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Aeromycobiota over banana plantations in Assiut Governorate and enzymatic producing potential of the most common species

AH Moubasher[1,2], AM Moharram[1,2], MA Ismail[1,2] and MH Abdel-Hafeez[2]
[1]Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt
[2]Assiut University Mycological Centre, Assiut University, Egypt

[*]Corresponding author: e-mail: ahamaumc@yahoo.com

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Abstract: Forty-nine species and 2 varieties belonging to 32 genera were recovered from the air of banana plantations during the period from spring 2007 to summer 2008 on glucose-Czapek's agar at 28°C and YpSs agar at 45°C. The total number of propagules caught on glucose-Czapek's agar was greatly exceeding those caught on YpSs agar. The fungal diversity on glucose-Czapek's agar was also higher than those on YpSs agar. The most common mesophilic fungi were species of *Aspergillus, Cladosporium, Alternaria, Penicillium, Cochliobolus, Acremonium* and *Setosphaeria*. The most common thermophilic and thermotolerant fungi were *Aspergillus, Neosartorya, Thermomyces,* and *Emericella*. Among the 8 isolates tested for enzyme-producing capabilities, *Setosphaeria rostrata, Cochliobolus lunatus* and *Fusarium solani* came the most potent in producing both exo- and endo- 1,4-β-glucanases, pectinases and L-asparaginase, respectively.

Keywords: fungi, banana, air, cellulases, pectinases, asparaginase.

Introduction

Airborne microorganisms originate from different natural sources such as soil, animals, and humans (Pósfai *et al.* 2003, Mouli *et al.* 2005, Fang *et al.* 2007). According to Lacey (1981), airborne fungal spores are originally created from plant, animal, and soil sources. Further to this, Moubasher (1993) advocated that airborne spores are mainly a contribution from vegetation rather than soil. Airborne microorganisms are known to be implicated in food spoilage (Tournas and Katsoudas 2005), damage of books and archival materials (Aira *et al.* 2007), biodeterioration of stones (Mohammadi and Krumbein 2008), and spread of plant and animal diseases (Rossi *et al.* 2005). Exposure to outdoor air microorganisms has also been associated with allergic respiratory symptoms, hypersensitivity reactions, asthma related death (Dales *et al.* 2004, Peternel *et al.* 2004). Airborne fungi have been studied by several workers in different places of the world, Shelton *et al.* 2002, Codina *et al.* 2008, on banana field in Jamaica (Meredith 1962), on groundnut field in India (Jadhav *et al.* 2010), air over *Ulmus americana* field in USA (Levetin and Dorsey 2006). In Egypt airborne fungi have been studied by several workers in different places; over citrus plantations in Assiut (Moubasher *et al.* 1971), over wheat fields in El-Minya (Mazen and Shaban 1983), inside citrus packing house at Assiut (Maghazy *et al.* 1987), over wild plants in Assiut (Hemida 2004), and over cultivated areas in Egypt (Awad 2005).

No information is available on fungal airspora of banana plantations in Assiut, Egypt, so the aim of the present paper was to study the airspora in banana plantations, to screen the most commonly-isolated species for the ability to produce some enzymes, namely, exo and endo-cellulases, pectinases and L-asparaginase.

Materials and Methods

Area of study

This study was carried out in six localities which are known to be rich in banana plantations in Assiut Governorate and these were El-Wasta, Bossra, Sahel-Saleem, El-Mattmer, Bani-Morr and Awlad Ibrahim. Six visits to each location were performed during the period from spring 2007 to summer 2008.

Isolation of airborne mycobiota

The exposed plate method was used to trap fungal spores over banana fields. Ten plates of 9 cm diameter were used for each exposure (5 plates for each of the two media used and 36 exposures were made). Glucose-Czapek's agar of the following composition (g/l; sodium nitrate 3.0; K_2HPO_4 1.0; $MgSO_4.7H_2O$ 0.5; KCl 0.5; $FeSo_4$ 0.01; glucose 10.0; agar 15.0) was used for the isolating mesophilic fungi at 28°C. Yeast peptone soluble starch agar YpSs (Cooney and Emerson 1964) of the following composition (g/l): Difco powdered yeast extract 4.0g; K_2HPO_4 1.0g; $MgSO_4.7H_2O$ 0.5g; soluble starch 15g; agar 15g, water ($^1/_4$ tap and ¾ distilled) up to 1000 ml) was used for the isolation of thermophilic and thermotolerant fungi at 45°C. Rose Bengal (1/30000) and chloramphinicol (0.5 mg/ml) were used as bacteriostatic agents in both media [Smith and Dawson 1944, Al-Doory 1980). The media were then autoclaved at 121°C for 15 min. The plates were exposed for 30 minutes; about 50 cm above the ground level.

Identification of fungi

The following references were used for the identification of fungal genera and species (purely morphologically, based on macroscopic and microscopic features): Raper and Fennell (1965), Ellis (1971), Samson and Tansey (1977), Pitt (1979), Moubasher (1993), Domsch *et al.* (2007), Salar and Aneja (2007).

Enzyme-producing abilities

Eight isolates representing seven genera commonly encountered from the air of banana plantations were screened for their abilities to produce 4 extracellular enzymes at 25°C.

Cellulases production

Detection of exo-1.4 ß-glucanase (C_1): The tested fungi were inoculated into Erlenmeyer conical flasks containing cellulase production medium with the composition (g/l): $(NH_4)_2SO_4$ 0.5, L-asparagine 0.5, KH_2PO_4 1.0, KCl 0.5, $MgSO_4.7H_2O$ 0.2, $CaCl_2$ 0.2, Yeast extract 0.5 and cellulose microcrystalline 10 (Eggins and Pugh (1962). The pH was adjusted to 7.0 using acetate buffer. The inoculated flasks were incubated at 28 °C for 7 days. Wells in the agar plates of the same medium as above were filled each with 100 µl of the crude culture filtrate and the plates were incubated at 28°C for 24 hr. The plates were then flooded with 1% iodine solution. Clear zone around wells indicates the hydrolysis of cellulose by the releasing exo- 1, 4 –β – glucanase (C_1).

Detection of endo-1.4 ß-glucanase (C_x): Utilization of soluble cellulose by the tested isolates was detected by growing them on a modified medium of Prasad and Verma (1979), which contained (g/l): $(NH_4)_2SO_4$, 2.1 carboxymethyl cellulose (CMC) 10, KH_2 PO_4 1.0 and $MgSO_4.7H_2O$ 0.5. The pH was adjusted to 7.0 using acetate buffer and the inoculated flasks were incubated at 28ºC for 7 days. Wells in the agar plates of the same composition were filled each with 100 µl of the crude culture filtrate and the plates were incubated at 28°C for 24 hr. The plates were then flooded with 1% iodine solution. Clear zone around wells indicates hydrolysis of cellulose by the releasing endo- 1, 4 –β –glucanase (C_x).

Pectinase production: The method described by Hankin *et al.* (1971) was adopted where the medium used was prepared in two separate portions (g/l):

Portion A: yeast extract, 1 g; pectin from citrus peel, 5 g; agar, 15g; distilled water, 500 ml; and its pH was adjusted to 7.0.

Portion B (mineral salt solution): composed (per litre) of: $(NH_4)_2SO_4$, 2 g; KH_2 PO_4, 4 g; Na_2 HPO_4, 6 g; $FeSO_4.7H_2O$, 1.0 mg; $CaCl_2$, 1mg; H_3 PO_4, 10 µg; $MnSO_4$, 10 µg; $ZnSO_4$, 70 µg; $CuSO_4$, 50 µg; MoO_3, 10 µg, distilled water 500 ml, pH was adjusted to 7.4.

After autoclaving at 121°C for 15 minutes, the two portions were mixed thoroughly. The isolates were inoculated into 250 ml Erlenmeyer's flasks each containing 50 ml broth medium and incubated at 28ºC for 7 days. Medium has the same composition (as above) solidified with agar was dispensed into 9 cm Petri dishes (20 ml per plate), and the plates were then inoculated with the crude culture filtrate in 1 cm diameter wells and incubated for 24 hr at 28°C. The plates were then flooded with 1% iodine solution and the developing clear zones around wells indicate the production of pectinase enzyme (Ammar *et al.* 1995).

L-asparaginase production: The modified method described by Gulati *et al.* (1997) was used in which the medium contained (g/l of distilled water): Glucose 2.0; L- asparagine 10.0; K_2PO_4 1.52; KCl 0.52; $MgSO_47H_2O$ 0.52; $CuNO_3$ $3H_2O$ trace; $ZnSO_47H_2O$ trace; Fe SO_47H_2O trace; 2 ml 2% phenol red (2 g phenol red dissolved in 100 ml 95 % ethanol) with the adjustment of pH to 6.2. The liquid medium was then dispensed into test tubes (10 ml each) and autoclaved at 121 ºC for 15 min. After cooling the test fungi were inoculated and incubated at 28ºC for 7 days. The appearance of pink color indicating L-asparaginase production have been reported. The color was assessed visually and its intensity was measured spectrophotometrically at a wave length of 546 and 600 nm (Howard and Schwartz 1968).

Results and Discussion

Fungi recovered from the air

A total of 49 species and 2 varieties belonging to 32 genera were recovered from the air of banana plantations during spring 2007 to summer 2008. The total number of propagules caught on glucose-Czapek's agar at 28°C (1895 CFUs /180 plates in 36 exposures, 30 minutes each) outnumbered those caught on YpSs agar at 45°C (740 CFUs). The fungal diversity on glucose-Czapek's agar was also higher (27 genera, 43 species + 1 variety) than those on YpSs agar (8, 11 + 1) (Tables 1 - 3).

Aspergillus was the most common genus on both media. *Aspergillus fumigatus, Thermomyces lanuginosus, Emericella nidulans* and *A. flavus* were the most common on YpSs medium.

Aspergillus (13 species and 2 varieties) was one of the most common genera in banana plantations contributing 20.1% and 65.3% of total fungi, and the count peak was registered in winter 2008 and spring 2007 but the trough in spring and summer 2007 on both glucose-Czapek's at 28°C and YpSs agar at 45°C respectively. *A. fumigatus, A. niger* were isolated in high frequency of occurrence; *A. flavus* and *A. terreus* were isolated in moderate frequency on glucose-Czapek's and YpSs agar. *A. ochraceus* was isolated in low frequency. The remaining species (*A. aculeatus, A. awamori, A. candidus, A. flavus* var. *columnaris, A. fumigatus* var. *albus, A. ochraceous, A. parasiticus, A. sclerotioniger, A. sydowii, A. tamarii, A. tritici* and *A. versicolor*) were of rare frequency. These results are in agreement with those obtained by many authors who found that *Aspergillus niger* was the most common airborne fungus over banana fields in Qena (El-Said and Abdel-Hafez 1995), over citrus plantation in Assiut (Moubasher *et al.* 1971, 2016), *A. fumigatus* was also common in the airspora in Assiut (Moubasher *et al.* 1981) and Qena (Abdel-Fattah and Swelim 1982), and *A. flavus* was isolated in high frequency from the air over banana fields in Qena (El-Said and Abdel-Hafez 1995), the air of wheat fields in El-Minya (Mazen and Shaban 1983) and New Valley (Abdel-Hafez *et al.* 2000) and over lentil field in Qena (Abdel-Hafez *et al.* 1990).

Thermomyces lanuginosus was one of the most common thermophilic fungi. It was isolated in high frequency from the air on YpSs at 45°C. It was reported from the airspora in Assiut (Moubasher *et al.* 1981), and Qena (Abdel-Fattah and Swelim 1982).

Cladosporium (*C. cladosporioides*) was isolated in moderate frequency. It emerged in 18 out of 36 exposures, yielding 44.9% of total count. Its CFUs irregularly fluctuated, giving its peak during winter 2008 whereas the trough was recorded in autumn 2007. In this respect, *C. cladosporioides* was isolated but with different numbers and incidences from the air over banana fields in Qena (El-Said and Abdel-Hafez 1995), and in Jamaica (Meredith 1962), over citrus plantation in Assiut (Moubasher *et al.* 1971, 2016), and over cultivated areas with chamomile and some vegetable plants in El-Fayoum Governorate, Egypt (Awad 2005).

Alternaria (*A. alternata*) was recovered in moderate frequency in 17 out of 36 exposures, yielding 4.8% of total CFUs on glucose-Czapek's agar at 28°C. Its peak was recorded in summer 2008 while the trough was in summer 2007. In this respect *Alternaria* was isolated in high frequency from the air over banana fields in Qena (El-Said and Abdel-Hafez 1995), over citrus plantation in Assiut (Moubasher *et al.* 1971, 2016), over tomato field in India (Lohare *et al*. 2009), and over cultivated areas with chamomile and some vegetable plants in El-Fayoum Governorate, Egypt (Awad 2005).

Penicillium (*P. chrysogenum* and *P. oxalicum*) was encountered in moderate frequency, in 13 out of 36 exposures, yielding 1.3% of total fungi at 28°C. The count peak was recorded in summer 2008. *P. oxalicum* was the most common *Penicillium* species; it was isolated in low frequency of occurrence in 9 out of 36 exposures. In this respect *P. oxalicum* and *P. chrysogenum* were also found common in the air of banana plantations in Jamaica (Meredith 1962), and from Egypt over cultivated areas with chamomile and some vegetable plants in El-Fayoum (Awad 2005), airspora in Assiut (Moubasher *et al.* 1981), and Qena Governorate (Abdel-Fattah and Swelim 1982).

Fusarium (*F. solani*) was isolated in low frequency. It was recorded in 10 out of 36 exposures, yielding small numbers of propagules on glucose-Czapek's agar (0.69% of total fungi). In contrast to our findings, *Fusarium* dominated in the air over banana fields in Qena (El-Said and Abdel-Hafez 1995), citrus plantations in Upper Egypt (Moubasher *et al.* 1971, 2016), over lentil field in Qena (Abdel-Hafez *et al*. 1990), and in the air over chamomile and some vegetable plants in El-Fayoum Governorate, Egypt (Awad 2005).

Cochliobolus (4 species) was isolated in low frequency. The most prevalent species was *C. lunatus* (anamorph: *Curvularia lunata*). *Curvularia* was isolated from banana soil in Assiut (Hemida *et al.* 2012), and from the phylloplane of banana plantations in Qena (El-Said 2001).

Acremonium (*A. strictum*) was isolated in low frequency. It was recorded in 7 out of 36 exposures, yielding 0.42% of total fungi on glucose-Czapek's agar and it was missing on YpSs. In this respect *A. strictum* was reported from the air over banana fields in Qena (El-Said and Abdel-Hafez 1995) and over citrus and grapevines plantations in Assiut (Moubasher *et al.* 2016).

Emericella (3 species) was identified in moderate frequency on YpSs and low on Glucose-Czapek's agar. It was recorded in 12 and 8 out of 36 exposures, accounting for 3.9% and 0.63% of total fungi, respectively. *E. nidulans* was the most common species. *Emericella* species were isolated from the air of Assiut (Moubasher and Moustafa 1974), from phylloplane and phyllosphere of banana plantations in Qena (El-Said 2001) and from soil of banana plantations in Assiut (Hemida *et al.* 2012).

Some other rare fungi were infrequently encountered either on Glucose-Czapek's agar namely, *Myrothecium verrucaria*, *Stachybotrys chartarum*, *Memnoniella echinata*, *Nectria inventa*, *Trichoderma harzianum*, *Trichothecium roseum*, *Ulocladium chartarum*, *Setosphaeria rostrata*, *Botryodiplodia theobromae*, *Nigrospora oryzae*, *Aureobasidium pullulans*, *Botryosporium* sp., *Microascus brevicaulis*, *Phoma epicoccina*, *Paecillium lilacinum*, *Rhizopus oryzae* and *Corynascus sepedonium* or on YpSs viz *Malbranchea cinnamomea*, *Melanocarpus albomyces* and *Rhizomucor pusillus*.

Enzyme-producing-potential of the most common airborne fungi of banana plantations

Cultures of biotechnological potential for the welfare of human society are targeted during the present work for the advantage of future research in this significant field. For this purpose, 8 common fungal isolates were screened for their abilities toward the production of four economically important enzymes:

1) Cellulases

As known, cellulase enzymes have application in the production of organic acid (Lue *et al*. 1997), detergents and other chemicals (Cao *et al*. 1997), and play a substantial role in pathogenicity and cell wall degradation of host plants by phytopathogenic fungi (Agrios 1978).

a) Exo-1, 4-β- glucanase (C_1)

This enzyme is applied in biotechnology in the production of fermentable sugars and ethanol (Olson and Hahn-Hagerdahl 1997). It has been used in the pulp and paper industry (Suurnakki *et al.* 2004), in the textile industry (Miettinen-Oinonen *et al.* 2004), and even in the food industry (Penttilä et al. 2004).

Four out of the 8 isolates tested showed positive results. Of the positive isolates, only one isolate (*Setosphaeria rostrata* AUMC 8764) showed moderate ability, while the other three isolates were of low ability (Table 4). In this respect, El-Said (2001) reported that the 34 fungal isolates recovered from leaf surfaces of banana plants cultivated in Qena, Upper Egypt were able to produce C_1 enzyme on solid media, but with different degrees. From these, 20 isolates showed high activity.

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Table 1: Counts of colony forming units (CFUs) (per 180 plates) and frequency of occurrence of fungi (FO) recovered from 36 exposures of the air of banana plantations during the seasons of study on glucose-Czapek's agar at 28°C

Seasons	Spring 2007			Summer 2007			Autumn 2007			Winter 2008			Spring 2008			Summer 2008		
Fungal taxa	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR
Acremonium strictum W. Gams	4.6	2	M	5.3	3	M	-	-	-	-	-	-	0.9	1	L	0.22	1	L
Alternaria alternata (Fries) Keissler	11.4	2	M	2.9	2	M	6.3	4	H	0.8	3	M	1.8	2	M	14.4	4	H
Aspergillus Link	38.6	6	H	35.3	6	H	61.3	6	H	12.0	6	H	23.8	4	H	22.0	6	H
A. aculeatus Lizuka	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	1	L
A. awamori Nakazawa	-	-	-	-	-	-	-	-	-	-	-	-	1.3	2	M	-	-	-
A. candidus Link	-	-	-	1.4	1	L	1.8	1	L	-	-	-	-	-	-	-	-	-
A. flavus Link	6.8	2	M	5.3	2	M	7.2	3	M	0.8	1	L	1.8	2	M	3.4	5	H
A. flavus var. *columnaris* Raper and Fennell	-	-	-	-	-	-	-	-	-	-	-	-	0.44	1	L	-	-	-
A. niger van Tieghem	22.7	5	H	18.6	5	H	45.5	6	H	10.8	6	H	19.8	3	M	15.9	6	H
A. ochraceus Wihelm	2.3	1	L	-	-	-	3.6	4	H	0.1	1	L	0.44	1	L	0.7	2	M
A. parasiticus Speare	-	-	-	1.4	1	L	0.9	1	L	-	-	-	-	-	-	-	-	-
A. sclerotioniger Samson and Frisved	-	-	-	1.4	1	L	-	-	-	-	-	-	-	-	-	-	-	-
A. sydowii (Bainier and Sartory) Thom and Church	-	-	-	1.4	1	L	-	-	-	-	-	-	-	-	-	-	-	-
A. tamarii Kita	2.3	1	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. terreus Thom	4.6	2	M	5.7	3	M				0.3	2	M	-	-	-	0.5	1	L
A. versicolor (Vuillemin) Tiraboschi	-	-	-	-	-	-	1.8	1	L				-	-	-	0.22	1	L
Aureobasidium pullulans (de Bary) Arnaud	2.3	1	R	-	-	-							-	-	-	-	-	-
Botryodiplodia theobromae Patouillard	-	-	-	-	-	-	0.9	1	L	0.1	1	L	-	-	-	-	-	-
Botryosporium sp. Corda	-	-	-	-	-	-	-	-	-	1.9	1	L	-	-	-	-	-	-
Cladosporium cladosporioides (Fresenius) de Vries	11.4	3	M	41.4	5	H	1.8	1	L	67.9	5	H	57.7	4	H	-	-	-
Cochliobolus Drechsler	-	-	-	-	-	-	4.5	3	M	0.1	1	L	0.9	1	L	5.6	5	H
C. australiensis (Tsuda and Ueyama) Alcorn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	1	L
C. cynodontis Nelson	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	2	M
C. lunatus Nelson and Haasis	-	-	-	-	-	-	3.6	2	M	-	-	-	0.44	1	L	2.9	4	H
C. spicifer Nelson	-	-	-	-	-	-	0.9	1	L	0.1	1	L	0.44	1	L	2.2	3	M
Corynascus sepedonium (Emmons) von Arx	-	-	-	-	-	-	-	-	-	8.1	2	M	1.3	1	L	-	-	-
Emericella Berkeley and Broome	-	-	-	5.3	2	M	-	-	-	0.2	2	M	-	-	-	1.6	4	H
E. nidulans (Eidam) Vuillemin	-	-	-	1.4	1	L	-	-	-	0.1	1	L	-	-	-	1.1	3	M
E. variecolor Berkeley and Broome	-	-	-	2.9	1	L	-	-	-	0.1	1	L	-	-	-	0.5	1	M
Fusarium solani (Martius) Saccardo	4.6	2	M	-	-	-	2.7	3	M	0.5	2	M	0.44	1	L	0.5	2	M
Melanospora zamiae Corda	-	-	-	-	-	-	-	-	-	0.1	1	L	0.44	1	L	-	-	-
Memnoniella echinata (Rivolta) Galloway	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.7	1	L
Microascus brevicaulis S. P. Abbott	-	-	-	-	-	-	-	-	-	3.1	2	M	-	-	-	-	-	-

Seasons	Spring 2007			Summer 2007			Autumn 2007			Winter 2008			Spring 2008			Summer 2008		
Fungal taxa	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	-	-	-	-	-	-	-	-	-	0.9	3	M	-	-	-	1.4	2	M
Nectria inventa Pethybridge	-	-	-	-	-	-	-	-	-	-	-	-	0.9	1	L			
Nigrospora oryzae O'Gorman, Fuller and Dyer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	2	M
Paecillium lilacinum (Thom) Luangsa-ard, Hywel-Jones and Samson	-	-	-	-	-	-	-	-	-	-	-	-	0.9	1	L	-	-	-
Penicillium Link	4.6	1	L	2.9	1	L	4.5	2	M	0.4	2	M	1.3	1	L	1.6	4	H
P. chrysogenum Thom	-	-	-	-	-	-	-	-	-	0.2	1	L	0.44	1	L	1.1	3	M
P. oxalicum Currie and Thom	4.6	1	L	2.9	1	L	4.5	2	M	0.2	2	M	0.9	1	L	0.5	2	M
Phoma epicoccina Punith, Tulloch and Leach	6.8	2	M	5.3	2	M	4.5	1	L	2.4	5	H	4.4	3	M	-	-	-
Rhizopus oryzae Went and Prinsen-Geerligs	-	-	-	1.4	1	L	-	-	-	0.3	1	L	0.9	2	M	-	-	-
Setosphaeria rostrata Leonard	-	-	-	-	-	-	0.9	1	L	-	-	-	-	-	-	34.1	2	M
Stachybotrys chartarum (Ehrenberg) Hughes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.3	4	H
Sterile mycelia (white, dark and yellow)	8.7	3	M	1.4	1	L	2.7	2	M	0.3	2	M	1.3	1	L	5.9	4	M
Trichoderma harzianum Rifai	4.6	1	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichothecium roseum (Persoon: Fries) Link	-	-	-	-	-	-	-	-	-	0.9	3	M	2.6	1	L	-	-	-
Ulocladium chartarum (Preuss) Simmons	-	-	-	2.9	1	L	1.8	2	M	-	-	-	0.44	1	L	-	-	-
Yeasts (white)	2.3	1	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total count	44 (100)	6	H	70 (100)	6	H	101 (100)	6	H	1007 (100)	6	H	227 (100)	6	H	446 (100)	6	H
No. of genera (28)	10			10			11			17			16			13		
No. of species (43 + 1 variety)	13			16			17			21			20+1			20		

CFUs= Colony forming units /30 plates in 6 exposures, 30 minutes each, % CFUs= calculated to total counts, FO= Frequency of occurrence, OR= Occurrence remarks: H= high (4, 5 and 6 exposures), M= moderate (2 and 3), L=low (1 exposures).

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Table 2: Counts of colony forming units (CFUs) (per 30 plates) and frequency of occurrence of fungi recovered from the air of banana plantations during the seasons of study on yeast powder soluble starch agar (YpSs) at 45°C

Seasons	Spring 2007			Summer 2007			Autumn 2007			Winter 2008			Spring 2008			Summer 2008		
Fungal taxa	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR	%CFU	FO	OR
Aspergillus	72.9	6	H	30.2	6	H	52.9	6	H	71.2	6	H	72.1	6	H	80.8	6	H
A. fumigatus var. *albus* Rai, Tewari and Agrawal	-	-	-	-	-	-	5.9	3	M	-	-	-	1.8	2	M	-	-	-
A. niger	67.5	6	H	17.9	6	H	36.8	6	H	56.4	6	H	60.6	6	H	72	6	H
A. flavus	-	-	-	-	-	-	4.4	2	M	3.6	3	M	2.4	3	M	7.2	4	H
A. terreus	4.8	5	H	12.3	4	H	2.9	2	M	7.3	4	H	6.1	5	H	1.6	2	M
A. tritici Mehrotra and Basu	0.6	1	L	-	-	-	2.9	2	M	-	-	-	1.2	2	M			
Emericella	4.8	3	M	3.8	2	M	8.8	2	M	6.4	3	M	1.8	1	L	0.8	1	L
E. nidulans	4.8	3	M	3.8	2	M	8.8	2	M	6.4	3	M	1.8	1	L	-	-	-
E. quadrilineata (Thom and Raper) Benjamin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	1	L
Malbranchea cinnamomea (Libert) van Oorschot and de Hoog	1.2	1	L	3.8	2	M	2.9	2	M	2.7	3	M	1.2	1	L	0.8	1	L
Melanocarpus albomyces (Cooney and Emerson) von Arx	-	-	-	-	-	-	10.3	3	M	1.8	2	M	1.2	2	M	1.6	1	L
Neosartorya fumigata	10.8	5	H	45.3	6	H	5.9	3	M	12.7	4	H	13.3	3	M	-	-	-
Rhizomucor pusillus (Lindt) Schipper	1.2	1	L	0.9	1	L	2.9	2	M	1.8	2	M	1.8	2	M	-	-	-
Sterile mycelia (white, dark and yellow)	2.4	2	M	12.2	4	H	8.8	3	M	4.5	3	M	3	2	M	12.8	6	H
Thermomyces lanuginosus Tsiklinsky	6.6	6	H	3.8	2	M	7.4	2	M	2.7	2	M	5.5	6	H	3.2	3	M
Total count (%)	166 (100)	6	H	106 (100)	6	H	68 (100)	6	H	110 (100)	6	H	165 (100)	6	H	125 (100)	6	H
No. of genera (8)	6			6			8			8			8			6		
No. of species (11 + 1 variety)	8			7			10 + 1			9			10 + 1			7		

CFUs= Colony forming units /30 plates in 6 exposures, 30 minutes each, % CFUs= calculated to total counts, FO= Frequency of occurrence, OR= Occurrence remarks: H= high (4, 5 and6 exposures), M= moderate (2 and 3), L=low (1 exposures).

Table 3: Counts (colonies / 180 plates in 36 exposures) and frequency of occurrence recovered from the air of banana plants on glucose-Czapek's agar at 28°C and Yeast powder soluble starch agar at 45°C,

Medium	Glucose-Czapek's agar				YpSs			
Fungal taxa	CFUs	%CFUs	FO	OR	CFUs	%TC	FO	OR
Acremonium strictum	8	0.42	7	L				
Alternaria alternata	90	4.8	17	M				
Aspergillus	381	20.1	33	H	483	65.3	36	H
A. aculeatus	6	0.3	1	R				
A. awamori	3	0.16	2	R				
A. candidus	3	0.16	2	R				
A. flavus	41	2.2	15	M	20	2.7	12	M
A. flavus var. *columnaris*	1	0.05	1	R				
A. fumigatus var. *albus*					7	0.95	5	R
A. niger	298	15.7	31	H	408	55.1	36	H
A. ochraceus	10	0.53	9	L				
A. parasiticus	2	0.11	2	R				
A. sclerotioniger	1	0.05	1	R				
A. sydowii	1	0.05	1	R				
A. tamarii	1	0.05	1	R				
A. terreus	11	0.59	8	L	43	5.8	22	H
A. tritici					5	0.7	5	R
A. versicolor	3	0.16	2	R				
Aureobasidium pullulans	1	0.05	1	R				
Botryodiplodia theobromae	2	0.11	2	R				
Botryosporium sp.	19	1.0	1	R				
Cladosporium cladosporioides	851	44.9	18	M				
Cochliobolus	39	2.1	10	L				
C. australiensis	2	0.11	1	R				
C. cynodontis	6	0.32	2	R				
C. lunatus	18	0.95	7	L				
C. spicifer	13	0.69	6	L				
Corynascus sepedonium	85	4.5	3	R				
Emericella	12	0.63	8	L	29	3.9	12	M
E. nidulans	7	0.37	5	R	28	3.8	11	M
E. quadrilineata					1	0.14	1	R
E. variecolor	5	0.26	3	R				
Fusarium solani	13	0.69	10	L				
Malbranchea cinnamomea					14	1.9	10	L
Melanocarpus albomyces					13	1.8	8	L
Melanospora zamiae Corda	2	0.11	2	R				
Memnoniella echinata	21	1.1	1	R				
Microascus brevicaulis	31	1.6	2	R				
Myrothecium verrucaria	15	0.79	5	R				
Nectria inventa	2	0.11	1	R				
Neosartorya fumigata					106	14.3	21	H
Nigrospora oryzae	3	0.16	2	R				
Paecillium lilacinum	2	0.11	1	R				
Penicillium	23	1.3	13	M				
P. chrysogenum	8	0.42	5	R				
P. oxalicum	15	0.79	9	L				
Phoma epicoccina	45	2.4	13	M				
Rhizomucor pusillus					10	1.4	8	L
Rhizopus oryzae	6	0.32	4	R				
Setosphaeria rostrata	153	8.1	3	R				
Stachybotrys chartarum	28	1.5	4	R				
Sterile mycelia	40	2.1	9	L	49	6.6	20	H
Thermomyces lanuginosus					36	4.9	21	H
Trichoderma harzianum	2	0.11	1	R				
Trichothecium roseum	15	0.79	4	R				
Ulocladium chartarum	5	0.26	4	R				

Medium	Glucose-Czapek's agar				YpSs			
Fungal taxa	CFUs	%CFUs	FO	OR	CFUs	%TC	FO	OR
Yeast (white)	1	0.05	1	R				
Total count	1895	100	36	H	740	100	36	H
No. of genera (33)	27				8			
No. of species (50 + 2 varieties)	43+ 1 variety				11 + 1 variety			

CFUs= Colony forming units /180 plates in 36 exposures collected in all seasons, % CFUs= calculated to total counts, FO= Frequency of occurrence, OR= Occurrence remarks: H= high (19 – 36 exposures), M= moderate (11- 18), L=low (6-10) and R= rare (1-5 samples).

b) Endo-1, 4-β- glucanase (Cx)

This enzyme is applied also in biotechnology in the production of fermentable sugars and ethanol (Van Wyk and Mohulatsi 2003), detergents and other chemicals (Cao *et al.* 1997). It has been used in the pulp and paper industry (Suurnakki *et al*. 2004), and in the textile industry (Miettinen-Oinonen *et al.* 2004).

Five out of the 8 isolates tested showed positive results. Of the positive isolates only 1 gave moderate ability*: Cochliobolus lunatus* AUMC 8357, whereas the other four isolates were of low ability. In this respect, El-Said (2001) reported that 11 out of 34 fungal isolates recovered from leaf surfaces of banana plants were high producers of C_x and these were *Alternaria alternata, Acremonium strictum, Chaetomium globosum, Curvularia pallescens, Fusarium oxysporum, Gibberella fujikuroi, G. zeae, Nectria haematococca, Setosphaeria rostrata, Stachybotrys chartarum* and *Trichoderma pseudokoningii.*

2) Pectinases

Pectinolytic enzymes are widely used in the food industry for juice and wine production (Semenova *et al.* 2006), and textile industries (Kashyap *et al.* 2001).

Five out of 8 isolates tested were pectinolytic. Of these, 1 isolate gave high production: (*Aspergillus flavus* AUMC 8301), two isolates were of moderate ability (*Cochliobolus lunatus* AUMC 8357, *Ulocladium chartarum* AUMC 8311), while two isolates were of low ability**.** Al-Khateeb (2004) studied the ability of 10 isolates of 5 fungal species isolated from reclaimed soil from Assiut, Egypt and found that all tested isolates were able to produce pectinase with variable degrees. The highest ability of pectinase production was that of *Fusarium oxysporum*.

3) L- asparaginase

L-asparaginase has been considered as a therapeutic agent against malignant tumors (Tozuka *et al.* 1997). This enzyme is well established, as it has remarkably induced remission in most patients suffering from acute lymphoblastic leukemia (Verma *et al.* 2007).

Seven isolates out of the 8 tested showed positive results, from which 1 gave high ability of production (*Fusarium solani* AUMC 8307). Five isolates performed moderate ability and only one isolate showed low ability. Seddek (2012) screened 214 endophytic fungal isolates and found that 162 (75.7%) isolates had the ability to produce L-asparaginase. All fungal isolates of *Alternaria, Cochliobolus, Cladosporium, Neosartorya,* and *Papulaspora* possessed this ability.

Table 4: Qualitative determination of cellulases, pectinases and L-aspraginase produced by the tested isolates.

Fungal taxa	AUMC No.	Cellulases (mm)		Pectinases (mm)	L-aspraginase		
		C_1	Cx		Visual	Absorbance	
						546 nm	600 nm
Aspergillus candidus	8373	-ve	-ve	-ve	+	0.584	0.148
A. ochraceus	8348	13 L	12 L	-ve	++	0.830	0.224
A. flavus	8301	14 L	12 L	25 H	++	0.628	0.084
Cladosporium cladosporioides	8380	-ve	-ve	12 L	-ve	0.121	0.032
Cochliobolus lunatus	8357	14 L	16M	19 M	++	0.717	0.135
Fusarium solani	8307	-ve	12 L	12 L	+++	0.922	0.128
Setosphaeria rostrata	8764	16M	14 L	-ve	++	0.795	0.078
Ulocladium chartarum	8311	-ve	-ve	16 M	++	0.725	0.208

For cellulases and pectinase: diameter of clear zones: > 20 mm= high production (H), 16 – 20 mm= moderate production (M), Up to 15 mm= low production (L), AUMC = Assiut University Mycological Centre.

Conclusion: The dark-coloured fungi e.g. *Cladosporium cladosporioides, Aspergillus niger* and *Alternaria alternata* outnumbered the hyaline ones. Environmental factors such as atmospheric conditions, high light intensity, and deep diurnal fluctuations of temperature and humidity may induce selective effects for the advantage of the dematiaceous fungi over the hyaline like. Thus the melanin-containing fungi are more adapted to survive the injurious effects of the atmospheric condition (Moubasher 1993). On the other hand, among

the 8 tested isolates for enzyme-producing capabilities, *Setosphaeria rostrata* was the most potent in producing both exo- and endo- 1,4- β-glucanases while *Cochliobolus lunatus* was the most pectinase-producer and *Fusarium solani* was the most L-asparaginase-producer.

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