Mycosphere 7 (3): 236–261(2016) www.mycosphere.org ISSN 2077 7019
Article
Doi 10.5943/mycosphere/7/3/1

Biodiversity and molecular characterization of yeast and filamentous fungi in the air of citrus and grapevine plantations in Assiut area, Egypt

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Moubasher AH, Abdel-Sater MA, Soliman Zeinab SM 2016 – Biodiversity and molecular characterization of yeast and filamentous fungi in the air of citrus and grapevine plantations in Assiut area, Egypt. Mycosphere 7(3), 236–261, Doi 10.5943/mycosphere/7/3/1

Abstract

A total of 218 species and 3 varieties belonging to 83 genera of filamentous and yeast fungi were recovered from the air of both citrus and grapevine plantations. A relatively higher numbers of genera and species were recovered from the air of citrus plantations compared with those recovered from grapevine plantations. The peak of total propagules of fungi caught from the air of citrus plantations was shown in February on both media and from the air of grapevine in December and August on DYM and DRBC, respectively. Their troughs were shown in June and October on DYM and DRBC, respectively for both citrus and grapevine plantations. The widest spectrum of species recovered from the air of citrus plantations was registered in June on both media and from the air of grapevine plantations in February and in April on DYM and DRBC, respectively. The air of citrus plantations shared the air of grapevine plantations in some highly encountered filamentous fungi on both media (*Cladosporium, Penicillium, Aspergillus* and *Alternaria*) or on one medium (*Cochliobolus, Fusarium, Myrothecium, Phoma* and *Pleospora*). Eighty-four fungal species were isolated from the air of citrus only, while 46 species were isolated from the air of grapevine only.Yeast fungi showed their peak of total propagules from the air of citrus plantations in October and April and from the air of grapevine plantations in June and December on DYM and DRBC, respectively. Fifteen genera and 26 species of yeasts were collected. Two genera of yeasts were encountered in high frequency on one medium and moderate or low on the other medium in the air of both citrus and grapevine plantations and these were *Cryptoccocus* (4 species) and *Rhodotorula* (3 species).

Key Words – Aerobiota – characterization – genotypic – phenotypic - seasonal fluctuation

Introduction

In the atmosphere many microbioparticles are present. These are fungal spores, pollen grains, insect parts. The study of aeromycology is important in plant pathology and in disease forecasting of plant diseases (Gregory 1973). Many fungal diseases of plants are spread by air. The deterioration of stored materials and the spoilage of foodstuffs are induced by growth of fungi which reach them from the air. Some fungal spores are regarded as important causes of allergic diseases such as bronchial asthma and allergic rhinitis. The fungal spores act passively as allergens acting on some individuals who have become sensitized (Moubasher 1993).

Submitted 19 February 2016, Accepted 30 April 2016, Published online 13 May 2016
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Aerobiological studies conducted in relation to respiratory allergic diseases (Hasnain et al. 1984, 1985a,b) revealed that the total air spora of the Auckland region and many of its fungal components show a strong tendency towards an increase during warmer months and a gradual decline during winter. It is well known that fungi require certain optimum conditions for each phase of their growth (Gregory 1973). Aeromycological researchs from the Middle East area are scattered, in Egypt (Moubasher & Moustafa 1974, Abu-El-Souod 1974, Abdel-Hafez et al.1986, Abdul Wahid et al.1996, Ismail et al. 2002), in Kuwait (Moustafa 1975, Khan et al. 1999), in Qatar (Al-Subai 2002), in Saudi Arabia (Abdel-Hafez 1984, Hasnain et al. 2005), in Yemen (El-Essawy et al. 1992), in Turkey (Sarica et al. 2002; Asan et al. 2004, Özkara et al. 2007), in Iran (Hedayati et al. 2005, Nourian et al. 2007) and in Jordan (Shaheen 1992, Al-Qura'n 2008).

Ben-Meir-Glueck (1952) isolated more than thirty different species from the air of orange groves and packing sheds and from the skins of fruits. These included *Penicillium italicum* and *P. digitatum,* which are the main incitants of citrus rot. Barkai-Gollan (1961) studied the air-borne fungi in citrus fruit packing houses and reported that *P. digitatum* and *P. italicum* predominated, whereas *Fusarium, Trichoderma, Colletotrichum* and *Diplodia* were encountered only occasionally. Moubasher et al. (1971) found that the fungal aerospora in citrus plantations was considerably different from that in the soil with *Cladosporium herbarum, Aspergillus niger, Curvularia* sp., *Alternaria alternata, Helminthosporium sativum* and *Fusarium* were the most abundant in the air of citrus plantations. Fifteen isolates from air sampled at the vineyard were identified as *A. niger* (88.2%) and 2 isolates were *A. carbonarius* (11.8%) (Díaz et al. 2009). In the aerospora of tomato plantations *Cladosporium* sp. was found to be the most dominant type followed by species of *Alternaria,* aspergilli, *Nigrospora, Torula* and *Curvularia* (Lohare et al. 2009).

There are several reports on the occurrence of yeasts in the air (Di Menna 1955, Turner 1966, Gregory 1973). Al-Doory (1967) found that species of *Cryptococcus, Rhodotorula, Sporobolomyces,* and *Debaryomyces* were the most dominant species from the air in San Antonia, Texas, U.S.A. *Rhodotorula mucilaginosa* and *Cryptococcus albidus* were the most dominant species followed by *Debaryomyces hansenii* isolated from the air of El-Minia city, Egypt while *Rhodotorula rubra, R. aurantiaca, Kluyveromyces marxianus, Torulaspora delbrueckii, Saccharomyces kluyveri,* and *Hansenula polymorpha* were of less frequency (Haridy 1992).

The present work was designed to investigate the diversity of filamentous and yeast (for the first time in this laboratory) fungi in the air of two economically–important plants (citrus and grapevine). Also, seasonal fluctuations of these fungi were carried out bimonthly during April 2008 to February 2009.

Materials & Methods

Sampling location

This study was carried out in Sahel-Saleem city at approximately 25 km south-east of Assuit city. Sampling was conducted bimonthly over a twelve - month period from April 2008-February 2009. Three different plantations of citrus in the suburbs of Sahel-Saleem city and three of grapevine in El-Khawaled village (about 6 Km to the east border of the river Nile), in the northeast of Sahel-Saleem city were selected.

Isolation of air -borne fungi

Five replicate plates of 9 cm diameter of each of two media (DYM and DRBC) were exposed for five minutes at a height of 60 cm above the ground level during the same hours of the day (10 am-2 pm) at each of the six sites. The plates were then sealed and brought back to the laboratory and incubated at 25ºC for 7-15 days, during which, the developing colonies were counted, isolated and identified. The meteorological data during the period of study were as follows: the maximum temperatures varied from 25ºC–46ºC, the relative humidity from 36-86%. A total of 36 exposures (3 farms of each of citrus and grapevine) were carried out bimonthly, with collectively 360 exposed plates.

Media used for isolation

The exposure plate method relied on two isolation media "yeast extract malt extract agar supplemented with dichloran (DYM) and dichloran rose bengal chloramphenicol agar (DRBC)" were used in this study:

1- Dichloran yeast extract malt extract agar (DYM): Yeast extract malt extract agar (Wickerham 1951) of the following composition was employed: (g/l) yeast extract 3.0, malt extract 3.0, peptone 5.0, glucose 10.0, agar 20.0. Chloramphenicol (250 µg/ml) was used as a bacteriostatic agent. This medium was modified in the present work after preliminary survey by addition of 1 ml/l of 2 mg of dichloran dissolved in 10 ml ethanol which restricts the growth of mucoraceous fungi without affecting the other species.

2- Dichloran rose bengal chloramphenicol agar (DRBC), (King et al. 1979): (g/l) peptone 5.0, KH_2 PO_4 1.0, Mg SO_4 0.5, glucose 10.0, agar 15.0, to which rose bengal (25 µg/ml) and chloramphenicol (100 µg/ml) as bacteriostatic agents (Smith & Dawson 1944, Al- Doory 1980) and dichloran (20 µg/ml) were used.

Identification of filamentous fungi

The following references were used for the identification of fungal genera and species (purely morphologically, based on macroscopic and microscopic features): Booth (1971), Ellis (1971, 1976), Pitt (1979); Domsch et al. (2007); Moubasher (1993), de Hoog et al. (2000), Schroers (2001), Samson & Frisvad (2004), Zare & Gams (2004), Leslie & Summerell (2006), Crous et al. (2007), Samson & Varga (2007), Simmons (2007), and De Seifert et al. (2011).

Identification of yeasts

Morphological characters

Formation of pseudomycelium and true mycelium (Wickerham 1951) and the ability to form ascospores on three sporulation media (corn meal agar, potato glucose agar and yeast extract malt extract agar at 25°C) (Barnett et al. 2000) were tested.

Physiological characters

Fermentation of 14 different sugars (D-glucose, D-galactose, maltose, Me-α-D-glucoside, sucrose, trehalose, melibiose, lactose, cellobiose, melezitose, raffinose, inulin, starch and D-xylose) and oxidative utilization of 36 carbon compounds were performed according to Barnett et al. (2000). Assimilation of 9 nitrogen compounds (potassium nitrate, sodium nitrite, ethylamine-HCl, L-lysine-HCl, creatine, creatinine, D-glucosamine, imidazole, or D-tryptophan) was determined (Suh et al. 2008).

Hydrolysis of urea, growth at high osmotic pressure, growth at different temperatures, growth in the presence of cycloheximide, diazonium blue B (DBB) test and production of extracellular starch-like compounds were tested.

Identification keys of Barnett et al. (2000) were followed to assign each isolate to its species level. Confirmations of these identifications were carried out using the molecular technique.

Molecular methods

Growth of the fungus and DNA extraction

The fungus was grown on Czapek yeast extract agar (CYA) plates and incubated at 25° C for 7 days (for filamentous fungal isolates) and on yeast extract malt extract agar (YMA) plates and incubated at 25° C for 2 days (for yeast isolates). A small amount of fungal growth was scraped and suspended in 100µl of distilled water and boiled at 100° C for 15 minutes and stored at -70° C, and sent to SolGent Company, South Korea, for PCR and rDNA sequencing.

Fungal DNA was extracted and isolated using SolGent purification bead in SolGent Company (Daejeon, South Korea). Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using primers ITS1, ITS4 as follow: universal primer ITS 1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then ampilification was performed using the polymerase chain reaction (PCR) (ABI, 9700). The PCR reaction mixtures were prepared using Solgent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mM dNTPs (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5U) 0.25µl, template1.0 µl, distilled water to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 1 min, with a final extension step of 72 °C for 5 min.

The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. Then the purified PCR products were reconfirmed (using size marker) by electrophoreses of the PCR products on 1% agarose gel. Then these bands were eluted and sequenced. Each sample was sequenced in the sense and antisense direction.

Phylogenetic analysis

Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained with those retrieved from GenBank database were subjected to Clustal W analysis using MegAlign (DNAStar) software version 5.05 for the phylogenetic analysis. Sequence data were deposited in GenBank and accession numbers are given for them.

Statistical analysis – Descriptive statistical analysis was employed (PC-ORD) to analyze mycobiotic data obtained in both habitats (McCune and Mefford 1999).

Results

Identification of fungal genera and species was performed using the morphological and microscopical characteristics in addition to the biochemical in case of yeasts (Table 1). In suspected isolates, molecular techniques [Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using primers ITS1, ITS4] were employed that either confirm the previous methods or disagree with them, and in the latter cases they are registered as unidentified (Table 2).

From the biochemical and physiological data presented in Table (1), it is obvious that only the two ascomycetous yeast species (*Candida parapsilosis* and *Meyerozyma guilliermondii*) could ferment D-glucose. None of basidiomycetous species could ferment any of the 14 sugars tested. In addition, of the ascomycetous species *M. guilliermondii* could also ferment sucrose and raffinose. On the other hand, the 10 basidiomycetous species tested could be differentiated from the ascomycetous species by their ability to hydrolyze urea and to give positive diazonium blue B reaction. Some other assimilation tests of carbon and nitrogen compounds could differentiate between species (Table 1).

A total of 176 and 139 species and 2 varieties belonging to 66 and 58 genera were recovered, respectively from the air of citrus and grapevine plantations (3 farms in the six trips) bimonthly during the period from April 2008 to February 2009. From these, 139 and 108 species and 2 varieties belonging to 59 and 46 genera were recorded on yeast extract malt extract agar supplemented with dichloran (DYM), and 135 and 89 species + 2 or 1 variety related to 58 and 44 genera on dichloran rose bengal chloramphenicol agar (DRBC) from the air of the two plants. The filamentous fungi constituted the greatest part of propagules (96.08% and 94.03% on DYM and 98.67% and 98.49% on DRBC) while yeasts contributed 3.92% and 5.97% and 1.33% and 1.51% of total fungi respectively (Table 3).

Table 1 Physiological comparison of the strains tested of the recorded species.

Species: **1** *Candida parapsilosis* AUMC7750, **2** *Meyerozyma guilliermondii* (=*Pichia guilliermondii)* (anamorph*:Candida guilliermondii*) AUMC7771, **3** *Cryptococcus flavescens* AUMC7794, **4** *C. magnus* AUMC 7793, **5** *Melanopsichium pennsylvanicum* AUMC7785, **6** *Pseudozyma hubiensis* AUMC7786, **7** *Rhodosporidium paludigenum* (anamorph: *Rhodotorula graminis*) AUMC7783, **8** *Rhodotorula glutinis* AUMC 7774, **9** *R. glutinis* AUMC 7776, **10** *Rhodotorula mucilaginosa* AUMC7778, **11** *R. mucilaginosa* AUMC7782, **12** *Sporidiobolus metaroseus (*anamorph: *Sporobolomyces roseus*) AUMC7788, **13** *Sporidiobolus ruineniae* (anamorph: *Sporobolomyces coprophilous*) AUMC7773, **14** *S. ruineniae* AUMC7781, **15** *Trichosporon asahii* AUMC7779.

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Fermentation																
D- glucose	F1	d	d	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	F5	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	F11	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation																
D-glucose	C1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	+	+	+	+	+	+	d	d	d	+	+	w	d	+	+
L-sorbose	C3	+	+	-	d	d	w	d	w	d	-	-	+	+	+	d
D-ribose	C5	-	+	d	d	+	d	+	d	+	+	+	+	+	+	+
D-xylose	C6	+	+	+	+	+	+	+	+	+	d	+	d	+	+	+
L-arabinose	C7	+	+	+	+	+	+									+
L-rhamnose	C9	-	+	+	+	+	-	d	-	-	+	+	-	d	+	+
Sucrose	C10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	C11	+	+	+	+	+	+	d	+	d	+	d	+	d	+	+
α, α-trehalose	C12	+	+	+	+	+	+	+	+	+	+	+w	+w	+	+	+
Methyl-α-D-glucoside	C13	+	+	+	+	+	+	d	d	-	-	-	d	d	-	+
Cellobiose	C14	-	+	+	+	d	+									+
Salicin	C15	-	+		d											
Arbutin	C16	-	+		d											
Lactose	C18	-	d	+	+	d	d	-	-	-	-	-	-	-	-	+
Raffinose	C19	-	+	+	+	+	+									-
Melezitose	C20	+	+	+	+	w	+	-	+	+	+	d	+	-	-	+
Inulin	C21	-	+	+	+	+	+	+	+	+	+	+	d	d	+	+w
Soluble starch	C22	-	+	+	+	+	+	-	+	+	d	-	d	-	-	+
Glycerol	C23	+	+		d											
Meso-erythritol	C24	d	d	w	-	d	+	-	-	-	-	-	+	-	-	+
Xylitol	C26	d	+		d											
D-glucitol	C28	+	+	d	d	d	+	+	d	+	+	d	d	+	+	+
D-mannitol	C29	+	+	+	+	+	+	+	+	+	+	+	-	+	+	d
Galactitol	C30	-	+	d	d	-	-	+	-	-	-	+w	-	+	+	-
Myo-inositol	C31	-	d	+	+	d	d	-	-	-	-	-	-	-	-	+
Glucono-d-lactone	C32	+	d	d	-	+	d	+	d	d	+	+	d	+	+	+
D-glucuronate	C36	-	d	+	+	d	+	-	-	-	-	-	d	-	-	+
D-galacturonate	C37	-	-	+	d	+	w	-	w	+	d	d	-	+	d	-
Succinate	C39	+	+		d											
Citrate	C40	+	+	+	+	d	+	d	+	+	+	d	d	+	d	+
Methanol	C41	w	w	-	-	-	w	-	-	-	w	-	-	-	-	-
Ethanol	C42	+	+	+	-	+	+	+	+	d	+	+	d	+	+	+
Propane 1,2 diol	C43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Butane 2,3 diol	C44	-	-	-	-	d	-	-	-	-	-	-	-	-	-	d
Quinic acid	C45	-	-	-	d	+	+	+	+	+	+	+w	+	+	+	-
Nitrogen compounds																
Nitrate	N1	-	-	-	+	+	+	+	+	+	-	-	+	+	+	-
Nitrite	N2	-	-	+	+	+	+	+	+	+	-	-	w	+	+	+
Ethylamine	N3	+	+	+	-	+	+	+	+	+	+	+	w	+	+	-
L-lysine	N4	+	+	+	-	+	+	-	+w	+	w	-	-	+	+	+
Creatine	N6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Species no.	S	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Creatinine	N7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-glucosamine	N8	-	-	+	w	+	+	-	-	-	-	-	-	-	-	-
Imidazole	N9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tryptophane	N10	-	-	+	-	-	d	w	d	d	+	+	-	-	-	+
Miscellaneous																
0.01% cycloheximide	O1	-	+	d	+	+	d	+	+	+	+	+	+	d	d	+
0.1 % cycloheximide	O2	-	+	w	-	+	d	+	+	+	d	+	w	d	d	+
50% D-glucose	O4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
60% D-glucose	O5	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10% NaCl	O6	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
16% NaCl	O7	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Starch formation	M1	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+
Urea hydrolysis	M3	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Diazonium blue B	M4	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.
Fermentation of 11 sugars gave negative results for all species tested and were omitted from the table.

From the fungi recovered on DYM, 123 and 96 species and 2 varieties belong to 47 and 37 genera of filamentous fungi and 16 and 14 species related to 12 and 9 genera of yeasts were isolated from the air of both plants, respectively. A total of 10555 and 9505 colony forming units (CFUs) were recovered from the air of citrus and grapevine plantations in 18 exposures (Table 2). The highest CFUs were recorded in the three farms collectively in February and December, while the lowest were registered in June on the two plantations (Figs. 1-4). The broadest spectrum of species in the three farms collectively was observed in June but the narrowest was noticed in February.

From the fungi recovered on DRBC, 120 and 83 species and 2 or 1 variety belong to 45 and 38 genera of filamentous fungi, and 16 and 8 species related to 12 and 6 genera of yeasts were isolated from the air of both plants respectively. A total of 9343 and 4644 CFUs were recovered (Table 2). The highest number of CFUs was recorded in the three farms collectively in February and August, while the lowest in October (Figs. 2 & 4). The broadest spectrum of species was observed in June and April in the three farms collectively (69 and 42 species) but the narrowest (14 and 9) in October.

Many genera of filamentous fungi were recorded in high frequency of occurrence but varied in their percentage counts from high (57.57% and 38.24%, and 53.41% and 26.29% on both media for citrus and grapevine plantations respectively) to low (0.24% and 0.26%, and 0.32% and 0.24%) and these were *Cladosporium, Penicillium, Aspergillus, Fusarium, Alternaria, Emericella, Myrothecium, Cochliobolus, Phoma, Pleospora, Setosphaeria, Botryodiplodia, Humicola, Petromyces* and *Rhizopus* (Table 3, Figs 1-4).

Cladosporium was the most common genus in the air of both plants, yielding considerably higher numbers of propagules in citrus plantations (53.41% - 57.57% of total fungi) than those in grapevine plantations (26.29% - 38.24%). Its count peak was regularly recorded in February in the air of citrus plantations on both media but irregularly in the air of grapevine plantations, in December and April on DYM and DRBC respectively. Its trough was registered in August and October on DYM and DRBC respectively in citrus air and in October on both media in grapevine air. In the air of citrus plantations, *C. cladosporioides* was the most common species, contributing about half of total fungi (46.78% - 53.80%). In the air of grapevine it yielded markedly less percentage counts (23.58% - 35.09%). *C. sphaerospermum* was recorded in high frequency in citrus air on both media but in grapevine air it was isolated in moderate and high frequencies on DYM and DRBC respectively. *C. herbarum* was isolated only from the air of grapevine plantation, in low frequency.

Table 2 The Assiut University Mycological Centre accession number (AUMC) of yeast and filamentous fungal [all belonging to Ascomycota except *Quambalaria cyanescens* to Basidiomycota (Ustilaginomycetes, Quambalariaceae)] strains isolated from air of citrus or grapevine plantations with accession GenBank numbers given together with the closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC number	Isolation source	Accession GenBank number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
Filamentous fungi							
5731	Grapevine air	JQ425375	585	EF634410=NRRL 35386T GU092949=NRRL 58014T	100 100	*Hamigera insecticola* *Hamigera inflata*	Peterson et al. 2010
6293	Citrus air	JQ425376	645	DQ317622= CBS357.73^{T}	100	*Quambalaria cyanescens*	de Beer et al. 2006
6707	Citrus air	JQ425377	556	HQ897807=DAOM:195309	93	*Pseudonectria pachysandricola*	Gräfenhan et al. 2011
5748	Grapevine air	JN393254	565	GQ352493=5GP/T EU833205=A1.9 FJ416300 =1002F2 GU595031 = H4307	99	*Aspergillus* sp. *Aspergillus aculeatus* *Aspergillus* sp. *Aspergillus* sp.	Moubasher & Zeinab Soliman 2011 (as *Aspergillus assiutensis*)
5716	Citrus air	JQ425381	558	GQ169452 = N11	100	*Pleosporaceae* sp.	
6241	Citrus air	JQ425386	596	HQ909075= GU291796=CBS125525^{T} AY924288 = AZ-32	100 99	*Bartalinia* sp. *Bartalinia pondoensis* *Bartalinia robillardoides*	Suryanarayanan et al. 2011, Marincowitz et al. 2010
Yeast fungi							
No 15 (dead)	Grapevine air	JQ425363	608	AY070006 = AS 2.2108 EU003482 = CBS 7683^{T}	100 99	*Sporidiobolus metaroseus* (anamorph: *Sporobolomyces roseus*)	Valerio et al. 2008
7788	Citrus air	JQ425365	582	AY015435=CBS5541 EU003482=CBS7683^{T}	90 89	*Sporidiobolus metaroseus (*anam: *Sporobolomyces roseus*)	Valerio et al. 2008
7785	Citrus air	JQ425368	777	AY740040	96	*Melanopsichium pennsylvanicum*	Stoll et al. 2005
7793	Grapevine air	JQ425369	612	AF190008 = CBS 140^{T}	89	*Cryptococcus magnus*	Fell et al. 2000
7776	Citrus air	JQ425370	618	HQ670677	99	*Rhodotorula glutinis*	Yang et al. 2011
7778	Citrus air	JQ425392	629	HQ909092=KDLYC24-1 AF444541= CBS 316^{T}	99	*Rhodotorula mucilaginosa*	Scorzetti et al. 2002
7781	Citrus air	JQ425394	610	AY015433 = CBS 5001^{T}	99	*Sporidiobolus ruineniae* (anamorph: *Sporobolomyces coprophilous*)	Fell et al. 2002
7783	Citrus air	JQ425395	616	AF444493= CBS 6567 AF444492= CBS 6566^{T}	99 99	*Rhodosporidium paludigenum* (anamorph: *Rhodotorula graminis*)	Scorzetti et al. 2002
7774	Grapevine air	JQ425397	618	HQ670677 EF194846 =MCCC2E00215	99	*Rhodotorula glutinis*	Yang et al. 2011

AUMC number	Isolation source	Accession GenBank number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7782	Grapevine air	JQ425399	633	HQ909092 = KDLYC24-1 AF444541 = CBS 316 ^{T}	99 98	*Rhodotorula mucilaginosa*	Scorzetti et al. 2002
7794	Grapevine air	JQ425400	539	FN428902 = IMUFRJ 51986 AM176643	99	*Cryptococcus flavescens*	Molnár & Prillinger 2005
7779	Grapevine air	JQ425402	553	AM900369 = YS124 FJ943429 = CBS 2479^{T}	99	*Trichosporon asahii*	
7773	Citrus air	JQ425373	613	AY015433 = CBS 5001^{T}	99	*Sporidiobolus ruineniae* (anamorph: *Sporobolomyces coprophilous*)	Fell et al. 2002
7786	Citrus air	JQ425374	987	DQ008954=CBS 10077^{T} HQ832814 = LH146	98 97	*Pseudozyma hubeiensis*	Wang et. al. 2006
7263	Citrus air	JQ425353	620	EF197943 = HK67-4 EF190231 = wwl-2 1	100	*Debaryomyces hansenii* (anamorph:*Candida famata*)	
7750	Citrus air	JQ425354	503	FJ872016 = CBS 604^{T}	100	*Candida parapsilosis*	
7771	Grapevine air	JQ425356	590	EF197814 = HK53 EU568971=CNRMA200500864	100	*Meyerozyma guilliermondii* (=*Pichia guilliermondii)* (anamorph:*Candida guilliermondii*)	Desnos-Ollivier et al. 2008
7264	Citrus air	JQ425359	635	EF197943= HK67-4 AB220029 = IFM 54258^{T}	100	*Debaryomyces hansenii* *D. nepalensis*	Moretti et al. 2007

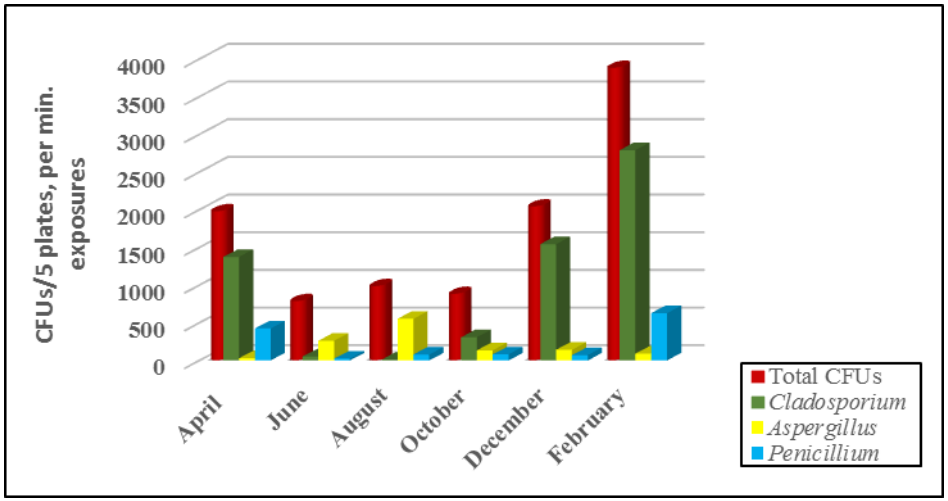

Fig. 1 – Bimonthly counts of common fungi in the air of citrus plantations on DYM during the period of study.

Aspergillus was one of the most common genera in both plants contributing relatively higher numbers of propagules in the air of grapevine plantations (25.26 % - 25.77 % of total fungi) than those from citrus plantations (11.47 % - 13.98 %). Its peak was regularly recorded in August in both plants on both media while its trough was registered in April and October on DYM and DRBC respectively in citrus air and in December on both media in grapevine. Fifteen species of *Aspergillus* were recorded from citrus air while two species were isolated from grapevine only. Species of *Aspergillus* belonging to section *Nigri* (*A. aculeatinus, A. aculeatus, A. brasiliensis* and *A. niger*) were the most common among its species in the air of grapevine plantations contributing relatively large proportions of *Aspergillus* count compared with those found in citrus air. On the other hand, *A. ochraceus* was the most common species in the air of citrus, contributing large numbers of propagules while it was recorded in moderate and low frequencies in grapevine air.

Alternaria was the second most frequent genus behind *Aspergillus* in grapevine air and after *Aspergillus* and *Cladosporium* in citrus air, yielding considerably higher numbers of propagules in the air of grapevine plantations (13.41%-17.44% of total fungi) than those from citrus plantations (2.69%-2.70%). Its peak was recorded in February and December in the air of citrus plantations on DYM and on DRBC respectively but regularly in December in the air of grapevine plantations on both media. Its trough was registered in August on both media in citrus air and in April and June on DYM and DRBC respectively in grapevine. In the air of both plantations, *A. alternata* was the most common, contributing considerably higher percentage counts of total fungi in grapevine air (10.45% - 13.65%) than those of citrus air (2.23% - 2.35%). *A. citri* was recorded in moderate and low frequencies in citrus air on DYM and DRBC respectively while it was missed in grapevine air. *A. astragali, A. nucis, A. raphani, A. tenuissima,* and *A. tuberculata* were isolated only from the air of grapevine plantations.

Fusarium was of high frequency in citrus air, yielding small numbers of propagules (1.22% - 2.92% of total fungi). In grapevine plantations, it was isolated in high or moderate frequency, contributing 1.67% - 0.84% of total fungi. Its peak was regularly recorded in June in the air of citrus plantations on both media but irregularly in the air of grapevine plantations, in October and August on DYM and DRBC respectively. Its trough was registered in October and April in citrus air on DYM and DRBC respectively and in June and February in grapevine air. In the air of citrus plantations, 9 species were identified of which *F. semitectum* was the most common species, contributing 0.56%-1.90% of total fungi. In the air of grapevine it was recorded in high or moderate frequency, yielding 0.43%-1.22% of total fungi. *F. equiseti* and *F. concolor* were recorded in high or moderate frequency in citrus air, while in grapevine air, *F. equiseti* was isolated in moderate frequency and *F. concolor* in rare frequency on DYM only. *F. camptoceras* and *F. torulosum* were isolated from citrus air only while *F. chlamydosporum, F. circinatum, F. konzum, F. proliferatum, F. pseudonygami* and *F.scripi* were isolated from grapevine air only.

Penicillium was one of the most common genera contributing noticeably higher numbers of propagules in the air of citrus plantations (12.38% and 19.99% of total fungi on DYM and DRBC respectively) than those from grapevine plantations (3.34% - 5.51%). Its peak was recorded in February and April on DYM and DRBC respectively in citrus air and in August on both media in grapevine air, while its trough was registered in June and October on DYM and DRBC respectively in citrus air and in December on both media in grapevine. In citrus air, *Penicillium olsonii* was the most common species contributing 7.54% - 10.24% of total fungi while it was missed in grapevine air. *P. citrinum* was isolated in high or moderate frequency from citrus air but was recorded in rare frequency on both media from grapevine air. On the other hand, *P. oxalicum* was the most common species in the air of grapevine, contributing relatively the largest proportions of *Penicillium* counts (3.03 % - 5.28 % of total fungi) while it was recorded in low frequency in citrus air. Fifteen species of *Penicillium* were recorded in citrus air while two species in grapevine only (Table 2).

Cochliobolus (2 species) was recorded in high frequency in the air of both plantations. The most prevalent species was *C. lunatus* followed by *C. australiensis. Curvularia* (4 species) was isolated in low frequency from the air of both plantations.

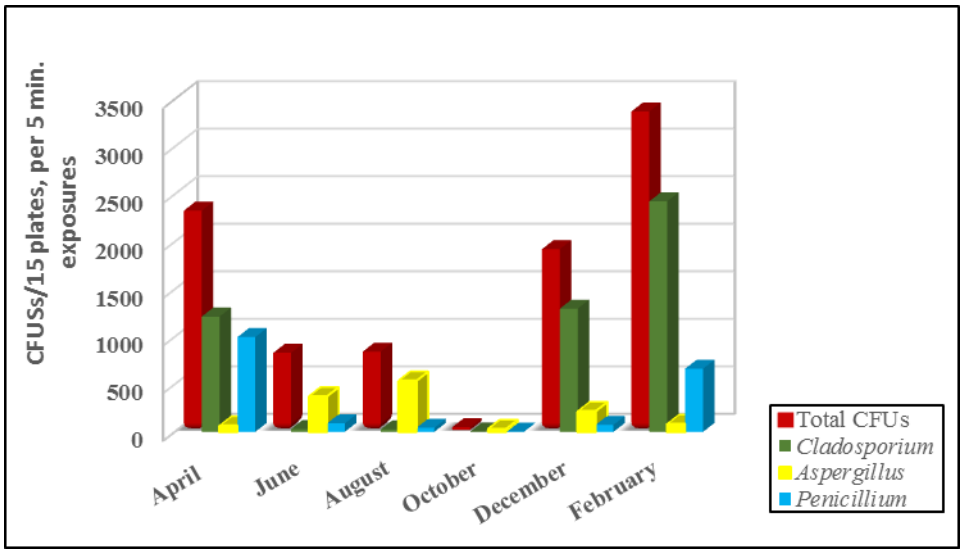

Fig. 2 – Bimonthly counts of common fungi in the air of citrus plantations on DRBC during the period of study.

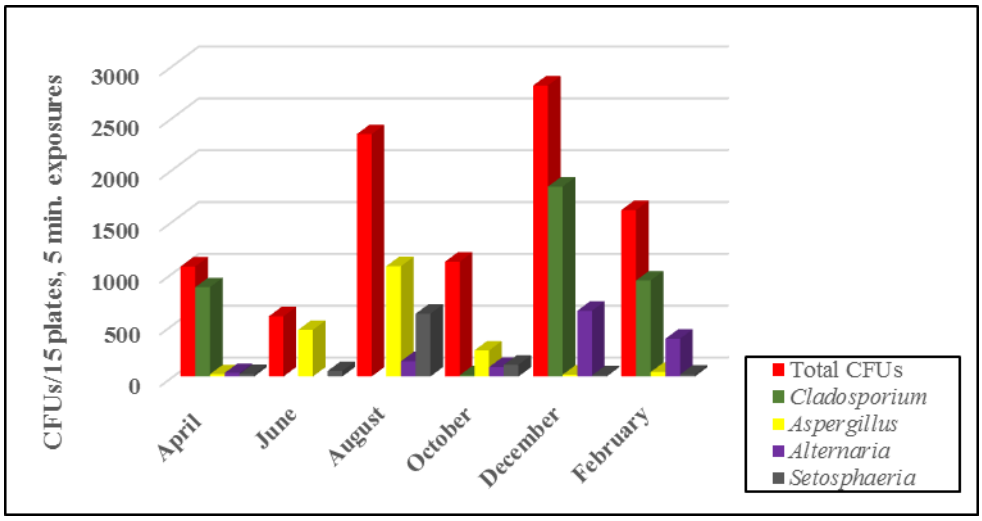

Fig. 3 – Bimonthly counts of common fungi in the air of grapevine plantations on DYM during the period of study.

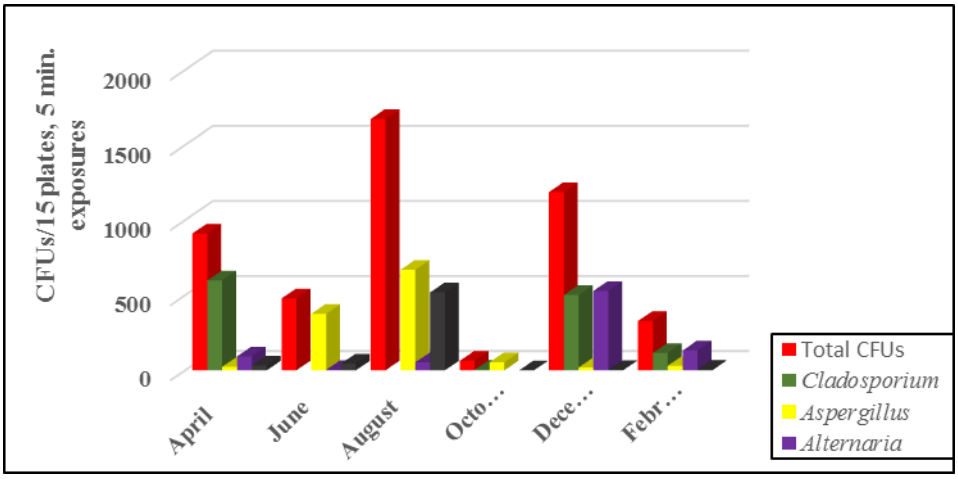

Fig. 4 – Bimonthly counts of common fungi in the air of grapevine plantations on DRBC during the period of study.

Table 3 Collective data of counts (CFU), percentage counts (CFU%) calculated to total fungi, frequency (F) and occurrence remarks (O) of fungi recovered from the air of citrus and grapevine plantations on DYM and DRBC agar media bimonthly, during the period from April 2008-February 2009 (counts of CFU calculated per 5 minutes exposures in each sample, collectively in 18 samples in each plantation).

	Citrus						**Grapevine**					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Filamentous fungi	10140	96.08	18 H	9219	98.67	18 H	8947	94.03	18 H	4574	98.49	18 H
Absidia	15	0.14	6M	6	0.06	3L				1	0.02	1R
A. cylindrospora	14	0.13	5M	6	0.06	3L				1	0.02	1R
Acremonium	1	0.01	1R	2	0.02	2R	11	0.12	4L	2	0.04	1R
A. fusidioides				1	0.01	1R				2	0.04	1R
A. hyalinulum				1	0.01	1R	9	0.09	3L			
Alternaria	285	2.70	18H	251	2.69	11H	1274	13.41	14H	810	17.44	12H
A. alternata	235	2.23	18H	219	2.35	11H	993	10.45	13H	634	13.65	11H
A. citri	28	0.27	5M	4	0.04	3L						
A. chlamydospora	8	0.08	5M	5	0.05	2R	88	0.91	7M	114	2.45	8M
Alternaria spp.	14	0.13	4L	23	0.24	4L	157	1.64	11H	55	1.18	8M
Aspergillus	1212	11.47	18H	1306	13.98	18H	1851	19.48	18H	1173	25.26	17H
A.aculeatinus							181	0.91	7M	125	2.69	5M
A. aculeatus	44	0.42	6M	25	0.27	4L	322	9.70	14H	183	3.94	14H
A. auricomus	8	0.08	1R	1	0.01	1R	3	0.03	2R			
A. brasiliensis	29	0.27	10H	93	0.99	9H	117	1.23	9M	152	3.27	10H
A. bridgeri	7	0.07	1R	3	0.03	1R						
A. calidoustus	8	0.08	2R	9	0.10	2R				1	0.02	1R
A. campestris	14	0.13	1R	4	0.04	1R						
A. candidus	3	0.03	1R	6	0.06	2R						
A. clavatus	6	0.06	4L	2	0.02	2R						
A. dimorphicus	59	0.56	6M	120	1.28	7M	3	0.03	2R	1	0.02	1R
A. flavus var. *columnaris*	3	0.03	2R	1	0.01	1R	1	0.01	1R			
A. insulicola	4	0.04	3L	5	0.05	2R						
A. japonicus	10	0.10	2R				17	0.18	2R	175	3.77	2
A. lacticoffeatus	1	0.01	1R	16	0.17	3L						
A. niger	186	1.76	17H	147	1.57	13H	1065	11.21	18H	467	10.66	16H
A. ochraceus	729	6.91	17H	700	7.49	14H	45	0.47	7M	13	0.28	4L
A. ostianus	18	0.17	4L	93	0.99	2R						
A. proliferans				7	0.07	1R				14	0.30	1R
A. robustus	9	0.09	4L	24	0.26	4L						
A. sclerotiorum	5	0.05	2R									
A. sulphureus	4	0.04	2R									

	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
A. sydowii	5	0.05	2R	1	0.01	1R	5	0.05	3R	2	O.04	2R
A. terreus	4	0.04	3L	7	0.07	3L	15	016	5L	7	0,15	6M
A. terreus var. *aureus*	6	0.06	2R	4	0.04	1R	63	0.66	4L	7	0.15	1R
A. tubingensis	37	0.35	3L	21	0.22	3L	11	0.12	1R	25	0.54	2R
A. ustus	1	0.01	1R	1	0.01	1R	1	0.01	1R			
A. versicolor	5	0.05	3L	2	0.02	2R	2	0.02	1R			
Bartalinia pondoensis	3	0.03	2R									
Basidiomycete sp.	9	0.09	3L	3	0.03	1R						
Beltrania querna	104	0.99	8M	97	1.04	5M						
Bipolaris				8	0.09	2 R	14	0.15	4 L	7	0.15	2R
B. papendorfii							10	0.11	4L	7	0.15	2R
B. subpapendorfii				8	0.09	2 R	4	0.04	1R			
Botryodiplodia theobromae	25	0.24	11H	12	0.13	7M	2	0.02	2R	3	0.06	3R
Botryotrichum sp.	1	0.01	1R	3	0.03	3L				8	0.17	2R
Cladosporium	6076	57.57	18H	4990	53.41	16H	3634	38.24	11H	1221	26.29	10H
C. cladosporioides	5678	53.80	18H	4371	46.78	12H	3335	35.09	11H	1095	23.53	9H
C. oxysporum	212	2.01	7M	269	2.88	8M	15	0.16	4L	31	0.67	6M
C. sphaerospermum	185	1.75	12H	349	3.74	14H	179	1.88	6M	89	1.92	9H
C. spongiosum	1	0.01	1R	1	0.01	1R	105	1.11	2R	2	0.04	1R
Clonostachys	6	0.06	2R	3	0.03	2R	3	0.03	2R			
C. rogersoniana	5	0.05	1R	1	0.01	1R						
C. rosea							3	0.03	2R			
Cochliobolus	93	0.89	10H	34	0.46	5M	222	2.34	13H	40	0.86	9H
C. australiensis	46	0.44	6M	4	0.04	2R	48	0.51	6M	15	0.32	5M
C. lunatus	47	0.45	10H	30	0.32	4L	172	1.81	8M	23	0.50	5M
C. tuberculatus							2	0.02	1R	2	0.04	2R
Curvularia	3	0.03	3 L	1	0.01	1 R	16	0.17	3 L	1	0.02	1R
C. clavata	3	0.03	3 L				15	0.16	3L	1	0.02	1R
Cylindrocladium intermedium	3	0.03	2R									
Dichocladosporium chlorocephalum	49	0.46	4L	17	0.18	5M	199	2.09	5M	13	0.28	4L
Embellisia didymospora							6	0.06	3L	3	0.06	2R
Emericella	190	1.80	15H	153	1.64	13H	53	0.56	7M	47	1.01	7M
E. dentata	15	0.14	3L	9	0.10	3L						
E. nidulans				3	0.03	1R	2	0.02	2R	1	0.02	1R
E. quadrilineata	1	0.01	1R	2	0.02	2R	3	0.03	3L	1	0.02	1R
E. rugulosa	1	0.01	1R	2	0.02	1R						
E. stella-maris	28	0.27	2R	8	0.09	4L						

	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
E. variecolor	144	1.36	13H	117	1.25	12H	46	0.48	4L	40	0.86	5M
Emericella sp.				11	0.12	2R	2	0.02	1R	3	0.06	1R
Eurotium amstelodami	2	0.02	2R	5	0.05	1R	1	0.01	1R			
Fennellia flavipes	6	0.06	2R	4	0.04	2R				1	0.02	1R
Fusarium	308	2.92	18H	114	1.22	13H	159	1.67	15H	39	0.84	8M
F. babinda	3	0.03	2R				2	0.02	1R	3	0.06	1R
F. camptoceras	17	0.16	3L	14	0.15	3L						
F. chlamydosporum							2	0.02	2R			
F. circinatum							3	0.03	2R			
F. concolor	28	0.27	9H	10	0.11	5M	4	0.04	1R			
F. equiseti	34	0.32	9H	18	0.19	8M	22	0.23	6M			
F. konzum							1	0.01	1R	1	0.02	1R
F. oxysporum				3	0.03	2R	1	0.01	1R			
F. proliferatum							2	0.02	2R	20	0.43	1R
F. pseudonygami							1	0.01	1R	2	0.04	1R
F. semitectum	201	1.90	18H	52	0.56	11H	116	1.22	12H	8	0.17	5M
F. solani	15	0.14	3L	11	0.12	2R				4	0.09	2R
F. subglutinans				2	0.02	1R	3	0.03	3R	1	0.02	1R
F. verticillioides	9	0.09	4L	4	0.04	2R	1	0.01	1R			
Humicola	24	0.23	8M	30	0.32	11H	8	0.08	4L	1	0.02	1R
H. fuscoatra	23	0.22	8M	30	0.32	11H	7	0.07	3L	1	0.02	1R
H. grisea	1	0.01	1R				1	0.01	1R			
Memmnoniella echinata	1	0.01	1R	1	0.01	1R						
Microascus brevicaulis	11	0.10	4L	5	0.05	1R	1	0.01	1R	3	0.06	1R
Microdochium dimerum							15	0.16	1R	5	0.11	2R
Mortierella alpina	3	0.03	2R	1	0.01	1R						
Mucor	9	0.09	6M	12	0.13	3L				1	0.02	1R
M. circinelloides	4	0.04	3L	2	0.02	1R						
M. hiemalis	4	0.04	3L	10	0.11	3L				1	0.02	1R
Myrothecium	113	1.07	12H	69	0.74	12H	46	0.48	9H	46	0.99	5M
M. roridum	4	0.04	2R	12	0.13	3L	8	0.08	3L			
M. verrucaria	109	1.03	11H	57	0.61	11H	38	0.40	7M	46	0.99	5M
Neosartorya fumigata							4	0.04	3L			
Nigrospora oryzae	8	0.08	4L	5	0.05	3L	8	0.08	4L	4	0.09	3L
Paecillium lilacinum							44	0.46	1R	53	1.14	1R
Penicillium	1307	12.38	18H	1868	19.99	16H	317	3.34	16H	256	5.51	10H
P. adametzioides	95	0.90	1R	35	0.37	1R				1	0.02	1R
P. aurantiogriseum	116	1.10	3L	245	2.62	3L						

	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
P. bilaii	33	0.31	2R									
P. brevicompactum	34	0.32	5M	57	0.61	3L	6	0.06	1R	2	0.04	1R
P. citrinum	114	1.08	9H	153	1.64	6M	1	0.01	1R	1	0.02	1R
P. corylophilum				2	0.02	1R				1	0.02	1R
P. crustosum	1	0.01	1R	3	0.03	1R						
P. cyaneum	4	0.04	2R									
P. duclauxii	15	0.14	4L	14	0.15	3L	4	0.04	3L			
P. expansum	2	0.02	1R				1	0.01	1R			
P. funiculosum	3	0.03	2R	4	0.04	2R						
P. glabrum				116	1.24	2R	1	0.01	1R			
P. granulatum				4	0.04	1R				1	0.02	1R
P. griseofulvum	38	0.36	5M	47	0.50	4L	1	0.01	1R			
P. implicatum	2	0.02	2R									
P. islandicum				1	0.01	1R	2	0.02	1R			
P. italicum				2	0.02	2R						
P. olsonii	796	7.54	9H	957	10.24	11H						
P. oxalicum	12	0.11	3L	12	0.13	4L	287	3.02	13H	245	5.28	9H
P. pinophilum	2	0.02	2R	6	0.06	3L	1	0.01	1R			
P. puberulum	18	0.17	3L	5	0.05	2R						
P. purpurogenum	17	0.16	7M	20	0.21	6M	12	0.13	5M	4	0.09	2R
P. verrucosum				7	0.07	2R						
P. vulpinum	3	0.03	2R	62	0.66	1R						
Petromyces flavus (ana: *Aspergillus flavus*)	19	0.18	9H	29	0.31	7M	13	0.14	5M	11	0.24	9H
Phoma	91	0.86	14H	46	0.49	6M	171	1.80	13H	63	1.36	8M
P. epicoccina	88	0.83	14H	46	0.49	6M	138	1.45	12H	57	1.23	8M
P. eupyrena							32	0.34	2R			
Phoma sp.	3	0.03	2R				1	0.01	1R			
Pleospora	45	0.43	10H	46	0.49	11H	35	0.37	7M	36	0.78	9H
P. tarda	43	0.41	10H	45	0.48	11H	35	0.37	7M	36	0.78	9H
Pochonia sp.	2	0.02	2R							1	0.02	1R
Pseudonectria pachysandricola	15	0.14	5M	3	0,03	1R						
Quambalaria cyanescens	8	0.08	1R	3	0.03	1R	10	0.11	2R	2	0.04	2R
Rhizopus oryzae	7	0.07	7M	1	0.01	1R	25	0.26	10H	23	0.49	3L
Sarcopodium araliae				2	0.02	2R						
Scytalidium sp.				2	0.02	1R	1	0.01	1R			
Setosphearia	48	0.46	8M	40	0.43	5M	763	8.03	17H	605	13.03	15H
S. rostrata	47	0.45	8M	40	0.43	5M	763	8.03	17H	603	12.98	15H
Stachybotrys chartarum	14	0.13	3L	6	0.06	2R	3	0.03	1R			

	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Stemphylium	4	0.04	4L	23	0.25	5M	11	0.12	2R	71	1.53	6M
S. sarciniforme	1	0.01	1R				8	0.08	2R	6	0.13	2R
Stemphylium spp.	3	0.03	3L	23	0.25	5M	3	0.03	1R	65	1.39	6M
Talaromyces stipitatus	1	0.01	1R	1	0.01	1R				6	0.13	1R
Toxicocladosporium irritans	1	0.01	1R	2	0.02	1R						
Trichoderma	6	0.06	6M	6	0.06	5M				4	0.09	3L
T. harzianum	1	0.01	1R	3	0.03	2R				1	0.02	1R
T. paraceramosum	3	0.03	3L	1	0.01	1R				3	0.06	2R
T. reesei	1	0.01	1R	2	0.02	2R						
Ulocladium				2	0.02	2R	19	0.20	2R	8	0.17	2R
U. atrum				1	0.01	1R	19	0.20	2R	8	0.17	2R
Volutella sp.	6	0.06	2R	1	0.01	1R						
Yeasts	414	3.92	16H	125	1.34	14H	557	5.86	15H	70	1.51	7M
Candida	2	0.02	2 R	5	0.05	3 L						
C. catenulate	2	0.02	2 R									
C. parapsilosis				5	0.05	3 L						
Cryptococcus	66	0.63	9 H	47	0.50	7 M	58	0.62	10 H	37	0.79	6 M
C. albidus	66	0.63	9 H	47	0.50	7 M	33	0.35	6 M	20	0.43	4 L
C. laurentii							17	0.18	4 L	17	0.37	6 M
Debaryomyces	20	0.19	4 L	10	0.11	5 M	7	0.07	4 L	2	0.04	1 R
D. hansenii	15	0.14	3 L	8	0.09	4 L	3	0.03	2 R	2	0.04	1 R
D. pseudopolymorphus	5	0.05	2 R	2	0.02	1 R	4	0.04	2 R			
Geotrichum	2	0.02	1 R	2	0.02	2 R						
G. citri-aurantii	2	0.02	1 R	1	0.02	1 R						
Hanseniaspora occidentalis	24	0.23	2 R	2	0.02	1 R						
Issatchenkia orientalis	4	0.04	1 R	3	0.03	2 R	1	0.01	1 R			
Melanopsichium pennsylvanicum	2	0.02	1 R	1	0.01	1 R						
Pichia	18	0.17	2 R	7	0.07	1 R	53	0.57	3 L	8	0.17	3 L
P. guilliermondii	17	0.16	1 R	7	0.07	1 R	53	0.57	3 L	8	0.17	3 L
Pseudozyma	2	0.02	1 R	9	0.09	1 R	5	0.05	1 R			
P. hubeinsis	2	0.02	1 R	9	0.09	1 R						
Rhodosporidium paludigenum	93	0.88	6 M	12	0.13	4 L						
Rhodotorula	63	0.59	10 H	18	0.19	5 M	428	4.58	9 H	18	0.39	3 L
R. aurantiaca	2	0.02	2 R									
R. glutinis	12	0.11	4 L	2	0.02	1 R	378	4.05	6 M	16	0.34	3 L
R. mucilaginosa	49	0.46	5 M	16	0.17	4 L	50	0.54	4 L	2	0.04	1 R
Sporidiobolus	119	1.14	5 M	5	0.05	3 L	5	0.05	3L	2	0.04	1 R
S. ruineniae	119	1.14	5 M	5	0.05	3 L	4	0.04	2 R	2	0.04	1 R

	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Total CFUs	10555	100	18H	9343	100	18H	9505	100	18H	4644	100	18H
No. of genera (83)	59			58			46			44		
No. of species + varieties (218+3)	139+2 var.			135+2 var.			108+2 var.			89+1 variety		

*O = Occurrence remarks: H = high, 9 - 18; M = moderate, 5 - 8; L = Low, 3 - 4; R = rare, 1 - 2 exposures. Fungal species that were isolated in one exposure were omitted from the table (but their frequency of occurrence and counts were included in statistical analysis).

Table 4a NMDS statistical analysis of data obtained from the frequency of occurrence of genera and species recovered from the two habitats on the 2 isolation media.

Parameter		**Citrus air**		**Grapevine air**	
		DYM	**DRBC**	**DYM**	**DRBC**
Genera vs Habitats	S	59	58	46	44
	E	0.900	0.892	0.889	0.890
	H	3.669	3.623	3.404	3.369
	D	0.9675	0.9644	0.9577	0.9551
	n	73	67		
	No of genera in one plant	27	23	14	9
	No of genera in 2 plants	32	35	32	35
Species vs Habitats	S	141	137	110	90
	E	0.917	0.922	0.909	0.904
	H	4.540	4.537	4.274	4.066
	D	0.9854	0.9854	0.9807	0.9764
	n	185	164		
	No of species in one plant	75	74	44	27
	No of species in 2 plants	66	63	66	63

S = Genera and species richness, E = Evenness of frequency of occurrence of genera or species in the samples, H = Shannon's index of genera or species diversity, $H = -\sum_i^s Pi \ln Pi$, D = Simpson's index of genera or species diversity, $D = -\sum_i^s Pi^2$.

Table 4b NMDS statistical analysis of data obtained from the total counts of all species (CFUs) recovered from the two habitats on the 2 isolation media.

Parameter		Citrus air		Grapevine air	
		DYM	DRBC	DYM	DRBC
Genera vs Habitats	E	0.415	0.384	0.528	0.543
	H	1.692	1.561	2.023	2.054
	D	0.6374	0.6536	0.7866	0.8155
Species vs Habitats	E	0.467	0.501	0.559	0.618
	H	2.314	2.463	2.618	2.780
	D	0.6969	0.7596	0.8406	0.8892
	Total counts of all species	10555	9343	9505	4644

Emericella (10 species and 3 unidentified) was isolated in high frequency from citrus air and in moderate frequency from grapevine air. *Emericella variecolor* was the most common species in citrus and grapevine plantations. It is worthy to mention that the isolation of *E. stella-maris* in this study from citrus air is a second world record after its first description in 2008.

Myrothecium (represented by *M. verrucaria*, *M. roridum* and *Myrothecium* sp.) was recovered in high frequency from the air of both plantations. *M. verrucaria* was the most common species followed by *M. roridum* in both plantations.

Botryodiplodia (*B. theobromae*) was recorded in high or moderate frequency from citrus air and in rare frequency from grapevine air. On contrary, *Setosphaeria* (*S. monoceras, S. rostrata* and *S. pedicillata*) was isolated in high frequency from grapevine air and in moderate frequency from citrus air, with *S. rostrata* being the main species, whereas *S. monoceras* was isolated from citrus air and *S. pedicillata* from grapevine air only.

Phoma (5 species and 1 unidentified) and *Pleospora* (*P. allii*, *P. herbarum* and *P. tarda*, teleomorphs of *Stemphylium vesicarium*, *S. herbarum* and *S. botryosum* respectively) were recorded in high frequency in the air of both plantations.The most common *Phoma* species was *P. epicoccina* (= *Epicoccum nigrum*) in both plantations, followed by *P. eupyrena* in grapevine plantations, while *Pleospora tarda* was the most frequent species isolated from both plantations and *P. herbarum* was more common in citrus plantations than grapevine.

Trichoderma (9 species and 1 unidentified) was encountered in moderate frequency from citrus air and in low frequency from grapevine air. The most common species were *T. harzianum* and *T.reesei* in citrus plantations and *T. paracemosum* in both plantations.

Rhizopus (*R. oryzae*) was recorded in high frequency from grapevine air and in moderate frequency from citrus air while *Mucor* (4 species with *M. circinelloides* being the most common in both air followed by *M. hiemalis*) was recovered in moderate frequency from citrus air and rare from grapevine plantations. *Humicola* (3 species) was isolated in high frequency from citrus air and in low frequency from grapevine air. *H. fuscoatra* was more common than *H. grisea* in both plantations. *Stachybotrys* (*S. chartarum* and synnematous species of *Stachybotrys*) was recorded in rare frequency from the air of grapevine while in low frequency from citrus air.

Nigrospora (*N. oryzae* and *N. sphaerica*) was identified in low frequency from the air of both plantations. *N. oryzae* was more frequent than *N. sphaerica*.

Acremonium (9 species and 1 unidentified) was isolated in low frequency from grapevine air and in rare frequency from citrus air. *A. strictum* was the most common species followed by *A. fusidioides* and *A. hyalinulum.* On contrary, *Microascus* (*M. brevicaulis* and *M. manginii*, teleomorphs of *Scopulariopsis. brevicaulis* and *S. candida* respectively) was isolated in low frequency from citrus air and in rare frequency from grapevine air.

Yeast fungi represented by 15 genera and 26 species were isolated from the air of both plantations. They showed their peak of total propagules caught from the air of citrus plantations in December on both media and from grapevine plantations in October and April on DYM and DRBC respectively, while their trough occurred regularly in April on both media in the air of citrus and in June and December on DYM and DRBC respectively in grapevine air.

Two genera of yeast fungi were encountered in high frequency on one medium and moderate or low on the other medium in the air of both plantations and these were *Cryptoccocus* (4 species) and *Rhodotorula* (3 species). On the other hand, two genera were recovered in moderate or low frequency from the air of citrus and low or rare frequency from grapevine air and these were *Debaryomyces* (2 species) and *Sporidiobolus* (*S. ruineniae*). Some yeast genera were recovered only from the air of citrus (*Ambrosiozyma, Candida, Geotrichum, Hanseniaspora, Rhodosporidium* and *Melanopsichium*) while *Sporobolomyces* and *Trichosporon* from grapevine plantations only (Table 2).

Cryptococcus was recovered in high or moderate frequency in both plantations. It contributed 0.50 % - 0.63 % of total fungi in citrus air and 0.62 % - 0.79 % in grapevine air. *C. albidus* was recovered in high or moderate frequency while it was recorded in moderate or low frequency in grapevine air. *C. carnescence, C. flavescens* and *C. laurentii* were isolated from grapevine air only.

Rhodotorula was recorded in high or moderate frequency in citrus air while in high or low frequency in grapevine air, constituting 0.59 %-0.19 % and 4.58 %-0.39 % of total fungi respectively. *R. mucilaginosa* was more common in citrus air than *R. glutinis* while the reverse occurred in grapevine air. *R. aurantiaca* was recorded in rare frequency from citrus air only.

Candida was isolated in citrus air in low or rare frequency, but was missed in grapevine air. *Candida catenulata* was recovered on DYM while *C. parapsilosis* was isolated on DRBC.
Debaryomyces was recorded in citrus air in moderate or low frequency while in low or rare frequency in grapevine air, contributing minute percentage counts (0.63 %-0.50 % of total fungi) in citrus air and (0.35 %-0.43 %) in grapevine air. *D. hansenii* was recovered in low frequency on both media in citrus air and in rare frequency in grapevine air. *D. pseudopolymorphus* was recorded in rare frequency in the air of both plantations.

Statistical analysis using NMDS method

Data presented in table (4a) reveal that the evenness of actual presence (frequency of occurrence) of genera and species was slightly higher in citrus air than in grapevine air on both media. Shannon's index (H) indicates that the diversity of genera and species was relatively higher in citrus than those in grapevine air. Simpson's index (D) indicates that the genera and species diversity was more proportionally important in citrus than in grapevine air. When the counts of all species were considered instead of frequency for calculations (Table 4b), Shannon's index (H) indicates that the diversity of genera and species was relatively higher in grapevine than those in citrus air and Simpson's index (D) indicates that the genera and species diversity was more proportionally important in grapevine than in citrus air.

Discussion

A total of 176 and 138 species and 3 varieties belonging to 66 and 58 genera were recovered respectively, from the air of citrus and grapevine plantations (3 farms in the six trips) bimonthly during the period from April 2008 to February 2009. Citrus plant has an advantage of being taller tree with dense evergreen foliage that may affect most environments around it more positively than grapevine. The filamentous fungi constituted the greatest part of propagules (96.08% and 94.03% on DYM and 98.67% and 98.49% on DRBC) while yeasts contributed 3.92% and 5.97% and 1.33% and 1.51% of total fungi in the two plantations respectively.

DYM regularly sustained a broader spectrum of genera and species in the air of the two plantations then DRBC (139 species + 2 varieties and 59 genera in citrus versus 108 species + 2 varieties and 46 genera in grapevine on DYM) and denser number of CFUs (20089 versus 13987).

The most common genera of filamentous fungi were *Cladosporium, Penicillium, Aspergillus, Fusarium, Alternaria, Emericella, Myrothecium, Cochliobolus, Phoma, Pleospora, Setosphaeria, Botryodiplodia, Humicola, Petromyces* and *Rhizopus*.

Cladosporium was the most dominant fungus in the air of both plants, yielding considerably higher numbers of propagules in citrus plantations than in grapevine. Its count peak was regularly

recorded in winter/spring months, while its trough was registered in summer/autumn months in the air of both plantations. *C. cladosporioides* was the most common species, contributing about half of total fungi in the air of citrus and about one-fourth to one-third in the air of grapevine. *C. sphaerospermum* came second in the air of both plantations, while *C. herbarum* was isolated only from the air of grapevine. In several aeromycological studies and in agreement with the current results, *Cladosporium* was considered as one of most abundant genera, if not, the most, abundant one reported all over the world such as Egypt (Moubasher & Moustafa 1974, El-Sherbeny 1987, Moubasher 1993, Ismail et al. 2002), Hellwan area, Egypt (Abdel Hameed et al. 2009), banana field in Qena (El-Said & Abdel-Hafez 1995), Uganda (Ismail et al. 1999), USA (Lacey 1981), Porto (Oliveira et al. 2005), Yemen (El-Essawy et al. 1992), Doha, Qatar (Al-Subai 2002), Eskisehir and Afyonkarahisar, Turkey (Asan et al. 2004, O¨zkara et al. 2007), Sari and Zanjan, Iran (Hedayati et al. 2005, Nourian et al. 2007), and Amman and Zarqa area, Jordan (Shaheen 1992, Abu-Dieyeh et al. 2010). *Cladosporium* spores are the dominant aerospora in hot climates (Takahashi 1997, Sen & Asan 2001, Al-Subai 2002, El-Morsy 2006). *Cladosporium* spores contributed the highest number (26.87% to the total sugarcane fields aerspora) in India (Ahire et al. 2010**)**, of aerospora in groundnut fields (47·32%) in Visakhapatnam, India (Mallaiah & Rao 1990), as well as of the aerospora (65·7%) in Hong Kong (Turner 1966). *C. cladosporioides* was the dominant species recorded of fungal aerospora in a coastal sandy belt of Orissa, India (Panda et al. 2009). According to Moubasher (1995) and Soliman (2012) comparison between the composition of air-borne fungal spores and soil mycobiota, and between air-borne spores and phyllosphere fungi reveal basic similarity between air- and phyllosphere borne fungi, which suggest that air-borne spores are mainly contributed by fungi developing on plant surface. This lends further support to Gregory's (1973) hypothesis that air-borne spores are basically a contribution from the vegetation rather than from the soil itself. This observation may show that some fungi such as *Cladosporium* spp. are strong competitive colonizers of the aerial plant materials unlike the case in soil whereas they are weak competitive colonizers of plant materials in soil, and hence they are more prevalent in air than in soil.

Soliman (2012) could deduce four patterns of correlation between the dominance (counts) of certain groups of fungi and the different studied sources in citrus and grapevine plantations, namely, soil air, phyllosphere, phylloplane, carposphere, carpoplane, and fruit juice. In soil pattern, the hyaline fungi e.g. *Penicillium, Aspergillus*, and *Fusarium* predominated over dark-coloured one e.g. *Cladosporium, Alternaria, Bipolaris*, and *Exerohilum*. In the air, phyllosphere and phylloplane pattern, the reverse occurs and the the dematiacous fungi outnumbered the hyaline ones. Soil is heavily populated by microorganisms and competition for existence is very severe. But the fungi are relatively protected from the injurious effects of atmospheric conditions: high light intensity, and deep diurnal fluctuations of temperature and humidity. These conditions may selectively favour the hyaline fungi over the dark-coloured (melanin-containing) fungi. In the air, phyllosphere and phylloplane pattern the environmental factors are markedly different which may induce selective effects for the advantage of the melanin-containing fungi over the hyaline ones. Melanin may protect fungi against stresses of solar UV and IR radiation.

Aspergillus contributed relatively higher numbers of propagules in the air of grapevine plantations (one-fourth of total fungi) than those from citrus plantations (11.47 % - 13.98 %). Its peak was regularly recorded in August in both plants. Species of *Aspergillus* belonging to section *Nigri* were the most common in the air of grapevine contributing relatively large proportions of *Aspergillus* count while, *A. ochraceus* was the most common species in the air of citrus, contributing large numbers of propagules. The dominance of these aspergilli agree with those obtained from the air of citrus plantations (Naim 1967, Moubasher et al. 1971), vineyard (Díaz et al. 2009), tomato plantations (Lohare et al. 2009), air at Assuit (Moubasher & Moustafa 1974, Abu-El-Souod 1974), air at Qena (Moubasher et al. 1981, 1982) and air at Zagazig (El-Sherbeny 1987).

Alternaria was the second most frequent (behind *Aspergillus*) in grapevine air and the third behind *Aspergillus* and *Cladosporium* in citrus air, yielding considerably higher numbers of propagules in the air of grapevine plantations than those from citrus plantations. Its peak was

recorded in winter months in the air of both plantations. *Alternaria* was isolated from the air of tomato plantations at Udgir, Latur District, India (Lohare et al. 2009), of the tea plantation area of Cachar District, Assam (Dutta *et al*. 2010), Brisbane, Queensland (Rees 1964), Hong Kong (Turner 1966) and Tulsa campus, USA (Levetin & Dorsey 2006), at Modinagar, India (Bhati & Guar 1979), at Zarqa area, Jordan (Abu-Dieyeh et al. 2010). *A. alternata* which was the most common species in the air of both plantations was also the dominant species in the air of citrus plantations (Moubasher et al. 1971) and the air of Assuit (Moubasher & Moustafa 1974) in Egypt, and Coastal Belt of Orissa in India (Panda et al. 2009).

Fusarium was of high frequency in citrus and in high or moderate frequency in grapevine air, yielding small numbers of propagules (0.84% - 2.92% of total fungi in both plantations. Its peak was regularly recorded in June in the air of citrus plantations on both media but irregularly in the air of grapevine plantations, in October and August on DYM and DRBC respectively. Nine species were identified in the air of citrus plantations and 11 species in grapevine air of which *F. semitectum* was the most common species. *F. equiseti* and *F. concolor* were recorded in high or moderate frequency in citrus air, while in grapevine air *F. equiseti* was isolated in moderate frequency and *F. concolor* in rare frequency. *F. camptoceras* and *F. torulosum* were isolated from citrus air only while *F. chlamydosporum*, *F. circinatum*, *F. konzum*, *F. proliferatum*, *F. pseudonygami* and *F. scripi* were isolated from grapevine air only. *Fusarium* dominated in the air of citrus plantations in Upper Egypt (Moubasher et al. 1971), tea plantation area of Cachar District, Assam, India (Dutta *et al*. 2010), air of Coastal Belt of Orissa, India (Panda et al. 2009), and atmosphere at Modinagar, India (Bhati & Guar 1979). *Fusarium* was also identified from the aerospora of Tulsa campus, USA (Levetin & Dorsey 2006) and from the air of Hong Kong (Turner 1966). It is worthmentioning that *Fusarium* was the most common genus in the soil of both plants. *F. solani* was the most common in the soil of both plants followed by *F. semitectum* in citrus plants, and by *F. oxysporum* and *F. babinda* in grapevine plants (Soliman 2012).

Penicillium was one of the most common genera contributing noticeably higher numbers of propagules in the air of citrus than those from the air of grapevine. Its peak was recorded in February and April on DYM and DRBC respectively in citrus air and in August on both media in grapevine air. In citrus air, *Penicillium olsonii* was the most common species while it was missed in grapevine air and *P. citrinum* was also isolated in high or moderate frequency from citrus air but was recorded in rare frequency from grapevine air. On the other hand, *P. oxalicum* was the most common species in the air of grapevine, contributing relatively the largest proportions of *Penicillium* counts while it was recorded in low frequency in citrus air. Fifteen species of *Penicillium* were recorded in citrus air while two species in grapevine only. *Penicillium* dominated in the air of Assuit (Moubasher and Moustafa 1974), the eastern desert of Egypt (Ismail *et al*. 2002), Hellwan area, Egypt (Abdel Hameed et al. 2009), tea plantation in Barak Valley, Assam, India (Dutta et al. 2010), Hong Kong (Turner 1966), Mondiagar, Uttar Pradesh, India (Bhati & Gaur 1979), Amman and Zarqa area, Jordan (Shaheen 1992, Abu-Dieyeh et al. 2010), Kuwait (Moustafa & Kamel 1979), Saudi Arabia (Abdel-Hafez 1984), and Qatar (Al-Subai 2002). *Penicillium* was recorded in the air of Tulsa campus, USA (Levetin & Dorsey 2006). *Penicillium italicum* and *P. digitatum* which are the main incitants of citrus rot were isolated from the air of orange groves and packing sheds (Ben-Meir-Glueck 1952) and air of citrus fruit packing houses, Israel (Barkai-Gollan 1961). *Penicillium expansum* and *P. solitum* were the most prevalent species while *Penicillium digitatum* was not common in the atmosphere of orchards and storage rooms of apples in Picardy and Pays de la Loire, France (Amiri & Bompeix 2005).

Cochliobolus (2 species) and *Myrothecium* (represented by *M. verrucaria*, *M. roridum* and *Myrothecium* sp.) were recorded in high frequency in the air of both plantations. *C. lunatus* and *M. verrucaria* followed by *C. australiensis* and *M. roridum* were the most prevalent species. *Curvularia* (4 species) was isolated in low frequency from the air of both plantations. *Cochliobolus lunatus* (=*Curvularia lunata*) was one of the most dominant species of the aerospora in a coastal sandy belt of Orissa, India (Panda et al. 2009). *Curvularia*, *Pithomyces*, and *Memnoniella* were

isolated from the aerospora in Jamaican banana plantations (Meredith 1961). *Curvularia, Epicoccum, Pithomyces, Drechslera* and *Nigrospora* were identified from *Ulmus americana* and *Quercus palustris* leaves, USA (Levetin & Dorsey 2006). *Drechslera (*with *D. spicifera, D. halodes* and *D. hawaiiensis* being the most prevalent), *Curvularia* (*C. lunata*), *Phoma* (*P. humicola, P. herbarum)* and *Chaetomium (C. globosum)* were the most frequent genera in the Qat (*Catha edulis*) phyllosphere, Yemen (Alhubaishi & Abdel-Kader 1991). *M. verrucaria* was isolated from the air at Assuit (Moubasher & Moustafa 1974).

Emericella (10 species and 3 unidentified) and *Botryodiplodia* (*B. theobromae*) were recorded in high frequency from citrus air and in moderate frequency from grapevine air. *Emericella variecolor* was the most common species in citrus and grapevine plantations. It is worthy to mention that the isolation of *E. stella-maris* in this study from citrus plantations is the second world record (Moubasher et al. 2013) after its first description in 2008 in the Mediterranean region from *Eucalyptus* leaf litter in Tunisia and hypersaline saltern water in Slovenia (Zalar et al. 2008). *Emericella* species were isolated from cocoa beans (Sanchez–Harvas et al. 2008). *E. variecolor* was isolated from the air of Assiut (Moubasher & Moustafa 1974). *B. theobromae* was isolated from decayed papaya fruits (Bagwan 2011) and from soil, Egypt (Mazen 1973). It was recorded as causal agent of mango and banana fruit-rot (El-Helaly et al. 1966).

Phoma (6 species with *P. epicoccina* being the most common) and *Pleospora* (3 species with *P. tarda* the most frequent) were recorded in high frequency in the air of both plantations. *Phoma* sp. was the most common in several varieties of grapevines, Madrid, Spain (González & Tello 2010) while *P. tarda* was isolated from air in citrus plantations in Upper Egypt (Moubasher et al. 1971), and from the air of Assuit (Moubasher & Moustafa 1974).

Setosphaeria (3 species) was isolated in high frequency from grapevine air and in moderate frequency from citrus air. *S. rostrata* was the main species, whereas *S. monoceras* was isolated from citrus air and *S. pedicillata* from grapevine air only. *Setosphaeria* was the most common fungus isolated from phyllosphere and phylloplane of banana plants cultivated in Upper Egypt (El-Said 2001).

Trichoderma (9 species and 1 unidentified) was recorded in moderate frequency from citrus air and in low frequency from grapevine air. The most common species were *T. harzianum* and *T. reesei* in citrus plantations and *T. paracemosum* in both plantations. *Trichoderma* was a component of the aerospora of Hong Kong (Turner 1966), and several varieties of grapevines, Madrid, Spain (González & Tello 2010), and was recovered from *Quercus* and *Acer* phylloplanes, Czech Republic (Guimarães et al. 2011). *T. harzianum* is well known as a biological control agent against plant pathogenic fungi.

Mucor (4 species) was recovered in moderate frequency from citrus air, and in rare frequency from grapevine plantations. *M. circinelloides* was the most common species followed by *M. hiemalis* in both plantations. *Rhizopus* (*R. oryzae*) was recorded in high frequency from grapevine air and in moderate frequency from citrus air. *Humicola* (*H. fuscoatra, H. grisea*, and *Humicola* sp.) was isolated in high frequency from citrus air and in low frequency from grapevine air. *H. fuscoatra* was more common than *H. grisea* in both plantations. *Stachybotrys* (*S. chartarum* and synnematous species of *Stachybotrys*) was recorded in rare frequency from the air of grapevine while in low frequency from citrus air.

Nigrospora oryzae and *N. sphaerica* were identified in low frequency from the air of both plantations, however the former was more frequent than *the* later. *Nigrospora* is a common component of the aerospora in Jamaican banana plantations (Meredith 1961), Nigeria (Cammack 1955). Also, *Nigrospora, Curvularia, Pithomyces, Phoma, Epicoccum* and *Neurospora* were the prevalent fungal genera recorded among the aerospora at Ibadan, Nigeria (Ogunlana 1975).

Acremonium (9 species and 1 unidentified) was isolated in low frequency from grapevine air and in rare frequency from citrus air. *A. strictum* was the most common species followed by *A. fusidioides* and *A. hyalinulum. Acremonium* was isolated from several varieties of grapevines, Madrid, Spain (González & Tello 2010).

Microascus (*M. brevicaulis* and *M. manginii*, teleomorphs of *Scopulariopsis. brevicaulis* and *S. candida* respectively) was isolated in low frequency from citrus air and in rare frequency from grapevine air. The two species were isolated from Egyptian soil (Moubasher 1993).

Of the 15 yeast genera (represented by 26 species) recovered, *Cryptoccocus* (4 species; *C. albidus*, *C. carnescence*, *C. flavescens* and *C. laurentii*) and *Rhodotorula* (3 species) were the most common, but *Debaryomyces* (2 species) and *Sporidiobolus* (*S. ruineniae*) were less common in the air of both plantations. Some yeast genera were recovered only from the air of citrus plantations (*Ambrosiozyma*, *Candida*, *Geotrichum*, *Hanseniaspora*, *Rhodosporidium* and *Melanopsichium*) while *Sporobolomyces* and *Trichosporon* from grapevine plantations only. The dominance of *Cryptococcus albidus* and *Rhodotorula mucilaginosa* in the air was reported by Di Menna (1955); Voros–Felkai (1966, 1967); Al–Doory (1967) and Haridy (1992). *Debaryomyces hansenii* was isolated from the air of El-Minia city, Egypt (Haridy 1992) and *Candida* sp. was recorded in the air of tea plantations of Barak Valley, Assam, India (Dutta et al. 2010).

The present study reveals that the dematiaceous fungi outnumbered the hyaline ones and Basidiomyceteous yeasts were dominant over ascomyceteous yeasts.

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