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Seasonal variation of air, soil and leaf surface fungi of broad bean and cellulolytic ability in Upper Egypt

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Seventy-five species and 3 species varieties belonging to 21 fungal genera were collected from air, soil and leaf surface of broad bean plant on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C. The results obtained from leaf surface (phyllosphere and phylloplane), soil and atmosphere were basically similar in the two types of media and the most common fungi were: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum* and *Drechslera neergaardii*. The monthly counts of these fungi on two types of media irregularly fluctuated giving maxima value at various months. *A. flavus* was the highest fungi that produced both exo- and endo-ß-1,4-glucanases among the 9 tested isolates. Maximum production of two enzymes by *A. flavus* was 8 and 6 days after incubation at 30°C with culture medium containing glucose and cellulose as carbon sources and sodium nitrate as nitrogen source and initially adjusted to pH 6.

Key words: Airborne fungi, soil and leaf surface, broad bean, cellulolytic ability.

INTRODUCTION

Food legumes play an important and diverse role in the farming systems and in the diets of poor people around the world. They are ideal crops for simultaneously achieving three developmental goals in targeted population reducing poverty, improving human health and nutrition, and enhancing ecosystem resilience. Broad bean (*Vicia faba* L.) is one of the most important winter crops of high nutritive value in the world as well as in Egypt. *Vicia faba*, which has several common names (fava bean, faba bean, horse bean and tic bean), is a species of bean (Fabaceae) native to north Africa and southwest Asia (Elwakil et al., 2009).

Broad bean can significantly improve the quality of fish and meat. High quality dried broad beans are processed into snack foods, vermicelli, starch, and spicy bean sauce /paste. Faba bean is an excellent source of protein (20-25%), calcium (0.15%), phosphorus (0.50%), lysine (1.5%) and methionine - cystine (0.5%), from dry weight (Rabey et al., 1992).

World production of broad bean varied during the last 10 years. China came first in the production. It produced 13033750 tones while Egypt production was 155554 tones (FAO, 2009).

Numerous investigations have been carried out on fungal flora of leaf surface, soil fungi and air borne fungi of several plants cultivated in many parts of the world by several researchers (Abdel-Hafez et al., 1990, 1995, 2003; Blakeman, 1991; Khallil and Abdel-Sater, 1993; El-Kholl et al., 1994; El-Said, 1994, 2001; El-Said et al., 2006; El-Said and Saleem 2008; Gunasekera, 2004; Moharram et al., 2004, 2010; Murace and Cellitin, 2005; Saleem et al., 2010, 2013).

Cellulose, a major polysaccharide constituent of plant cell walls, is a 1,4 linked linear polymer of 8000~12000

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Gglucose units. Three major enzymes are involved in the degradation of cellulose to glucose which are endoglucanase (endo-1,4-d-glucanasem EG), cellobiohydrolase (exo-1,4-d-glucanasem CBH) and β-glucosidase (1.4-d-glucosidase, BG). EG acts in random fashion, cleaving linked bonds with in the cellulose molecule; CBH removes cellobiose units from the non reducing ends of the cellulose chain and BG degrades cellobiose and cello-oligosaccharides to glucose (Saha, 2004).

The aim of the present investigation was to study the seasonal variations of fungus flora of leaf surface, airborne fungi and soil in *Vicia faba* field cultivated in Oena governorate and cellulolytic activity of some fungal isolates and the effect of some environmental and nutritional factors on cellulase production.

MATERIALS AND METHODS

During the growing season of broad bean crop which extended from December 2011 to April 2012, a broad bean field in South Valley University in Qena city in Upper Egypt was selected to study the mycoflora of leaf surface (phyllosphere and phylloplane) and soil, as well as the airborne fungi over the broad bean field. Samples were collected fortnightly and two media types were used: dicholran chloramphenicol malt extracts agar (DCMA) Andrews and Pitt (1986) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C King et al. (1979).

Determination of phyllosphere fungi

The dilution plate method was used as employed by El-Said et al. (2006).

Determination of phylloplane fungi

The determination of phylloplane was as employed by El-Said et al. (2006).

Determination of airborne fungi

The "exposed plate" method was used to trap fungal spores over broad bean field during growing season. Six plates of 9 cm diameter were used for each exposure (3 plates for each type of medium). The plates were exposed at 10-11 a.m., for 15 min every 15 days (Abdel-Hafez et al., 1990).

Determination of soil fungi

The dilution-plate method as described by El-Said (1994) was used for estimation of soil fungi. 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 (Raper and Fennell, 1965; Ellis, 1971, 1976; Domsch et al., 1980; Pitt, 1985) and calculated per g dry soil.

Screening of fungal isolates for cellulase production

Nine species (the most common species) belonging to 5 genera were screened for their abilities to produce exo- and endo-β-1,4-glucanase (C_1 and C_x enzymes, respectively). Isolates were cultured on Eggins and Pugh medium (1962). Cultures were incubated at 28°C for 7 days. Using sterile cork borer, 10 mm diameter, discs were cut to inoculate 50 ml sterile liquid medium (in 250 ml Erlenmeyer flasks) of Eggins and Pugh medium (1962) for exo-glucanase production and Prasad and Verma medium (1979) for endo-glucanase. The cultures were incubated at 28°C for 7 days. The cultures were filtered and the filtrates were used to detect the activity of the enzymes as follows:

Detection of exo-β-1, 4-glucanase (C_1 enzyme)

Using a sterile cork borer, 3 cavities (10 mm diameter) were made in plates containing solid Eggins and Pugh medium (1962). A 0.1 ml of culture filtrate was dropped in each of these cavities followed by incubation at 28°C for 24 h, then the plates were flooded with chloroiodide of zinc solution and the uncolored zone gave a measure of cellulolytic power of isolates.

Detection of endo-β-1.4-glucanase (C_x enzyme)

Ten millimeters cavities were cut in plates containing solid medium of Dingle et al. (1953), filtrate obtained from 7 days old fungal cultures grown on Prasad and Verma (1979) medium was dropped in each cavity. After 24 h of incubation at 28°C, plates were flooded with chloroiodide of zinc solution and the clear zones around cavities were measured.

Factors affecting cellulase production

The effect of different ecological and nutritional factors on production of cellulase enzymes (C_1 and C_x) by *Aspergillus flavus* was shown. Since this species was found to be highly active in cellulase production so this species was used for this study. The previous isolate was grown on liquid medium (Deacon, 1985). Fifty milliliters of the medium were dispensed into each 250 ml Erlenmeyer flask and each flask was inoculated with an agar mycelial disc (10 mm diameter) of the mould obtained from 7 days old fungal cultures growing on the solid basal medium. Experiments were done to indicate the best conditions which produce a good deal of the enzyme as well as of the best expense.

Effect of temperature and time course

The inoculated flasks were incubated at 20, 30 and 40°C for 14 days and harvested at 48 h intervals. Culture fluid were filtered and centrifuged at 5000 r.p.m. for 10 min, the clear supernatants were assayed for enzyme activity.

Effect of pH values

The test isolate (*A. flavus*) was grown on the basal medium of Deacon (1985). The initial pH of the medium was adjusted with 0.1 N NaOH or 0.1 N HCL to different values ranging from 2 to 14. After inoculation with *A. flavus*, cultures were incubated at 30°C for 8 days for C_1 and C_x, respectively. At the end of incubation period the cultures were filtered, centrifuged at 5000 r.p.m. for 10 min and the clear supernatants were assayed for cellulase activity.

Effect of different carbon sources

The basal medium (Deacon, 1985) with pH 8 (the best pH for cellulase production) was supplemented with 1% of one of the following carbon sources: glucose, fructose, lactose, sucrose, cellulose, starch and carboxymethyl cellulose. The flasks were inoculated with *A. flavus* and incubated at 30°C (the best temperature of C_1 and C_x enzymes production) for 8 days (the best incubation periods for C_1 and C_x enzymes, respectively) and the cultures were filtered. After centrifugation the filtrate was used to detect the cellulase activity.

Effect of different nitrogen sources

To determine the effect of nitrogen source on cellulase production, sodium nitrate in the basal medium was replaced by the same amount of various nitrogen compounds such as: sodium nitrite, potassium nitrate, yeast extract, ammonium sulphate, ammonium nitrate and peptone in addition to sodium nitrate as a control. Cultures were incubated at 30°C for 8 days and the cultures were filtered, centrifuged and the filtrates were used for detection of cellulase activity.

Assay for cellulase activity (C_1 and C_x enzymes)

The method described by Nelson (1944) and modified by Naguib (1964) was employed. The amount of reducing sugars produced was estimated by determining the optical density (absorption spectrum) at 700 nm wave length with a spectrophotometer model (Spectronic ® Genesys ^{TM} 2PC USA). A standard curve was plotted using aqueous solution of D-glucose.

RESULTS AND DISCUSSION

The monthly total counts of phyllosphere and phylloplane surface fungi of broad bean on plates of DCMA and DRBC irregularly fluctuated giving peaks during March and March, respectively.

Thirty-four species and 3 species varieties belonging to 15 genera were collected from phyllosphere (10 genera and 20 species + 2 varieties) and phylloplane (12 genera and 21 species +1 var.) of broad bean leaves on DCMA and DRBC at 28°C (Table 1). The most common fungi of two substrates on the two types of media were: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum* and *Drechslera neergaardii*. The monthly counts of the above fungal species were widely varied and fluctuated irregularly giving maxima during different months (Figures 1 and 2). El-Said (2001) isolated *Alternaria alternata*, *Alternaria citri*. *A. flavus*, *A. fumigatus*, *A. niger*, *Chaetomium globosum*, *Cladosporium cldosporioides*, *Cochliobolus lunatus*, *Mycosphaerella tassiana*, *Setosphaeria rostrata* and *Stachybotrys chartarum* from leaf surface of banana leaves. Also, El-Said et al. (2006) found that the most common fungi isolated from 60 samples of leaf surface of broad bean on DCMA and DRBC at 28°C were: *Alternaria petroselini*, *A. citri*, *Aspergillus flavus*, *A. niger*, *C. cladosporioides* and *C. sphaerospermum*. Also, 25 species and one species variety belong to 17 genera from leaf surface of broad bean on dichloranchloram-phenicol-malt extract agar (DCMA) at 28°C and the most common species were *A. alternata*, *C. cladosporioides* and *C. sphaerospermum*. All fungal species recovered from leaf surface of broad bean on the two types of media were previously isolated but with different inciden-ces from leaf surface of several plants growing or cultiva-ted in many parts of the world (Abdel-Hafez et al., 1990, 1995; Abdel-Sater, 1993; El-Kholl et al., 1994; Murace and Cellitin, 2005; Moharram et al., 2010; Saleem et al., 2010, 2012).

Forty species and 1 variety representing 14 genera were collected from the air above broad bean field on plates of DCMA (14 genera and 29 species) and DRBC (10 and 32+1var.) at 28°C (Table 2). The monthly counts of fungi on DCMA and DRBC in the atmosphere over broad bean field irregularly fluctuated and widely varied between 50-12 and 100-200 colonies/6 plates in 2 expo-sures of 10 min each giving peaks during February and February, respectively (Figure 3). The most common fun-gi on the two types of media were: *A. citri*, *Alternaria petroselini*, *Alternaria pluriseptata*, *A. flavus*, *A. melleus*, *A. niger*, *C. sphaerospermum*, *Curvularia richardiae*, *D. neergaardii* and *P. chrysogenum*. The monthly counts of these fungi were widely varied and irregularly fluctuated giving maxima during various months (Figure 3). Some species were prevalent on one type of media such as*: Emericella nidulans* and *Mucor circinelloides* on DRBC. Abdel-Hafez et al. (1990) observed that the most preva-lent fungi in the air over lentil field were: *A. alternata*, *A. flavus*, *A. niger*, *Cladosporium herbarum*, *Cochliobolus spicifer*, *Curvularia pallescens*, *Fusarium moniliforme*, *F. oxysporum*, *Myrothecium verrucaria*, *Penicillium chrysogenum* and *Stachybotrys chartarum.* Abdel-Hafez et al. (1995) isolated fifty species and 3 varieties representing 26 genera were collected from the air above sugarcane field and the most common fungi were: *A. alternata, A. flavus*, *A. niger*, *A. terreus*, *P. chrysogenum* and *P. oxalicum.* Patel (2008) carried out aeromyco-logical studies on tomato (*Lycopersicon esculentum* Mill. and *Solanum melongana* L.) fields at Nashik (M. S.) during two seasons. Spores of *Cladosporium*, *Alternaria*, *Curvularia*, *Helminthosporium*, *Aspergillus*, *Penicillium*, *Fusarium* and *Periconia* were found in maximum percentage in the total air-spora. More spore catch was found in the month of January, March and August, September. During the period of investigations, 66 types of fungal spores were observed. The maximum numbers of spores were found in the second season. Low temperature, high relative humidity and moderate and alternate spell of rain show effect on release and dissemination of air borne fungal spores. Chavan (2012) studied the occurrence of Ascomycetes fungal spores over a paddy field and observed that the spores belonging to groups Deuteromcotina contributed 66.61%, Basidiomycotina 8.89%, Ascomycotina 24.56% and other types 0.35% of the total airspora. The most prevalent genera were *Curvularia*, *Fusarium*, *Helminthosporium*, *Phoma*, *Nigrospora*, *Alternaria* and *Cladosporium*. Several of the above species were also frequently isolated from the air of some Egyptian localities (Abdel-Hafez, 1989; Abdel-Sater, 1990; Abdel-Hafez et al., 1990, 1993, 1995; Patel, 2008; Chavan, 2012).

Thirty-one species and 1 variety belonging to 12 genera were collected from soil of broad bean field on plates of DCMA (9 genera and 24 species + 1 var.) and DRBC (11 and 24 + 1 var.) at 28°C (Table 3). The monthly counts of fungi on DCMA and DRBC in soil of broad bean field irregularlyfluctuatedandgivingpeaksduringdifferentmonths

Table 1. Total counts of phyllosphere (per g fresh leaves) and phylloplane (120 leaf segments) fungi, number of cases of isolation (NCI), occurrence remarks (OR) and relative importance value (RIV) of the fungal genera and species on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C.

Genera and species	Phyllosphere				Phylloplane					
	DCMA		DRBC		DCMA			DRBC		
	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR	RIV	TC	NCI&OR	RIV
Alternaria	333.4	2L	666.7	2L	27	4M	55.1	16	5M	60.5
A. alternata (Fries) Keissler	-	-	-	-	3	1R	11.7	1	1R	10.7
A. chlamydospora Mouehaeca	-	-	-	-	2	1R	11.1	3	1R	12
A. cinerariae Hori & Enjaji	-	-	-	-	3	1R	11.7	-	-	-
A. citri Ellis &Pierce	166.7	1R	-	-	-	-	-	-	-	-
A. petroselini (Neergaard) Simmons Comb. nou.	166.7	1R	166.7	1R	11	3M	36.1	8	2L	25.2
A. pluriseptata (Karst. & Hart) Jorsted	-	-	500	1R	8	3M	34.5	4	3M	32.6
Aspergillus	7166.7	10H	12999.8	10H	81	9H	135.3	88	9H	147.5
A. deflectus Fennell & Raper	166.7	1R	2833.3	1R	-	-	-	2	1R	11.3
A. flavus Link	999.9	3M	2833.3	1R	21	6H	71.7	23	9H	105
A. fumigatus Fresenius	2166.6	3M	2833.3	4M	-	-	-	14	3M	39.2
A. japonicus Saito	-	-	333.3	1R	2	1R	11.1	4	1R	12.6
A. melleus Yukawa	-	-	833.3	1R	-	-	-	1	1R	10.7
A. niger Van. Tieghem	3333.5	8H	3000	6H	57	9H	121.8	43	9H	118.1
A. parasiticus Speare	-	-	-	-	1	1R	10.6	-	-	-
A. sulphureus (Fres.) Thom & Church	-	-	333.3	1R	-	-	-	1	1R	10.7
A. terreus var. *aureus* Thom & Raper	500	1R	-	-	-	-	-	-	-	-
Cladosporium	39666.6	6H	36500	8H	24	3M	43.4	18	2L	31.8
C. cladosporioides (Fres.)de Vries	37833.3	6H	11166.7	3M	7	2L	23.9	10	2L	26.5
C. sphaerospermum Penzig	1833.3	3M	25333.3	5M	17	3M	39.5	8	2L	25.2

Table 1. Contd.

Genera and species	Phyllosphere				Phylloplane					
	DCMA		DRBC		DCMA			DRBC		
	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR	RIV	TC	NCI&OR	RIV
Curvularia richardiae Alcorn	166.7	1R	166.7	1R	3	2L	21.7	-	-	-
Drechslera	333.4	2L	500.1	3M	16	4M	48.9	5	3M	33.3
D. indica (Rui, Wadhwani & Twari) Mouchacca	166.7	1R	166.7	1R	1	1R	10.6	1	1R	10.7
D. neergaardii Danguash	166.7	1R	333.4	2L	15	4M	48.4	4	3M	32.1
Emericella nidulans var. *dentata* Sandhu & Sandhu	-	-	-	-	9	1R	0.2	4	1R	12.6
Fusarium	166.7	1R	333.3	1R	3	2L	21.7	4	1R	12.6

Table 1. Contd.

F. culmorum W.G. Smith (Sacc)	-	-	-	-	-	-	-	4	1R	12.6
F. merismoides Corda	-	-	-	-	3	2L	21.7	-	-	-
F. oxysporum Schlecht	166.7	1R	333.3	1R	-	-	-	-	-	-
Melanopsmma pomiformis (Pres.ex. Fr.) Sacc	-	-	333.3	1R	-	-	-	-	-	-
Mucor circinelloides Van Tiegh	166.7	1R	-	-	2	2L	21.1	2	2L	21.3
Myrothecium roridium Tode ex Fries	-	-	-	-	1	1R	10.6	-	-	-
Paecilomyces variottii Bain	333.3	1R	-	-	-	-	-	-	-	-
Penicillium	12166.8	4M	4833.3	2L	10	2L	25.6	13	2L	28.5
P. aurantiogriseum Dierckx	833.3	1R	-	-	-	-	-	-	-	-
P. camembertii Thom	9333.4	2L	1166.7	1R	-	-	-	12	1R	17.8

Table 1. Contd.

Genera and species	Phyllosphere				Phylloplane					
	DCMA		DRBC		DCMA			DRBC		
	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR	RIV	TC	NCI&OR	RIV
P. chrysogenum Thom	833.3	1R	3666.6	2L	5	1R	12.8	1	1R	10.7
P. chorylophilum Dierckx	166.7	1R	-	-	-	-	-	-	-	-
P. duclauxii Delacroix	666.7	1R	-	-	5	1R	12.8	-	-	-
P. funiculosum Thom	333.4	2L	-	-	-	-	-	-	-	-
Rhizopus oryzae Went & Prisen Geerligs	-	-	-	-	1	1R	10.6	2	1R	11.3
Stachybotrys atra var. *microspora* Mathur Sankhla	1 66.7	1R	-	-	-	-	-	-	-	-
Sterile mycelium	-	-	166.7	1R	-	-	-	-	-	-
Trichothecium roseum (Pres.) Link ex Gray	-	-	-	-	2	2L	21.1	1	1R	10.7
Total counts	60667		56499.9		179			153		
Number of genera 15	10		8		12			10		
Number of species 34+3var.	20+2var.		18		21+1var.			21+1var.		

*OR = Occurrence remarks: H = high occurrence from 6-10 cases, M = moderate occurrence from 3-5cases, L = low occurrence 2 cases and R = rare occurrence 1 case.

(Figure 4). The most common fungal genera on the two types of media were: *Alternaria* (5 species), *Aspergillus* (7), *Cladosporium* (4), *Emericella* (1) and *Fusarium* (6). The most prevalent species on the two types of media were: *A. flavus*, *A. fumigatus*, *A. niger*, *C. cladosporioides*, *C. sphaerospermum* and *Emericella nidulans* var. *dentata* (Table 3). Abdel-Hafez (1994) found that the most common species in the Egyptian soils on glucose-, cellulose- and 50% sucrose-Cazpek's agar were: *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *E. nidulans* var. *dentata*, *E. nidulans* var. *lata*, *Penicillium chrysogenum*, *P. puberulum* and *Rhizopus stolonifer.* On the other hand, the most frequently encountered species in Bahreen soils were: *A. alternata*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *E. nidulans*, *E. nidulans var. dentata*, *F. oxysporum*, *Nectria haematococca*,

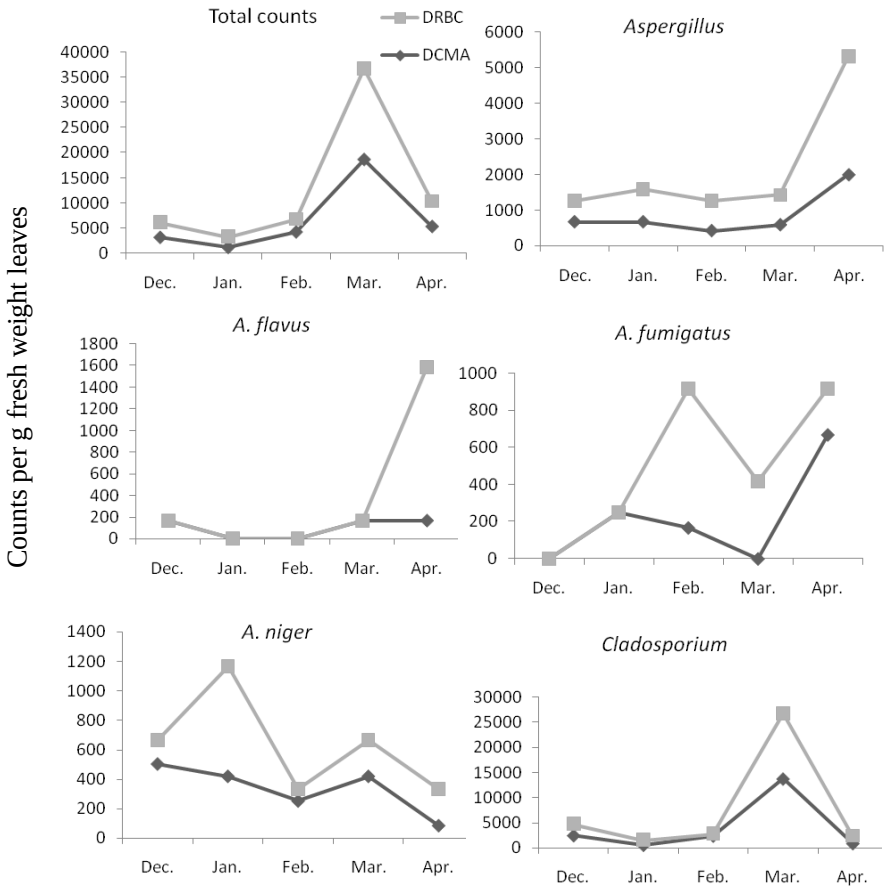

Figure 1. Monthly counts (per g fresh leaves) of common phyllosphere fungi of *Vicia faba* on DCMA and DRBC at 28°C.

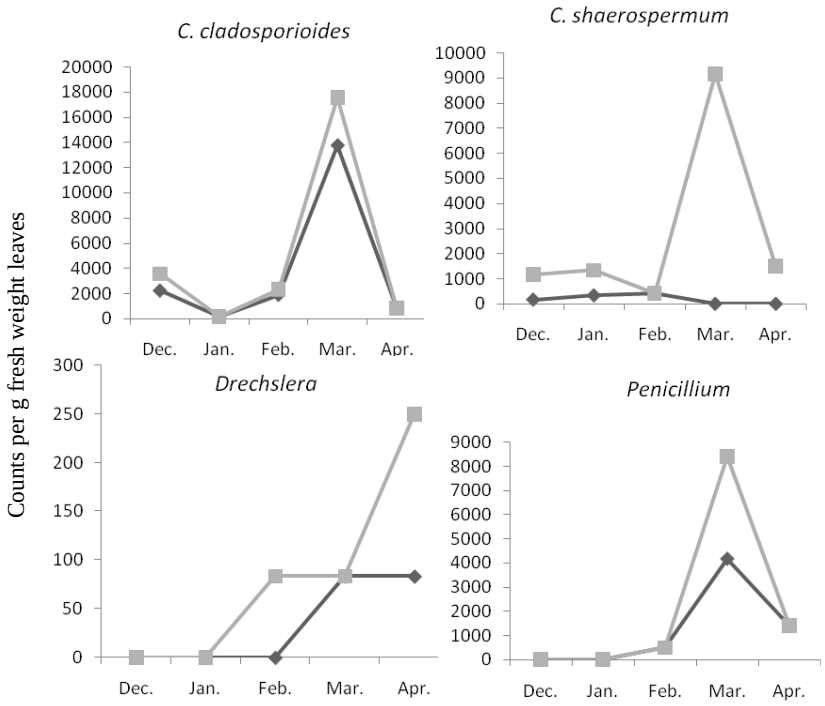

Figure 1. Contd.

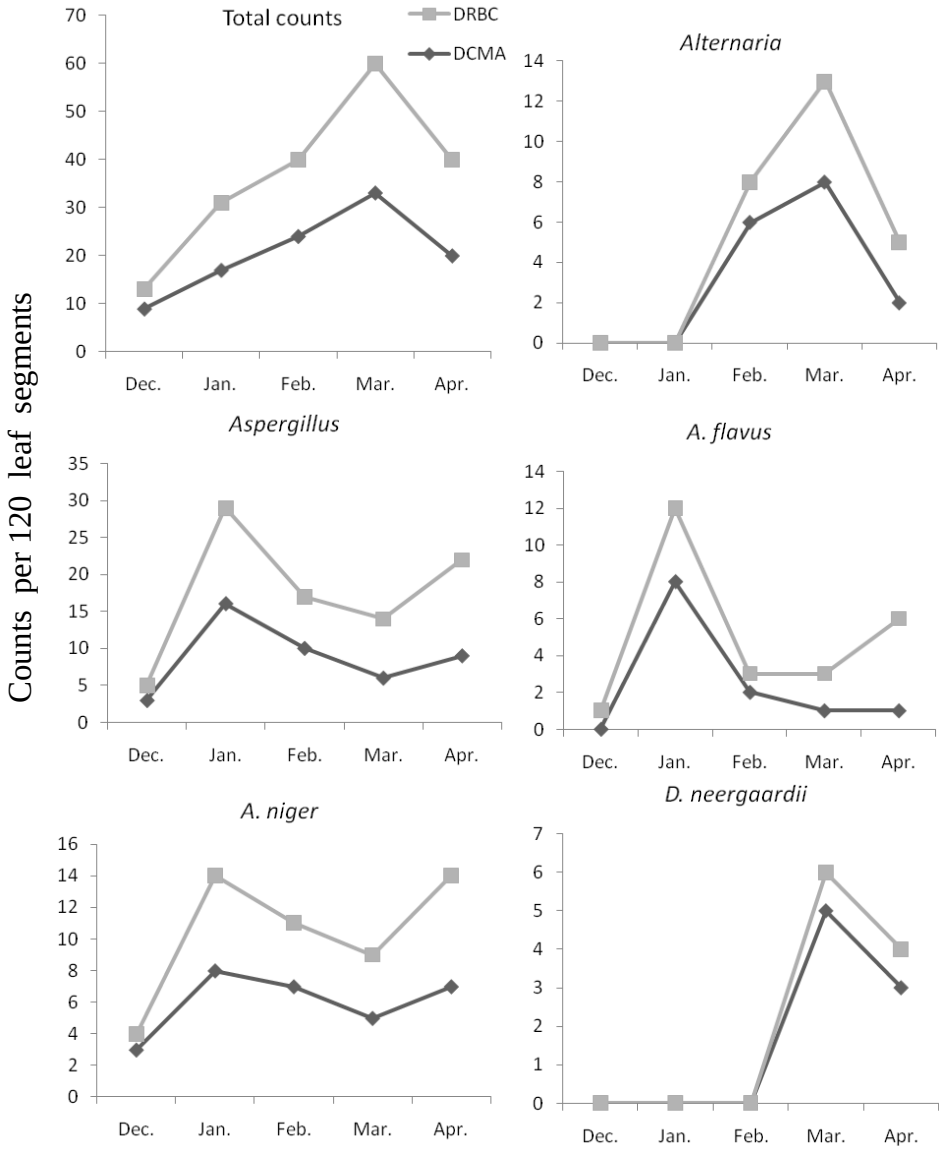

Figure 2. Monthly counts (per 120 leaf segments) of common phylloplane fungi of *Vicia faba* on DCMA and DRBC at 28°C.

Table 2. Total counts (TC, calculated per 30 plates in 10 exposures of 15min), number of cases of isolation (NCI, out of 10) and occurrence remarks (OR) of various fungal genera and species recovered from air of *Vicia faba* field on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C.

Genera and species	DCMA		DRBC	
	TC	NCI&OR	TC	NCI&OR
Alternaria	221	9H	147	8H
A. alternata (Fries) Keissler	1	1R	-	-
A. chlamydospora Mouehaeca	7	1R	18	1R
A. citri Ellis &Pierce	8	1R	13	3M
A. petroselini (Neergaard) Simmons Comb. nou.	157	7H	56	4M
A. pluriseptata (Karst. & Hart) Jorsted	25	2L	55	4M
A. rahpani Grosves Skolko	-	-	4	1R

Table 2. Contd.

Genera and species	DCMA		DRBC	
	TC	NCI&OR	TC	NCI&OR
A. tenuissima (Kunze:Pers.) Wilshire	6	1R	-	-
A. triticina Prasada & Prabhu	17	1R	1	1R
Aspergillus	108	10H	83	8H
A. flavus Link	9	5M	6	1R
A. fumigatus Fresenius	-	-	14	1R
A. japonicus Saito	-	-	7	1R
A. melleus Yukawa	3	2L	11	3M
A. niger Van. Tieghem	90	10H	40	6H
A. parasiticus Speare	3	2L	1	1R
A. sulphureus (Fres.) Thom & Church	1	1R	3	1R
*A.*sydowii (Bain. & Start.)Thom &Church	1	1R	3	1R
Cladosporium	155	4M	143	5M
C. cladosporioides (Fres.)de Vries	-	-	74	4M
C. sphaerospermum Penzig	155	4M	69	3M
Cochliobolus lunatus Nelson & Haasis	1	1R	-	-
Curvularia richardiae Alcorn	8	3M	4	1R
Drechslera	60	7H	22	6H
D. indica (Rui, Wadhwani & Twari) Mouchacca	2	2R	1	1R
D. neergaardii Danguash	58	7H	21	6H
Emericella nidulans var. *dentata* Sandhu &Sandhu	-	-	13	3M
Epicoccum purpurascens Ehrenb. ex Schlecht	3	2L	-	-
Fusarium	17	3M	121	5M
F. chlamydosporum Wollen weber & Reinking	-	-	15	1R
F. equiseti (Corda) Sacc	-	-	16	1R
F. lateritium Nees' Syst.	-	-	2	1R
F. merismoides Corda	-	-	22	1R
F. oxysporum Schlecht	7	2L	2	1R
F. poae (Peck) Wollenweber	10	2L	9	2L
F. scripi Lambotte & Fautr	-	-	55	2L
Gibberella fujikuroi (Sawada) Ito	2	1R	1	1R
Mucor	25	1R	25	3M
M. circinelloides Van Tiegh	-	-	25	3M
M. hiemalis Wehmer	25	1R	-	-
Penicillium	130	6H	95	4M
P. camembertii Thom	1	1R	1	1R
P. chrysogenum Thom	104	5M	77	3M
P. chorylophilum Dierckx	25	1R	2	1R
P. funiculosum Thom	-	-	15	1R
Stachybotrys parvispora Hughes, Mycol Pap.	6	2L	-	-
Sterile mycelia	8	2L	2	1R
Ulocladium	4	2L	-	-
U. alternariae (Cooke) Simmons	1	1R	-	-
U. chlamydosporum Mouchacca	3	2L	-	-
Total counts	748		654	
Number of genera 14	14		10	
Number of species 40+1 var.	29		32+1var.	

*OR = Occurrence remarks: H = high occurrence from 6-10 cases, M = moderate occurrence from 3-5 cases, L = low occurrence 2 cases and R = rare occurrence 1 case.

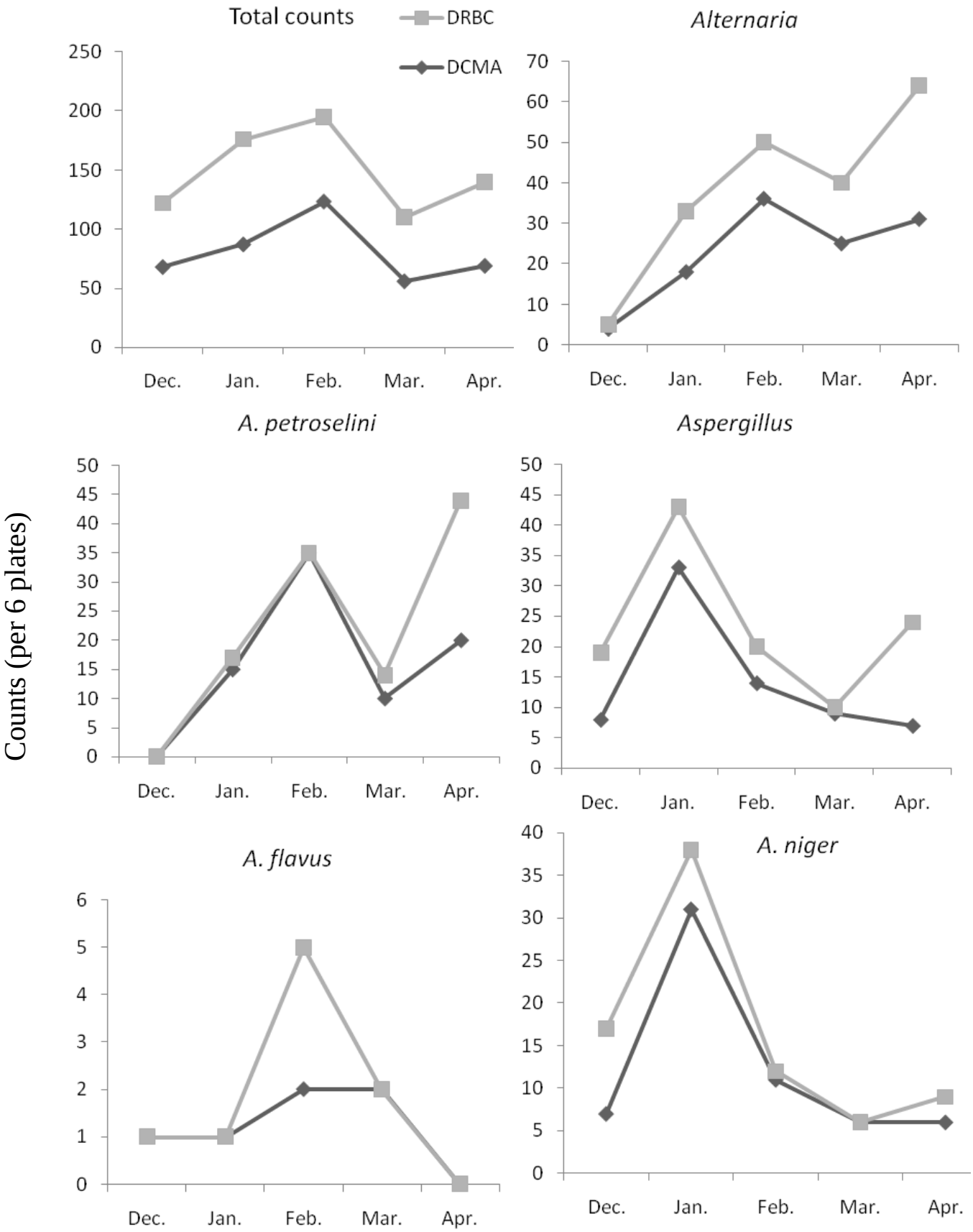

Figure 3. Monthly counts (per 6 plates) of common airborne fungi on DCMA and DRBC at 28°C.

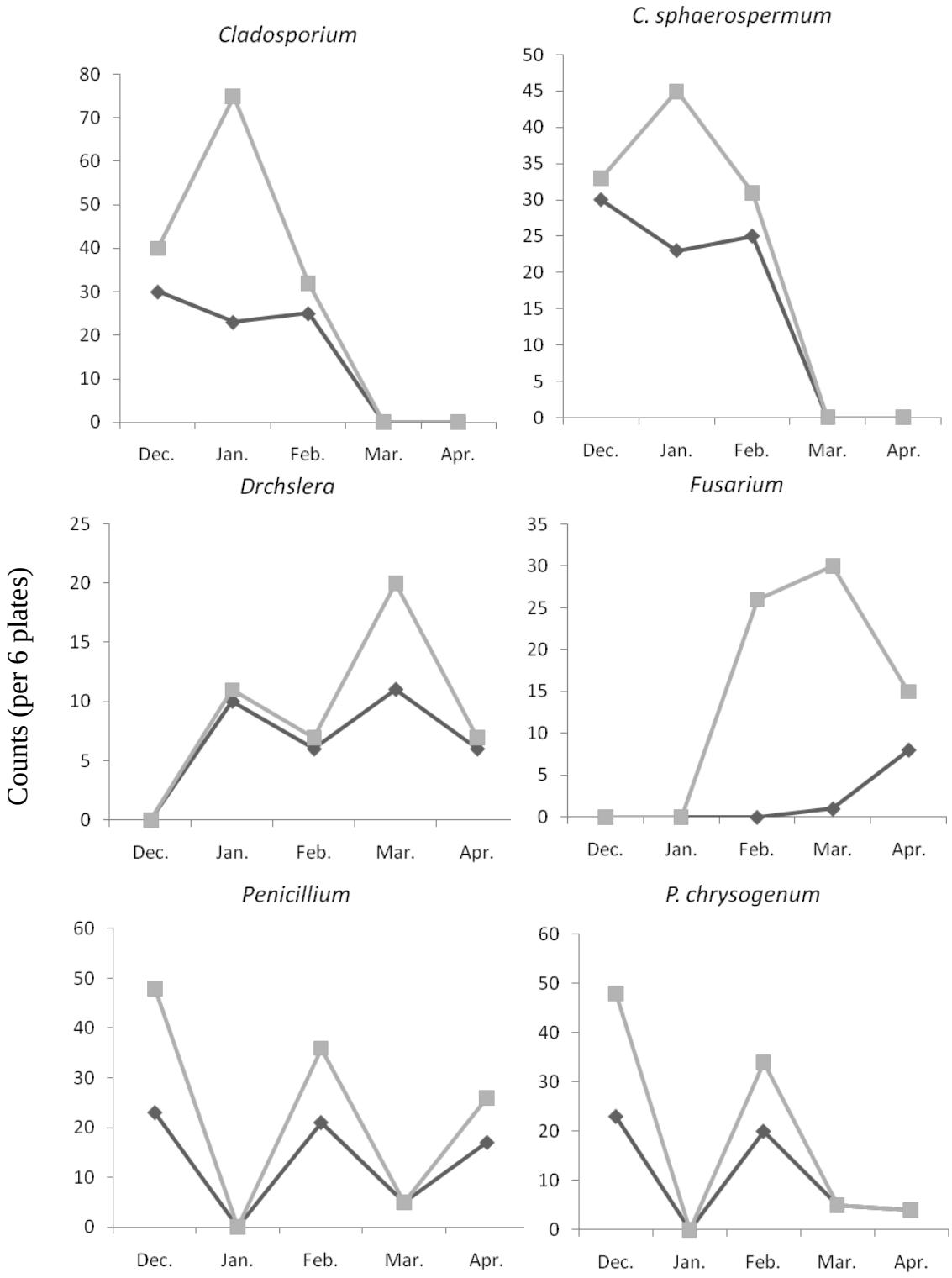

Figure 3. Contd.

P. chrysogenum and *P. corylophilum* (El- Said, 1994). El-Said and Saleem (2008) isolated sixty three species and 5 varieties belonging to 30 genera from cultivated, saline and desert soil in Western region in Libya. The most prevalent species from the three types of soils were: *A. alternata*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *E. nidulans*, *F. oxysporum*, *M. tassiana*, *Nectria haematococca* and *P. chrysogenum.* The above species were isolated

Table 3. Total counts (TC, calculated per 1 g soil), number of cases of isolation (NCI, out of 10) and occurrence remarks (OR) of various fungal genera and species recovered from soil of *Vicia faba* field on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rose bengal chloramphenicol agar (DRBC) at 28°C.

Genera and species	DCMA		DRBC	
	TC	NCI&OR	TC	NCI&OR
Alternaria	14333.4	3M	10000	2L
A. alternata (Fries) Keissler	3333.3	1R	-	-
A. chlamydospora Mouehaeca	1666.7	1R	-	-
A. petroselini (Neergaard) Simmons Comb. nou.	1666.7	1R	6666.7	2L
A. pluriseptata (Karst. & Hart) Jorsted	1000	1R	3333.3	1R
A. triticina Prasada & Prabhu	6666.6	1R	-	-
Aspergillus	71666.7	6H	205000.2	7H
A. cremeus Kwon & Fennell	1666.7	1R	-	-
A. flavus Link	23333.4	3M	50000	2L
A. fumigatus Fresenius	6666.6	2L	23333.4	3M
A. japonicus Saito	26666.7	2L	25000	1R
A. melleus Yukawa	-	-	1666.7	1R
A. niger Van. Tieghem	13333.3	2L	85000.1	5M
A. parasiticus Speare	-	-	20000	1R
Botryotrichum atrogriseum Van Beyma	-	-	21666.7	2L
Cladosporium	115000.1	8H	61666.7	6H
C. cladosporioides (Fres.)de Vries	98333.4	3M	3333.3	1R
C. cucumerinum Ellis & Arth	3333.4	2L	8333.3	2L
C. oxysporum Berk & Curt	5000	1R	6666.7	1R
C. sphaerospermum Penzig	8333.3	2L	43333.4	3M
Curvularia richardiae Alcorn	1666.7	1R	-	-
Drechslera neergaardii Danguash	6666.6	2L	5000	2L
Emericella nidulans var. *dentata* Sandhu &Sandhu	5000	1R	8333.4	3M
Fusarium	15000.1	4M	41666.8	3M
F. equiseti (Corda) Sacc	-	-	8333.3	1R
F. oxysporum Schlecht	6666.7	1R	6666.7	1R
F. poae (Peck) Wollenweber	5000	2L	11666.7	2L
F. sambucinum Fukel	1666.7	1R	11666.7	1R
F. scripi Lambotte & Fautr	-	-	1666,7	1R
F. tricinctum (Sacc) Corda.	1666.7	1R	1666.7	1R
Gibberella fujikuroi (Sawada) Ito	-	-	1000	1R
Myrothecium	65000	2L	95000	2L
M. roridum Tode ex Fries	1666.7	1R	-	-
M. verrucaria (Alb. & Sch.) Dit.	63333.3	2L	95000	2L
Penicillium	3000	2L	60000	2L
P. chrysogenum Thom	1333.3	1R	60000	2L
P. chorylophilum Dierckx	1666.7	1R	-	-
Stemophyilum botryosum Wallroth	-	-	3333.3	1R
Sterile mycelium	-	-	166.7	1R
Total counts	297333.5		512833.6	
Number of genera 12	9		11	
Number of species 31 + 1 var.	24+1var.		24+1var.	

*OR= Occurrence remarks: H = high occurrence from 6-10 cases, M = moderate occurrence from 3-5cases, L= low occurrence 2 cases and R = rare occurrence 1 case.

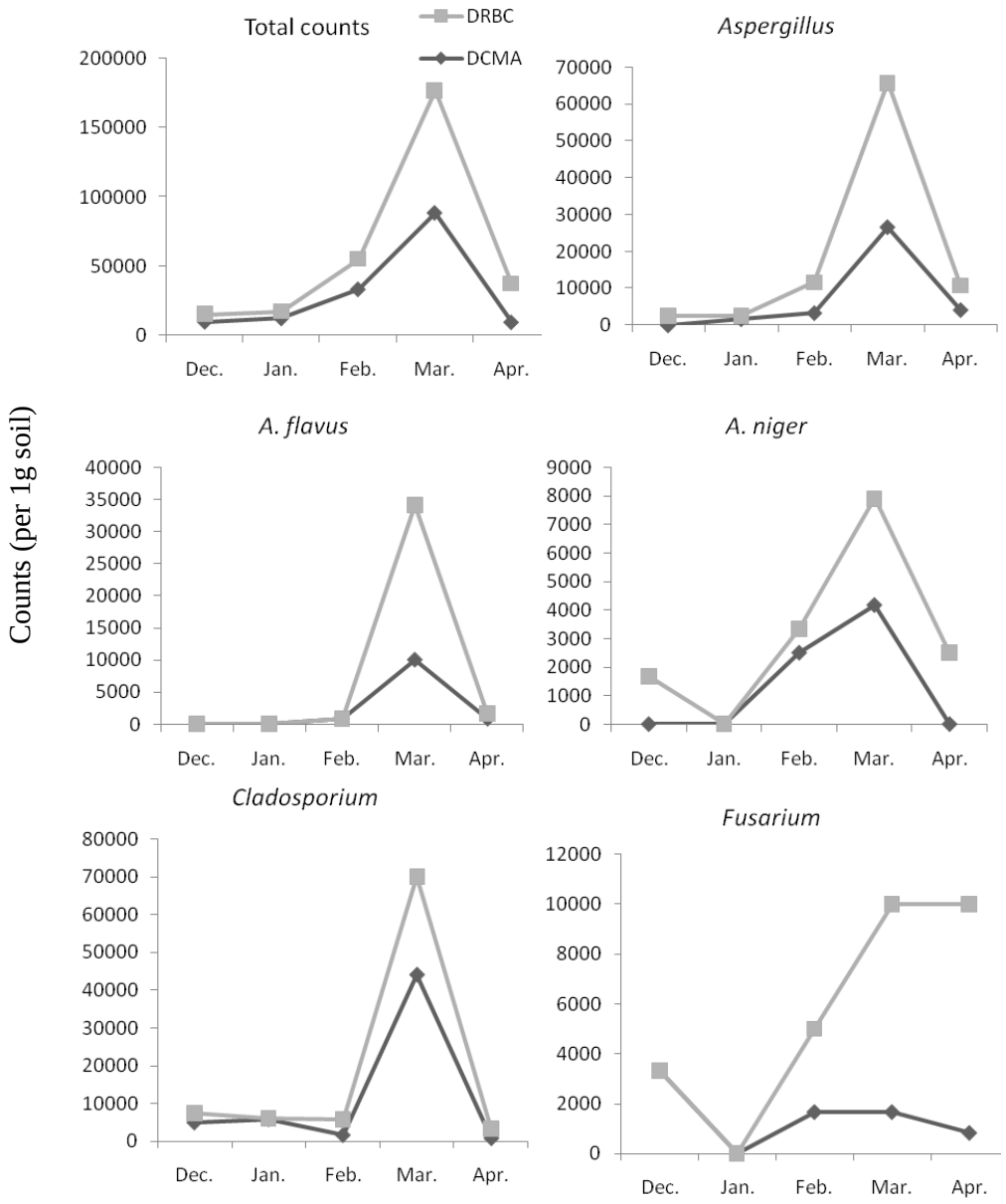

Figure 4. Monthly counts (per 1 g soil) of common soil fungi on DCMA and DRBC at 28°C.

Table 4. Degree of cellulolytic activities (calculated as diameter of clear zone in mm) of the most common fungal isolates.

Fungal isolate	Exo-ß-1,4-glucanase (C_1)	Endo-ß-1,4-glucanase (C_X)
Alternaria citri	29H	21W
A. petroselini	31H	24M
Aspergillus flavus	42H	29H
A. fumigatus	30H	25M
A. niger	26M	23W
Cladosporium cladosporioides	27M	20W
C. sphaerospermum	20W	18W
Drechslera neergaardii	26M	18W
Penicillium chrysogenum	33H	22W

*Activity remarks H = high 29 - 42 mm, M = moderate 24 - 28 mm and W = Week 20 - 23 mm.

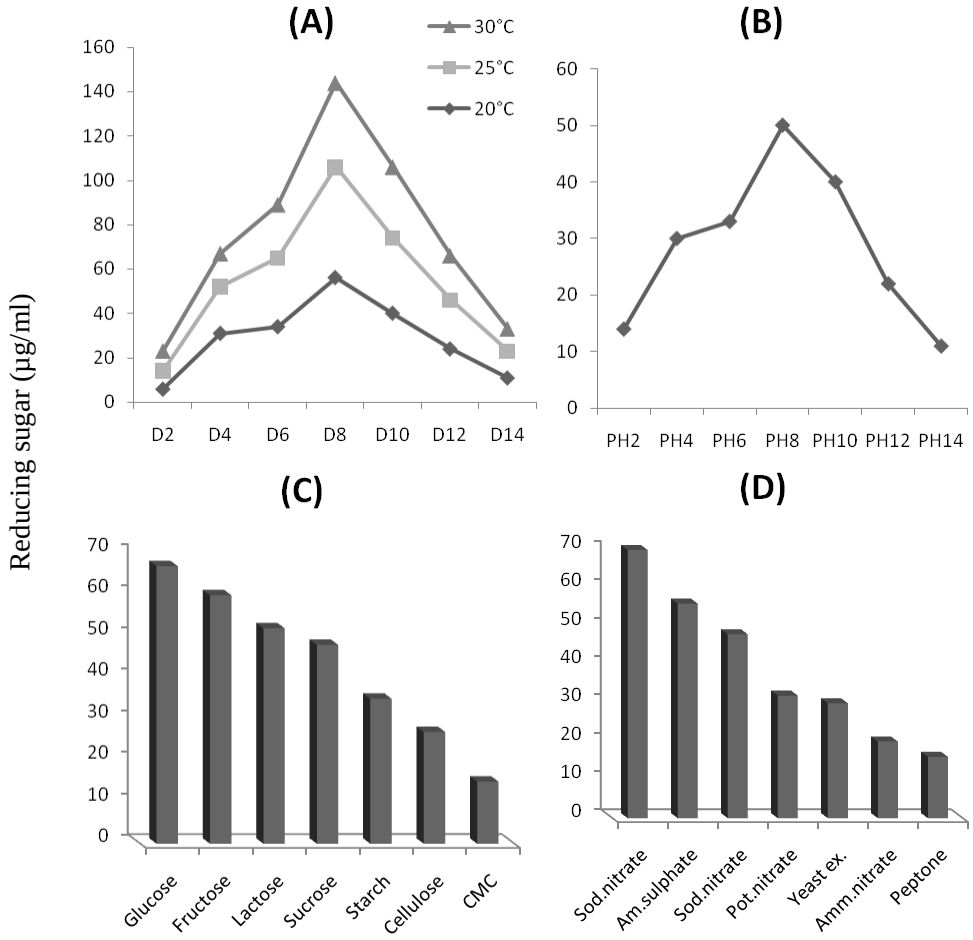

Figure 5. Effect of time course and temperature (A), pH values (B), carbon sources (C) and nitrogen sources (D) on production of exo-ß-1,4-glucnase by *A. flavus*.

with different numbers and frequencies from various soils in many places of the world by several workers (Abdel-Hafez et al., 1990; Moubasher and Mazen, 1991; Abdel-Hafez, 1994; Karl and Iain, 2004 and El-Said and Saleem, 2008).

Cellulolytic activities of some fungal isolates

Nine species (most common species) belonging to 5 genera were screened for their abilities to produce C_1 and C_x enzymes on solid media and proved to be active to utilize cellulose but with different degrees (Table 4). Five isolates (55.6% of total isolates) showed high activity in production of C_1 enzyme only and these were: *A. citri*, *A. petroselini*, *A. flavus*, *A. fumigatus* and *P. chrysogenum*. On the other hand, one fungal isolate exhibited high activity on production of C_X enzyme only and this was *A. flavus.*

Three and two isolates (33.0 and 22.2% of total isolates) were found to be of moderate production of C_1 and C_X enzymes, respectively, while one and six isolates (11.1 and 66.6% of total isolates) were of weak cellulolytic activity. Most of the above fungal isolates were reported as cellulase producers, but with variable capabilities by several workers (Abraha and Gashe, 1992; Abdel-Hafez et al., 1995; Moharram et al., 1995, 2004; Berlin et al.,2005; Rashid et al., 2009; Sohila et al., 2009; Saleem et al. 2010,2013).

A. flavus was the highest fungi in the production of endo and exo-ß-1,4glucanase in this investigation so it was chosen for further studies to achieve the most favorable environmental and nutritional conditions for C_1 and C_x enzymes production.

Maximum production of exo and endo-β-1,4-glucanase by *A. flavus* was obtained after 8 and 6 days of incubition at 30°C with culture media containing glucose and cellulose as a carbon sources and sodium nitrate as nitrogen source and the culture medium was initially adjusted to pH 6 (Figures 5 and 6). These findings are almost in agreement with those reported by El-Said et al. (2006). They found that *F. oxysporum* was the highest fungi in producing endo-β-1,4-glucanses among the 70 tested isolates obtained from 60 samples leaves of *Vicia faba*.

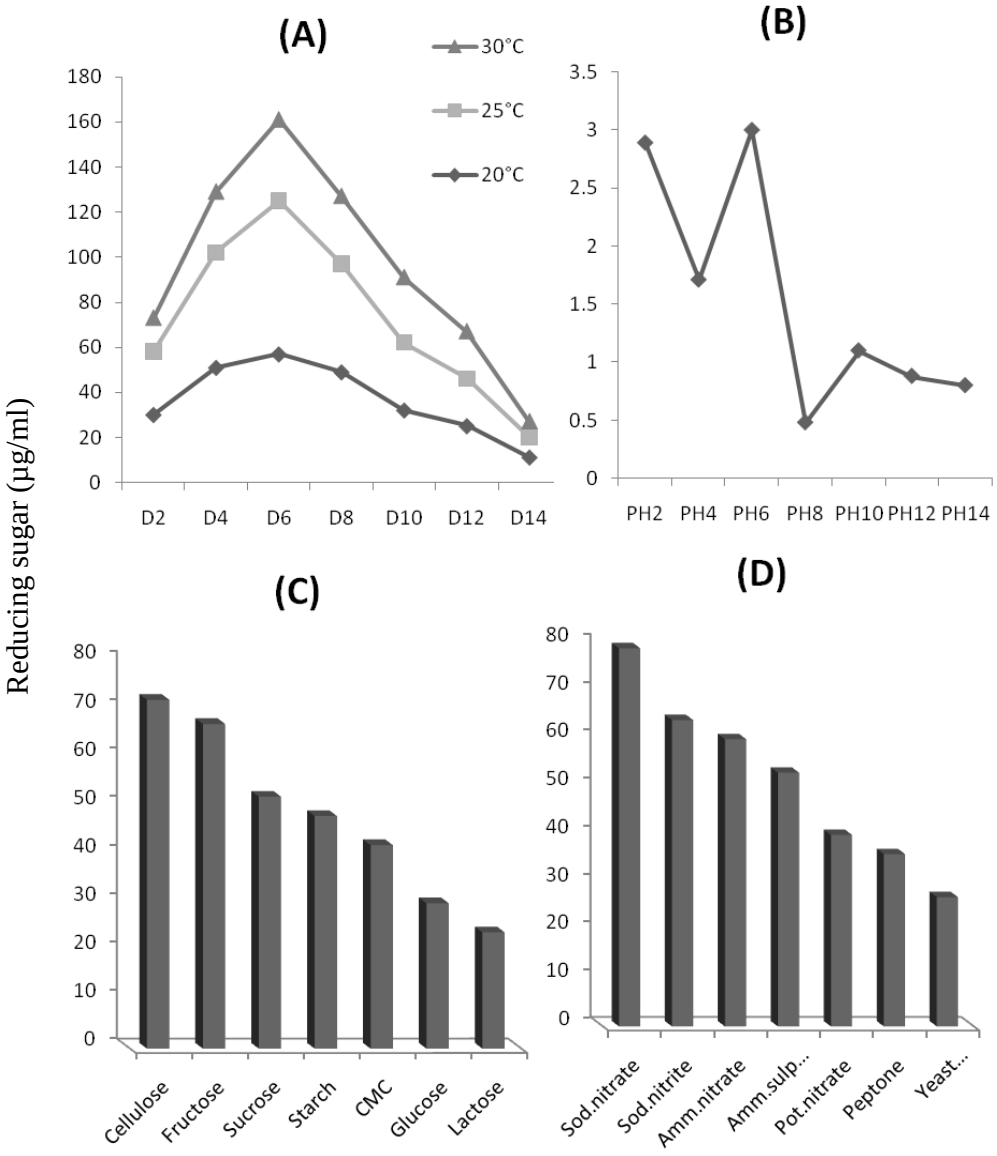

Figure 6. Effect of time course and temperature (A), pH values (B), carbon sources (C) and nitrogen sources (D) on production of endo-ß-1,4-glucnase by *A. flavus*.

Maximum production of endo-β-1,4- glucanses by *F. oxysporum* was achieved after 8 days of incubation at 30°C with culture medium containing carboxymethyl cellulose as carbon and peptone as nitrogen source and initially adjusted to pH 6. Immanuel et al. (2007) studied the effect of environmental factors on production of cellulase enzyme by *A. fumigatus* and *A. niger*. They reported that the optimum pH for cellulase production was 6 to 7 and optimum temperature was about 40°C. El Said and Saleem (2008) found that maximum production of endo-ß-1,4 glucanase by *Cheatomium globosum* was achieved 6 days after incubation at 30°C with incorporation of maltose as carbon source and NH_4NO_3 as nitrogen source in the culture medium which is initially adjusted to pH 6. Recently Saleem et al. (2013) found that maximum production of exo- and endo-ß-1,4 glucanase by *Mucor circinelloides* and *A. flavus* was achieved 6 days after incubation at 30°C with incorporation of fructose or sucrose as a sole carbon source and potassium nitrate or sodium nitrate as a sole nitrogen source, respectively in the basal medium initially adjusted to pH 6.

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