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Ecological and Physiological Studies on Soil Fungi at Western Region, Libya

A. H. M. El-Said and A. Saleem*

Botany Department, Faculty of Science, South Valley University, Qena, Egypt

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Sixty three species and 5 varieties belonging to 30 fungal genera were collected from 75 soil samples. Cultivated (29 genera and 58 species + 5 var.), desert (22 and 35 + 2 var.) and saline soil (21 and 41 + 1 var.) fungi were recovered on glucose-, cellulose- and 50% sucrose-Czapek's agar at 28°C. The most common genera were *Alternaria*, *Aspergillus*, *Emericella*, *Fusarium*, *Mycosphaerella*, *Nectria* and *Penicillium*. The most prevalent species from the three types of soils on the three types of media were *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Fusarium oxysporum*, *Mycosphaerella tassiana*, *Nectria haematococca* and *Penicillium chrysogenum*. *Chaetomium globosum* was in the top of fungi in producing endo-β-1,4-glucanases among the 42 tested isolates obtained from soils on cellulose-Czapek's agar. Maximum production of this enzyme by *C. globosum* obtained after 6 days of incubation at 30°C with culture medium containing maltose as a carbon source and ammonium nitrate as a nitrogen source and pH initially adjusted to 6.

KEYWORDS : Cellulolytic ability, Soil fungi

Mycoflora of soils have been paid a considerable attention during the last forty years. The subject has been investigated from various points of view, but most of the work in this respect has been concerned with those fungi inhabiting cultivated, desert and saline soils in many parts of the world (Abdel-Hafez *et al.*, 1991; Abdel-Hafez, 1994; El-Said, 1994; Ozerskaya *et al.*, 2004; Lalley and Viles, 2005).

Cellulose, a major polysaccharide constituent of plant cell walls, is a -1,4 linked linear polymer of 8000~12000 glucose units. Three major enzymes are involved in the degradation of cellulose to glucose are endoglucanase (endo-1,4-d-glucanase, EG), cellobiohydrolase (exo-1,4-d-glucanase, CBH), and β-glucosidase (1,4-d-glucosidase, BG). EG acts in random fashion, cleaving linked bonds within the cellulose molecule; CBH removes cellobiose units from the nonreducing ends of the cellulose chain and BG degrades cellobiose and cellooligosaccharides to glucose (Saha, 2004). Several fungi such as members of *Aspergillus*, *Penicillium*, *Trichoderma*, *Chaetomium* and some other moulds of Mucors and dematiaceous hyphomycetes produced cellulolytic enzymes as reported by several researchers (Nelly, 1991; Abdel-Hafez, *et al.*, 1995, 2003; Moharram *et al.*, 2004; El-Said *et al.*, 2005, 2006; Vasil'chenko *et al.*, 2005). This investigation aimed to study the distribution and occurrence of various groups of fungi in cultivated, desert and saline soils as well as the ability of fungal isolates to produce cellulase enzyme under different environmental and nutritional conditions.

*Corresponding author <E-mail : hussienelsaid@yahoo.com>

Materials and Methods

Cultures. Twenty-five soil samples of each of cultivated (Nos. 1-25), desert (Nos. 26-50) and saline (Nos. 51-75) were collected from different localities in Western region in Libya according to the method described by Johnson and Curl (1972). The geographical feature of El-Gaffara plain is refer that it is a big region in Libya, as it is covers more than 17,000 km^2. It takes a triangle shape with apex at the east near Al-khums town. The north is parallel with the Mediteranean sea coast and about 275 km long. The western side forming the western borders of the Republic and about 150 km long (Fig. 1).

Chemical analysis of soil samples. Organic matter content (OM) was determined by Walkely and Black method (Jackson, 1958). The amount of total soluble salts per one g oven-dry soil (TSS) was calculated according to Jackson (1958). A pH meter (Orior Research model 601T/digitalionalyzer) was used for the determination of soil pH according to Jackson (1958). Carbonate (CO_3^{--}) and bicarbonate (HCO_3^-) were determined directly in the soil by back-titration (Hydrochloric acid digestion) according to the method described by Jackson (1958). Soluble chloride (Cl^-) was estimated by applying the silver nitrate titration method using potassium chromate as an indicator (Jackson, 1958). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined by titration method (Schwarzenbach and Biederman, 1948). The cations such as sodium (Na^+) and potassium (K^+) were determined by using Carl Zeiss flame photometer method (Williams and Twine 1960).

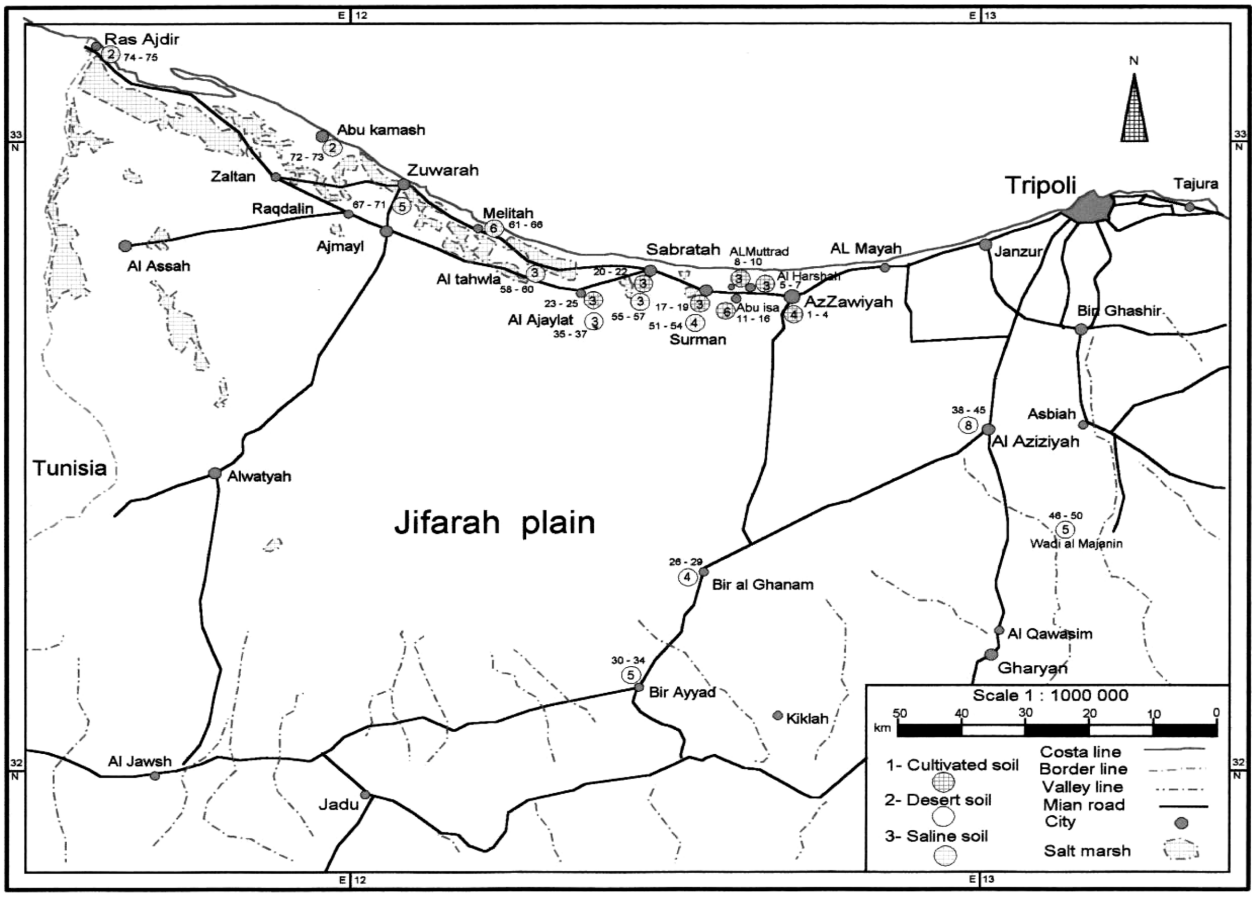

Fig. 1. A map showing the different sites Western region in Libya from which the soil samples were collected. *Dr. Ali Ayad Ben-Hamed. Geographic department. National Atlas of Jamahiriya. 1977, pp. 33-34.

Estimation of soil fungi. The dilution-plate method as described by Johnson and Curl (1972) was used for estimation of soil fungi. Modified Czapek's Dox agar medium was employed (g/*l*: glucose 10.0 or cellulose powder 20.0 or sucrose 500.0, sodium nitrate 3.0, magnesium sulphate 0.5, potasium chloride 0.5, potassium dihydrogen phosphate 1.0, ferrous sulphate 0.01, agar 15), in which glucose or powdered cellulose or sucrose were used for the isolation of glucophilic, cellulose-decomposing and osmophilic (or osmotolerant) fungi, respectively. These media were supplemented with rose bengal (0.1 mg/*ml*) and chloramphenicol (0.05 mg/*ml*) in order to suppress bacterial growth. The plates were incubated at 28°C for 5~10 days during which the developing fungi were counted, identified (purely morphologically, based on macro- and microscopic characters) and calculated per g dry soil.

Screening of fungal isolates for cellulase production. Forty-one species and 1 species variety representing 26 genera were screened for their abilities to produce endo-β-1,4-glucanase (Cx enzyme). Isolates were cultured on Eggins and Pugh medium (1962) and pH was adjusted to 5.4 using acetate buffer. Cultures were incubated at 28°C for 7 days. Using a sterile cork borer (10 mm diameter) discs were cut to inoculate 50 *ml* sterile liquid medium (in 250 *ml* Erlnmeyer conical flasks) of Prasad and Verma medium (1979) for endo-β-1,4-glucanase. After 7 days incubation at 28°C the cultures were filtered and the filtrates were used to detect the activity of Cx enzyme.

Detection of endo-β-1,4-glucanase (Cx enzyme). Using a sterile cork borer three cavities (10 mm diameter) were made in plates containing solid medium of Dingle *et al.* (1953) for detection of endo-β-1,4-glucanase. 0.1 *ml* of culture filtrate was dropped in each of these cavities followed by incubation at 28°C for 24 hours, then the plates were flooded with chloroiodide of zinc solution and the clear zone gave a measure for cellulolytic power of isolates.

Factors affecting cellulase production. The effect of different ecological and nutritional factors on production of Cx enzyme by *Chaetomium globosum* was studied, since this species was found to be highly active in endo-glucanase production.

Effect of temperature and time course. Inoculated flasks were incubated at 20, 30 and 40°C for 14 days and harvested at 48 hours intervals. Culture fluids were filtered and centrifuged at 5,000 rpm for 10 min. The clear supernatents were assayed for C_x enzyme activity.

Effect of pH value. *C. globosum* was grown on the basal medium of Deacon (1985). The initial medium was adjusted with 0.1 N NaOH or 0.1 N HCl to different values of pH ranging from 2 to 12. After inoculation, cultures were incubated at 30°C (the best temperature for endoglucanase activity) for 6 days (the best incubation period), then filtered, centrifuged at 5000 rpm for 10 min and the clear supernatents were assayed for C_x enzyme activity.

Effect of different carbon sources. For estimation the effect of different carbon materials on endoglucanase activity, the carboxymethylcellulose (CMC) in cellulose-Czapek's medium was replaced with the same weight of different carbon sources such as: clover straw, filter paper, maltose, powdered cellulose, wheat bran, wheat straw and yeast extract. The inoculated flasks were incubated at 30°C for 6 days and the cultures were filtered. After centrifugation the clear filtrate was used to detect the C_x enzyme activity.

Effect of different nitrogen sources. To determine the effect of nitrogen source on Cx enzyme activity, sodium nitrate (3 g/*l*) in cellulose-Czapek's medium was replaced with the same amount of various nitrogen compounds such as; $NaNO_2$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$ and $CaNO_3$. Cultures in flasks were incubated at 30°C for 6 days, then filtered, centrifuged and the clear filtrate was used for the detection of endoglucanase activity using the method described by Naguib (1964).

Results and Discussion

The moisture contents of the soil samples tested varied a low value (0.1~3.6%), a moderate value (0.3~6.4%) and a high value (1.29~15.5%). The highest value (15.5%) occurred in the cultivated soil sample No. 22 collected from Sabratah under *Solanum lycopersicum*. Abdel-Sater (1987) found that the water content of 25 soil samples collected from different habitats of each of cultivated, desert and saline soils in Egypt fluctuated between a low value (2.4~9.9%), a moderate value (10~15.2%) and a high value (15.3~21.9%). The moisture contents of soil samples collected from Bahreen ranged between 0.1~1.1% (El-Said, 1994).

The soil samples were generally poor in their organic matter content, but the cultivated soil was the richest (0.18~1.71% of dry soil) followed by desert (0.1~0.57%) and saline soils (0.01~0.54%). The present observations almost agree with the result obtained from different types of soil in some Arab countries (Moubasher *et al.*, 1985; Abdel-Sater, 1987; Abdel-Hafez *et al.*, 1989a, b, 1990a, b, 1991, 1995; El-Said, 1994).

The highest value of total soluble salts was detected in saline soil (1.02~8.89%). These high amounts of salts were not found in the cultivated (0.06~0.85%) and desert (0.01~0.48%) soils. Similar results were recorded by Abdel-Sater (1987) who found that the total soluble salts in the samples of cultivated, desert and saline soil collected from Egypt ranged between 0.13~1.69%, 0.03~1.6% and 6.62~18.63%, respectively. Also, Abdel-Hafez *et al.* (1991, 1995) recorded that the total soluble salts collected from Egypt fluctuated between 2.2~4.7% and 0.18~0.30%. El-Said (1994) reported that the amount of total soluble salts fluctuated between 2.3~4.7% in cultivated soils of Bahreen.

The amount of carbonates, bicarbonates and chlorides in the samples tested fluctuated markedly from 2.01~7.60%, 0.23~2.04% and 0.02~0.24%; 3.21~7.75%, 0.32~2.02% and 0.001~0.32%; and 6.09~7.49%, 0.18~1.72% and 0.02~1.31% in cultivated, desert and saline soils, respectively. Abdel-Sater (1987) recorded that the amount of carbonate, bicarbonate and chlorides in the samples of cultivated, desert and saline soils collected from Egypt ranged between 2.26~5.4%, 0.36~1.5% and 0.07~0.68%; 1.65~5.88%, 0.23~1.02% and 0.14~3.9%; and 4.2~5.94%, 0.18~1.93% and 0.36~4.14%, respectively.

The amount of elements in cultivated, desert and saline soils were: Ca: 0.03~0.73, 0.05~0.2 and 0.09~2.85 mg; Mg: 0.03~0.19, 0.02~0.35 and 0.03~1.0 mg; K: 0.07~0.27, 0.11~0.53 and 0.10~0.89 mg and Na: 0.03~0.09, 0.02~0.46 and 0.1~0.79 mg/g dry soil, respectively. Abdel-Sater (1987) found that the amount of elements in cultivated, desert and saline soils collected from Egypt were: Ca: 0.3~0.75, 0.03~2.67 and 0.07~3.75; Mg: 0.13~0.54, 0.02~0.54 and 0.013~1.23; K: 0.02~0.27, 0.02~0.51 and 0.05~0.88; and Na: 0.16~4.8, 0.12~8.05 and 2.35~39 mg/g dry soil, respectively.

pH values of cultivated, desert and saline soils were ranged between 4.5~7, 6.4~7.2 and 6.4~7.3, respectively. Abdel-Sater (1987) found that the pH values of cultivated, desert and saline soils gathered from Egypt fluctuated between 7.2~8.9, 6.9~7.4 and 7.2~8.8, respectively. Similar observations were obtained by Abdel-Hafez *et al.* (1989b, 1991, 1995) and by El-Said (1994).

Sixty three species and 5 varieties belonging to 30 genera were collected from 75 soil samples. These fungi recovered from cultivated (29 genera and 58 species + 5 var.), desert (22 and 35 + 2 var.) and saline (21 and 41 + 1 var.) soils on glucose-, cellulose- and 50% sucrose-Czapek's agar at 28°C (Tables 1, 2 and 3). The most common genera were: *Alternaria* (2 species), *Aspergillus* (11

Table 1. Average total count (calculated per g dry soil in every sample), number of cases of isolation (NCI, out of 25 cases) and occurrence remarks (OR) for fungal genera and species recovered from 25 cultivated soil samples on glucose, cellulose and 50% sucrose-Czapek's agar at 28°C

Genera & species	Glucose			Cellulose			50% Sucrose		
	ATC	NCI	OR	ATC	NCI	OR	ATC	NCI	OR
Acremonium strictum W. Gams				100	2	R			
Alternaria alternata (Fries) Keissler	100	1	R	100	2	R	340	4	L
Aspergillus	29600	25	H	11880	23	H	32000	25	H
A. candidus Link	180	2	R				260	2	R
A. carneus (V. Tiegh.) Blochwitz	220	3	L						
A. flavus Link	9720	25	H	3480	18	H	6740	24	H
A. flavus var. *columnaris* Raper & Fennell	60	1	R	100	2	R	100	2	R
A. fumigatus Fresenius	100	1	R	2280	9	M	280	4	L
A. niger Trieghern	9340	22	H	4220	19	H	12380	25	H
A. ochraceus Wilhelm	1180	7	M	600	7	M	760	8	M
A. sydowii (Bainier. Sartory)	240	3	L				260	3	L
A. terreus Thom	3880	14	H	1200	7	M	6940	20	H
A. terreus var. *africanus* Fennel &Raper	200	3	L				380	3	L
A. terreus var. *aureus* Thom, Raper	540	4	L				600	4	L
A. ustus (Bainier) Thom. Tiraboschi	2400	10	M				920	6	M
A. versicolor (Vuill.) Tiraboschi	240	3	L						
A. wentii Wehmer	1300	10	M				2480	17	H
Botryotrichum atrogriseum Van Beyma	860	5	L	1540	5	L	100	1	R
Chaetomium globosum Kunze	140	3	L	740	4	L			
Cladosporium	140	3	L	200	3	L	400	3	L
C. cladosporioides (Fres.) de Vries	140	3	L	200	3	L	240	3	L
C. sphaerospermum Penzig							160	2	R
Cochliobolus	200	2	R	620	5	L	200	2	R
C. hawaiiensis Alcorn, Trans				300	4	L			
C. lunatus Nelson & Haasis	100	2	R				100	2	R
C. spicifer Nelson	100	2	R	320	4	L	100	2	R
Cunninghamella echinulata (Thaxt.) Thaxt. ax. Blak.	160	2	R						
Emericella	11800	24	H	3560	18	H	5100	16	H
E. nidulans (Eidam) Vuill.	11100	23	H	3560	18	H	5100	16	H
E. nidulans var. *dentata* Sandhu & Sandhu	460	5	L						
E. nidulans var. *lata* (Thom & Rapper) Subram	240	3	L						
Fusarium	3420	7	M	3620	8	M	920	8	M
F. dimerum (Corda) Sacc.				820	5	L			
F. moniliforme Sheldon	300	4	L						
F. oxysporum Shelecht.	3120	7	M	2380	7	M	700	8	M
F. poae (Peck) Wollenweber				420	3	L	220	3	L
Gibberella	500	4	L	120	1	R			
G. acuminata Wollenweber				120	1	R			
G. intricans Wollenw.	500	4	L						
Humicola	600	4	L	5760	13	H			
H. brevis (Gilman et Abbott) Gilman et Abbott	160	3	L	1460	6	M			
H. grisea Taaen	440	4	L	4300	13	H			

+ 4 var.), *Emericella* (1+2), *Fusarium* (4), *Mycosphaerella* (1), *Nectria* (1) and *Penicillium* (7). The most prevalent species from the three types of soils on the three types of media were: *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Fusarium oxysporum*, *Mycosphaerella tassiana*, *Nectria haematococca* and *Penicillium chrysogenum*. The above species were isolated with different numbers and frequencies from various soils in many places of the world by several workers (Abdel-Hafez *et al.*, 1990a, b; Moubasher and Mazen, 1991; Abdel-Hafez, 1994; Karl and Iain, 2004; Lalley and Viles, 2005 and several others). Abdel-Hafez *et al.* (1991) found that the most common species in the Egyptian soils on glucose-, cellulose- and 50% sucrose-Czapek's agar were: *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *E. nidulans* var. *dentata*, *E. nidulans* var. *lata*, *P. chrysogenum*, *P. puberulum* and *Rhizopus stolonifer*. On the other hand, the most frequently encountered species in Bahreen soils recovered on glucose-, cellulose- and 50% Sucrose-Czapek's agar were *A. alternata*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *E. nidulans*, *E. nidualns* var. *dentata*, *F.*

Table 1. Continued

Genera & species	Glucose			Cellulose			50% Sucrose		
	ATC	NCI	OR	ATC	NCI	OR	ATC	NCI	OR
Eurotium							15060	24	H
E. amstelodami Mangin							4540	18	H
E. chevalieri Mangin							10100	24	H
E. rubrum Konig, Spiekermann. Bremer							420	6	M
Mucor	160	2	R	120	2	R	100	2	R
M. circinelloides Van Tieghem				60	1	R			
M. racemosus Fresenius	160	2	R	60	1	R	100	2	R
Mycosphaerella tassiana (Albertini, Schweinitz) Ditmer ex Steudel	1460	10	M	280	4	L	1240	13	H
Myrothecium verrucaria Bainier				180	2	R			
Nectria haematococca Berkeley, Brown	14080	22	H	10640	21	H			
Paecilomyces variotii Bainier							260	4	L
Penicillium	10660	22	H	2840	16	H	13640	21	H
P. chrysogenum Thom	8520	21	H	3980	16	H	12400	21	H
P. citrinum Thom	360	4	L				160	3	L
P. corylophilum Dierckx	300	4	L	300	4	L	220	2	R
P. funiculosum Thom	240	3	L				240	3	L
P. puberulum Bainier	1240	8	M	560	6	M	480	8	M
P. purpurogenum Stoll							140	2	R
Phoma glomerata (Corda) Wollenweber, Hochapfel	100	1	R						
Pleospora herbarum (Fr.)Rabenh.ex.Ces &de Not	140	3	L	540	5	L	100	2	R
Rhizopus stolonifer (Ehrenb.) Lindt	80	2	R						
Scopulariopsis breviculis (Sacc.) Bainier	100	2	R						
Scytalidium lignicola Pesante.	100	2	R	180	3	L	100	2	R
Setosphaeria rostrata Leonard	100	2	R	300	4	L			
Stachybotrys chartarum (Ehrenb.: Lindt) Hughes	180	2	R	400	5	L			
Sterile mycelia (White & dark color)	100	1	R	220	3	L	220	3	L
Syncephalastrum racemosum (Cohn) Schroeter	160	2	R				220	2	R
Torula herbarum (Pers.) Link	60	1	R						
Ulocladium	880	5	L				100	1	R
U. alternariae (Cke) Simmons	260	2	R				100	1	R
U. botrytis Preuss	240	3	L						
U. chartarum (Preuss) Simmons	320	3	L						
U. tuberculatum Simmons	60	1	R						
Gross total count	75860			45940			70100		
Number of genera = 29	25			20			16		
Number of spcies = 58 + 5 var.	45 + 5 var.			31 + 1 var.			34 + 3 var.		

ATC = Average total count (per g dry soil); NCI = Number of cases of isolation (out of 25); OR = Occurrence remarks: H = High occurrence, from 13~25 (out of 25); M = Moderate occurrence, from 6-12 cases; L = Low occurrence, from 3-5 cases; R = Rare occurrence, from 1 or 2 cases.

oxysporum, *N. haematococca*, *P. chrysogenum* and *P. corylophilum* (El-Said, 1994).

Eurotium was recovered from the three types of soils on plates of 50% sucrose-Czapek's agar and these were: *Eurotium amestelodami, E. chevalieri* and *E. rubrum*. Members of *Eurotium* are well known osmophilies as reported by some workers (Abdel-Hafez *et al.*, 1989a, b, 1990a, 1991, 1995; El-Said, 1994; El-Said *et al.*, 2005).

Cellulolytic activities of fungal isolates. All fungal isolates (42 isolates) were screened for their abilities to produce endo 1,4-β-glucanase (CMase or Cx enzyme) on solid medium proved to be active to utilize cellulose, but with different degrees (Table 4). Ten isolates (23.80% of total isolates) showed high cellulolytic activity for endo-β-1,4-glucanase and these were *A. alternata*, *A. flavus*, *A. fumigatus*, *C. globosum*, *Cladosporium cladosporioides*, *F. oxysporum*, *Mucor racemosus*, *Papulaspora immersa*, *R. stolonifer* and Sterile mycelia. The moderately cellulolytic isolates included 16 isolates (38.09% of total isolates) and the most important isolates being: *A. niger*, *A. sydowii*, *E. nidulans*, *F. poae*, *P. chrysogenum*, *P. puberulum* and *Phoma glomerata.* While, sixteen isolates (38.09% of total isolates) were found to be weak cellulolytic activities which comprised for examples: *Aspergillus ochraceus*, *Cochliobolus spicifer*, *Myrothecium verrucaria*, *N. haematococca*, *Setosphaeria rostrata* and *Ulocladium botrytis* and others. Most of the above fungal isolates were reported as cellulase producers, but with variable capabilities by several workers (Abraha and

Table 2. Average total count (calculated per g dry soil in every sample), number of cases of isolation (NCI, out of 25 cases) and occurrence remarks (OR) for fungal genera and species recovered from 25 desert soil samples on glucose, cellulose and 50% sucrose-Czapek's agar at 28°C

Genera & species	Glucose			Cellulose			50% Sucrose		
	ATC	NCI	OR	ATC	NCI	OR	ATC	NCI	OR
Acremonium strictum W. Gams	40	1	R						
Alternaria alternata (Fries) Keissler	300	3	L	700	4	L	2780	11	M
Aspergillus	16480	22	H	8780	18	H	21860	25	H
A. flavus Link	10180	18	H	4880	16	H	6580	18	H
A. fumigatus Fresenius	960	4	L	2800	7	M	4380	8	M
A. niger Trieghern	3320	18	H	340	4	L	6660	23	H
A. ochraceus Wilhelm	340	3	L	260	3	L	220	4	L
A. sydowii (Bainier. Sartory)	140	2	R						
A. terreus Thom	880	7	M	500	4	L	2180	13	H
A. terreus var. *aureus* Thom & Raper	120	2	R				940	7	M
A. ustus (Bainier) Thom Tiraboschi	540	3	L				900	6	M
Chaetomium globosum Kunze				880	5	L			
Cochliobolus spicifer Nelson, Hassis							60	2	R
Emericella	3240	13	H	1120	7	M	4220	16	H
E. nidulans (Eidam) Vuill.	2720	13	H	1120	7	M	4220	16	H
E. nidulans var. *lata* (Thom & Raper) Subram	520	2	R						
Eurotium							15180	19	H
E. amstelodami Mangin							7000	15	H
E. chevalieri Mangin							8180	18	H
Fusarium oxysporum Shelecht.	160	1	R	160	2	R			
Humicola grisea Traaen				480	4	L			
Mucor	2980	12	M	3320	17	H	740	3	L
M. circinelloides Van Tieghem	640	3	L						
M. hiemalis Wehmer	1180	6	M						
M. racemosus Fresenius	1160	6	M	3320	17	H	740	3	L
Mycosphaerella tassiana (Albertini, Schweinitz) Ditmer ex Steudel	80	1	R				20	1	R
Nectria haematococca Berkeley, Brown	120	2	R						
Papulaspora immersa Hotson				1020	3	L			
Penicillium	7560	16	H	7480	23	H	11280	16	H
P. chrysogenum Thom	7120	16	H	7400	23	H	11000	16	H
P. citrinum Thom							140	2	R
P. puberulum Bainier	440	3	L	80	2	R	140	3	L
Poma glomerata (corda) Wollenweber, Hochapfel	100	2	R	260	3	L	60	1	R
Pleospora herbarum (Fr.) Rabenh.ex Ces& de Not	240	2	R	720	4	L	420	2	R
Rhizopus stolonifer (Ehrenb.) Lindt	120	1	R				200	1	R
Scytalidium lignicola Pesante.	80	1	R	300	3	L	80	2	R
Stachybotrys chartarum (Ehrenb.: Lindt) Hughes	360	2	R						
Sterile mycelia (White & dark color)	4620	11	M	2320	8	M	2720	11	M
Syncephalastrum racemosum (Cohn) Schroeter.	680	3	L				1060	2	R
Torula herbarum (Pers.) Link				620	4	L	520	3	L
Ulocladium	720	2	L	660	4	L	400	1	R
U. alternariae (Cke) Simmons				240	2	R	400	1	R
U. botrytis Preuss	500	2	L	420	3	L			
U. chartarum (Preuss) Simmons	220	2	L						
Gross total count	37880			28820			61600		
Number of genera = 22	16			14			15		
Number of spcies = 35 2 var.	26 + 2 var.			20			23 + 1 var.		

ATC = Average total count (per g dry soil); NCI = Number of cases of isolation (out of 25); OR = Occurrence remarks: H = High occurrence, from 13-25 (out of 25); M = Moderate occurrence, from 6-12 cases; L = Low occurrence, from 3-5 cases; R = Rare occurrence, from 1 or 2 cases.

Gashe, 1992; Abdel-Hafez *et al.*, 1995; Moharram *et al.*, 1995, 2004; El-Said, 2001; Berlin *et al.*, 2005).

C. globosum was in the top of fungi in producing of endo 1,4-β-glucanase (Cx enzyme) in this investigation. Maximum production of the enzyme by *C. globosum* was achieved 6 days after incubation at 30°C with the incorporation of maltose as carbon sucrce and NH_4NO_3 as nitrogen source in the culture medium which is initially

Table 3. Average total count (calculated per g dry soil in every sample), number of cases of isolation (NCI, out of 25 cases) and occurrence remarks (OR) for fungal genera and species recovered from 25 saline soil samples on glucose, cellulose and 50% sucrose-Czapek's agar at 28°C

Genera & species	Glucose			Cellulose			50% Sucrose		
	ATC	NCI	OR	ATC	NCI	OR	ATC	NCI	OR
Acremonium strictum W. Gams	1160	7	M	800	4	L	120	2	R
Alternaria	1900	12	M	860	6	M	1260	13	H
A. alternata (Fries) Keissler	1900	12	M	860	6	M	1160	13	H
A. raphani Grooves, Skolko							100	2	R
Aspergillus	21620	25	H	10340	22	H	22340	25	H
A. candidus Link	80	1	R				140	3	L
A. flavus Link	10300	25	H	4580	19	H	8580	24	H
A. fumigatus Fresenius	380	2	R				20	1	R
A. niger Trieghern	5700	25	H	3700	16	H	10340	25	H
A. ochraceus Wilhelm	860	8	M	280	2	R	420	7	M
A. sydowii (Bainier. Sartory)	200	1	R	120	2	R			
A. terreus Thom	2220	10	M	1120	9	M	1700	10	M
A. terreus var. *aureus* Thom & Raper	680	3	L						
A. ustus (Bainier) Thom. Tiraboschi	1200	9	M	540	3	L	1140	14	H
Botryotrichum atrogriseum Van Beyma	360	3	L	220	2	R	80	3	L
Chaetomium globosum Kunze	100	1	R	1380	9	M			
Cladosporium cladosporioides (Fres.) de Vries	240	2	R	280	3	L	380	2	R
Cochliobolus spicifer Nelson				280	2	R	180	2	R
Emericella nidulans (Eidam) Vuill.	3500	16	H	1260	8	M	2040	10	M
Eurotium							8300	25	H
E. amstelodami Mangin							1680	15	H
E. chevalieri Mangin							6340	25	H
E. rubrum Konig, Spiekermann. Bremer							280	9	M
Fusarium	1220	6	M	1500	7	M	680	3	L
F. dimerum (Corda) Sacc.				280	4	L			
F. moniliforme Sheldon	620	4	L				180	2	R
F. oxysporum Shelecht.	600	4	L	1220	6	M	500	2	R
Mucor	760	3	L	620	5	L	540	5	L
M. hiemalis Wehmer	340	3	L				320	4	L
M. racemosus Fresenius	420	2	R	620	5	L	220	2	R
Mycosphaerella tassiana (Albertini, Schweinitz) Ditmer ex Steudel	2760	15	H	2640	10	M	2140	10	M
Nectria haematococca Berkeley, Brown	7420	22	H	5780	23	H	900	8	M
Penicillium	10200	20	H	7780	17	H	10960	22	H
P. chrysogenum Thom	9820	20	H	7100	17	H	10500	22	H
P. citrinum Thom	60	1	R						
P. corylophilum Dierckx				100	2	R	460	3	L
P. duclauxi Delacroix	40	1	R						
P. puberulum Bainier	280	2	R	580	3	L			
Phoma	40	1	R	200	1	R			
P. humicola Gilman & Abbott									
P. glomerata (corda) Wollenweber, Hochapfel	40	1	R	200	1	R			
Pleospora herbarum (Fr.) Rabenh.ex Ces & de Not.	500	4	L	780	4	L	100	2	R
Rhizopus stolonifer (Ehrenb.) Lindt	80	1	R	60	1	R	60	2	R
Scytalidium lignicola Pesante.	2840	8	M	1400	2	R	780	4	L
Stachybotrys chartarum (Ehrenb.: Lindt) Hughes	2500	11	M	6460	20	H	160	2	R
Sterile mycelia (White & dark color)	440	3	L	40	1	R	40	1	R
Torula herbarum (Pers.) Link	600	1	R	100	1	R	1380	7	M
Ulocladium	100	2	R	180	3	L	80	1	R
U. botrytis Preuss	80	1	R	100	2	R			
U. chartarum (Preuss) Simmons	20	1	R	80	2	R	80	1	R
U. tuberculatum Simmons	80	1	R						
Gross total count	58340			42960			52520		
Number of genera = 21	19			20			19		
Number of spcies = 41 + 1 var.	32 + 1 var.			29			31		

ATC = Average total count (per g dry soil); NCI = Number of cases of isolation (out of 25); OR = Occurrence remarks: H = High occurrence, from 13-25 (out of 25); M = Moderate occurrence, from 6-12 cases; L = Low occurrence, from 3-5 cases; R = Rare occurrence, from 1 or 2 cases.

Table 4. Activity of carboxymethyl cellulase (C_x) of different fungal species isolated on cellulose-Czapek's agar at 28°C

Organisms	Diameter of clear zone (mm)
Acremonium strictum	17 M
Alternaria alternate	22 H
Aspergillus flavus	24 H
A. flavus var. *columnaris*	11 W
A. fumigatus	21 H
A. niger	18 M
A. ochraceus	14 W
A. sydowii	18 M
A. terreus	13 W
A. ustus	17 M
Botryotrichum atrogriseum	16 M
Chaetomium globosum	27 H
Cladosporium cladosporioides	20 H
Cochliobolus hawaiiensis	13 W
C. spicifer	14 W
Emericella nidulans	18 M
Fusarium dimerum	16 M
F. oxysporum	21 H
F. poae	18 M
Gibberella acuminata	17 M
Humicola brevis	13 W
H. grisea	12 W
Mucor circinelloides	16 M
M. racemosus	22 H
Mycosphaerella tassiana	13 W
Myrothecium verrucaria	14 W
Nectria haematococca	15 W
Papulaspora immersa	20 H
Penicillium chrysogenum	19 M
P. corylophilum	17 M
P. puberulum	18 M
Phoma glomerata	18 M
Pleospora herbarum	17 M
Rhizopus stolonifer	24 H
Scytalidium lignicola	12 W
Setosphaeria rostrata	14 W
Stachybotrys chartarum	12 W
Sterile mycelia (white)	22 H
Torula herbarum	17 M
Ulocladium alternaria	12 W
U. botrytis	14 W
U. chartarum	13 W

Degree of C_x activity; High activity, H = from 20~28 mm; Moderate activity, M = 16~19 mm; and Weak activity, W = 11~15 mm.

adjusted to pH 6 (Figs. 2 and 3). These findings are almost in agreement with those reported by Sandhu and Kalra (1985) and Kalra and Sandhu (1986). They noticed that the maximum production of C_1 and C_x enzymes produced by *Trichoderma longibrachiatum* and *T. harzianum* was achieved after 5~7 days of incubation at 27°C but with the incorporation of 1% lactose in culture medium which initially adjusted to pH 5. They also found that CMC and malt extract were favourable for the enzyme

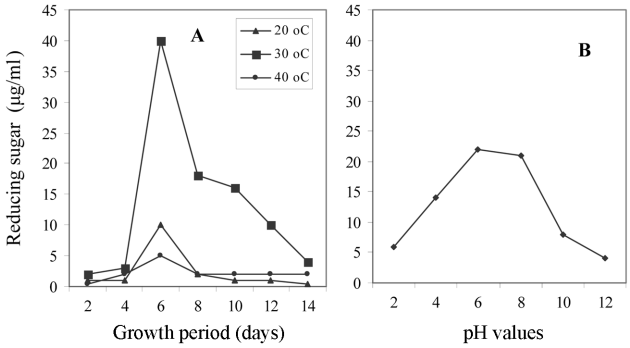

Fig. 2. Cellulase production by *C. globosum* in cultures incubated at different temperatures for different periods (A) and in cultures initially adjusted to different pH values (B).

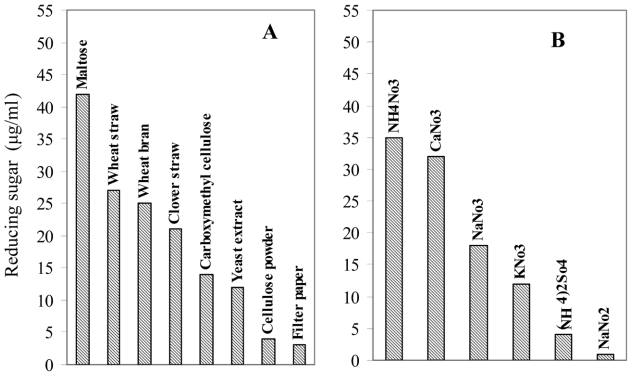

Fig. 3. Cellulase production by *C. globosum* in culture media containing different carbon (A) and nitrogen (B) sources.

production. The maximum production of exo- and endo-β-1,4-glucanase by *C. globosum* and *Trichoderma viride* were after 6 and 8 days of incubation at 25°C with culture medium containing wheat bran as a carbon source and peptone as nitrogen source and initially adjusted to pH 6 (Abdel-Hafez *et al.*, 1995; El-Said, 2001). Recently, El-Said *et al.* (2006) found that maximum production of endo-β-1,4-glucanase by *F. oxysporum* could be achieved after 8 days of incubation at 30°C with the incoporation of carboxymethylcellulose as a carbon source and peptone as nitrogen source in the culture medium which initially adjusted to pH 6.

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