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# Monthly and Seasonal Distribution of Dustborne Fungi in Atmospheric of El-Beida City (Libya)

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**Abstract:** An investigation of the quantity of dustborne microbes in 5 different locations of El-Beida City, Libya was carried out to assess the level of dustborne pathogens in different locations and studied the monthly and seasonal distribution for dustborne fungi. A total of 719 mould colony were collected. Mould colonies were assigned to 10 genera and 13 species. The highest abundance was attributed to *P. chrysosgenium* with a percentage of 29.1% of the total colony count followed by *P. digitatum* 20.2%, *R. nigricans* 16.4%, *F. solani* 10.7%, *T. roseum*, 9.9%, *A. alternata* 3.8% and *A. niger* 3.6%. *P. chrysosgenium* showed one peak in June while *A. alternata* and *P. digitatum* peaked in June. *R. nigicans* peaked in April and June and *F. solani* in April. However, *T. roseum* showed almost the same abundance from January to May and then in August to November. With respect to seasons it was found that in Summer, *A. alternata*, *P. digitatum* and *R. nigicans*, in Autumn *P. chrysogenum* and *T. roseum*. In Spring, *A. niger* and *F. solani*. Winter had the least number of total colony. *P. chrysogenum* were the most prevalent fungal genera. In addition most of fungi isolated were important aeroallergens and phytopathogen.

**Key words:** Fungi, dustborne, seasonal distribution, phytopathogen, El-Beida, Libya

## INTRODUCTION

Of an estimated 2 billion metric tons of dust that move some distance in Earth's atmosphere each year, approximately 75% originates from the Sahara and Sahel regions of Africa (Goudie and Middleton, 2001; Moulin *et al*., 1997; Perkins, 2001). Among the wide variety of biological particles present in the atmosphere, there is a very significant number of fungal spores. Fungi live as saprophytes on organic material or as parasites (mainly plant pathogens), so the majority of fungal spores in the air atmospheric come from farms, forest stands, soil, water and decomposing plant matter. Fungi are correlated with high environmental burdens have been shown to be affected by various factors such as wind, moisture and temperature and air pollution leading to variations with respect to species and quantities from one season to another. Fungi thrive better in moist and warm places. Fungal spores in aquatic environments may be transferred to the air by wave action. The concentration of atmospheric fungal spores has been linked to wind, humidity, temperature, rainfall, altitude, vegetation and various specific reservoirs of contamination. In addition, fungal propagative units may be dispersed in the air by insects (Kerssies, 1993). According to Lacey (1981), airborne fungal spores are originally created from plant, animal and soil sources; however, some researchers believed that airborne spores are mainly a contribution from vegetation rather than soil (Gregory, 1973; Moubasher, 1993). The number of fungal airspora and their diversity vary with time of day, weather, season, geographical location and the presence of local spore sources (Lacey, 1981). Some species of fungi such as *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium brevicompactum*, *P. chrysogenum*, *Aspergillus candidus*, *A. niger* and *A. versicolor* can provoke extreme allergic reactions in humans (Simeray *et al*., 1995) and these bioaerosols may cause eye and sinus irritation, sore throat, headache, fatigue and dizziness (Buttner *et al*., 2001). Mould growth may contribute to sick-building syndrome as well as to allergy and other environmental health problems (Singh, 2001). Fungal propagules can serve as infectiveagents of plant diseases and moreover, airborne fungi cause spoilage of foods and are responsible for many adverse health effects; the mycotoxins which they produce may affect humans and animals. This study represents the first investigation of air-borne fungal spore population of the El-Beida area, Libya and represents a baseline for future studies.

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## MATERIALS AND METHODS

**A study area:** El-Beida is a city in Northwestern Libya (Fig. 1a). It is the capital of the Al-Gabal Al-Akhdar Province. It lies 44'-30'21° East longitude and 45'-59'32° North latitude with an area of 11.429 $km^2$. The city has a population of 356,876 according to El-Beida Province (2011 estimate). 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.

**Collection of house dust samples:** Five sites (Fig. 1b) were selected for collecting samples of dust from outdoor of home over four seasons during the period from April 2013 up to March 2014 in Winter (December to February), Spring (March to May), Summer (June to August) and Autumn (September to November). Site 1 is characteristics with less populated areas, site 2 is highly population Densely populated areas), site 3 is local near from abattoirs, butcheries, sawmills and ateliers that polluted the environment. While site 4 is local in the center of city and dominated by restaurants whereabouts. Site 5 is local in north of city and covered with some herbs, shrubs and dominated by trees whereabouts plantations. Three homes in one site were selected randomly. Three glass watch were put outdoor of the home in 10 first days from each month and the dust samples were mixed to give a composite sample for each home. Each dust sample was kept in clean specimen bottles 10 mL PE at 4-6°C until processed.

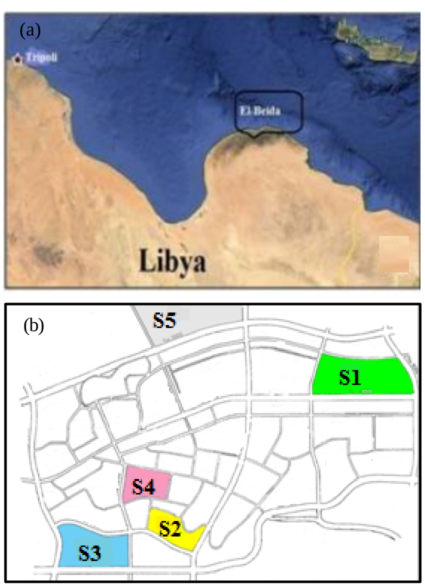


Fig. 1: a) Map of Northeast Libya and location El-Beida City and b) different sites for study

**Meteorological measurements:** Daily meteorological data were supplied by the Weather Record Department at El-Beida weather station.

**Isolation of fungi:** Samples were analyzed for fungi that can be cultured using a procedure described. Total 1 g of individual samples was weighed and suspended in 10 mL sterile distilled water. From the above suspension, 1 mL was then diluted to 0.01 this process is repeated to yield dilutions 0.001. A 1 mL of the diluted sample ($10^{-1}$, $10^{-2}$) can be put on the surface of solidified medium (PDA, MEA) and spread evenly throughout. This procedure was carried out in duplicate. Then, Petri dishes were incubated at 28°C for 5-7 days. Count (C) of Colony Forming Units per g dust (CFU/g dust) and percentage of total count (%) were calculated.

**Identification of fungi:** Cultural characters were assessed visually and by microscopic examination. Pure culture of the fungal isolates were identified according to the following descriptive manuals (CMI, 1996; Barnett and Hunter, 1972; Hoog and Guarro, 1995; Larone, 1995; Ainsworth *et al*., 1995; Alexopoulos *et al*., 1996; Toussoun and Nelson, 1976; Davis *et al*., 2007).

## RESULTS

**Meteorological measurements:** Table 1 represented the average meteorological measurements in El-Beida City. The results indicated that temperatures degrees were increased gradually through the period from April till September, 2013. The average measurements of temperatures recorded 24.3°C. Metrological data showed that humidity was higher during November to March, while wind speed recorded a higher level at June and July (18.2-18 m $sec^{-1}$). 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.

The 270 dishes exposed yielded 917 colonies of 13 fungi. Table 2 shows the number of the colonies and frequency of each fungal genus in the city of El-Beida and the five collection sites. Table 1 shows the number of the colonies and frequency of each fungal genus in the city of El-Beida and the five collection sites. The seven most frequent airborne fungi isolated were: *P. chrysogenum* (29.1%), *P. digitatum* (20.2%), *R. nigricans* (16.4%), *F. solani* (10.7%), *T. roseum* (9.9%), *A. alternata* (3.8%) and *A. niger* (3.1%). In this research, *A. alternate*, *F. solani*, *P. chrysogenum*, *R. nigricans*, *T. roseum* and *U. botrytis* were isolated from all sites while *C. cladosporioides* was

Table 1: Average temperature, RH %, precipitation and wind speed in atmosphere of El-Beida City

| Months | Average temperature (°C) | Total precipitation (mm) | Average humidity (%) | Average wind (m $sec^{-1}$) |
|---|---|---|---|---|
| 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 |

Table 2: Total numbers of isolates of dust-borne fungal species at five sites in El-Beida City

| Fungal genera and species | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Total | Frequency (%) |
|---|---|---|---|---|---|---|---|
| *A. alternata* | 3.0 | 6.0 | 120.0 | 3.0 | 3.0 | 27 | 3.80 |
| *A. niger* | - | 1.0 | 4.0 | 7.0 | 14.0 | 26 | 3.60 |
| *C. cladosporioides* | - | - | 3.0 | - | - | 3 | 0.40 |
| *F. oxysporum* | 5.0 | 1.0 | - | - | 1.0 | 7 | 1.00 |
| *F. solani* | 11.0 | 5.0 | 36.0 | 12.0 | 13.0 | 77 | 10.7 |
| *Mucor* sp. | 1.0 | - | - | 1.0 | - | 2 | 0.28 |
| *P. chrysogenum* | 21.0 | 59.0 | 74.0 | 24.0 | 31.0 | 209 | 29.1 |
| *P. digitatum* | 15.0 | 26.0 | 94.0 | - | 10.0 | 145 | 20.2 |
| *Penicillium* sp. | 9.0 | - | 7.0 | 2.0 | 5.0 | 23 | 3.10 |
| *R. nigricans* | 29.0 | 19.0 | 19.0 | 23.0 | 28.0 | 118 | 16.4 |
| *T. harzianum* | - | - | - | - | 2.0 | 2 | 0.27 |
| *T. roseum* | 5.0 | 11.0 | 12.0 | 29.0 | 14.0 | 71 | 9.90 |
| *U. botrytis* | 1.0 | 2.0 | 4.0 | 1.0 | 1.0 | 9 | 1.25 |
| Total | 100.0 | 114.0 | 265.0 | 102.0 | 122.0 | 719 | - |
| Frequency (%) | 13.9 | 15.9 | 36.9 | 14.2 | 17.0 | - | - |

Table 3: Monthly distribution and number of colonies of dust borne fungi isolated in El-Beida City

| | Months | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fungal genera and species | Jan. 2014 | Feb. 2014 | Mar. 2014 | Apr. 2013 | May. 2013 | Jun. 2013 | Jul. 2013 | Aug. 2013 | Sep. 2013 | Oct. 2013 | Nov. 2013 | Dec. 2013 | Total |
| *A. alternata* | - | - | - | 4.0 | - | 12.0 | - | - | 1.0 | 1.0 | 9.0 | - | 27 |
| *A. niger* | - | 2.0 | 10.0 | 2.0 | 1.0 | 2.0 | 7.0 | | 1.0 | 1.0 | - | - | 26 |
| *C. cladosporioides* | - | - | - | - | - | - | - | - | - | - | 3.0 | - | 3 |
| *F. solani* | 5.0 | 7.0 | 17.0 | 1.0 | 10.0 | 4.0 | 11.0 | - | 6.0 | - | 10.0 | 6.0 | 77 |
| *F. oxysporum* | 1.0 | 2.0 | - | - | - | - | - | - | - | 1.0 | 3.0 | - | 7 |
| *Mucor* sp. | - | - | 2.0 | - | - | - | - | - | - | - | - | - | 2 |
| *Penicillium* sp. | 1.0 | - | - | - | - | 1 | - | - | 13.0 | 8.0 | - | - | 23 |
| *P. chrysosgenium* | 3.0 | 1.0 | 12.0 | 11.0 | 1.0 | 56.0 | 14.0 | 18.0 | 27.0 | 18.0 | 48.0 | - | 209 |
| *P .digitatum* | - | 20.0 | 15.0 | 14.0 | 13.0 | 22.0 | 20.0 | 17.0 | 20.0 | 14.0 | - | - | 145 |
| *R. nigricans* | 1.0 | 1.0 | 28.0 | 10.0 | 1.0 | 28.0 | 15.0 | 25.0 | - | - | 9.0 | - | 118 |
| *T. harzianum* | - | - | - | - | - | - | 1.0 | - | - | 1.0 | - | - | 2 |
| *T. roseum* | 11.0 | 1.0 | 12.0 | 5.0 | 1.0 | - | - | 8.0 | 1.0 | 16.0 | 16.0 | - | 71 |
| *U. botrytis* | 1.0 | 3.0 | 2.0 | - | - | - | - | - | - | - | - | 3.0 | 9 |
| Frequency (%) | 3.2 | 5.1 | 14.3 | 6.5 | 3.8 | 17.4 | 9.5 | 9.5 | 8.2 | 8.3 | 13.6 | 1.3 | - |

isolated only in site 3 and *T. harzianum* only in site 5. *F. oxysporum* was isolated in site 1, 2 and 5 and *Mucor* sp. was isolated in site 1 and 4. The fungi *A. niger*, *Penicillium digitatum* and *Penicillium* sp. were found in all sites excepted site 1, 4 and 2, respectively. Regarding the density of fungi in sites we noticed that site 3 was the more contamination of fungi (265 colonies) while site 1 was the less contaminated of fungi (100 colonies). The higher diversity 11 species of fungi was found in site 5 and the lowest diversity 9 species was found in sites 2 and 4.

Table 3 shows the monthly distribution of each genus. *P. chrysosgenium* was found in all months excepting December. Also *P. digitatum* was found in all months except November till February. *T. harzianum* was isolated in July and October while *C. cladosporioides* isolated only in November and *Mucor* sp. only in March. The maximum frequency of fungi were recorded in June (17.4%) and the lowest frequency of fungi recorded in December (1.3% frequency). The remaining genera were randomly present throughout the year.

Figure 2 showed that the decrease in the fungal diversity in all months. The maximum number of colonies

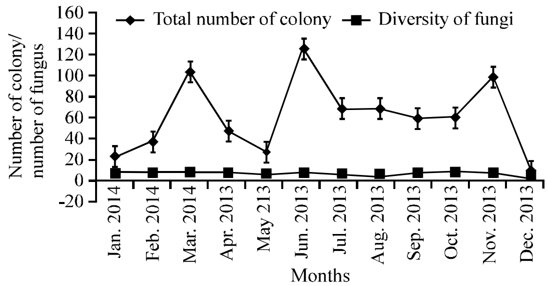


Fig. 2: Monthly variations in total colony count and number of fungal species in each month

Table 4: Seasonal distribution and number of colonies of dustborne fungi isolated in El-Beida City

| Fungal genera and species | Seasons Spring | Summer | Autumn | Winter | Total |
|---|---|---|---|---|---|
| *A. alternata* | 4.0 | 12.0 | 11.0 | - | 27 |
| *A. niger* | 13.0 | 9.0 | 2.0 | 2.00 | 26 |
| *C. cladosporioides* | - | - | 3.0 | - | 3 |
| *F. oxysporum* | - | - | 4.0 | 3.00 | 7 |
| *F. solani* | 28.0 | 15.0 | 16.0 | 18.00 | 77 |
| *Mucor* sp. | 2.0 | - | - | - | 2 |
| *P. chrysogenum* | 24.0 | 88.0 | 93.0 | 4.00 | 209 |
| *P. digitatum* | 42.0 | 59.0 | 24.0 | 20.00 | 145 |
| *Penicillium* sp. | - | 1.0 | 21.0 | 1.00 | 23 |
| *R. nigricans* | 39.0 | 68.0 | 9.0 | 2.00 | 118 |
| *T. harzianum* | - | 1.0 | 1.0 | - | 2 |
| *T. roseum* | 18.0 | 8.0 | 33.0 | 12.00 | 71 |
| *U. botrytis* | 4.0 | - | - | 5.00 | 9 |
| Total | 174.0 | 261.0 | 217.0 | 67.00 | 719 |
| Frequency (%) | 24.2 | 36.3 | 30.2 | 9.32 | - |

were recorded in June (125 colonies) and the lowest number of colony recorded in December (9 colonies). The first peak of colony count and species diversity was in March then decreased to May. The maximum peak was observed in June. Also in November the peak of colony count was observed the decreased in December.

The result of seasonal distribution was tabulated in Table 4. The highest reproduction level was observed in Summer (261 colonies, 36.3%) followed by Autumn (217 colonies, 30.250). The most reproductive fungus species were *P. chrysogenum* in Autumn and Summer with 88 and 93 colonies, respectively, *P. digitatum* and *R. nigricans* in Summer and Spring in with 59 and 42, 68 and with 39 colonies for both fungi, respectively. *C. cladosporioides* and *Mucor* sp. were recorded in Autumn and Spring, respectively. *A. alternata* was found in all seasons excepting Winter.

## DISCUSSION

Knowledge of species and density of dustborne 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. Thirteen species of fungi including *P. chrysogenum*, *P. digitatum*, *R. nigricans* and *F. solani*. *Pencillium* sp., *R. nigricans*, *F. solani*, *T. roseum* and *A. alternata* were the most prevalent and appeared to be the most common species in the all of the sites. Soil is an important source for airborne fungi. A study on soil fungi in El-Beida by El-Gali (2014) showed that the most abundant genera were *A. alternata*, *A. niger*, *Fusarium* and *Pencillium* sp.

The Penicillium species have been identified as important causative agents of extrinsic bronchial asthma (Shen and Han, 1998). Furthermore, it has been reported that the most common genera namely *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* should always be considered as a cause of fungal allergy (Peat *et al*., 1993). Alternaria which also known to be allergenic and is one of the most common fungi worldwide. Downs *et al*. (2001) reported that Alternaria allergens contributed to severe asthma in regions where exposure to the fungus was high. *Fusarium* was the second most abundant species in the findings with a total contribution of 11.7% divided between 2 identified species (Table 2). Although, *Fusarium* was reported to be one of the most abundant species in the atmosphere of Tel-Aviv, Israel (Kessler 1953, 1957).

In the collections site 3 the greatest number of the airborne fungi genera was isolated (Table 2). This result due to local near from abattoirs, butcheries, sawmills and ateliers that polluted the environment. In site 1 was isolated a smaller number of airborne fungi, these results suggesting the less populated area and new buildings. The second large number of total colonies was recorded in site 5 which covered with some herbs, shrubs and dominated by trees where abouts plantations that stimulate the sporulation of the fungi.

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*., 2009; Khan *et al*., 1999).

Many researchers have reported significant effects of meterological factors on the concentration of aerospora (Agarwal *et al*., 1969; Di Giorgio *et al*., 1996; Bandyopadhyay *et al*., 1991; Asan *et al*., 2004). Far from considering correlations with a certain climatic parameter, the climatic year of the El-Beida area can be divided into two periods which can be repeatedly overlapped: the first is the rainfall temperate period including October to March and the second is the dry high temperature period including May to September. Moreover, the vegetation and mainly the annuals are flourishing between January and March. Therefore, the first peak of spore colony count and species diversity was March (Fig. 2). This is easily explained as it comes after the 3 months of the highest rainfall and moderate temperature, December till February, so the soil is moistened enough and the vegetation is in its maximum growth and diversity. Basically, soil and vegetation are considered the main sources contributing to airborne fungal spores (Moustafa, 1971; Gregory, 1973; Abdel-Gawad, 1984; Moubasher and Al-Subai, 1987). The sudden rise of fungal count in June due to the country exposure to dust storms in Summer 2013 which increasing the contamination by dust borne fungi. This result was harmony with the results was obtained by Fareid (2011). The second peak was in November period (Fig. 2). This peak is also due to the conditions following the rainfall encountered in October and also to the relatively moderate temperature and humid climate. Even though rainfall was too low but still enough to moisten the soil and to cause perennial shrubs and bushes to thrive and in consequence fungal growth to flourish after the extended dry hot conditions from May to September. Usually rainy months have maximum frequency and concentration of fungal spores due to the favourable growth and sporulation conditions for fungi and the availability of suitable substrates (Kakde *et al.*, 2001).

In the present study, although some degrees of seasonal variations of the major genera were detected, the most notable ones were Autumn the *Penicillium* sp. and *T. roseum* genus in that they were in higher numbers in the Autumn and in Summer, respectively. Rainfall and relative humidity almost always have profound effects on the level of fungi spores. It has been stated that *A. alternata* levels absent in Winter as opposed to *P. chrysogenum* and *P. digitatum* levels which may be high in Autumn and Summer despite the fact that they may be found in the atmosphere all year round (Al-Suwaine *et al.*, 1999; Doory, 1984). The results also showed that overall the total number of fungi colonies decrease in winter but in contrast to the results of other studies, Penicillium reproduces more in Winter and Summer.

## CONCLUSION

The present study suggests that the city of El-Beida, as in any of the mountain dwellings in the region, harbors various species of fungi due to its warm and rainy climate and very rich flora. It is of significance that the findings may be of use with regard to the diagnosis and prophylaxis of allergic diseases thought to be resulting from airborne fungi and this should be born in mind when using allergic tests the spectrum of the fungal genera examined in this region. This study may thus be of considerable assistance to scientists and clinicians working in this field in adopting preventive measures and/or selecting an appropriate antigen for diagnostic purposes.

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