

## Aeromycological analysis of allergenic airborne fungi in Qazvin, Iran

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(Received: 15 October 2016; Revised: 11 December 2016; Accepted: 4 January 2017)

### Abstract

**Background and Purpose:** Airborne fungi are one of the most important agents responsible for triggering allergic reactions such as rhinitis and severe asthma. This study was conducted to analyze and monitor the prevalence and distribution patterns of atmospheric fungal aerosols in the air of Qazvin during winter of 2012.

**Materials and Methods:** In the current descriptive study, the incidence and diversity of potentially allergenic airborne fungi were determined using two times sampling interval in 25 different locations of Qazvin city by Petri dish trapping technique and exposure of 10- cm diameter plates of Sabouraud's dextrose agar medium plus chloramphenicol to the air.

**Results:** A total of 2867 fungal colonies were counted on 156 Petri dishes. Of the identified 18 microfungi genera, *Cladosporium* spp. was the most frequently isolated genera representing 30.9% of isolates, followed by 30.9% *Penicillium* spp. (27.3%), *Aspergillus* spp. (24.5%), *Alternaria* spp. (3.3%), *Rhizopus* spp. (3.1%), and other fungal genera.

**Conclusion:** The high prevalence, high quantity and variety of allergenic airborne fungi in the air of Qazvin showed that people residing in this area are exposed to health hazards. Furthermore, reduction of exposure to bio-aerosols containing these outdoor fungi is necessary to improve the health of individuals, especially those sensitive to fungal-induced diseases like asthma.

**Keywords:** Airborne fungi, *Aspergillus*, Bioaerosol, *Cladosporium*, Fungal allergy, Outdoor fungal spores, *Penicillium*

➤ How to cite this paper:

Ghiasian SA, Maghsood AH, Aghamirian MR. Aeromycological analysis of allergenic airborne fungi in Qazvin, Iran. *Curr Med Mycol.* 2016; 2(3): 5-9. DOI: [10.18869/acadpub.cmm.2.3.5](https://doi.org/10.18869/acadpub.cmm.2.3.5)

### Introduction

Air pollution is closely related to human health and the pollutants may be of either natural or artificial origins. Natural pollutants include natural dust and dirt, bacteria, fungi, and plant pollens. Fungi are among those pollutant organisms that under certain conditions can become pathogenic for humans and animals. Otomycosis, keratomycosis, chronic bronchitis, emphysema, asthma, and allergy are among the complications caused by airborne fungi [1]. The importance of fungi in producing respiratory tract allergies becomes more prominent due to the vital need of humans to air.

Fungi can act as outdoor and indoor respiratory allergen sources, and it is evident that the frequency and variety of indoor fungi are indirectly influenced by outdoor ones [2]. The exact incidence of fungal respiratory allergies is unknown, but it is estimated to be 20-30% among atopic subjects [3] and 3-10% in the general population [2]. It was shown that airborne fungi have specific immunoglobulin E (IgE), which induce type I allergic respiratory

reactions like rhinitis and asthma in atopic individuals [3].

In spite of allergic reactions, some airborne fungi can create systemic infections in humans under certain conditions [1]. Yoda (2003) found a statistically significant difference between asthma in first-year high school students and increased levels of specific IgE antibodies against fungal spores of *Penicillium*, *Cladosporium*, and *Aspergillus* [4]. The sensitivity, allergy, and anaphylactic reactions were also reported in people with a history of long-term inhalation of moulds and yeasts [5].

The quality and quantity of airborne microorganisms vary with time of day, year, and location [6]. Furthermore, the concentration of spores in the air depends on the wind speed, as well as air temperature and humidity [7]. Nowadays, evidence from several epidemiological studies repeatedly showed the important role of outdoor fungi in incidence of respiratory diseases. It is proved that soil is the basic source of airborne fungi [1]. Recently, a field trial study on the incidence of saprophytic

soilborne fungi in a vast region of Qazvin Province revealed that soil sources hold a wide range of fungi, especially ones associated with allergy [8].

Furthermore, our previous study on airborne mycoflora of Qazvin in summer of 2007 demonstrated high contamination of air with a wide variety of allergenic fungi [9]. Moreover, the high frequency of mycotoxin-producing fungal species in cow feed proved that most of the foods and feeds have the potential chance for contamination with these airborne fungi, and subsequently with mycotoxins [10, 11].

The aim of this study was to further evaluate the atmospheric fungal content in winter season of Qazvin and complete the aeromycological profile of the region. The mycoflora data collected in this investigation can help establish a standard as reference for future studies and may be useful in the development of preventive and educational strategies.

## Materials and Methods

Qazvin is a province located in the North-West of the capital of Iran, Tehran. The average summer temperature ranges from 25°C to 35 °C and the humidity is up to 50%. Qazvin covers an area of 50 km<sup>2</sup> and is divided into 25 regions.

A total of 150 air samples were obtained from 25 locations by Petri dish trapping technique as a quantitative method, during January-March 2012. Sampling intervals were two times per day, at 8 o'clock in the morning and 4 in the afternoon. Every time, 25 Petri dishes were exposed to air in the middle of every month. The fungal spore content of the air was determined by inactive sampling through settling 10-cm diameter plates of Sabouraud's dextrose agar (BioMeri ux, Marcy-1, Etoil, France) containing 0.05 g/L of chloramphenicol (SC). The plates were horizontally exposed to air at about 1.5 m height for 20 min. The plates were kept in a 30°C incubator and examined one or two weeks for

possible growth, although the majority of the cases were detected within the first days of incubation.

The identification of fungal mold species was based on the macroscopic and microscopic characteristics of the isolates according to the methods of Watanabe. In brief, after growth of the fungi was established, different types of colonies were subcultured on SC plates for further identification. The isolates were examined microscopically and microscopically in order to determine the colonial features and morphological structures, including growth rate, colony size, surface and reverse color, as well as characteristics of conidiogenous cells in lactophenol cotton blue.

Regarding the yeast fungi, Corn Meal Agar media were used to determine the types of the grown yeasts. The yeasts were primarily isolated in SC media and cultured in Corn Meal Agar media plus Tween-80 for species determination. The results were expressed as colony forming units (CFU) per sample.

## Statistical analysis

Statistical analyses were performed using SPSS, version 9.0. The results were statistically analyzed using One-way analyses of variance (ANOVA) and t-test. Analyses of variance (ANOVA) and Tukey's multiple comparison tests were applied to compare the means of CFU counts. Student's t-test was applied to verify the effect of the winter months on CFU count.

## Results

In this survey, mycological analyses revealed that all the examined samples were positive for fungal growth. The incidence of different types of fungal genera (mold and yeast) isolated from air samples in winter is reported in Table 1. In 150 of

**Table 1.** Distribution of airborne fungi according to three months of winter

Fungal genera	(CFU mean)/Month						Total	
	Jan.		Feb.		Mar.		No	%
	No	%	No	%	No	%		
<i>Cladosporium</i>	297	30.8	296	30.9	294	31.1	887	30.9
<i>Penicillium</i>	264	27.4	262	27.3	256	27.1	782	27.3
<i>Aspergillus</i>	237	24.6	231	24.1	236	25	704	24.5
<i>Alternaria</i>	31	3.2	32	3.3	33	3.5	96	3.3
<i>Rhizopus</i>	32	3.3	28	2.9	28	3	88	3.1
<i>Fusarium</i>	14	1.45	17	1.8	16	1.7	47	1.6
<i>Ulocladium</i>	12	1.25	13	1.35	11	1.2	36	1.22
<i>Mucor</i>	12	1.25	13	1.35	10	1.05	35	1.2
<i>Acremonium</i>	8	0.83	9	0.94	7	0.74	24	0.9
<i>Trichothecium</i>	8	0.83	8	0.83	8	0.85	24	0.9
<i>Candida</i>	6	0.62	7	0.73	8	0.85	21	0.74
<i>Drechslera</i>	7	0.73	7	0.73	6	0.64	20	0.71
<i>Rhodotorula</i>	6	0.62	7	0.73	6	0.64	19	0.67
<i>Scopulariopsis</i>	7	0.73	6	0.63	5	0.53	18	0.63
<i>Chrysosporium</i>	6	0.62	6	0.63	6	0.64	18	0.63
<i>Mycelia Sterilia</i>	5	0.52	6	0.63	6	0.64	17	0.61
Unknown yeasts	6	0.62	5	0.52	5	0.53	16	0.56
<i>Geotrichum</i>	5	0.52	6	0.63	4	0.42	15	0.53
	963	100	959	100	945	100	2867	100

**Table 2.** Distribution of airborne fungi according to the day time of sampling

Fungal genera	(CFU mean)/day time of sampling				Total	
	Morning		Evening		No	%
	No	%	No	%		
<i>Cladosporium</i>	445	30.9	442	30.9	887	30.9
<i>Penicillium</i>	389	27.1	393	27.5	782	27.3
<i>Aspergillus</i>	355	24.7	349	24.4	704	24.5
<i>Alternaria</i>	46	3.2	50	3.5	96	3.3
<i>Rhizopus</i>	43	3	45	3.1	88	3.1
<i>Fusarium</i>	23	1.6	24	1.7	47	1.6
<i>Ulocladium</i>	19	1.32	17	1.2	36	1.22
<i>Mucor</i>	17	1.2	18	1.26	35	1.2
<i>Acremonium</i>	13	0.90	11	0.77	24	0.9
<i>Trichothecium</i>	13	0.90	11	0.77	24	0.9
<i>Candida</i>	11	0.73	10	0.71	21	0.74
<i>Drechslera</i>	11	0.73	9	0.64	20	0.71
<i>Rhodotorula</i>	10	0.7	9	0.64	19	0.67
<i>Scopulariopsis</i>	9	0.62	9	0.64	18	0.63
<i>Chrysosporium</i>	9	0.62	9	0.64	18	0.63
<i>Mycelia Sterilia</i>	9	0.62	8	0.57	17	0.61
Unknown yeasts	8	0.55	8	0.57	16	0.56
<i>Geotrichum</i>	8	0.55	7	0.51	15	0.53
	1438	100	1429	100	2867	100

the studied air samples, a total of 2867 fungal colonies with 18 genera were isolated. The 18 isolated fungal genera and their incidences are presented in Table 1.

Furthermore, the prevalence of different types of isolated mold and yeast fungi according to the time of sampling (morning and evening) are provided in Table 2. The collected samples from morning time showed a considerably higher frequency than those of evening time (Table 2). The same increasing trend of CFUs was noticeable for three months of January, February, and March (Table 1). Based on the mean rates of prevalence, members of the genus *Cladosporium* spp. were the most frequent (30.9%) followed by *Penicillium* (27.3%), *Aspergillus* (24.5%), and *Alternaria* (3.3%).

Furthermore, 14 other fungal genera were isolated infrequently (Table 1). The lowest prevalence was related to *Geotrichum* species with 0.53% of the total isolations. *Mycelia sterilia* were a group of fungal isolates without any fruiting body. However, mycological analyses revealed that moniliaceous fungi were significantly more predominant than dematiaceous ones (57% vs. 36%;  $P < 0.001$ ). The distribution of the fungal spores in the air of different parts of the city and in different hours of the day was almost equal and there was no significant difference among them ( $P > 0.05$ ). Additionally, there was no significant difference in the mean of CFU of the isolated fungi during the three months of winter ( $P > 0.05$ ). There were no statistical differences in the mean of CFU of the isolated fungi between different sampling day times ( $P > 0.05$ ).

## Discussion

Fungal spores are almost always present in the air, but their quantity and quality vary according to

the time of day, climate, geographical situation, and the presence of spore sources in the environment [1]. Fungal spores can trigger severe asthmatic attacks in susceptible persons. For diagnosis of allergy to fungi, several recombinant allergens and standardized extracts are needed. Due to the variety of allergenic fungi and complexity of their extracts, preparation of pure and standard extracts is difficult [3]. Thus, the first step should be an aeromycological analysis of the allergenic airborne fungi of every region.

Regarding the presence of airborne fungi, the residing place of individuals is of great importance. It was established that *Aspergillus fumigatus* is frequently found in compost waste, and allergic bronchopulmonary aspergillosis is more common in people who live around composts [12]. Environmental exposure to aspergilli and increasing number of patients with immune deficiency has led to an increase in the incidence of fatal invasive aspergillosis, and at present, this fungus is the most common infectious fungus among saprophytic fungi [13].

In the current study, the most dominant fungal genera isolated from the air samples were *Cladosporium* spp. (30.9%), *Penicillium* spp. (27.3%), *Aspergillus* spp. (24.5%), and *Alternaria* spp. (3.3%). Hedayati et al. (2005) upon conducting a study on indoor and outdoor airborne fungi in houses of asthmatic patients stated that the most commonly found fungi in order of frequency were *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* [14]. This concordance between the main fungal genera isolated in this study with the isolated fungi from Sari, Iran, shows that in spite of higher relative humidity and higher temperature of Mazandaran Province in comparison with Qazvin Province, the variety and incidence of the first four main fungal genera are to some extent similar.

In the study carried out by Cetinkaya et al. on the incidence of airborne fungal spores in houses in Turkey, the most common fungi were *Cladosporium* (31.9%), *Aspergillus* (18.6%), *Penicillium* (15.5%), and *Alternaria* (13%) [15]. Denning et al. described some epidemiological evidence that associated severity of asthma with allergenic fungi, like *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria*, present in the air of the city and houses in the UK. They also stated that severe fungal-induced asthma is on the rise [16].

The present study confirms the hypothesis suggesting a similarity between a variety of isolated allergenic fungi from soil of Qazvin district [8] and airborne ones obtained from the same area. Since air humidity and climatic conditions are almost constant in different months of winter, the distribution of the fungal spores in the air of different parts of the city and in different hours of the day were almost equal and there was no significant differences among them. This is comparable with the results of de Ana et al. who showed that *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* were present in the air of indoor and outdoor of asthmatic patients' house during all seasons in Spain [17].

In this study, *Cladosporium* species were widely distributed in the air. In fact, they were the most frequently found species during different months of year. This dematiaceous fungus was frequently observed during the current study (~31%), and its ability to propagate extensively and being airborne make it an important fungal allergen. The high incidence of black fungi isolated in this study is in accordance with our previous study on soil-borne fungi, in which ~30% of the total fungal isolation were related to *Cladosporium* spp. [8]. Furthermore, *Alternaria*, *Ulocladium*, and *Drechslera* species were also the other members of black fungi isolated infrequently in this study, the first and second one can be very allergenic and the latter is mostly a plant pathogen [18].

*Aspergillus* and *Penicillium* species are the two important and omnipresent soil-borne fungi that are usually present in the air, as well; in the current inquiry, they outnumbered the other detected fungi by 52% of the total. Regarding *Aspergillus* spp., a variety of species such as *A. flavus* are the causative agents of allergy in atopic individuals and are able to produce mycotoxins and some diseases, including onychomycosis, mycetoma, otomycosis, and keratomycosis.

Some species such as *A. fumigatus* can cause pulmonary aspergillosis in patients with defective immune system or even in immunocompetent

persons [10, 11, 18]. The prevalence of *Penicillium* spp. (27.3%), as the second predominant airborne fungus in Qazvin, is in sharp contrast with findings of Shams-Ghahfarokhi et al., in which *Penicillium* spp. accounted for 13.8% of the fungal isolates recovered from air samples of the outdoor environment in Tehran, Iran [19].

Of the identified potential pathogens, *Mucor* and *Rhizopus* spp. were two fast growing Zygomycetes, isolated from 4.3% of the air samples, which can cause infection in malnourished, immunocompromised, or severely burned patients.

In this study, *Scopulariopsis* species were among the fungi with the lowest frequency of isolation (0.63%). This soil-borne fungus is most often in association with onychomycosis, otomycosis, and occasionally with respiratory tract in drug addicts and immunosuppressed patients. Most species can release antimony compounds and arsenic gaseous compounds, characterized by garlic-like odor production, which can cause arsenic poisoning [21].

Although detection of allergenic and potentially pathogenic fungi in the air does not necessarily indicate that all may cause problems, it addresses the potential risk of diseases and sensitivity in individuals. This study can possibly reveal allergenic airborne fungi engaged in a variety of allergic reactions in vulnerable people in Iran. Awareness of the epidemiology of the common fungal species may promote early identification of systemic opportunistic fungal infections in immunocompromised cases. Furthermore, the results of this study provide a better perception of the incidence pattern of airborne fungi, which may be important for allergists, physicians, as well as epidemiologists.

### Author's contribution

MR.A. designed and managed the research, SA.G. performed the tests. AH.M. analyzed data and edited the final manuscript.

### Conflicts of interest

Authors declare that there is no conflict of interest.

### Financial disclosure

The authors received no external funding for this study.

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