6
A. tubingensis	1								
Aspergillus AY-1	1	1	1			1	1		
Aspergillus AY-2	1	1		1					
Beauveria bassiana	1	1	1						
Chaetomium senegalense	1	1	1						
Cladosporium exile	1	1			1	1		1	
Clonostachys solani	1	1	1			1		1	
Curvularia spicifera	2	1	1			2	2		
Fusarium	**23**	**20**	**14**	**3**	**3**	**16**	**13**	**1**	**2**
F. oxysporum	13	11	8	2	1	11	10		1
F. solani	10	9	6	1	2	5	3	1	1
Macrophomina phaseolina	7	3			3	3	2	1	
Penicillium olsonii	1	1			1	1		1	
Pseudo allescheria boydii	1	1		1		1			1
Rhizopus microsporus	1	1		1		1	1		
Sarocladium kiliense	1	1	1			1	1		
Scopulariopsis fimicola	1								
Stemphylium botryosum	5	5	3	2		3	3		
Verticillium fungicola	1	1		1		1			1
Yeast spp.	10	7	3	1	3	6	4	2	
Total isolates	**120**	**94**	**51**	**25**	**18**	**66**	**37**	**16**	**13**
No. of genera	**17**	**16**	**10**	**8**	**8**	**14**	**9**	**6**	**5**
No. of species	**31**	**28**	**17**	**15**	**9**	**23**	**14**	**9**	**8**

Note: H = high producers: ≥ 20 mm, M = moderate: 11-19 mm, L = <11 mm