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RESEARCH ARTICLE

Isolation and identification of airborne fungi in Indoor/Outdoor of Home in El-Beida City (Libya)

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Abstract

In Libya, there is not enough information about microbial contaminants in indoor/outdoor environments. Microbial monitoring of such environments is important for the quality of human life. The aim of this study was identification of fungi in indoor and outdoor of home environment. Opened plates were used for isolation of fungi in the air of El-Beida city. Twenty-one fungal species were identified. A total of 5431 colonies with 14 genera and 5375 colonies with 15 genera of fungi were identified from indoor and outdoor of home respectively. In addition most of fungi isolated were important aeroallergens and phytopathogen.

INTRODUCTION

There are multi-thousands of recognized species of fungi. They are found in soil, in water, on animals, on vegetation, in humans, and in almost every part of the environment. Anemophilous fungi are spread by the atmospheric air. Fungal spores are always present in the air, with rain and snow washing down most if not all spores from the air, and sunshine and wind cause an increase in the atmospheric distribution of such spores (AL-Doory, 1984). Molds are essential components of our planets ecosystem providing decomposition of many organic substances necessary to plant, animal, and human life (Davis, 2001). It is well known that fungi can survive in almost every region and climate (Asan, et al., 2002; Carpenter, 1977). It has been reported that airborne fungi are the most common microorganisms that have adverse effect on human health causing asthma, rhinitis and dermatitis besides they are considered as a source of plant and animal pathogens (McGinnis, 1980 ; Al-Doory, 1984; Agrawal, et al., 1996; Burge, et al., 2000). Fungi generally grow as saprophyte in the soil and facultative parasite on its hosts and they spread into the atmospheric air by means of several factors. It is important for phytopathologists that a large proportion of the fungi transported in the atmospheric air are phythopathogens. Over 10,000 types of fungi in nature are pathogens in plants (Agrios 2005). Besides being plant pathogens, most of the fungi cause pneumonia, hay fever and allergic diseases. This fact draws the attention of aerobiologists due to the increase in recent years in the density of fungi in the atmospheric air and the allergic diseases they cause (Larsen and Gravesen, 1991; Pasanen, 1992; Šen and Asan, 2001; Fareid, 2011; Muhsin and Adlan, 2012; Awad, et al., 2013). Many scientists and allergists began to study the presence and type of fungal spores in both indoor and outdoor air, using various collection methods (Gomez de Ana, et al., 2006; Awad, et al., 2013).. The main purpose of this study was to determine the prevalence of anemophilous fungi in the atmospheric air of indoor and outdoor of El-Beida city in order to contribute to the knowledge of density of airborne fungi and distribution in the region.

MATERIALS AND METHODS

Sampling locations: El-Beida is in the northern part of Libya. It is situated on Algabal Alakhdar. 45' - 59' 32° N and long. 44' - 30' 21° E, about (30 km) from the coast. The town is set on hill at an average elevation of 624 m above the level sea. During the year there are two distinct seasons, wet and dry. The wet season is from November to April and the dry season is between May and September. The mean annual rainfall is 1.2 mm, and the temperature varies between 12.8 and 25ºC.

Experimental design

The present work had the following experimental design: five sites (Table 1), two types of air samples indoor air (AIS) and outdoor air (AOS), both studied in each sample. we chose three homes at each of five sites, thus working at a total of fifteen locations. We used the same hour of the day (9:00 a.m. to 12:00 noon) for each of the five sites. The study was conducted to a 12-month period, (April, 2013 to March, 2014) are presented here. Three replicate plates of each of the two media were exposed at each incubation temperature (25 ºC) at each of the locations once a week.

Isolation and taxonomic study of fungi

Two media, Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), were used for collecting samples to observe any possible effect of media on the collection of the fungal spores. Preliminary studies indicated the need to suppress bacterial growth, consequently, 20 U of penicillin per ml and 40 U of streptomycin per ml were added to each of the media. The exposed plates were incubated initially for 3 days at 25 ºC, the colonies were counted, and the plates were left for further incubation, with daily colony counts made for up to 7 days. This was found necessary from preliminary studies, which indicated a need for continued incubation up to 14 days at 25 ºC for the development of slow-growing organisms.

The fungal colonies on each plate were counted and the mean number of colonies on each medium at each site were recorded. Representative plates from each of the locations were used in the characterization study. Each colony was coded before sub-culturing for identification purposes. The taxonomic identification of fungi was performed considering the morphological characteristics of the vegetative mycelium and the reproductive structures (CMI. 1966; Barnett and Hunter, 1972; Koneman and Roberts, 1992; Hoog and Guarro, 1995; Larone, 1995; Ainsworth, et al., 1995; Alexopoulos, et al., 1996; Nelson and Toussun, 1997; Ellis, et al., 2007). Some of the fungal colonies were identified by genera only, but Most were fully identified by species.

RESULTS

At the end of this study, a total number of twenty one species belonging to fifteen genera were isolated from indoor and outdoor samples in different sites were indicated that in Table (1). This highlights the exact locations where the samples were obtained and the characteristics of it such as Densely populated areas, or Less populated areas, Pollution environmental and the Restaurants whereabouts or Trees whereabouts. Designations were also assigned to each location for easy reference in other table presentations and discussion.

Table 1: Sampling Sites, Characteristic of Locations and Designations

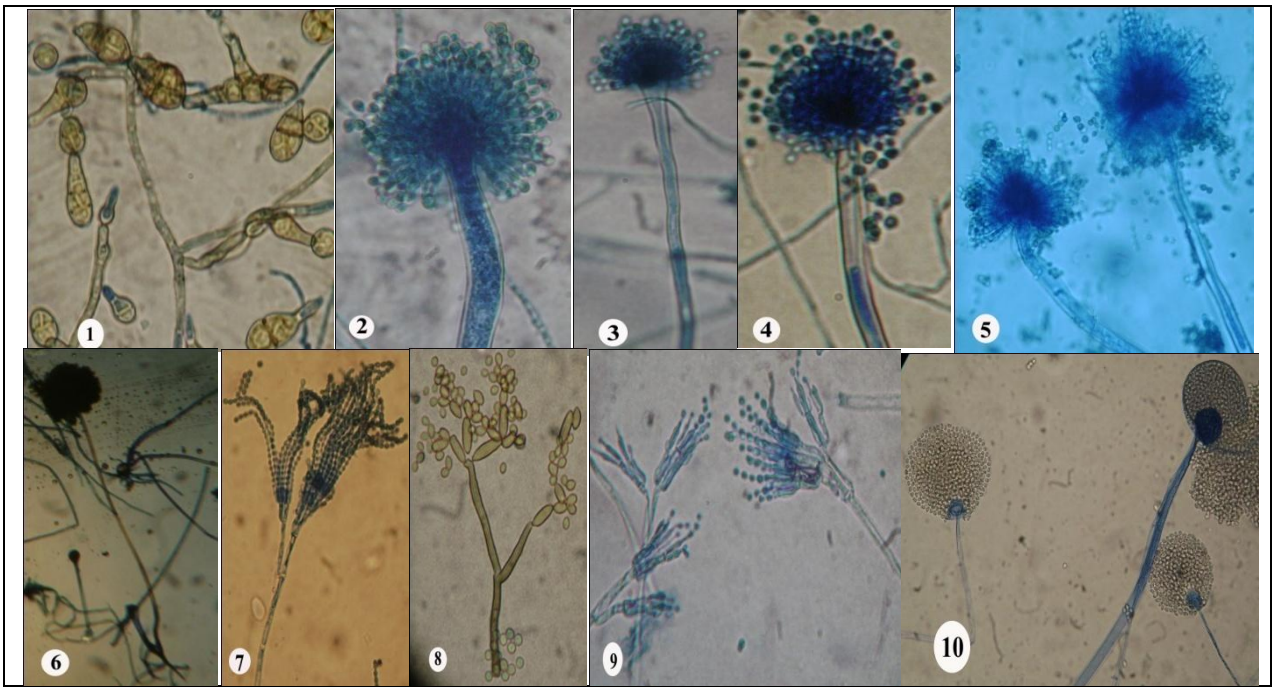
LOCATIONS	CHARACTERISTICS	DESIGNATIONS
D2- 4	Less populated areas	Site 1
C3- 45	Densely populated areas	Site 2
C3- 4	Pollution environmental	Site 3
C3- 42	Restaurants whereabouts	Site 4
C2-1	Trees whereabouts	Site 5

In the surveys carried out once a month for 12 months using Petri plate method, 10806 fungal colonies were isolated from a total of 360 Petri dishes and colonies were counted and identified in order to detect the incidence frequencies of fungi. The most common fungi isolated were *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus A. niger*, *A. terreus* , *Cheatomium murorum*, *Cladosporium cladosporioides*, *Cruvularia* sp., *Fusarium solani*, *F. oxysporum Mucor* sp., *Penicillium chrysogenum*, *P. digitatum*, *Penicillium* sp., *Phoma* sp., *Rhizopus nigricans*, *Rhizoctonia solani*, , *Trichoderma harzianum*, *Trichothecium roseum* and *Ulocadium botrytis.* Non sporulating strains were grouped in Mycelia sterilia. Micromorphological structures of the predominant fungi are illustrated in Fig. (1).

Concerning of contamination level, data in Table (2) showed that This highlights the specific fungal species isolated from each sample site. Level of contamination of each site was expressed in percentage of the total number of fungal species obtained from the study which was twenty (20) species in all.

Table 2 : Types of fungi identified at specific sample sites and level of contamination.

Location	Fungal genera and species	Level of Contamination
Site 1	*A. alternata*, *A. flavus*, *A. niger*, *C. murorum*, *C. cladosporioides*, *Curvularia* sp, *F. oxyspporum*, *F. solani*, *Mucor* sp, *P. chrysogenum*, *P. digitatum*, *Penicillium* sp, *Phoma* sp, *R. nigricans*, *T. harzianum*, *T. roseum*, *U. botrytis*.	85
Site 2	*A. alternata*, *A. flavus*, *A. niger*, *C. murorum*, *C. cladosporioides*, *Curvularia* sp, *F. oxyspporum*, *F. solani*, *Mucor* sp, *P. chrysogenum*, *P. digitatum*, *Penicillium* sp, *Phoma* sp, *R. nigricans*, *T. harzianum*, *T. roseum*, *U. botrytis*.	85
Site 3	*A. alternata*, *A. flavus*, *A. niger*, *A. terreus*, *C. murorum*, *C. cladosporioides*, *Curvularia* sp, *F. oxyspporum*, *F. solani*, *Mucor* sp, *P. chrysogenum*, *P. digitatum*, *Penicillium* sp, *Phoma* sp, *R. nigricans*, *R. solani*, *T. harzianum*, *T. roseum*, *U. botrytis*.	95
Site 4	*A. alternata*, *A. flavus*, *A. niger*, *A. terreus*, *C. murorum*, *C. cladosporioides*, *Curvularia* sp, *F. oxyspporum*, *F. solani*, *Mucor* sp, *P. chrysogenum*, *P. digitatum*, *Penicillium* sp, *Phoma* sp, *R. nigricans*, *T. roseum*, *U. botrytis*.	85
Site 5	*A. alternata*, *A. flavus*, *A. niger*, *A. fumigatus*, *C. murorum*, *C. cladosporioides*, *Curvularia* sp, *F. oxyspporum*, *F. solani*, *Mucor* sp, *P. chrysogenum*, *P. digitatum*, *Penicillium* sp, *Phoma* sp, *R. nigricans*, *R. solani*, *T. harzianum*, *T. roseum*, *U. botrytis*.	95



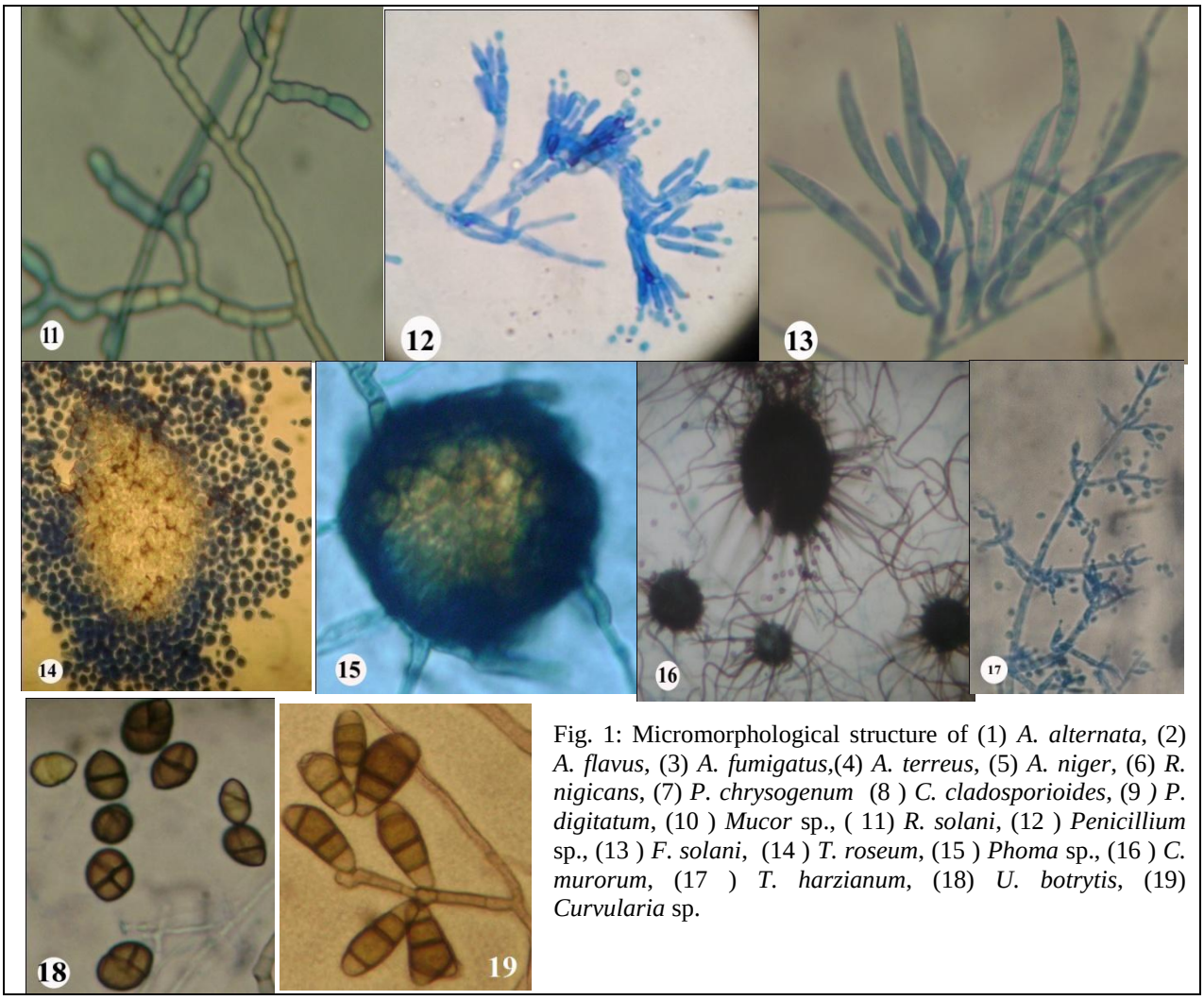


Fig. 1: Micromorphological structure of (1) *A. alternata*, (2) *A. flavus*, (3) *A. fumigatus*,(4) *A. terreus*, (5) *A. niger*, (6) *R. nigicans*, (7) *P. chrysogenum* (8) *C. cladosporioides*, (9) *P. digitatum*, (10) *Mucor* sp., (11) *R. solani*, (12) *Penicillium* sp., (13) *F. solani*, (14) *T. roseum*, (15) *Phoma* sp., (16) *C. murorum*, (17) *T. harzianum*, (18) *U. botrytis*, (19) *Curvularia* sp.

The number of colony of fungi in the samples of AIS was smaller in site 1 and in site 2 and bigger in site 3, site 5 and site 4. On the other hand, for the AOS samples, the number of colonies showed differences only on the air of site 1, was smaller than other sites. It has been observed that in all the sites the outdoor air had a bigger number of colonies of fungi when compared with the indoor air excepted in site 3. The ratio AIS:AOS of colonies of fungi for the different sites analyzed also showed differences, being smaller in site 2 and bigger in site 3 (Table 3).

Table 3: Ratio between indoor and outdoor number of fungi colony. AIS = air indoor sample; AOS = air outdoor sample; I:O = ratio between indoor and outdoor samples. The values represent means of five replications and the Standard Error (SE) was estimated by sample from each place.

Samples	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
	NCF	**NCF**	**NCF**	**NCF**	**NCF**
AIS	908	957	1385	1054	1127
SE	10.90	11.57	15.54	11.97	13.96
AOS	942	1168	981	1069	1215
SE	13.27	22.18	15.67	16.23	18.20
I:O	0.96	0.82	1.41	0.99	0.93

The values from indoor and outdoor ratio of number of fungi colony forming m-3 air), I:O = < 1.5 environment in proper condition; I:O = 1.5 environment regular condition; I:O = >2 environment not proper condition.

The genera *A. alternata*, *A. niger*, *C. cladosporioides*, *F. solani*, *Mucor* sp., *P. chrysogenum*, *P. digitatum*, *Phoma* sp., *R. nigricans*, *T. roseum* and *U. botrytis* were found in all the AIS and AOS air samples of five sites. *A. flavus* was found in all AOS samples but in the AIS samples this species was not found in site 1 and site 2 samples. Among the four most common fungi found, the population of *C. cladosporioides* was biggest in all the AIM and AOM samples while *P. digitatum*, was biggest in all the AIM and AOM samples except for the site 5. Also *P. chrysogenum* was biggest in all the AIM and AOM samples except for the site 3. *A. alternata* was the smaller in site 2 than the other site.

The biggest number of *P. digitatum*, *C. cladosporioides* and *A. alternata* population was observed in the AOS air samples, and the spices *P. chrysogenum* in the AOS air samples (Table 4). On the other hand, some fungi were found in a few AIS and AOS samples, In the AIS samples the genus *Penicillum* sp. was found in all AIS samples of the five sites. *R. solani* occurred only in AOS samples of site 3 and site 5, While *A. fumigatus* was found only in AIS for site 5 and *A. terreus* was found in AIS samples for site 3 and site 4.

Others genera found such as *C. murorum* and *Curvularia* sp. were not found in AOS of site 2, site 3 and site 4. Also *F. oxysporum* was not in AIS of site 1 and site 5. Non-identified (Mycelia sterila) fungi were found in the AIM and AOM samples of the five analyzed sites, because it was not possible to obtain reproductive structures in the several conditions tested (Table 4).

Table 4 : Numbers of colony forming of fungi isolated from air of indoor (AIS) and outdoor (AOS) at different locals.

Fungal genera and species	Site 1		Site 2		Site 3		Site 4		Site 5	
	AIS	AOS	AIS	AOS	AIS	AOS	AIS	AOS	AIS	AOS
A. alternata	98	155	74	136	155	144	112	179	86	183
A. flavus	-	21	-	10	7	26	3	30	7	59
A. fumigatus	-	-	-	-	-	-	-	-	40	-
A. niger	75	16	20	12	67	6	55	8	39	10
A. terreus	-	-	-	-	10	-	14	-	-	-
C. murorum	2	5	5	-	4	-	9	-	6	4
C. cladosporioides	150	117	170	237	197	181	181	221	199	323
Curvularia sp.	11	-	62	-	156	29	111	-	210	8
F. oxysporum	-	7	1	6	1	2	2	9	-	13
F. solani	51	79	70	72	63	103	54	87	68	133
Mucor sp.	3	8	10	2	32	7	1	15	8	13
Mycelia sterilia	4	1	2	1	13	1	4	1	2	1
P. chrysogenum	143	97	119	145	188	57	135	181	142	142
P. digitatum	110	226	141	396	192	271	120	192	62	148
Penicillum sp.	56	-	71	-	77	-	63	-	71	-
Phoma sp.	23	40	11	11	12	22	4	24	4	33
R. nigricans	78	55	96	51	111	51	84	44	102	47
R. solani	-	-	-	-	-	2	-	-	-	2
T. harzianum	2	2	-	2	3	-	3	-	-	1
T. roseum	75	62	86	49	64	31	68	32	58	43
U. botrytis	27	51	19	38	33	48	31	46	23	52
Total	908	942	957	1168	1385	981	1054	1069	1127	1215

The relative frequency of fungi varied according to the species, sites and samples. The species that occurred most in the site 1 were, in decreasing order, in the AIS samples *C. cladosporioides* (16.5%), *P. chrysogenum* (15.7%), *P. digitatum* (12.1%), *A. alternata* (10.8%), *R. nigicans* (8.6%) and *A. niger* (8.23%); as for the AOS samples, *P. digitatum* (23.9%), *A. alternata* (16.4%), *C. cladosporioides* (12.4%), *P. chrysogenum* (10.3%) and *F. solani* (8.37%). In the site 2, in the AIS samples the species were *C. cladosporioides* (17.8%), *P. digitatum* (17.4%), *P. chrysogenum* (12.4%), *R. niricans* (10.0%), *T. roseum* (9.0%) and *A. alternata* (7.73%), and in the AOS samples the species *P. digitatum* (33.9%), *C. cladosporioides* (20.3%), *P. chrysogenum* (12.4%), *A. alternata* (11.6%) and *F.*

solani (6.16%). In the site 3, the most frequent fungi species in the AIS were *C. cladosporioides* (14.2%), *P. digitatum* and *P. chrysogenum* (13.9%, 13.6%), respectively and 11.2%, 11.3% for both fungi *A. alternata* and *Curvularia* sp. and in the AOS samples, *P. digitatum* (27.7%), *C. cladosporioides* (18.5%), *A. alternata* (14.7%), *F. solani* (10.5%), and *P. chrysogenum* (5.82%). In the site 4 there was predominance of the species *C. cladosporioides* (17.2%), *P. chrysogenum* (12.8%), *P. digitatum* (11.4%), *A. alternata* (10.6%), *Curvularia* sp. (10.5%) and *R. nigricans* (8.0%%) in the AIS samples, and the species *C. cladosporioides* (20.7%), *P. digitatum* (18.0%), *P. chrysogenum* (16.9%), *A. alternata* (16.7%), *F. solani* (8.14%), and *U. botrytis* (4.30%) in the AOS samples. In the AIS samples of site 5, the dominant fungi were *Curvularia* sp. (18.6%), *C. cladosporioides* (17.7%), *P. chrysogenum* (12.6%), *R. nigicans* (9.05%), *A. alternata* (7.63%) and (6.30%, 6.03%) for both fungi *Penicillum* sp. and *F. solani* respectively. While the species which was dominant in AOS samples *C. cladosporioides* (26.6%), *A. alternata* (15.1%), *P. digitatum* (12.2%), *P. chrysogenum* (11.7%), *F. solani* (10.9%) and *A. flavus* (4.90%). The frequency of Some of genera was less than one such as *C. murorum* , *R. solani* and *T. harzianum* in AIS and AOS samples in all locals. The frequency values for the species were calculated based on the total number of fungi in the AIS and AOS samples (Table 5).

Table 5: Fungal isolated from indoor air samples (AIS) and outdoor (AOS) in different locals, represented by percentage of each species.

Fungal genera and species	Site 1		Site 2		Site 3		Site 4		Site 5	
	AIS	AOS	AIS	AOS	AIS	AOS	AIS	AOS	AIS	AOS
A. alternata	10.8	16.4	7.73	11.6	11.2	14.7	10.6	16.7	7.63	15.1
A. flavus	-	2.22	-	0.86	0.51	2.66	0.28	2.81	0.62	4.90
A. fumigatus	-	-	-	-	-	-	-	-	3.55	-
A. niger	8.23	1.7	2.09	1.03	4.84	0.61	5.22	0.75	3.46	0.82
A. terreus	-	-	-	-	0.72	-	1.33	-	-	-
C. murorum	0.22	0.53	0.52	-	0.29	-	0.85	-	0.53	0.33
C. cladosporioides	16.5	12.4	17.8	20.3	14.2	18.5	17.2	20.7	17.7	26.6
Curvularia sp	1.21	-	6.48	-	11.3	3.00	10.5	-	18.6	0.66
F. oxysporum	-	0.74	0.10	0.51	0.07	0.20	0.19	0.84	-	1.07
F. solani	5.62	8.37	7.31	6.16	4.55	10.5	5.12	8.14	6.03	10.9
Mucor sp	0.33	0.85	1.04	0.17	2.31	0.72	0.09	1.40	0.71	1.07
Mycelia sterilia	0.44	0.11	0.21	0.09	0.94	0.10	0.38	0.09	0.18	0.08
P. chrysogenum	15.7	10.3	12.4	12.4	13.6	5.82	12.8	16.9	12.6	11.7
P. digitatum	12.1	23.9	14.7	33.9	13.9	27.7	11.4	18.0	5.50	12.2
Penicillum sp	6.17	-	7.42	-	5.56	-	6.0	-	6.30	-
Phoma sp	2.53	4.24	1.15	0.94	0.87	2.25	0.38	2.25	0.35	2.72
R. nigricans	8.6	5.83	10.0	4.37	8.01	5.21	8.0	4.12	9.05	3.87
R. solani	-	0.21	-	-	-	-	-	-	-	0.16
T. harzianum	0.22	0.21	-	0.17	0.22	-	0.28	-	-	0.08
T. roseum	8.26	6.57	9.0	4.20	4.62	3.17	6.45	3.0	5.15	3.54
U. botrytis	3.0	5.40	2.0	3.25	2.40	4.90	2.94	4.30	2.04	4.28
Total	100	100	100	100	100	100	100	100	100	100

DISCUSSION

The present study was started on April 2013, up to the end in March, 2014. This study is based on the isolation, identification and assessment of mycoflora from indoor and outdoor of home in different localities of El-Beida in Libya. Libya is a country with very little rainfall occurrence per year. Along the time, the country experiences

sandstorms thus increasing the propensity of indoor household fungal contamination. Two previous studies on fungal flora in air samples in Benghazi and Shahat in Libya conducted in 2010 reported higher concentrations of fungal colonies in the air conditioner in all locations, with *Penicillium* and *Rhizopus* in outdoor samples of Benghazi (Hamady, 2010), *Cladosporium cladosporioides* in indoor samples of Shahat (Ibrahim, 2010) predominating the isolated colonies. Similarly our works indicated that there was a total of 21 fungal species isolated from indoor and outdoor these mycoflora were represented in the following genera *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cheatomium mourum*, *C. cladosporioides*, *Curvularia* sp, *Fusarium oxysporum*, *F. solani*, *Mucor* sp, *Penicillium chrysogenum*, *P. digitatum*, *Penicillium* sp, *Phoma* sp, *Rhizopus nigricans*, *Rhizoctonia solani*, *Trichoderma harzianum*, *Trichothecum roseum* and *Ulocladium botrytis*. Non-identified (Mycelia sterila) fungi were collected in separated group. Our study showed that *Penicillium* , *Cladosporium*, and *Alternaria* were the most common fungi recovered inside and outside the homes, these finding are in agreement with the finding of several other researchers (Katz, et al., 1999 ; Dharmage, et al., 2002 ; Unlu, et al., 2003 and Hedayati, et al., 2005). It is well known that spores of species of *Aspergillus*, *Cladosporium*, and *Penicillium* generated in damp building can cause bouts of asthma and or/ rhinitis among atopic occupants; as well as having a role in such individual cases of allergic disease. Fungi are now seen as having a wider role in respiratory ill health (Gambale, et al. 1981; 1982 ; Lacaz, 2002). Soil is an important source for airborne fungi. A study on soil fungi in Trabzon by Soylu (1997) showed that the most abundant genera were *Penicillium*, *Aspergillus*, *Cladosporidium* and *Fusarium*. El-Gali (2014) reported that the soil of El-Beida contained *A. alternata*, *A. niger*, *Cheatomium*, *Fusarium* spp and *Pencillium* spp, *Phoma* sp. and others fungi. Intensively isolated *Cladosporium*, *Alternaria*, *Phoma*, *Penicillium* and *Aspergillus* are pathogens in cultivated plants besides being important fungal allergens and they are the most common fungus species found in the atmospheric air (Agrios, 2005; Kaarakainen, et al., 2008; Topbas, et al., 2006). Prevailing winds, vegetation, medical and house waste storage areas, active population structure and high altitude are thought to be contributory effect to the higher fungal density in the locations compared to other locations (Asan et al., 2002; Bandyopadhyay, et al., 1991; Di Giorgia, et al., 1996; Pasanen, 1992; Šimsekli, et al., 1997). Cladosporium species may be found in agricultural areas where vegetation is more intense, while the density of *Penicillium* and *Aspergillus* may be higher in locations closer to dumping areas (Kaarakainen, et al., 2008). Cladosporium causes fractures and burns in the needle-shaped leaves of conifers, leaf blight in tomato (*C. fulvum*), scaby and offshoot blight in peach (*C. cladosporioides*), scaby and gumnosis in cucumber (*C. cucumerinum*), root decay and blights in pea (Agrios, 2005). The spores of *Alternaria*, which comes the second, exist in the air and soil and cause leaf blots, blights, dampingoff, trunk decays, burl and fruit decays especially in vegetables and foliage plants. *Phoma*, another fungus intensively isolated in the study, exists together with other weak parasitic pathogens and causes blackleg disease and root decay in zucchini (telemorph, Leptosphaeria, maculans) and *phoma* disease in tomato. *Penicillium* causes rots in damaged and ripe post-harvest fruits and several infections in cereals and legume stored under high humidity and low temperature. *Aspergillus* is responsible for mold and decays in cereals and legume and rots in fruits like peach and strawberry. The fungi isolated in the air are significant due to the diseases they cause in humans and animals, and production of the mycotoxins as well as efficiency and quality loss in plants.

CONCLUSION

In this study carried out for the first time in order to determine the fungi in the atmospheric air and their density in El-Beida, it is significant for aerobiologists and phythopathologists that isolated fungi are consistently followed due to the diseases, especially allergy, they cause in humans and animals and the loss they cause in the efficiency and quality of plants.

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