

## Common environmental allergenic fungi causing respiratory allergy in North of Iran

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### Key Words:

Airborne fungi; *Cladophialophora*; *Alternaria*; fungal allergy.

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### Abstract

The aim of this study was to isolate and identify airborne fungi from locations in Babol city, Babolkenar forest and a beach on the Caspian Sea (all in North part of Iran) in spring and summer. Of 126 samples collected, the most clinically important isolated fungi were *Mucor* spp. (18.6%) in the forest, *Alternaria* spp. (13.4%) at the beach; and *Cladophialophora* spp. (15.8%), *Mucor* spp. (11.7%) and *Alternaria* spp. (10.6%) in the urban areas. Non-sporulating fungal isolates were grouped in *Mycelia sterilia*. The concentration of air spora in the forest location was significantly greater than the other locations ( $p < 0.05$ ). In this study, the highest quantities of isolated fungi were found in the summer. The results showed that *Alternaria* spp., *Cladophialophora* spp. and *Mucor* spp. were the most predominant fungi isolated in the different locations. Since some allergenic fungi have been shown to play a role in the appearance of clinical signs in allergic conditions, the results of this study can be used by physicians and veterinarians for the diagnosis and treatment of allergies.

### Introduction

Fungi are among the most common organisms in all environments. They are common in the air, soil, water and decaying vegetation throughout the world (Menezes *et al.*, 2004). The concentration and type of fungal spores in the atmosphere change in a 24 h period and from one season to another (Wang *et al.*, 2001). Airborne fungal concentration has a profound influence on the respiratory health of both humans and animals (Zureik *et al.*, 2002). Sensitivity to allergenic fungi may develop in normal individuals who are chronically exposed to the conidia of certain fungi at work, resulting in allergic diseases such as bronchial asthma, allergic rhinitis and atopic dermatitis (Burge and Rogers, 2000; Terui *et al.*, 2000). Some genera of airborne fungal spores such as *Alternaria*, *Aspergillus* and *Cladophialophora* are which the worldwide and are generally considered as an important cause of allergic diseases (Kurup, 2000). Due to increasing awareness of the relationship between airborne fungi and allergies in individuals with asthma, many microbiologists and allergists have begun to examine the distribution and type of fungal spores in both indoor and outdoor environments (Rainer *et al.*, 2000; Troutt and Levetin, 2001). Since studies on air spora have rarely been performed in Iran, this study was conducted

to determine both the geographical and seasonal distributions, and the daily and intradiurnal concentrations of airborne fungi in and around Babol city at seven different locations.

### Materials and Methods

The air sampling was conducted in seven locations in and around Babol city. These were five different urban locations (north, south, west, east and central region), a beach location (Caspian sea) and a forest location (Babolkenar). The sampling was performed three times a day; at 8 am at the forest location, between 12 noon and 14 pm in the urban locations and at 18 pm at the beach location during the last three days of each month in spring and summer. The amounts of air spora were determined by exposing a Petri dish containing Sabouraud glucose agar to air for 15 min. The media were incubated at 25°C for 7 to 10 d, and the quantity of fungal spores was calculated as colony forming units (CFU/plate/15 min). For fungal identification, each colony was mounted in lactophenol-cotton blue and examined under a light microscope to determine the colonial features and morphological structures. Fungal genera were then identified based on micro- and macro-morphology, reverse and surface coloration and colony size grown on Czapek-Dox agar, malt extract

agar and potato dextrose agar media. Differential tests such as the urease test and API 20c kit (RapID, Remel, Lenexa, Kansas, USA) were used as well. All chemicals and media were purchased from Merck (Darmstadt, Germany). For statistical analysis, a chi-square test was performed to reveal the differences between different locations, months and seasons with respect to quantities of isolated air spora. *P* value less than 0.05 was considered statistically significant.

## Results

Table 1 shows the number of colonies and frequency of each fungal genus in and around the city of Babol in the seven collection locations. The seven most frequently isolated fungi were: *Mucor* spp., *Cladophialophora* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Rhizopus* spp. In this study, the most common isolated fungus in the north, south, east, west and central regions of Babol were *Cladophialophora* spp. (22.9%), *Mucor* spp. (23.6%), *Alternaria* spp. (10.7%), *Cladophialophora* spp. (21.1%) and *Mucor* spp. (24.8%), respectively, whereas the most frequently isolated fungus in the forest and beach locations were *Mucor* spp. (18.6%) and *Alternaria* spp. (13.4%) respectively. Statistical analysis showed significant difference between the forest location when compared with the other locations ( $p < 0.05$ ). The seasonal and monthly distribution of colonies of airborne fungi is summarized in Table 2. The highest level of fungal reproduction was observed in summer (58.8%). The most frequently isolated fungi in different months were *Alternaria* spp. (18.2%) in

April, *Alternaria* spp. (15.3%) in May, *Penicillium* spp. (5.2%) in June, *Mucor* spp. (48.3%) in July, *Rhizopus* spp. (5.8%) in August and *Cladophialophora* spp. (33.4%) in September.

## Discussion

In recent years, aerobiologists have shown a great interest in airborne fungi due to both their constant presence in the air and the increase in allergies caused by them (Rylander and Carvalheiro, 2006; Khosravi *et al.*, 2009). In this study, *Cladophialophora* spp. was the most frequent genus detected in different urban locations, followed by *Mucor* spp. and *Alternaria* spp. In addition, the above-mentioned fungi were shown to be the predominant genera at the beach and forest locations. Reports and surveys of airborne fungi from all parts of the world have appeared in the literature. Average numbers obtained in 19 studies show that five fungal genera (*Cladophialophora*: 29%, *Alternaria*: 14%, *Penicillium*: 9%, *Aspergillus*: 6% and *Aureobasidium*: 5%) are predominant in the atmospheric air (Mete, 2001; Scholte, 2002; Asan, 2003; Colakoglu *et al.*, 2003). These data are similar to those found in our work that showed the *Cladophialophora* and *Alternaria* species as the most common airborne fungi in and around Babol city. Depending on geographic and climatic conditions, the prevalence of an allergic response to these fungi might be as high as 30% (Horner *et al.*, 1995). Among the allergenic fungi, sensitization to the *Cladophialophora* and *Alternaria* species was considered to be a risk factor for the development of wheezing and asthma and has been associated with

**Table 1:** Number of colonies and geographic distribution of different airborne fungal genera isolated from the city of Babol, Iran in seven different locations.

Genus	Location													
	North		South		East		West		Center		Forest*		Seaside	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Mucor</i>	11	4.3	86	23.6	8	1.8	16	3.1	121	24.8	204	18.6	11	8.7
<i>Rhizopus</i>	6	2.4	1	0.3	1	0.2	6	1.2	28	5.7	6	0.5	1	0.9
<i>Aspergillus</i>	26	10.3	17	4.7	32	7	24	4.7	16	3.3	1	0.1	12	9.4
<i>Penicillium</i>	16	6.3	27	7.4	17	3.7	16	3.1	9	1.8	10	0.9	13	10.2
<i>Cladophialophora</i>	58	22.9	20	5.5	22	4.8	109	21.1	119	24.4	0	0	15	11.8
<i>Alternaria</i>	51	20.2	43	11.8	49	10.7	32	6.2	46	9.5	15	1.4	17	13.4
<i>Ulocladium</i>	0	0	0	0	0	0	9	1.7	0	0	0	0	0	0
<i>Trichothecium</i>	6	2.4	1	0.3	7	1.5	2	0.4	0	0	1	0.1	1	0.9
<i>Mycelia sterilia</i>	48	18.9	124	34.1	227	49.7	152	29.5	120	24.6	857	78.1	30	23.6
<i>Pseudoallescheria boydii</i>	0	0	0	0	8	1.8	0	0	0	0	0	0	0	0
<i>Helminthosporium</i>	1	0.4	0	0	3	0.7	8	1.6	0	0	0	0	9	7.1
<i>Paecilomyces</i>	0	0	0	0	0	0	25	4.8	1	0.2	0	0	0	0
<i>Fusarium</i>	17	6.7	29	7.9	3	0.7	48	9.3	0	0	3	0.3	3	2.4
Dematiaceous fungi	7	2.8	9	2.5	73	15.9	67	12.9	16	3.3	0	0	14	11
<i>Cryptococcus</i>	0	0	6	1.6	2	0.4	0	0	1	0.2	1	0.1	0	0
<i>Rhodotorula</i>	4	1.6	1	0.3	5	1.1	1	0.2	7	1.4	0	0	1	0.9
<i>Geotrichum</i>	2	0.8	0	0	0	0	1	0.2	3	0.7	0	0	0	0
Total	253	100	364	100	457	100	516	100	487	100	1098	100	127	100

\* The concentration of air spora in the forest location was significantly more than the other regions ( $p < 0.05$ ).

**Table 2:** Monthly distribution and number of colonies of airborne fungi isolated from Babol city.

Genus	Month											
	April		May		June		July		August		September	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Mucor</i>	15	2.5	14	4.8	15	3.2	372	48.3	4	0.8	37	5.5
<i>Rhizopus</i>	1	0.2	0	0	1	0.2	6	0.8	29	5.8	12	1.8
<i>Aspergillus</i>	10	1.7	10	3.4	3	0.6	54	7	12	2.4	39	5.8
<i>Penicillium</i>	6	1	15	5.1	24	5.2	1	0.1	17	3.4	45	6.8
<i>Cladophialophora</i>	51	8.4	19	6.5	4	0.9	41	5.3	3	0.6	225	33.4
<i>Alternaria</i>	110	18.2	45	15.3	20	4.3	0	0	27	5.4	51	7.6
<i>Ulocladium</i>	0	0	0	0	0	0	0	0	9	1.8	0	0
<i>Trichothecium</i>	3	0.5	2	0.7	0	0	7	0.9	6	1.2	0	0
<i>Mycelia sterilia</i>	282	46.7	125	42.5	358	77.5	228	29.6	321	64.6	244	36.2
<i>Pseudoallescheria boydii</i>	0	0	0	0	0	0	8	1	0	0	0	0
<i>Helminthosporium</i>	6	1	15	5.1	0	0	0	0	0	0	0	0
<i>Paecilomyces</i>	25	4.1	1	0.3	0	0	0	0	0	0	0	0
<i>Fusarium</i>	29	4.8	9	3.1	13	2.8	16	2.1	26	5.2	8	1.2
Dematiaceous fungi	56	9.2	37	12.6	11	2.4	28	3.6	42	8.5	12	1.8
<i>Cryptococcus</i>	5	0.8	0	0	2	0.4	3	0.4	0	0	0	0
<i>Rhodotorula</i>	3	0.5	1	0.3	7	1.5	6	0.8	1	0.2	1	0.1
<i>Geotrichum</i>	1	0.2	1	0.3	4	0.9	0	0	0	0	0	0
Total	603	100	294	100	462	100	770	100	497	100	674	100

No significant difference was observed between different months nor between summer and spring.

severe cases of asthma and respiratory arrest (Khosravi *et al.* 2009a, b). A recent study on 4962 respiratory patients showed that 19% of the allergic population reacted to at least one fungal extract, as determined by means of a skin test, and more than 66% of these fungal-sensitized patients reacted to the *Alternaria* extract (Mari *et al.*, 2003). The *Cladophialophora* spp. genus contains important allergenic fungal species that have been reported to cause allergic diseases in nearly all climactic zones. Of the allergic population, 5 to 30% display IgE antibodies against this mold. Sensitization to *Cladosporium* has often been associated with severe asthma and less frequently with chronic urticaria and atopic eczema (Simon-Nobbe *et al.*, 2006). Myszkowska *et al.* (2002) noted that 4 to 7% of the European population showed sensitivity to *Alternaria* and *Cladophialophora* spores and these were also the predominant genera found in their study.

The relative humidity of the air and temperature are important factors in fungal growth in different areas (Di Giorgio, 1996). In the present study, although some degree of seasonal variation was detected in the major genera, the most notable ones were *Mucor* spp. (21.3%) and *Cladophialophora* spp. (13.8%) in summer and *Alternaria* spp. (12.8%) and *Cladophialophora* spp. (5.4%) in spring. The highest quantities of fungi were isolated in the summer. There have been many reports concerning the monthly (seasonal) variation of fungi in an outdoor environment in different countries. Most reports show that the peaks of concentration of fungi are recorded during the summer and early fall months of July, August and September (Larsen and Gravesen, 1991; Liao *et al.*, 2004). In the present study, the highest geometric mean number was recorded in July, followed by September and April, which is fairly consistent with

other studies. In conclusion, the present study showed that the city of Babol and environs harbored various genera of fungi due to its warm and rainy climate. Among them, *Alternaria* spp., *Cladophialophora* spp. and *Mucor* spp. were the most predominant isolated fungi from the different locations. Our findings could be used in the diagnosis and prophylaxis of allergic diseases and in allergy testing using the spectrum of the fungal genera isolated from this region.

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