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## Study of the Airborne Fungal Spores in Rosetta, Egypt

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### Abstract

In this study, fungal spores in the atmosphere of Rosetta, Egypt were studied for one year (August 2015 to July 2016) using a Hirst type volumetric pollen trap. An annual spore index equals to 8023 was recorded during the studied period. The maximum records were in August 2015, May and July 2016. *Alternaria*, *Cladosporium*, *Tilletia*, *Stemphylium*, *Chaetomium*, *Aspergillus/Penicillium*-type, *Drechslera*-type, *Mycosphaerella*, and *Epicoccum* represent the main spore producers organized in relation to their abundance. A total of nine fungal spore genera with minimum 10-day mean equal to or greater than 0.1 spores/m<sup>3</sup> of air are involved to construct an approximate spore calendar. This aeropalynological study was compared with others elsewhere in the world. Correlation analysis between spore counts and different meteorological parameters (temperature, rainfall, and relative humidity) as well as a number of allergic patients were studied. It was obvious that most of the recorded fungal spores have allergenic properties, especially from April till August. This study revealed that the air in Rosetta district is polluted and care must be taken in fruit and food storage especially during the summer period.

**Keywords:** Egypt; Rosetta; Spore calendar; Aerobiology; Spore allergy; Fungal spores

### Introduction

Allergy has become one of the most annoying health problems that have prevailed worldwide during the last decades [1,2]. Allergic reactions can result from different kinds of antigen-bearing agents such as foods, insect stings, soaps, pets, feathers, fibers, cosmetics, pollen and spores [3]. Pollen and spore allergy is considered the most typical form of allergic disease. Several works on allergy involving pollen allergy have been done all over the world. In Egypt, there are some works regarding pollen and spore allergy has been done. However, this is the first aeropalynological study done in Rosetta along the Mediterranean coast. This study deals with the investigation of fungal spores distributed in the atmosphere of Rosetta, and

their relation to allergy. Also, investigate the influence of different meteorological parameters on spore concentration.

### Materials and Methods

Rosetta is a port city of the Nile Delta, located 65 km east of Alexandria, Al-Behaira Governorate (**Figures 1 and 2**). Rosetta is located between latitudes 31° 12' N to 31° 28' N and longitudes 30° 16' E to 30° 32' E.

The climate data was obtained from the Egyptian Meteorological Authority in Cairo, Egypt. We can describe Rosetta district by being hot and dry in most of the months with moderate relative humidity. The maximum temperature reaches 32.2°C in August and the minimum temperature is 10.8°C in January. The relative humidity ranges from 66% in May to 73% in August. The rate of precipitation in the studied area is relatively low along the studied year with maximum rainfall in November (58.7 mm/month).



Figure 1: Location of the studied area, Rosetta in Egypt.



**Figure 2:** Location of the studied area, Rosetta in the delta region.

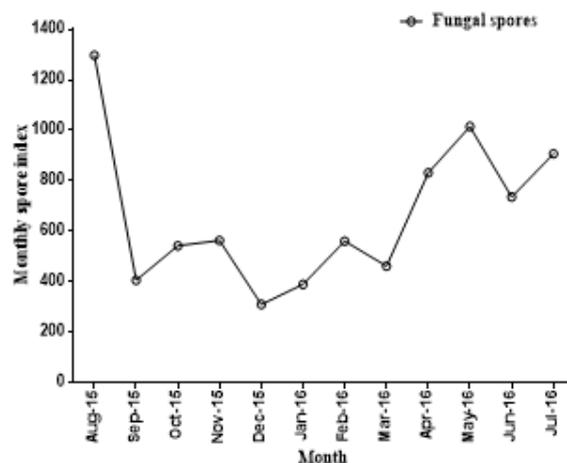
In the present study, airborne fungal spores in Rosetta atmosphere during the period from August 2015 until July 2016 were studied using Hirst type volumetric pollen trap supplied by Burkard Scientific (UK). This trap was operated on the roof of Basha Specialist Hospital, Rosetta, Egypt. The height of the hospital is about 18 m above the ground. The coordinates of Basha Hospital are 31° 23' 53" North, 30° 24' 44" East. The mechanism of the sampler is that a vacuum pump draws 10 l/min airflow through an orifice continuously oriented towards the wind [4]. Spores are impacted on an adhesive coated transparent plastic tape (Melinex) supported on a drum with a fixed circumference driven by a 7-jewel clockwork movement. The drum rotated past the orifice at 2 mm/hour. The drum is changed weekly [3]. The sampling method used is by Hirst 1952 [5]. The Melinex tape is coated with 10% gelvatol and then the adhesive mixture (Vaseline and wax). After a week, the tape was cut into 48 mm or 24 hours segments and then mounted on slides using glycerin jelly. The slides were examined under a light microscope according to the methodology proposed by Spanish Aerobiology Network, REA [4] as four longitudinal horizontal sweeps per slides were counted at a magnification of 400X. Spore counts should be expressed as the daily mean count per cubic meter of air. An approximation to spore calendar was constructed by following Spieksma's model [5], which transforms 10-day mean spore concentrations into a series of classes represented as columns of increasing height. The dominant fungal spores with minimum 10-day mean equal to or greater than 0.1 spores/m<sup>3</sup> of air are only included in the calendar. We acquired the data on numbers of patients suffering from chest diseases during the studied year (August 2015-July 2016) from Local Rosetta Hospital in Rosetta, Egypt.

The data analysis involved the multiple regression analysis between the environmental parameters (maximum, minimum and average temperature, relative humidity and total rainfall) and the number of trapped spores, as well as the number of the patient. We use the Pearson product-moment correlation coefficient [6] between the transformed values of the environmental parameters and number of patients, and also the number of spores at confidence limit 95%. Correlation coefficient ( $r$ ) is such that  $-1 < r < +1$  and probability value ( $P$ ) of less than 0.05 was considered significant.

## Results

**Fungal spores (*Alternaria*, *Aspergillus/Penicillium*-type, *Chaetomium*, *Cladosporium*, *Drechslera*-type, *Epicoccum*, *Mycosphaerella*, *Stemphylium* and *Tilletia*)**

The study of the aerospora during the period from August 2015 to July 2016 in Rosetta, Egypt exhibited an annual spore index equals to 8023, belonging to 13 genera and a group of unidentified fungal spores arranged in alphabetic order. The maximum monthly spore index was in August 2015 (1297), there are also two other peaks found in May and July 2016 with 1015 and 907 respectively (**Figure 3 and Table 1**). The minimum monthly spore index was in December 2015 (310), (**Figure 3**). The highest number of fungal spores came from *Alternaria* with total (38.0%). There are four genera of fungal spores represented by a very small count; those are *Beltrania*, *Curvularia*, *Pithomyces* and *Tetraploa*. The monthly spore indices of different spore genera are given in (**Table 1**). An approximation to a spore calendar is constructed for the nine dominant genera (**Figure 4**).



**Figure 3:** Monthly spore index during the period from August 2015 to July 2016 in Rosetta, Egypt.

The following taxa produced the greatest amount of fungal spores:

### *Alternaria*

*Alternaria* is considered the largest genus among all fungal spores. It accounts for 38% of total spore assemblage throughout the studied year (**Table 1**). Its highest peak was found in July with the maximum record was in the first decade (**Figure 4**). August and June also contain high records of *Alternaria* spores. The lowest record occurred in December (**Figure 5a and 5b**).

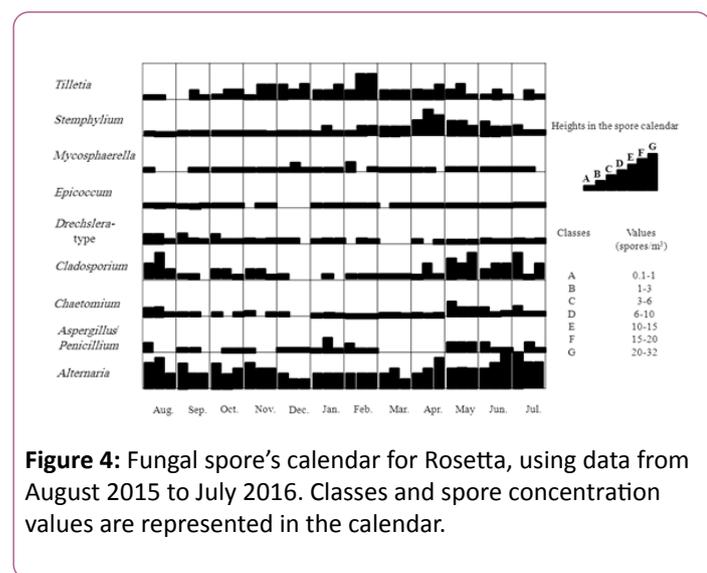
### Aspergillus/Penicillium-type

The annual spore index of *Aspergillus/Penicillium*-type 198 (2.47%) (Table 1 and Figure 5c). The highest peaks were found in

January and May while the minimum records were in March and April (Figure 4).

**Table 1:** Monthly fungal spore index spanning the period from August 2015 to July 2016 in Rosetta, Egypt.

Month/spore	15-Aug	15-Sep	15-Oct	15-Nov	15-Dec	16-Jan	16-Feb	16-Mar	16-Apr	16-May	16-Jun	16-Jul	Yearly Influx	Percentage*
<i>Alternaria</i>	356	197	248	289	91	114	120	137	289	233	409	566	3049	38
<i>Aspergillus/Penicillium</i> -type	11	3	18	2	12	45	25	0	0	55	15	12	198	2.47
<i>Beltrania</i>	0	0	0	2	0	0	1	0	0	0	0	1	4	0.05
<i>Chaetomium</i>	48	6	7	5	3	7	11	15	15	80	27	28	252	3.14
<i>Cladosporium</i>	189	11	53	33	3	4	6	12	58	264	93	173	899	11.21
<i>Curvularia</i>	2	1	3	0	1	0	0	0	1	0	0	1	9	0.11
<i>Drechslera</i> -type	34	25	4	21	5	5	2	1	3	4	4	16	154	1.92
<i>Epicoccum</i>	11	8	9	7	1	6	7	3	14	10	4	6	86	1.07
<i>Mycosphaerella</i>	4	7	10	20	30	27	12	6	3	12	7	4	142	1.77
<i>Pithomyces</i>	2	1	0	1	0	0	1	0	0	0	0	0	5	0.06
<i>Stemphylium</i>	12	2	20	19	4	21	37	56	204	134	97	20	636	7.93
<i>Tetraploa</i>	2	1	0	2	0	2	0	1	1	1	0	1	11	0.14
<i>Tilletia</i>	11	17	37	88	93	70	259	61	78	75	34	19	842	10.49
Undefined fungal spore	615	117	104	75	67	89	80	170	166	147	46	60	1736	21.64
<b>Monthly influx</b>	<b>1297</b>	<b>406</b>	<b>543</b>	<b>564</b>	<b>310</b>	<b>390</b>	<b>390</b>	<b>462</b>	<b>832</b>	<b>1015</b>	<b>736</b>	<b>907</b>	<b>8023</b>	



**Figure 4:** Fungal spore's calendar for Rosetta, using data from August 2015 to July 2016. Classes and spore concentration values are represented in the calendar.

### Chaetomium

*Chaetomium* exhibits an annual spore index equals to 252 (3.14%), (Table 1). The highest peaks were in August and May (Figure 5d). The lowest record was in December (Figure 4).

### Cladosporium

The annual spore index of *Cladosporium* was 899 (11.21%), (Table 1). It is the second largest spore (Figure 5e and 5f). The maximum peak was in May, August and July also have high records. The minimum record was in December (Table 1 and Figure 4).

### Drechslera-type

The annual spore index of *Drechslera*-type was 154 (1.92%), (Table 1). The highest peaks were in August and October while the minimum record was in March (Table 1 and Figure 4) and (Figure 5g and 5h).

### Epicoccum

The annual spore index of *Epicoccum* was 86 representing 1.07% of the total catch (Table 1). The highest record was in April, (Figure 5i) whereas December showed the minimum record (Table 1).

### Mycosphaerella

The annual spore index of *Mycosphaerella* was 142 representing 1.77% of the total assemblage (Table 1). The

highest records were in December and January (Figure 5j). The minimum record was found in April (Table 1).

### Stemphylium

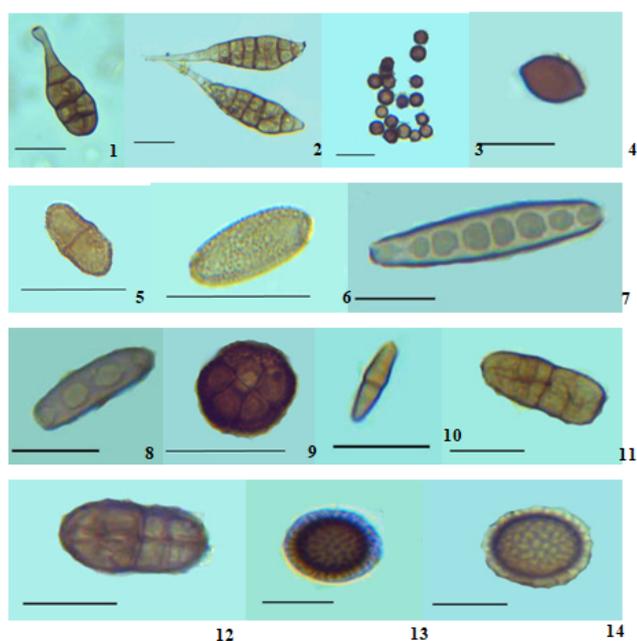
The annual spore index of *Stemphylium* was 636 that accounts to 7.93% of the total assemblage (Table 1). *Stemphylium* showed a maximum peak in April (Figure 4). The minimum record was in December (Table 1 and Figure 5k and 5l).

### Tilletia

*Tilletia* comes in third place among the largest spores count. The annual spore index was 842 representing 10.49% of the total catches (Table 1). The highest peak was in February as represented; which mainly concentrated in the second and third decades (Figure 4). The minimum record was in August (Table 1) (Figures 4 and 5m and 5n).

## Data Analyses

The Multiple regression analysis between numbers of patients recorded in the local Rosetta hospital during the study period and the environmental parameters show that the correlation coefficient value equals 0.815 at probability <0.02. This means that there is a significant correlation between the environmental parameters (minimum temperature, total rainfall, and relative humidity) and a number of patients. Fungal spores gave a significant correlation coefficient with the environmental parameters (average temperature, total rainfall, and relative humidity) equals 0.671 at probability <0.167.



**Figure 5:** Plate 1: (Scale bar=20 µm); a,b: *Alternaria* spp.; c: *Aspergillus*/*Penicillium*-type; d: *Chaetomium* sp.; e,f: *Cladosporium* spp.; g,h: *Drechslera*-type; i: *Epicoccum* sp.; j: *Mycosphaerella* sp.; k,l: *Stemphylium* sp.; m,n: *Tilletia* sp.

## Discussion

It has been clear that allergy becomes a social problem on all continents. Thus, it is so important to know the properties of allergens and mechanisms of pollen/spore allergy to help in the protection of allergic diseases [7-9]. Accordingly, studies must include both qualitative and quantitative composition of the airborne pollen/spores in any given area. In this study, the concentration of aerospora in Rosetta region was studied for one year to investigate an approximate spore calendar.

Fungal spores and particles are found in both outdoor and indoor environments, and exposure to fungi has been proved to stimulate allergic diseases [10]. Spores are often found in outdoor air throughout the year, and they exceed the pollen population by 100- to 1000-fold or more, that depends on environmental factors, such as water, nutrients, temperature and wind [5,11]. In this study, the high spore count can be attributed to the presence of many fruit trees which may be rotted by temperature and humidity. The transport and dispersal of airborne fungal spores are affected by several meteorological parameters [12,13]. In our study, the numbers of spores are increased from April to August as the temperature increased and rainfall decreased; temperature is involved in the release of conidia and in the colonization processes [14]. On the contrast, there is a decrease in spore count from September to March which may be associated with the increase in rainfall in most months and a relative decrease in temperature; rainfall washes out spores from the atmosphere. The multiple regression analysis showed that there is a significant correlation between the environmental parameters (average temperature, total rainfall, and relative humidity) and a number of spores. Several studies indicated that airborne fungal spores are considered very significant sensitizing factors in allergic respiratory diseases such as asthma and rhino-conjunctivitis [15-17].

The spores of different fungi have been trapped in this study. The identified fungal spores belong to 13 genera. Spores of *Beltrania*, *Curvularia*, *Pithomyces* and *Tetraploa* are found with very small annual spore indices in the assemblage equal to 4, 9, 5 and 11 (Table 1). These four genera of fungal spores probably come to the assemblage by accident; they might be attached with insects and transferred to the trap.

*Alternaria* is one of the most allergenic fungal spores worldwide that predominates in the atmosphere of many countries. In the present study, *Alternaria* spores were the most common fungal spores trapped from the atmosphere of Rosetta; their annual spore index was 3049 (38.0%, (Table 1)). The most common allergenic fungal spores in Iraq, Kuwait, Turkey, and Saudi Arabia are *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp. and *Ulocladium* spp. [18-21]. The report from Greece mentioned that *Cladosporium* spp., *Alternaria* spp. and *Ustilago* spp. were the most allergenic fungal spores affect patients [22]. Sensitization to *Alternaria* and other species was found to be associated with severe and potentially fatal episodes of asthma [15,23]. The spores of *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Drechslera* are considered important allergens of the world that spread in warm, humid and dry

climates [24-26] indeed these species are found in Rosetta district and almost increased in such climate.

*Aspergillus* and *Penicillium* species are considered closely related genera. They are found in outdoor air and are also known as major indoor fungi [27]. In the United Kingdom, they are dominated in the atmosphere in autumn and winter, but their peak was in the autumn [28]. In the present study, *Aspergillus*/*Penicillium*-type spores are relatively moderate; their annual spore index equals to 198 (**Table 1**) and reaches their maximum count in January and May [29]. Found that *Aspergillus* and *Penicillium* are associated with current asthma. Natural exposure of *Penicillium* species spores can induce both immediate and late asthma in sensitive people [30].

*Chaetomium* spores have occurred in a relatively moderate annual spore index in the present study (252) (**Table 1**). Several species are known to produce mycotoxins [31,32] and are recognized as human allergens [33]. It is reported that *Chaetomium globosum* has type I and III allergens [34], but the exposure to airborne spores may be rather limited because these spores are not easily aerosolized. Spores of *Cladosporium* species are spread worldwide and are considered the dominant airborne spores in several areas, particularly in temperate climates [35] In the present study; this species comes in second place among all the fungal spores found (**Table 1**) [36,37]. Its annual spore index was 899 and found that the most abundant allergenic fungal spores in Thessaloniki, Greece are *Alternaria* and *Cladosporium*, similarly to other areas worldwide [38,39]. Spores of *Cladosporium* are released during both wet and dry conditions and dispersed by rain splash. A test of allergic patients will have to involve spores of *Cladosporium* and *Alternaria*, particularly in summer [40].

*Drechslera* type is found in the present assemblage with a relatively moderate annual spore index equals to 154 (**Table 1**). It is indicated that the prevalence of skin test reactivity to members of *Drechslera*, *Exserohilum*, and *Bipolaris* is significant [41-43] also detected a significant reactivity to *Drechslera*-type spores in Finnish asthmatic children.

*Epicoccum nigrum* which is the only species in this genus is found frequently in air assemblages [44]. *Epicoccum* spores occurred with a relatively small amount in the atmosphere of the present study; their annual spore index was 86 (**Table 1**). The study in Northern Portugal from 2005 to 2007 showed that *Epicoccum* spores were presents almost all year round [45]. In

Poland, *Epicoccum* spores increased toward summer and autumn [46]. These studies some way match with our data as the spores of *Epicoccum* is nearly distributed throughout the whole year. *Epicoccum* is usually found on dead parts of many plants, seeds and soft fruits [47]. Several studies show skin test and RAST (Radio Allergy Sorbent Test) reactivity to *E. nigrum* ranging from 20% to 30% in atopic subjects [48-50]. In Europe, *Epicoccum* affects between 5% and 15.4% of the population causing allergy [51,52].

Spores of *Mycosphaerella* are reported in a relatively moderate annual spore index in the air assemblage of Rosetta equals to 142 (**Table 1**). Spores produced by *Mycosphaerella* species cause leaf diseases of many plants such as Eucalyptus [53].

One of the allergenic fungal spores found in the atmosphere is *Stemphylium* [54]. The spores of *Stemphylium* trapped from Rosetta are considered relatively high annual spore index (636) (**Table 1**) they come after spores of *Alternaria*, *Cladosporium* and *Tilletia* found that the most abundant airborne fungal spores in Timișoara city, România are *Cladosporium*, *Alternaria*, *Epicoccum*, *Helminthosporium* type, *Fusarium/Leptosphaeria* type, *Stemphylium* and *Curvularia*; these taxa are considered a group of cosmopolitan fungi. *Stemphylium* is found on decaying plants and in the soil. It is responsible for causing Type I allergies [55].

The third abundant fungal spores trapped in the atmosphere of Rosetta are *Tilletia* spores; their annual spore index was 842 (**Table 1**) [32]. Considered *Tilletia* one of the allergenic fungal spores. Some aerobiological studies have revealed the occurrence of *Tilletia* spores in the atmosphere of ecologically diverse locations.

The obtained data on the number of patients suffering from chest problems during the studied year in Rosetta, Egypt was compared with the number of spores trapped during this period (**Table 2**) As a result, we can notice the effect of fungal spores on a number of patients in some months. However allergenic pollen beside pollution and dust may also affect the number of the patient [56]. The Multiple regression analysis between numbers of patients and the environmental parameters shows that there is a significant correlation between the environmental parameters (minimum temperature, total rainfall, and relative humidity) and a number of patients; which in turn effect on the number of spores in the atmosphere [57].

**Table 2:** Numbers of patients having chest problems versus a number of airborne fungal spores during the year of study in Rosetta, Egypt.

Month	15-Aug	15-Sep	15-Oct	15-Nov	15-Dec	16-Jan	16-Feb	16-Mar	16-Apr	16-May	16-Jun	16-Jul
Fungal spores	1297	406	543	564	310	390	561	462	832	1015	736	907
Number of patients	360	322	240	270	351	466	465	330	420	320	225	226

This study reveals how much the fungal spores affect the health of the peoples in this area and increase their chest problems. This attributed to unhealthy storage of the food and fruit in this area causing rotting of them. For that, we advise the

peoples there to take care of the storage process to eliminate such problems.

## Conclusion

According to the spore calendar of Rosetta (Northern Egypt), the identified fungal spores that spread in the atmosphere of Rosetta are also found in different Mediterranean cities and also different areas around the world. The most effective allergenic fungal spores in Rosetta are *Alternaria* and *Cladosporium* then, *Tilletia*, *Stemphylium*, *Aspergillus*, *Penicillium*, *Drechslera*-type, and *Epicoccum*. These types affect the number of patients in Rosetta, but also there are other factors that have great influence on the allergenic patient, most probably dust and pollution besides the number of airborne pollen. This study indicates that the air in Rosetta district is polluted and care must be taken in food and fruit storage especially during the summer period to decrease the causes of allergy in this area.

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