

Seasonal Distribution of Indoor and Outdoor Fungi in the Air of El-Beida City, Libya.

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Abstract: The main objective of the present study was to assess culturable airborne fungal colony, and types in different seasons. Open Petri Dish with two media PDA and MEA were used. Culturable airborne fungal concentrations were collected indoors and outdoors of 15 homes in different localities from April 2013 to March 2014 in Libya. The greatest colony were found in the Autumn and Summer seasons, while the lowest was recorded in Winter. *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium solani*, *Penicillium chrysogenum* and *Penicillium digitatum* were the predominant genera indoors and outdoors, and the abundance of genera varied by season.

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1. Introduction

The term "Air-borne fungi" is namely that the spores of fungi carrying by atmospheric air. The intensity of fungi spores increases depending on air pollution (Asan et al., 2002). Nevertheless, the number of air spore and diversity vary with time of day, weather, season, geographical location, flora combination (Asan et al., 2002; Di Giorgio et al., 1996; Menezes et al., 2004) and the presence of local spore sources (Lacey, 1981). According to Lacey (1981), and El-Gali (2014) airborne fungal spores are originally created from plant, animal, and soil sources. Common molds like *Alternaria*, *Aspergillus*, *Penicillium*, *Mucor*, *Drechslera*, and *Cladosporium*, which occur frequently in many environments, are reported to cause plant diseases (Agrios, 2005).

In other studies the moulds reported causes human diseases such as rhinitis and allergic asthma (Bokhari & Parvez, 1995; Li and Kendrick, 1995; Gomez de Ana et al. 2006). Many studies have been carried out on the distribution air borne fungi in both indoor and indoor environment in the air of some cities were published. (Pastuszka et al. 2000; Hedayati, et al., 2005; Topbas, et al. 2006; Sabariego, et al., 2007; Abu-Dieyeh, et al., 2010). Abdel-Hafez et al. (1987) and Nourian, et al. (2007) showed that the maximum airborne fungi were in winter and the minimum in the summer. The highest level of airborne fungi was recorded in Autumn (Muhsin and Adlan, 2012) and in Summer (Fang, et al. 2005). In Denmark, Frankel, et al. (2012) reported the indoor fungi Peaked in Summer, while in Egypt, Awad, et al. (2013) found the greatest fungal air concentrations in the autumn and spring season.

The present study was conducted in the center of El-Beida, a city in the north region in Libya,

Altitude is 624 m and it is surrounded by rich forests and flora. The primary livelihood of the city people is agriculture and products such as cereals, fruit, vegetable and olive are grown in the in nearby regions around the city. El-Beida reflects the characteristics of Mediterranean climate, which is hot and dry in summers and warm and rainy in winters and it is windy almost every day throughout the year. Our purpose was to determine the genus, quantity and seasonal distributions of airborne fungi that may be important causative allergens. No study of this kind has previously been conducted in this region.

2. Material and Methods

Sampling Strategy and home selected

Air samples were collected indoors and outdoors, from 15 homes were selected with no obviously visible fungal growth or existing water leaks. The samples were taken between 09.00 a.m. and 12.00 p.m. through four seasons of 1 year between April 2013 and March 2014.

Sampling of Fungi

Using the Open Petri Dish Method air samples were collected. Indoor samples were obtained at a height of 1.5 m, the human breathing zone, above the floor level in the middle of the main common room or living room while outdoor comparison samples were taken approximately 5 m outside the entrance doors or on (window, balcony or terrace) according to the location of homes.

Opened Petri dish plates containing Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) with Chloramphenicol were used at the level of breathing height for each room and outdoor environment for 15 min. The plates were incubated at 25°C for 7-14 days before colonies present were counted and identified up to the genus level or

species. The Fungal pure culture was made for each of the isolated fungi and were identified by mycological techniques based upon gross cultural and microscopic morphology according to the criteria published in the specialized literature on the (CMI. 1966; Barnett and Hunter, 1998; McGinnis, 1980; Hoog and Guarro, 1995; Larone, 1995; Ainsworth et al., 1995; Alexopoulos et al., 1996; Nelson and Toussun, 1997; Ellis et al., 2007). Also research articles and other related literature fungal Identification and illustrations were made up to the Genera and Species level.

Diversity of fungi

To study the diversity of all fungi in all survey seasons, we used Simpson method (Muhsin, 1987):

$$\text{Diversity (Dv)} = 1 - \sum \{(p1)^2 + (p2)^2 + \dots + (pi)^n\}$$

Where Dv: diversity factor

$$P: \frac{\text{No. Colony of fungus}}{\text{No. Total colony of all fungi}}$$

No. Total colony of all fungi

Meteorological measurements

Temperature and relative humidity were measured during each sampling event. Also wind speed and rainfall records were obtained from Omer Al-Mukhtar University Meteorological station. During this study, The average measurements of temperatures recorded 24.3°C. relative humidity ranged between 50 and 80% , wind speed records varied between 11.2 and 18.2 m/s and rainfall between 1.1- 3.5 mm. El-Beida reflects the characteristics of Mediterranean climate, which is hot and dry in summers and warm and rainy in winters and it is windy almost every day throughout the year.

3. Results

Metrological data (Table 1) showed that moisture in the air was higher during winter, precipitation in Autumn and Winter from September 2013 till March 2014, while mean temperature and wind variability in summer.

During the air sampling phase of the investigation, 540 indoor samples and 540 outdoor samples were collected using opened plate method. All of these plates showed fungal growth. Table 2 and 3 shows the frequency of different fungal genera isolated from indoor and outdoor of air home. A total of 5431 colonies with 20 species and 5375 colonies with 18 species of fungi were identified from indoor and outdoor respectively over four seasons.

There was great difference in fungal isolations among seasons The seasonal distribution of colonies of airborne fungi is summarized in Table 1 and 2, and shows that the highest reproduction level was observed in Autumn (1701 colonies, 31.3% and 1562 colonies, 29.1%) from indoor and outdoor respectively. The lowest reproduction level was recorded in Winter (1025 colonies, 18.9% from indoor) and (1069 colonies, 19.9% from outdoor).

Table 1. Average Temperature, RH% , Precipitation and Wind speed in Atmosphere of El-Beida

Months	Temp. (C°)	Precip. (mm)	R.H (%)	Wind (m/sec)
April 2013	20.3	1.3	60	13.4
May 2013	23.4	-	57	12.9
June 2013	27.0	-	53	18.2
July 2013	26.9	-	55	18.0
August 2013	27.2	-	62	13.6
September 2013	25.9	1.1	65	14.5
October 2013	22.3	2.0	68	13.0
November 2013	17.1	2.8	75	12.0
December 2013	14.7	3.0	80	11.8
January 2014	10.8	2.3	75	12.1
February 2014	12.0	3.5	80	11.2
March 2014	15.8	2.6	69	11.2

The most reproductive fungus species in indoor air were *C. cladosporioides* in Autumn and Winter with 529 and 320 colonies, respectively, followed by *Curvularia* sp and *A. alternata* in Summer with 326 and with 310 colonies, respectively. While in outdoor samples distribution at all seasons we noticed *P. digitatum* was recorded 453 and 426 colonies in Summer and Autumn respectively, followed by *C. cladosporioides* (373 colony) in Autumn.

The diversity of fungal convened in samples every season from the year seasons was studied. After calculated the coefficient of fungal diversity, the data were tabulated in Table (4). We noticed that the samples of Summer and Autumn was more fungal diversity (1.8 and 1.1) respectively, followed by Spring samples (0.9) while, the diversity of winter samples was recorded 0.8. Also the indoor fungal diversity 2.4 was larger than outdoor diversity 2.2.

The greatest frequency count was attributed to *C. cladosporioides* with 32.4%, 31.1% in Winter and Autumn, respectively followed by *A. alternata* with 19.9 and *P. chrysogenum* with 17.5% in Summer and Spring, respectively (Fig. 1). *P. digitatum* achieved the highest percentage of total colony count (31.1%, 27.3%) in Summer and Autumn, respectively, while *C. cladosporioides* had the second highest percentage with 23.9%, 21.5% in Autumn and Winter, respectively, and the third with 18.7% for *A. alternata* in Summer and *P. chrysogenum* in Winter (Fig. 2).

Table 2: Seasonal distribution and number of colonies of indoor fungi isolated in El-Beida city

Fungal genera and species	Seasons				Total	Frequency (%)
	Spring	Summer	Autumn	Winter		
<i>A. alternata</i>	52	310	148	15	525	9.7
<i>A. flavus</i>	-	-	17	-	17	0.31
<i>A. fumigatus</i>	20	20	-	-	40	0.74
<i>A. niger</i>	100	91	23	37	256	4.7
<i>A. terreus</i>	-	-	24	-	24	0.44
<i>C. murorum</i>	24	-	-	2	26	0.48
<i>C. cladosporioides</i>	48	-	529	320	897	16.5
<i>Curvularia</i> sp	105	326	119	-	550	10.1
<i>F. oxysporum</i>	2	2	-	-	4	0.07
<i>F. solani</i>	102	132	46	26	306	5.63
<i>Mucor</i> sp	19	9	7	19	54	1.0
<i>Mycelia sterilia</i>	-	15	3	7	25	0.46
<i>P. chrysogenum</i>	207	196	196	128	727	13.4
<i>P. digitatum</i>	163	130	179	153	625	11.5
<i>Penicillium</i> sp	27	112	104	95	338	6.22
<i>Phoma</i> sp	17	8	20	9	54	1.0
<i>R. nigricans</i>	136	103	156	76	471	8.7
<i>R. solani</i>	-	-	-	-	-	-
<i>T. harzianum</i>	8	-	-	-	8	0.15
<i>T. roseum</i>	91	67	90	103	351	6.46
<i>U. botrytis</i>	26	37	40	30	133	2.45
Total	1147	1558	1701	1025	5431	100.0
Frequency (%)	21.1	28.7	31.3	18.9		

Spring (March – May), Summer (June - August), Autumn (September – November) , Winter (December – February).

Table 3: Seasonal distribution and number of colonies of outdoor fungi isolated in El-Beida city

Fungal genera and species	Seasons				Total	Frequency (%)
	Spring	Summer	Autumn	Winter		
<i>A. alternata</i>	161	226	250	160	797	15.2
<i>A. flavus</i>	53	-	93	-	146	2.8
<i>A. fumigatus</i>	-	-	-	-	-	-
<i>A. niger</i>	14	15	15	8	52	1.0
<i>A. terreus</i>	-	-	-	-	-	-
<i>C. murorum</i>	9	-	-	-	9	0.17
<i>C. cladosporioides</i>	234	257	373	215	1079	20.6
<i>Curvularia</i> sp	1	21	-	15	37	0.71
<i>F. oxysporum</i>	20	9	-	8	37	0.71
<i>F. solani</i>	173	95	88	118	474	9.1
<i>Mucor</i> sp	19	22	1	3	45	0.86
<i>Mycelia sterilia</i>	2	3	-	-	5	0.1
<i>P. chrysogenum</i>	135	89	200	198	622	11.9
<i>P. digitatum</i>	251	453	426	103	1233	22.9
<i>Penicillium</i> sp	-	-	-	-	-	-
<i>Phoma</i> sp	29	28	15	58	130	2.5
<i>R. nigricans</i>	38	86	88	36	248	4.7
<i>R. solani</i>	4	-	-	-	4	0.08
<i>T. harzianum</i>	5	-	-	-	5	0.1
<i>T. roseum</i>	92	85	-	40	217	4.14
<i>U. botrytis</i>	46	69	13	107	235	4.5
Total	1286	1458	1562	1069	5375	100
Frequency (%)	23.9	27.1	29.1	19.9		

Spring (March – May), Summer (June - August), Autumn (September – November) , Winter (December – February).

Table 4: Coefficient of Fungal Diversity during the seasonal study.

Seasons	Dv		Total
	Indoor	Outdoor	
Spring	0.6	0.3	0.9
Summer	0.6	1.2	1.8
Autumn	0.8	0.3	1.1
Winter	0.4	0.4	0.8
Total	2.4	2.2	

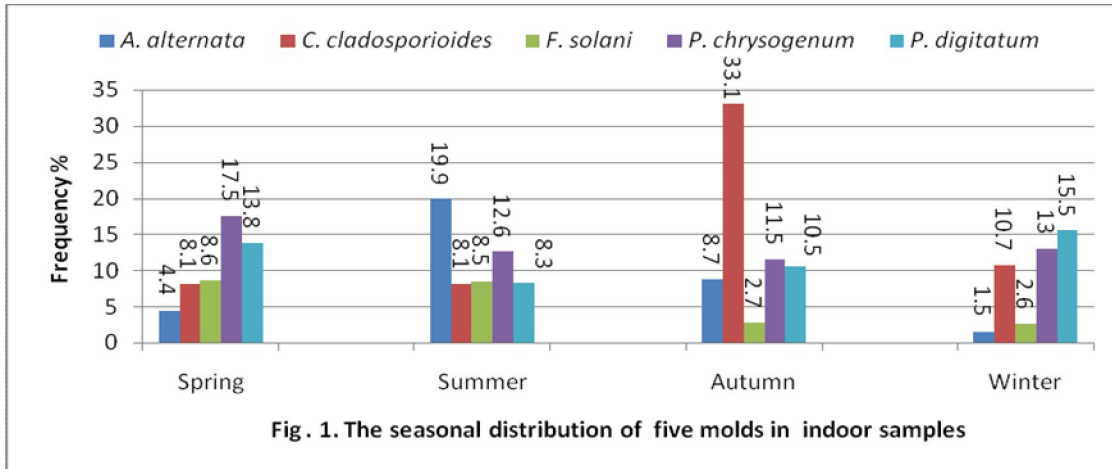


Fig. 1. The seasonal distribution of five molds in indoor samples

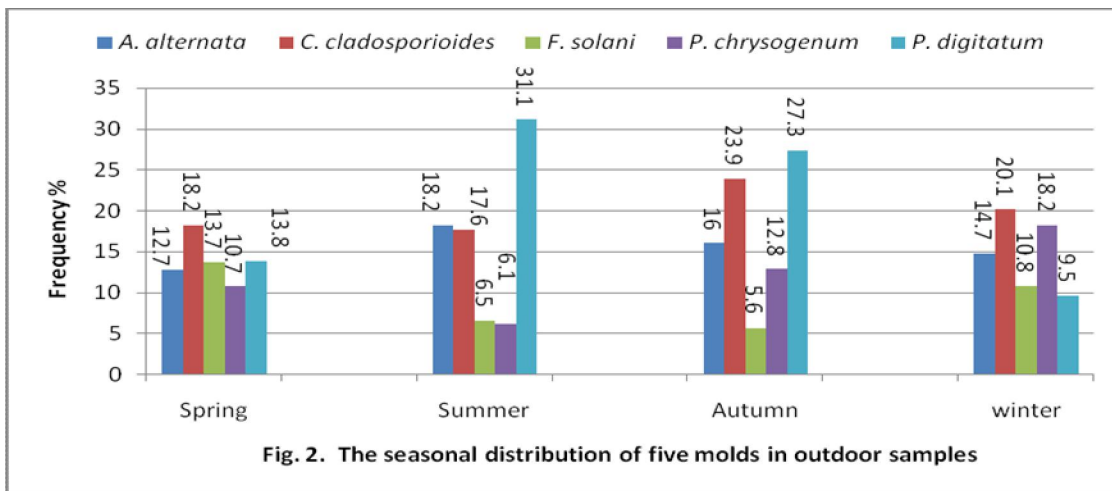


Fig. 2. The seasonal distribution of five molds in outdoor samples

4. Discussions

Knowledge of species and density of outdoor airborne fungi in a given environment can be especially important in the diagnosis and treatment of various allergic diseases. This study was therefore conducted in El-Beida which, compared to other parts of Libya, has different features in terms of climate, geography and flora. Twenty one species of fungi including *A. alternata*, *Aspergillus* spp, *C. cladosporioides*, *Curvularia* sp., *Fusarium* spp,

Pencillium spp., *R. solani*, *R. nigicans*, *T. roeeum* and *U. botrytis* were identified in indoor and outdoor. *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Pencillium* were the most prevalent and appeared to be the most common genera during the study.

Some genera of airborne fungal spores such as *Alternaria* and *Cladosporium* and *Pencillium* are found throughout most of the world. Spores are generally considered to be important causes of both allergic rhinitis and allergic asthma; mould allergy,

however, is the least understood and studied of the major forms of inhalant allergy (Peat, et al 1993; Shen and Han, 1998; Katz, et al., 1999 ; Dharmage, et al., 2002 ; Unlu, et al., 2003 and Hedayati, et al., 2005).

Soil, animal and plant is an important source for airborne fungi (Lacey,1981). A study on soil fungi in El-Beida by El-Gali (2014) showed that the most abundant genera were *A. alternata*, *A. niger*, *Fusarium* spp and *Penicillium* spp. Also, in this study some of isolated fungi such as *Alternaria*, *Cladosporium*, *Fusarium* and *Penicillium* were phytopathogenic in cultivated plants besides being important fungal allergens and they are the most common fungus species found in the atmospheric air (Nelson, et al.1994; Agrios, 2005; Topbas et al., 2006; Kaarakainen et al., 2008).

In the present study, although some degrees of seasonal variations of the major genera were detected, the most notable ones were Autumn. the *Alternaria*, *Cladosporium* and *Penicillium* genus in that they were in higher numbers in the Autumn and in Summer, respectively. High concentrations of *Alternaria*, *Cladosporium* and *Penicillium* in the autumn are associated with the decaying vegetable materials (Chakraborty, et al. 2000). Rainfall and relative humidity almost always have profound effects on the level of fungi spores (Celenk, et al. 2007; Dubey, et al. 2011). Our results also showed that overall the total number of fungi colonies decrease in winter. In December, January and February temperature was lower than in the previous months.

Although humidity and wind were high enough, low temperatures caused a decrease in spore concentration during these months, which have a negative effect on the content of these particles in the air (Sabariego, et al., 2007; Abu-Dieyeh, et al., 2010) and a high relative humidity which produces an absorption of water by the spores, making them heavier and less transportable by air (González Minero et al., 1994). *Cladosporium* was found in the highest concentration in the months with low temperatures, as precipitation seemed to optimize their sporulation (Dubey, et al. 2011). Increase the concentration of fungi in winter in indoor samples may be attributed to less opening of windows and heating systems that increased temperature inside the homes to make favorable for spore growth.

Regarding the diversity, the Autumn and Summer seasons were highest Diversity (DV) in fungi followed by Spring samples while Winter was lowest. Fungal it indicated to difference of contamination sources due to difference of sites and environment (Jawetz, et al., 1989).

5. Conclusion

Indoor culturable airborne fungi were found in concentrations relatively similar to those outdoors of the homes. Fungal concentration and composition varied by season indoors and outdoors concentrations. Knowledge on airborne fungi is important as it is considered a potential public health problem and data can be used as a base to develop criteria for assessing indoor air quality in Libya. Studies regarding fungal concentrations of El-Beida city as in any of the costal dwellings in the region, harbors various species of fungi due to its warm and rainy climate and very rich flora are needed in the future with regard to the diagnosis and prophylaxis of allergic diseases thought to be resulting from airborne fungi.

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