First Report About The Atmospheric Fungi in El-Beida City, Libya.

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Abstract: Air and dust monitoring were conducted at representative El-Beida, Libya indoor/outdoor homes in order to identify and enumerate fungal common in indoor and outdoor environment. A total of fifteen home were assessed between April 2013 and March 2014, yielding a total of 270 samples from indoor homes, outdoor and dust samples for each month. Indoor samples had a maximum number of isolates fungi than outdoor, while dust samples contain the less number of isolates fungi. The large number of fungal colonies was recorded in site 3 followed site 5, in Autumn season followed by Summer then Spring, in February month followed by June then April and on MEA medium through the study.

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1. Introduction

Fungi, or moulds, are an abundant group of micro-organisms that play a crucial role in the degradation process of organic matter and organisms in the environment. Approximately one quarter of the Earth's biomass is made up of fungi and approximately 100,000 species of fungi have been identified (Whitehead 2007). Although moulds are fully functional in the outside environment, they can potentially pose a significant threat to health when transferred into the home. They are microscopic organisms that form visible colonies or other structures when growing on a suitable substrate under favorable environmental conditions (Stetzenbach and Buttner, 2005). Fungi reproduce through the production of microscopic spores, many of which are dispersed by wind, rainfall, and physical disturbance. Most people spend over 90% of their lives indoors: in houses, offices, and schools (Abdel Hameed and Farag, 1999) where they are exposed to some indoor environmental factors (bioaerosol) that influence their health and physical condition. Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some substances harmful for health. These are different kinds of toxic metabolism products, for example mycotoxins (Flannigan, 2001; Pieckova and Kunova, 2002; Daisey et al., 2003). Epidemiological studies show that too high concentration of microorganisms in the air can be allergenic; however, sometimes even very low concentrations of some particular microorganisms can cause serious diseases. There have been several studies of airborne fungi in different parts of the world (Hedayati et al., 2005; Gomez de Ana et al., 2007; Lingnell, 2008; Suerdem and Yildirim, 2009; Abu-Dieyeh et al., 2010; Caballero et al., 2010; Fareid, 2011; Muhsin and Adlan, 2012; Awad et al.; 2013).In Libya, the airborne mould flora has only been studied in the cities areas of some Libyan Governorates (Hamadi, 2010; Ibrahim, 2010).

This study was intended to be viewed as an initial investigation into airborne and dustborne fungal profiles of homes El-Beida city into localities, monthly and seasonal variation and culture media relationship of abundance and genera identified. information gathered may also help to address lack of knowledge and inform local guidelines on indoor microbial air quality under El-Beida conditions.

2. Materials and Methods

Location of study:

El-Beida is a city located in the northeast of Libya (Fig. 1-A), and most of its territory covering an area of 11.429 km² at a distance of 25 km from the coast. The city lends its name to the snow fallen at winter gave the ground bright white color (El-Beida = White). Altitude is 624 m and it is surrounded by rich forests and flora (Fig. 1-B). 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. This work was started in February 2013 to March 2014. Five different locations were selected in El-Beida city to install "Spore Trapper" in order to trap the fungal spores from the atmospheric air. Map of study area locations are shown color on the map in Fig. (1-C). Five locations selected in El-Beida were (Site1) Less populated areas = green color, (Site 2) Densely

populated areas = yellow color, (Site 3) Pollution environmental = blue color, (Site 4) Restaurant whereabouts = pink color, (Site 5) Trees whereabouts = grey color.

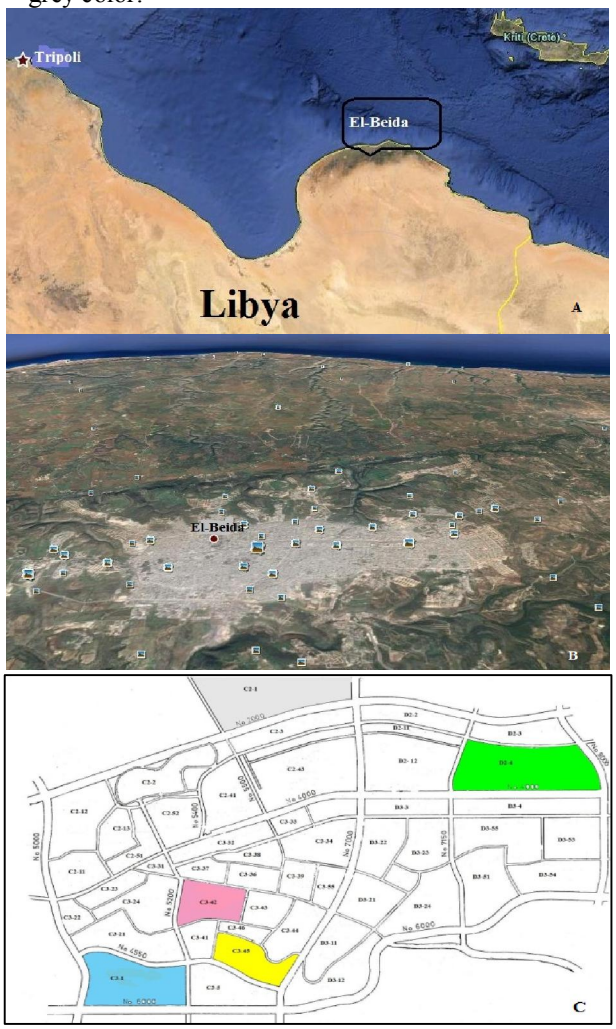

Fig. 1. Map of El-Beida city and locations of study

During the year there are two distinct seasons, wet and dry. The wet season is from November to March and the dry season is between April and October. The mean annual rainfall is 1.2 ml, and the temperature varies between 18.3 ºC and 27 ºC. Three homes were chosen at each of five sites, thus working at a total of 270 samples/month in five locations. The same hour of the day (9:00 a.m. to 12:00 noon) were used for each of the five sites. The study was conducted to a 12-month period. 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 of fungi: for isolation of fungi we used to methods:

Opened plates containing of PDA and MEA media were used for isolation of fungi in the air of indoor and outdoor of 15 homes. The plates will opened for 3 hours, then wrapped with Parafilm, and incubated inverted under 12 hr artificial daylight at room temperature for 7 to 14 days prior to examination. (Yassin & Almouqatea, 2010).

Dilution method: In this technique, the dust collecting in glass watch after 10 days. These samples were supplied in sealed. Three subsamples of approximately 50 mg (the actual mass was recorded) were added individually to 10 mL of sterile distilled water and suspended by vortexing at medium speed. Three serial dilutions of these stock suspensions were made subsequently using an adaptation of the standard technique reviewed by Malloch (1981); the first was made by diluting 1 mL of stock suspension in 9 mL of sterile distilled water and the second was made by diluting 1 mL of the first serial dilution in 9 mL of sterile distilled water Four aliquots of 1 mL each were taken from each of the two sets of dilutions and dispensed individually into Pyrex 100 mm Petri dishes. Three Petri plates were set up in this manner for each of the two stock suspensions. PDA and MEA were used. The medium was mixed with the dilution aliquot by gently swirling the Petri plates prior to solidification. Plates were wrapped with Parafilm (Alcan) and then incubated under 12 hr artificial daylight at room temperature (24 °C) for 7 days.

Identification of fungi

Where possible, fungi were identified to the genus level directly from colonies on PDA media using well-established techniques of macroscopic and microscopic examination and standard reference works for the identification of moulds using lactophenol blue stain (HiMedia- India). A portion of the obtained culture was placed and teased out into a clean glass slide upon a drop of lactophenol cotton blue using sterile inoculating needles and covered with clean coverslip. It was then viewed under the microscope using x4, x10 and x40 objectives. The light microscope depended on studying the morphological characteristic (growth, pigments, colony color) and microscopic characteristic (mycelium, spores, etc.) which was compared to the mycological atlas for confirmatory identification such as CMI (1966); Larone, (1995); Nelson & Toussun, (1976); Singh *et al*., (1991) and Ellis, *et al*., (2007). Many isolates were further identified to species level using appropriate monographs.

3. Results

The present study was conducted to elucidate the distribution pattern of airborne and dustborne fungi over four seasons of the year 2013- 2014 in relation to

some metrological parameters in El-Beida city, northern Libya.

A total of 11,525 fungal colonies were isolated from a total of 270 Petri dishes/month in samples of air and dust during 12 months. The isolated fungi were classification in twenty one species belonging to sixteen genera as well as yeast. The highest number of isolated fungi (5431 colonies) were detected in indoor samples followed by (5375 colonies) in outdoor and (719 colonies) in dust samples (Fig. 2).

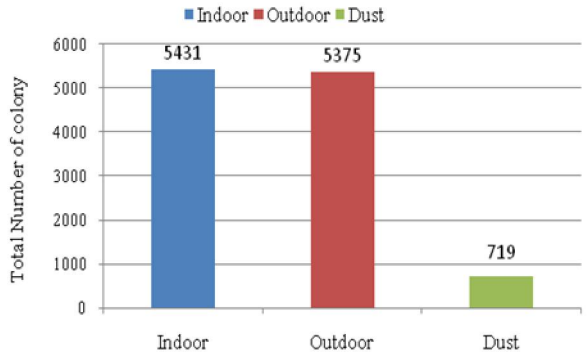

Fig. 2. Total number of fungal colonies in air and dust samples

Figure 2. highlights sites variation in number of fungal colonies in indoor, outdoor and dust. It show the highest levels were detected in Site 3 to have a characteristic Pollution environmental areas followed by Site 5 to have a characteristic Trees whereabouts and Site 4 to have a characteristic Restaurants whereabouts, while the lowest levels were detected in Site 1 to have a characteristic Less populated areas.

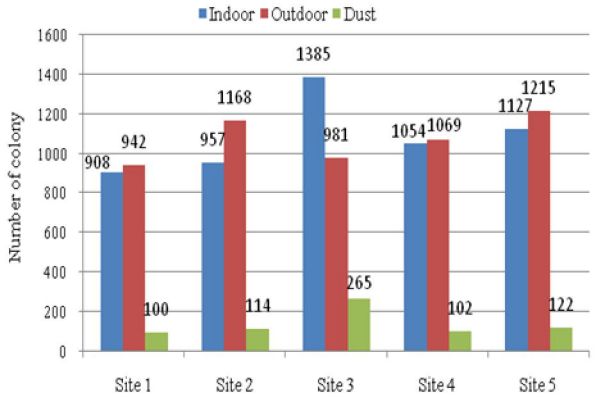

Fig. 2. Total number of fungal colonies in five sites

Concerning with the distribution of fungi in seasons, the most notable were in Autumn, Summer then Spring while in Winter detected less levels in indoor and outdoor samples, while in dust samples, Summer recorded the highest level Autumn and Spring then Winter. The abundance of genera varied by season and region (Fig. 3).

In the Spring and Winter season was highest Diversity (DV) in fungi and the Autumn season was lowest. The references showed that the isolated fungi from samples in this study caused disease and that made it a environmental pollution causing agents, addition with the dust. The increase and decrease in percentage of isolated fungi during the study may be due to the change in the temperatures and relative humidity in all the months throughout the year.

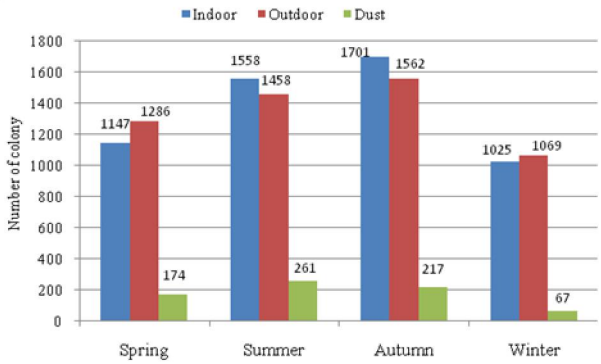

Fig. 3. Seasonal distribution of air and dust fungi

The agar open plate method in direct isolation was more efficient to isolate different fungi in airborne and dustborne than the dilution method. It is also noticed that the efficiency of the method differed according to the isolated fungi. Malt extract Agar (MEA) media was more effective to isolated fungi than Potato Dextrose Agar (PDA) in airborne samples, while in dust samples the media PDA was the best (Fig. 4).

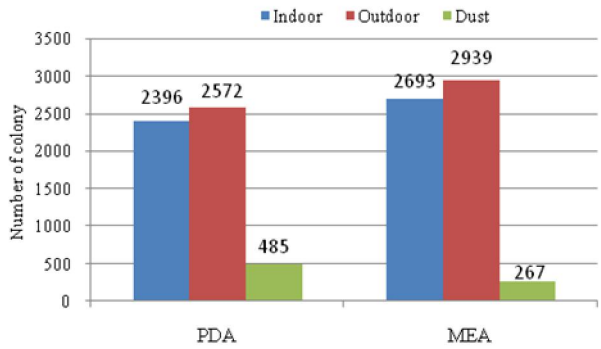

Fig. 4. Number of colonies on PDA and MEA media

The highest fungal density was recorded in March, June, and November in indoor, outdoor and dust samples whereas fungal density was the lowest in January and December in more sample (Fig. 5). November had a maximum number of colony but the minimum was recorded in December for all samples.

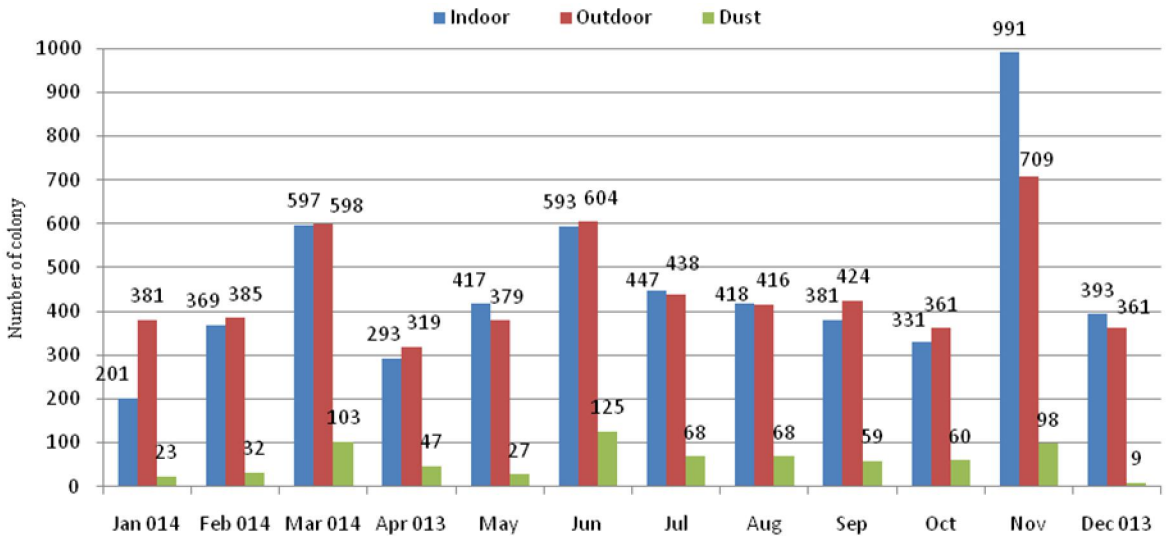

Fig. 5. Monthly fungal density in air and dust samples

Regarding the isolated fungi from air and dust samples collected from different localities during the season 2013 – 2014 were identified as *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus Aspergillus niger, Aspergillus terreus*, *Cheatomium murorum*, *Cladosporium cladosporioides, Cruvularia* sp, *Fusarium oxysporum*, *Fusarium solani*, *Mucor* sp, *Mycelia sterilia, Penicillium chrysogenum*, *Penicillium digitatum*, *Penicillium* sp, *Phoma* sp, *Rhizopus nigricans*, *Rhizoctonia solani*, *Trichoderma harzianum, Trichothecium roseum*, *Ulocladium botrytis* and some of yeasts as *Rhodotorula mucilaginosa* and other unidentified yeast (Table 1).

The prevalence of specific fungal genera was determine for indoor, outdoor and dust by calculating the presented in smples in which each genus was found. The top five most commonly detected fungi in indoor air were *C. cladosporioides* (16.5%), *P. chrysogenum* (13.4%), *P. digitatum* (11.5%), *Curvularia* sp (10.1%) and *A. alternata* (9.7%). In outdoor the most dominant fungi were *P. digitatum* (22.9%), *C. cladosporioides* (20.6%), *A. alternate* (15.2%), *P. chrysogenum* (11.9%) and *F. solani* (9.1%). The species *P. chrysogenum* (29.1%), *P. digitatum* (20.2%), *R. nigricans* (16.4%), *F. solani* (10.7%) and *T. roseum* (9.9%) were dominant in dust samples.

Some of fungal genera regarded as toxigenic were isolated from indoor, outdoor and dust samples of homes such as *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp., (Table 1).

Table 1. Frequency of isolates fungi from air and dust samples.

Fungi	Indoor	Outdoor	Dust
A. alternata	9.7	15.2	3.8
A. flavus	0.31	2.8	-
A. fumigatus	0.74	-	-
A. niger	4.71	1.0	3.6
A. terrus	0.44	-	-
C. murorum	0.48	0.17	-
C. cladosporioides	16.5	20.6	0.4
Curvularia sp	10.1	0.71	-
F. oxysporum	0.07	0.71	1.0
F. solani	5.63	9.1	10.7
Mucor sp	1.00	0.86	0.28
Mycelia sterilia	0.46	0.1	-
P. chrysogenum	13.4	11.9	29.1
P. digitatum	11.5	22.9	20.2
Penicillum sp	6.22	-	3.1
Phoma sp	1.00	2.5	-
R. nigricans	8.7	4.7	16.4
R. solani	-	0.08	-
T. harzianum	0.15	0.1	0.27
T. roseum	6.46	4.14	9.9
U. botrytis	2.45	4.5	1.25
Yeast:			
R. mucilaginosa	+	+	+
Unidentified yeast	+	+	+
+: Found, -: Not found			

From this study noticed that different growth and diversity of fungi. The following figures are describing growth and diversity of fungi in different samples on both media. Some of fungi overgrowth other fungi and others were non sporulating (Fig. 6).

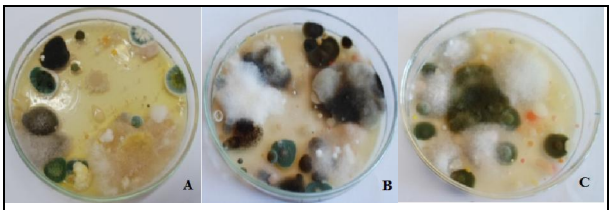

Fig. 6: Plate A and B show an examples of colonies obscured by overgrowth, and thus may be difficult to identify; Plate B and C show examples of non-sporulating sterile mycelia (White colonies).

In Figure (7) was noticed different concentration of fungal population in indoor samples from low, moderate and highly concentration on PDA medium.

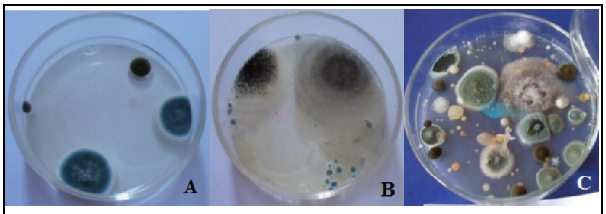

Fig. 7: Examples of indoor air sample results showing low fungal concentration (Plate A), moderate concentration (Plate B) and high concentration (Plate C) on PDA medium

On the other hand, the same results were observed on MEA medium. Different concentrations growth and different diversity was show in Figures (8) and (9).

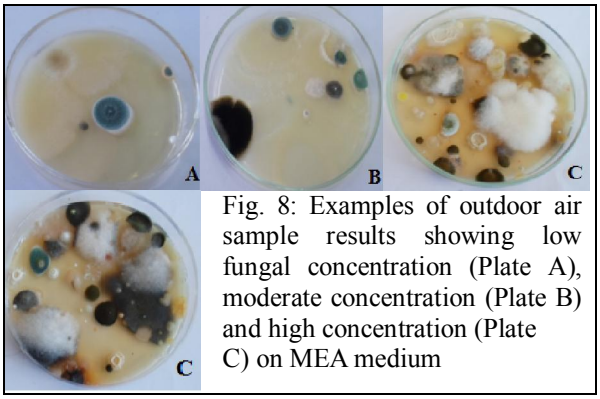

Fig. 8: Examples of outdoor air sample results showing low fungal concentration (Plate A), moderate concentration (Plate B) and high concentration (Plate C) on MEA medium

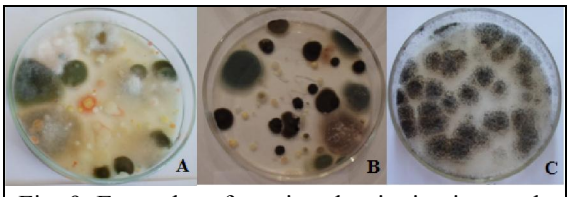

Fig. 9: Examples of varying density in air sample results: Plate A shows a highly diverse sample, Plate B and C show low and very low diversity from air samples with apparent single dominant species on each on MEA medium.

Regarding dust samples the results of isolation from dilution technique show that decreased fungal diversity and number of colonies (Fig. 10) from all samples.

Yeast also were presented in this study. They different in growth and color from pink, yellow and cream (Fig. 11).

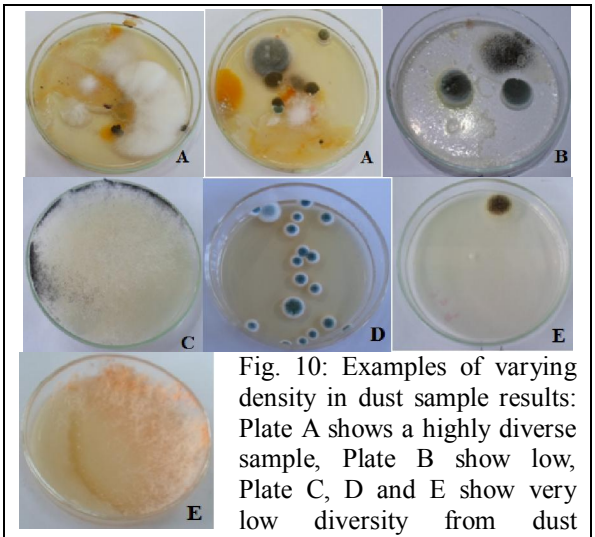

Fig. 10: Examples of varying density in dust sample results: Plate A shows a highly diverse sample, Plate B show low, Plate C, D and E show very low diversity from dust samples with apparent single dominant species on each on PDA medium.

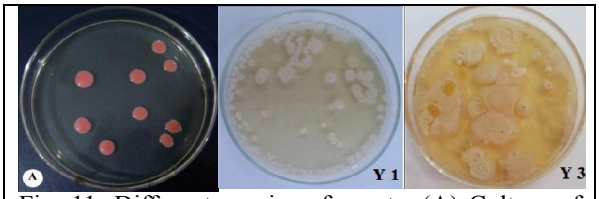

Fig. 11: Different species of yeasts, (A) Culture of *Rhodotorula mucilaginosa*. Y_1, and Y_2 unidentified species

4. Discussion

All of the fungi isolated in this study were first described as airborne, although they have also been reported from soil borne (El-Gali, 2014). The most frequent species were *C. cladosporioides, P. chrysogenum, P digitatum, A. alternata* and *F. solani.*

This result is consistent with previous studies in Benghazi (Hamadi, 2010) and Shahat (Ibrahim, 2010). On the Other hand the pathogenic potential of some of the environmental fungal isolates such as *A. alternata, C. cladosporioides, F. oxysporum, F. solani, P. digitatum* and *R. solani* located in the outdoor of home vicinity of site 5 who characterized by tree whereabouts. It is important for phytopathologists that

a large proportion of the fungi transported in the atmospheric air are phytopathogens. Over 10,000 types of fungi in nature are pathogens in plants (Agrios, 2005). These great differences could be due to geographic location, fungal growth substrates in different countries as well as different sampling methods used (Adhikari, et al., 2004). Moreover, the human activities in agriculture and industry might also increase the fungal frequency in the atmosphere

The highest number of isolated fungi were detected in indoor samples followed by outdoor and dust samples in all localities. These results are in agreement with results of many earlier investigators, Hedayati, et al. (2005); Gómez de Ana, et al. (2006); Chew, et al. (2003) and Caballero, et al. (2010). These results may be due to high humidity in indoor, It has been proposed that fungal flora inside homes are representative of the outdoor atmosphere, since airborne spores penetrate through doors and windows (Chew, et al., 2003).

Regarding season, In the present study some degrees of seasonal variations of the major genera were detected, the most notable were Autumn, Summer and Spring and it less in Winter in indoor and outdoor samples respectively, this result was argument with Shelton, et al (2002), while in dust samples it recorded Summer, Autumn, Spring and then Winter Both the indoor and outdoor populations were largest in the fall and summer and smallest in the spring and summer. The higher outdoor concentrations in the summer and fall may reflect higher temperatures and humidities and resulting increases in microbiological activity in these periods.

The increase in fungal density in March, June and November plays a significant role in seasonal distribution and the highest fungus isolation was observed in these months in indoors, outdoors and Dust samples. And the lowest level was detected in December. It is also noted that the lowest minimum temperature and relative humidity was in the month of January and December, which may account for the lowest counts of colonies from samples at all sites.

Two media were used in this study, PDA medium and MEA medium to isolates fungi from air and dust samples. The results showed that MEA medium was more effective to isolates fungi from indoor and outdoor samples. Whereas PDA was most to isolates fungi from dust samples. The graphs indicating the total number of colonies isolated at all sites after incubation. This results agreement with Ogunlana (1975). Ho reported there were no marked differences observed with respect to the two media, However, it was noted that the high incidence of growth in MEA medium put it at a relatively higher incidence rate. However, there are marked differences shown by a few of the fungal genera especially fungal group which grew better in the MEA medium (Ogunlana, 1975). Malt extract agar is a broad-spectrum growth medium commonly used for culture of mold in indoor environments (ACGIH, 1999). The results showed that the isolated fungi from samples in this study caused disease and that made it a environmental pollution causing agents, addition with the dust.

Conclusions

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 phytopathologists 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. El-Beida is a dusty city in dry seasons. Regular meticulous cleaning is very important to prevent dust accumulation at indoor environment and consequently the propagation of indoor fungi.

6. Acknowledgements

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References

1. Whitehead K. Fungal and Bacterial Populations of Visually Contaminated Bathroom Shower Surfaces Before and After Cleaning with Sodium Hypochlorite. American Society for Microbiology. 2007. Abstract Q-449.
2. Stetzenbach, L.D. and Buttner, M.P. Toxic mold litigation. Lawyers & Judges Co., Inc. 2005; 1-15.
3. Abdel Hameed A.A.. and Farag S.A. An indoor bio-contaminants air quality. International Journal of Environmental Health Research 1999; 9: 313
4. Flannigan, B. Microbial Aerosols in Buildings: Origins, Health Implications and Controls. Proceedings of the II International Scientific Conference: Microbial Biodegradation and Biodeterioration of Technical Materials, 11-27, Łódź, Poland, 2001
5. Pieckova E., and Kunova Z. Indoor fungi and their ciliostatic metabolites. Ann Agric Environ Med. 2002; 9:59.
6. Daisey, J.M., Angell, W.J., and Apte, M.G. Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information. Indoor Air 2003;13, 53,

7. Hedayati, M.T.; Mayahi, S.; Aghili, R. and Kayvan, G. Airborne Fungi in Indoor and Outdoor of Asthmatic Patients' Home Living in the City of Sari, Iran J Allergy Asthma Immunol, 2005; 4(4): 189-191.
8. Gómez de Ana, S.; Torres-Rodríguez, J.M.; Alvarado Ramírez, E. Mojal García, S. and Belmonte- Soler, J. Seasonal Distribution of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* species Isolated in Homes of Fungal Allergic Patients. J Investig Allergol Clin Immunol 2006;16(6): 357-363
9. Lingnell, U. Characterization of microorganisms in indoor environments. Publication of the National Health Institute, Kuopio, Finland. ISSSN. 2008; 1458 – 6290.
10. Suerdem, T.B. and Yildirim, I. Fungi in the atmospheric air of Çanakkale province in Turkey. African Journal of Biotechnology, 2009;8(18):4450-4458.
11. Abu-Dieyeh, M.H.; Barham, R.; Abu-Elteen, K.; Al-Rashidi, R. and Shaheen, I. Seasonal variation of fungal spore populations in the atmosphere of Zarqa area, Jordan. Aerobiologia, 2010; 1-14.
12. Caballero, C.P.; Palma, I.M.C.; Pacheco, M.L.; Marrufo, M.G. and Franco, C.Q. Indoor-outdoor fungal-aerosols ratios of domestic homes in Merida, Mexico. Ingeniería 2010;14(3):169-175.
13. Fareid, M.A. Indoor mycoflora in household dust and human health. Nature and Science, 2011;9(10): 27-36.
14. Muhsin, T.M. and Adlan, M.M. Seasonal distribution pattern of outdoor airborne fungi in Basrah city, southern Iraq Journal of Basrah Researches (Sciences), 2012; 38(1): 90 98.
15. Awad, A.A.; Gibbs, S.G.; Tarwater, P.M. and Green, C.F. Coarse and fine culturable fungal air concentrations in urban and rural homes in Egypt. Int. J. Environ. Res. Public Health (2013); 10: 936-949.
16. Hamadi, E.M.A. Microbial contamination in ambient air in Benghazi area. M. Sc. Thesis submitted to Benghazi University. 2010.
17. Ibrahim, S.A.E. Study of estimation of rates of microbial contamination in ambient air in Shahat area. M. Sc. Thesis submitted to Bengahzi University. 2010.
18. Yassin, M.F. and Almouqatea, S. Assessment of airborne bacteria and fungi in an indoor and outdoor environment. Int. J. Environ. Sci. Tech., 2010;7(3): 535- 544.
19. Malloch, D.W. Moulds: Their Isolation, Cultivation and Identification. University of Toronto Press, Toronto, Canada. 1981; 97 pp
20. CMI. ″Commonwealth Mycological Institute″ Description of pathogenic fungi and bacteria. Kew, Surry, England. 1966.
21. Larone, D.H. Medically Important fungi: a guide to identification. Washington: AMS Press. 1995.
22. Nelson, P.E. and Toussoun, T.A. A pictorial guide to the identification of *Fusarium* species. 1976; 43 pp.
23. Singh, K.; Frisual, J.C.; Thrane, U. and Mathur, S.B. An illustrated of some seed-borne Aspergilli, Fusaria, Penicillia and their mycotoxins. Jordbrugs forlaget. Frederik Sberg, Denmark. 1991.
24. Ellis, D.; Davis, S.; Alexiou, H.; Handke, R. and Bartely, R. Descriptions of medical fungi. Second Edition. Adelaide, Australia. 2007.
25. El-Gali, Z.I. Comparison of natural soil sterilization methods and their effects on soil inhabitant fungi. Nat. Sci., 2014;12(4):72-78.
26. Agrios, G.N. Plant Disease Caused by Fungi, in: Agrios G.N (ed.) Plant Pathology. Elsevier Academic Press, USA, (2005); pp 386-615.
27. Adhikari, A.; Sen, M.M.; Gupta, S.B. and Chanda, S. Airborne viable, non-viable and allergenic fungi in a rural agricultural area of India. Sci Total Environ., (2004); 326: 123–41.
28. Chew, G.L; Rogers, C.; Burge, H.A.; Muilenberg, M.L. and Gold, D.R. Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics. Allergy. 2003;58:13-20.
29. Shelton, B.G.; Kimberly, H.; Flanders, D.W. and Morris, G.K. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl. Environ. Microbiol. 2002;8: 1743-1753.
30. Ogunlana, E.O. Fungal Air Spora at Ibadan, Nigeria. Applied Microbiology, 1975;29(4): 458-463**.**
31. American Conference of Governmental Industrial Hygienists (ACGIH) "Sample Analysis." In J.M. Macher, ed. Bioaerosols: Assessment and Control. Cincinnati, OH, 1999; pp. 6-1 - 6-13.

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