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Profiles of airborne fungi in schools of Saudi Arabia in relation to the allergy problems and respiratory diseases

A. M. Al-Qurashi

Department of Microbiology, College of Medicine, King Faisal University, P.O. Box 2114, Saudi Arabia.
e-mail:dr.alqurashi@hotmail.com

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Abstract

There is increasing concern about the exposure to fungal aerosols in occupational environments and associated respiratory allergic diseases and asthma. A large number of students and teachers stay for long time in schools around the world. Pulmonary function impairments and higher frequency of respiratory symptoms have been reported in schools. Fungi seem to be an important causative factor of pulmonary and allergic diseases. However, it appears that adequate information on the fungal aerosols from schools are largely lacking. Aerobiological data were collected by semi-quantitative methods in indoor and outdoor of the three schools in Al-Khobar city in the Eastern Region of Saudi Arabia. The results of outdoor study showed the presence of 38 species of 21 genera of culturable fungi. Amongst them *Alternaria, Cladosporium, Aspergillus, Penicillium, Epicoccum* and *Stachybotrys* were isolated with higher seasonal frequencies and constituting 65% of total outdoor culturable fungal count. The results of indoor study revealed 31 species of 18 genera of culturable fungi; amongst them *Aspergillus, Penicillium, Cephalosporium, Cladosporium* and *Fusarium* were isolated in higher seasonal frequencies and represented 58.3% of total indoor culturable fungal counts. We also observed that mycoflora of an indoor environment depended on both the fungal spores coming from outside and the capacity of the fungi to colonize in different sub-layers found indoors. Moreover, out of fifteen species tested for skin allergy in experimental animals fourteen exhibited positive skin reactivity and ten species of all isolates observed gave characteristic post mortem lesions in mice. We suggest that increased culturability of fungi inside the classrooms might have important implications because of the potential increase in the release of allergens from viable spores and pathogenicity of viable fungi, particularly in immuno-compromised individuals.

Key words: Airborne fungi, allergy, respiratory diseases, Saudi Arabian schools.

Introduction

Exposure to certain fungi (molds) can cause human illness. Molds cause adverse human health effects through 3 specific mechanisms: generation of a harmful immune response (e.g., allergic or hypersensitivity pneumonitis), direct infection by the organism and toxic-irritant from mold by-products. On the average, man inhales approximately 10 m^3 of air per day [23], containing among others a large number of fungal spores, probably the largest constituents of microorganisms in bioaerosol, ranging from 3 to 30 µm in size [38]. Fungal spores of >10 µm sizes are deposited in the nasopharynx and are associated with nasal and ocular symptoms, generally called as hay fever [22]. Spores < 10 µm size, especially those of < 6 µm size, can be transported to the lower airways and lungs where they can cause allergic reactions sometimes manifest as asthma [28]. Higher frequencies of airborne fungal spores were recorded by many workers from occupational environments [18-20, 29, 36] including a few studies from classrooms of schools.

Fungi are common in indoor and outdoor environmental polluters, and fungal spores constitute a significant fraction of airborne bio-particles [39]. They are often 100-1000 times more numerous than other airborne bioparticles like pollen grains [12]. Information on the airborne fungal flora both outdoor and indoor of the student classrooms will be useful while dealing with the occupational health problems like respiratory allergy and incidence of pulmonary function impairment symptoms among the students and workers in the schools. There are few reports about airborne fungi and fungal aeroallergens in eastern region in Saudi Arabia. It appears that there is no available report on student classrooms in this region. The present study is anticipated to fill this knowledge gap.

The examination and characterization of typical fungal distributions in a particular region can be helpful in identifying association between domestic fungal sensitization and the diagnosis; as well as prevention of seasonal allergic diseases. The objectives of the present study are therefore; firstly, the survey of airborne fungal spores in outdoor and indoor environments in student classrooms in Al-Khobar; secondly, to estimate the degree of potential health concern resulting from the observed levels; and finally, to formulate the suggestions for prevention of fungi-related allergic diseases.

Materials and Methods

Determination of airborne fungi: Airborne fungi were determined using gravity plate methods[15]. Six Petri plates containing modified Czapek's agar medium formula of Thom and Raper [40] were exposed to air at 10 - 11 p.m. for 15 minutes inside and outside of three student classrooms of three different schools in Al-Khobar city of the Eastern Region of Saudi Arabia, weekly for a period of ten months in 2005. Modified Czapeck's plus rose Bengal and streptomycin were added in the medium as bacteriostatic agents. The plates were then incubated for 5-7 days at 28°C. The developing fungal colonies were counted and identified [7, 10, 11, 13, 14, 25, 26, 30, 31].

Ratios of indoor to outdoor concentrations (I/O ratios): I/O ratios were calculated to compare the distribution of indoor and outdoor concentrations and assess the potential sources of airborne fungi [27].

Sorensen's quotient of similarity (Q.S.): The similarity between outdoor and indoor culturable fungi was determined by Sorensen's quotient (Q.S.): $Q = (2\ J/T) \times 100$, where J is the number of taxa in common between in- and outdoor fungi and T is the total number of taxa of in- and outdoor fungi. The fair values of Q.S. may express a moderate or fair affinity between out- and indoor culturable fungi [37].

Experimental animals: Ninety six male mice of ages ranging from 20-26 days and individual average weight 22-27 g were obtained from Veterinary Medicine College.

Nasal instillation test: Fifty ml portions of modified Czapex-Dox liquid medium supplemented with one ml aliquots of living spore suspension of *A. alternata, C. herbarum, C. oxysporium, A. flavus, A. fumigatus, A. sydowii, P. corylophilum, C. roseogriseum, F. moniliforme, E. nigrum, S. atra, R. nigricans*, *M. geophila, P. notatum* and *T. viride* were used as inocula into 25 ml Erlenmeyer flasks. The flasks were incubated at 28°C for 21 days, vigorously shaken by mechanical shaker for two minutes and then filtered through fibrous cotton. One ml of spore suspension of each selected species was instilled in the nose of a group of 6 rats day after day. In addition, one group was also instilled with 1 ml of medium without spores and used as control. All of these groups were reared for 35 days from last instillation dosage. Clinical symptoms and post mortem (PM) lesions of the internal organs were noticed in all experimental animals whether they died during the incubation period.

Skin test: The prick method 24 was used for skin test. The test was done for the antigenic extracts of 15 selected fungal species (*A. alternata, C. herbarum, C. oxysporium, A. flavus, A. fumigatus, A. sydowii, P. corylophilum, C. roseogriseum, F. moniliforme, E. nigrum, S. atra, R. nigricans*, *M. geophila, P. notatum* and *T. viride*). In similar groups of rats normal saline and histamine were used for the skin test as placebo and active controls, respectively.

Results

Outdoor and indoor fungi: In the outdoor study we isolated a total of 38 species of 21 genera of culturable fungi. Amongst them *Alternaria* (4 species), *Cladosporium* (3 species) *Aspergillus* (7 species), *Penicillium* (4 species), *Epicoccum* (2 species) and *Stachybotrys* (1 species) were isolated with higher seasonal frequencies and constituting 65% of total outdoor culturable fungal count. *Cephalosporium roseogriseum*, *Helminthosporium solani, Curvularia lunata, Trichothecium roseum* and *Neurospora* sp. were isolated in moderate seasonal frequencies and constituted 19.8% of total outdoor culturable fungal count. The remaining genera of outdoor fungi had lower and rare seasonal frequencies and represented 15.2% of total outdoor culturable fungal count (Table 1).

The indoor study revealed 31 species of 18 genera of culturable fungi and amongst them *Aspergillus* (6 species), *Penicillium* (3 species), *Cephalosporium* (1 species), *Cladosporium* (2 species) and *Fusarium* (1 species) were isolated in higher seasonal frequencies and represented 58.3% of total indoor culturable fungal count. Indoor fungi with moderate seasonal frequencies were *Alternaria, Stachybotrys atra, Monilia geophila, Trichoderma viride*, *Epicoccum* and *Rhizopus nigricans*, comprising 28% of total indoor culturable fungal count. The rest of the genera was recorded as lower and rare indoor fungi (Table 1).

The highest I/O ratios were observed for *C. roseogriseum* (2.35), *A. fumigatus* (2.0), *Trichoderma viride* (1.66), *A. carbonarius* (1.44), *A. flavus* (1.42) and *P. corylophilum* (1.27) (Table 1). Similarity between out- and indoor culturable fungi was determined by Sorensen's Q.S. and a collective fair value was found to be 72.5.

Animal tests: Skin test reactivity to fungal antigens was found in 62 cases out of 90 animals tested. *C. herbarum* exhibited the highest positive skin reactivity (90% of tested animals) followed by *A. alternata* (80%), *C. oxysporium* (70%), *M. geophila* (70%), *P. corylophilum* (70%), *A. sydowii* (60%) and *P. notatum* (60%). The remaining tested fungi produced 40% of skin reaction to tested animals (Table 2).

Characteristics PM lesions were observed in 28 out of 90 experimental animals. *C. roseogriseum, A. fumigatus* and *S. atra* exhibited the maximum positive PM reactivity, accounting collectively 90, 80 and 60% of tested animals, respectively. Other fungal genera showed 4% of PM responses to tested animals. PM reactivity was associated with different lesions including pneumonia, fibrinous peritonitis, signs of hepatitis, urates in ureter, enteritis, inflammatory of tests and congestion of liver (Table 2).

Discussion

The results of the present study revealed that the average fungal concentrations in the outdoors were generally higher than those in the indoors. The most common outdoor fungal species isolated were *Alternaria alternata, Cladosporium herbarum, C. oxysporium, E. nigrum, S. atra, A. sydowii* and *P. corylophilum.* On the other hand most common species of indoor fungi were identified as *P. corylophilum, C. roseogriseum, C. herbarum, F. moniliforme, A. flavus* and *A. fumigatus.* The fungi isolated only from indoor environment included *F. moniliforme, M. geophila, D. microsporus* and *A. candidus.*

Outdoor airborne fungi sometimes influence the levels of airborne concentration in indoors. On the other hand, the number and types of culturable fungi in indoor air depend on air exchange with the outside and the presence of indoor spore sources. Without such sources, the air spores will consist of the same species as the outdoor in the same proportion, but their numbers are usually smaller. Our data showed that I/O ratios were mostly below one, which suggests that the indoor inhalation exposure to airborne fungi is largely influenced by outdoor airborne fungal concentration.

Table 1. Mean total count (mean number of colonies/plate in all periods of exposure) and frequency of occurrence (F.O.) of out- and indoor fungi isolated from students classrooms in Al-Khobar.

Fungal species	Outdoor fungi		Indoor fungi		I/O
	M.T.C	F. O.	M.T.C	F. O.	ratio
Total count	861.7		526.7		0.61
Total *Alternaria* spp.	124.9	H	34.1	M	0.27
Alternaria alternata	47.5	H	25.0	M	0.53
A. brassicola	27.2	M	9.1	L	0.33
A. citri	16.0	L	0.0	-	-
A. longipes	11.6	L	0.0	-	-
A. raphani	22.6	M	0.0	-	-
Total *Cladosporium* spp.	115.7	H	45.0	H	0.39
Cladosporium cladosporioides	24.9	M	0.0	-	-
C. herbarum	49.5	H	34.3	H	0.69
C.oxysporium	36.2	H	10.7	L	0.29
C. sphaerospermum	5.1	R	0.0	-	-
Total Aspergilli	103.6	H	95.3	H	0.92
Aspergillus candidus Link.	0.0	-	4.1	R	-
A. clavatus Desm	2.3	R	0.0	-	-
A. flavus Link	17.6	M	25.0	H	1.42
A. fumigatus Fresenius	12.1	L	23.8	H	2.0
A. carbonarius	10.0	L	14.4	L	1.44
A. petrakii (Voros)	11.7	L	2.0	R	0.17
A.sydowii	31.2	H	26.2	H	0.84
A. versicolor (Vuill.) Tiraboschi	18.7	M	0.0	-	-
Total *Penicillia*	89.8	H	85.0	H	0.95
P. chrysogenus Thom	17.7	L	13.8	L	0.78
P. corylophilum	41.7	H	52.9	H	1.27
P. italicum Whemer	6.1	R	0.0	-	-
P. notatum Westling	24.3	M	18.3	M	0.75
Total *Epicoccum*	67.0	H	24.7	M	0.37
Epicoccum nigrum	57.0	H	19.6	M	0.34
E. purpurascens	10.0	L	5.1	R	0.51
Stachybotrys atra	59.2	H	28.0	M	0.47
Fusarium moniliforme	0.0	-	30.0	H	-
Cephalosporium roseogriseum	22.0	M	51.9	H	2.35
Helminthosporium solani	53.3	M	0.0	-	-
Curvularia lunata var. *aeria*	47.2	M	2.2	R	0.05
Trichothecium roseum	25.9	M	3.0	R	0.12
Neurospora sp.	21.8	M	14.9	L	0.68
Monilia geophila	0.0	-	25.6	M	-
Total *Rhizopus*	0.0	-	32.0	M	-
Rhizopus nigricans	0.0	-	19.2	M	-
R. oryzae	0.0	-	12.8	L	-
Trichoderma viride	12.9	R	21.4	M	1.66
Sepedonium chrysospermum	32.1	L	0.0	-	-
Drechslera	28.1	L	2.0	R	0.07
Myrothecium verrucaria	21.6	L	0.0	-	-
Syncephalastrum racemosum	17.0	L	15.6	L	0.92
Verticillium lateritium	9.5	L	3.0	R	0.32
Doratomyces microsporus	0.0	-	10.6	L	-
Mucor sphaeosporus	6.0	R	3.0	R	0.5
Humicola grisea	12.6	R	0.0	-	-
Torulomyces langena	8.3	R	-	-	-
Botryotrichum atrogriseum	5.0	R	0.0	-	-

F.O.= number of cases of isolation out of total number of exposures (40), H = higher frequency (number of cases of isolation $\geq$ 20), M = moderate frequency (number of cases of isolation between 19-10), L = lower frequency (number of cases of isolation between 9-5), R = rare frequency (number of cases of isolation between $\leq$ 4)

Notably, our results present an extremely high I/O ratio of *C.roseogriseum, A. fumigatus, T. viride, A. carbonarius* and *P. corylophilum*. This result in addition to other results that recorded fungal species isolated only from indoor indicates that indoor environment provides more favorable conditions for the survival of these fungal spores and should be examined for its health implications. High I/O ratio of *Aspergillus* and *Penicillium* species was reported in suburban and urban indoor homes in China [27].

In the present study the similarity between outdoor and indoor culturable fungi calculated was 72.5% and this may reflect moderate or fair affinity between out- and indoor culturable fungi. This is because a lower air exchange rate is usually expected for classrooms (the only air exchange is through air-conditions allover the year due to the extreme environmental conditions). Our results are in agreement with studies of Horner *et al.* [17] and Shelton *et al.*[35] but disagree with the findings of Adhikari [2] and Picco and Rodolfi [29].

Twenty five genera including forty four species for indoor and outdoor culturable fungi were identified during this investigation. *Aspergillus* (14.3% of total out-and indoor count), *Penicillium* (12.6%), *Cladosporium* (11.6%), *Alternaria* (11.4%), *Epicoccum* (6.6%), *Stachybotrys* (6.3%), *Cephalosporium* (5.3%) and

Table 2. Skin test reactivity and PM pathogenigicity to fungal antigens (the infective dosage was 25 spores/g of body weight) among 96 mice.

Tested fungi	Post nasal instillation % Skin allergy	% P M test	% of positive reaction	P M lesions
Alternaria alternata	80	0.0	80	
A. flavus Link	40	20	60	Pneumonia
A. fumigatus	20	80	100	Pneumonia
A .sydowii	60	30	90	Pneumonia and fibrinous peritonitis
Cephalosporium roseogriseum	0.0	90	90	Pneumonia, signs of hepatitis and urates in ureter
Cladosporium herbarum	90	0.0	90	
C.oxysporium	70	0.0	70	
Epicoccum nigrum	20	40	60	Pneumonia and enteritis
Fusarium moniliforme	60	10	70	Pneumonia
Monilia geophila	70	0.0	70	
Penicillium corylophilum	70	20	90	Pneumonia, enteritis and urates in ureters
P. notatum	60	20	80	Pneumonia and urates in ureters
Rhizopus nigricans	30	40	70	Pneumonia and inflammatory of tests
Stachybotrys atra	20	60	80	Pneumonia, enteritis and congestion of liver
Trichoderma viride	50	0.0	50	
Control	0.0	0.0	0.0	

Fusarium (2.2%) were the predominant fungal genera and appeared in ≥50% of the isolated fungi in outdoor and indoor environments. The most prevalent culturable fungi reported in this study are in consistent with previous studies [1, 21, 34].

The use of antigenic extracts of the most prevalent fungal species, *A. alternata*, *C. herbarum*, *C. oxysporium*, *A. flavus*, *A. fumigatus*, *A. sydowii*, *P. corylophilum*, *C. roseogriseum*, *F. moniliforme*, *E. nigrum*, *S. atra* and *R. nigricans* collectively contributed 54.8% of the total fungal counts in this study in addition to *M. geophila*, *P. notatum* and *T. viride* which were isolated in moderate frequencies from indoor, can be recommended for the primary skin-prick testing of tested animals while evaluating the mold allergen sensitivity.

Exposure to certain fungi (molds) can cause human illness. Molds cause adverse human health effects through 3 specific mechanisms: generation of a harmful immune response (e.g., allergy or hypersensitivity pneumonitis), direct infection by the organism and toxic-irritant effects from mold by-products. Our results authenticated positive skin test in 68.9% of tested animals while 31.1% of tested animals exhibited positive PM reactivity associated with different lesions as a result from nasal instillation of these fungal species. These results are in agreement with the Institute of Medicine reports [8, 9].

C. herbarum showed the highest positive skin test and suggests that this species is one of the most potent allergens, followed by *A. alternata*, *C. oxysporium*, *M. geophila* and *P. corylophilum.* Other species as allergens showed lesser percentage (20%). Also in earlier studies [3, 6, 16, 33] *A. alternata*, *C. oxysporium*, *Aspergillus flavus*, *Penicillium notatum, Mucor, Rhizopus, Curvularia, Phoma* and *Candida* isolated from the air either outdoor or indoor exhibited positive skin reactivity to patients at different percentage and different degrees. Variation of skin test results may be due to variations in the preparation of fungal extracts, standardization of method and interpretation of skin test reactivity [4]. In this context, numbers of new fungal species as allergens have been hypothesized and continually been discovered.

A. fumigatus and *S. atra* showed the highest positive PM reactivity in the present investigation. *A. fumigatus, Penicillium* spp., *R. nigricans* and *Pullularia spp*. were identified from affected birds during study on fungal infection on lungs and air sacs of poultry [32]. Also it has been reported [5] that a variety of fungi in addition to *A. fumigatus* produced asthma. The pilot data from this study will help in understanding human exposure to airborne fungi in relatively clean student classrooms.

Increased culturability of fungi in inside the classrooms is significant because it may lead to the increased allergen release from spores and some culturable fungi may cause infections in immunocompromised individuals. Future investigations are needed to examine the effects of these exposures on the related health problems.

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