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Assessment of Indoor Air Fungi in Western-Anatolia, Turkey

Zafer Cetinkaya[1], Fatma Fidan[2], Mehmet Unlu[2], Ismet Hasenekoglu[3], Levent Tetik[2] and Reha Demirel[4]

SUMMARY This study was conducted to determine fungal spores in the indoor air of the houses in the city of Afyon, Western-Anatolia, Turkey. We investigated the seasonal properties of mould spores in 10 houses of Afyon over a period of one year. Viable moulds were recovered from all 10 houses. Twenty seven different moulds were isolated and identified from the indoor air of the houses. The most common genus was *Cladosporium* spp. (31.9%), followed by *Aspergillus* spp. (18.6%), *Penicillium* spp. (15.5%), *Alternaria* spp. (13.0%) and other species (21.0%). The mould concentration was higher in the kitchens than in other parts of the houses such as the living rooms and bedrooms ($p < 0.05$). The fungal flora of the air in the Afyon city region has a seasonal variation. All fungal species had their highest prevalence in summer and their lowest in winter, but only *Aspergillus* spp. had a significant seasonal variation ($p = 0.012$). Viable moulds are common in the houses of Afyon. Reducing these indoor fungi is necessary to improve the health of individuals with fungal-induced diseases like asthma.

Fungi are ubiquitous and can utilize many different substances for growth. Most fungal growth in domestic environments is accompanied by domestic dampness.[1] The majority of the indoor airborne fungal population is derived from outdoor sources, in particular from the regional vegetation, which is known to strongly affect the nearby airborne fungal concentration. However, when suitable conditions are present (relative humidity, temperature, air exchange rate) fungi may also flourish on indoor man-made structures. The deposition of fungal spores in the lungs and their effect on human health depends on their virulence, genera and species, concentrations, and sizes.[2]

A large number of studies have linked exposure to airborne fungi to various health problems.[3,4] More than 80 genera of fungi have been associated with symptoms of respiratory tract allergies.[5] *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium* are amongst the most common allergenic genera. Metabolites of fungi including toxins and volatile organic compounds are also believed to irritate the respiratory system.[6] These allergens, invade the respiratory system and mostly cause rhinitis, conjunctivitis and bronchial asthma, but occasionally also urticaria and systemic anaphylaxis.[7] The relationship of asthma attacks with fungal spores was first mentioned by Charles Blackley in 1873, and fungal spores taken from the respiratory tract as a cause of asthma were first demonstrated by Storm Van Leeuwen in 1924.[8] Mould spores are prevalent in houses and flourish in the presence of certain growth conditions, especially humidity. Most of the mould surveys were performed in the outdoor environment in spite of the fact that the more relevant site of harmful allergen exposure is in-

From the [1]Afyon Kocatepe University, Faculty of Medicine, Department of Microbiology; [2]Department of Pulmonary Medicine; [3]Ataturk University, Kazım Karabekir Faculty of Education, Department of Biology; [4]Afyon Kocatepe University Faculty of Medicine, Department of Public Health, Afyon, Turkey.
Correspondence: Zafer Cetinkay
E-mail: zfcetinkaya@hotmail.com

doors. It is generally found that prevention of contact of patients, who have allergic complaints, with the allergens is important to control the disease.

The aim of this study was to investigate the established fungal flora of the houses in Afyon and the seasonal variation of the patient's exposure to fungal spores.

MATERIALS AND METHODS

Study area

Afyon city is located in southwestern Turkey. The weather is very cold and rough in winter, and hot and arid in summer. The air pollution in the city is of concern.

Selection of the samples and study design

A sample of 10 non-air conditioned houses was randomly selected for this study, because weather and house conditions are similar. The study was carried out during the 12 month period between June 2001 and July 2002. Sampling of indoor fungi was done monthly for one year. The final results were evaluated for each season: winter (December-February), spring (March-May), summer (June-August) and autumn (September-November).

The samples were collected from the following areas: bedroom, as this is where people spend approximately 6–9 hours per day; living room, as this is where people spend their time either watching TV or relaxing; and the kitchen, as food preparation especially promotes fungal growth. Therefore, allergens present in the bedroom, living room and kitchen have potential to have an affect on human health.

Humidity and monthly air temperature data were obtained from the regional meteorological station.

Collection of fungi

The petri-plate method was used to trap the fungal airspora. Peptone-dextrose agar with 30 ml/l streptomycin and 30 ml/l rose bengal was used to catch the fungal spores and to eliminate bacteria and *Actinomycetes* and to retard the development of rapidly growing fungal colonies. The samples were caught one meter above the floor in the living room, kitchen and bedroom by exposing the plates for 45 minutes after which they were brought to the laboratory. The plates were incubated at 25°C for 10-15 days and examined periodically. At the end of the incubation period, the colonies that had appeared on the agar surface were counted, and examined under low magnification. *Penicillium* and *Aspergillus* species were transferred to Czapek-Dox agar for identification of the species. The genera were identified by Malt agar. The identification of the fungi was based on their macroscopic and microscopic features following the keys methods.[9,10]

Fungal species

The fungal species that were captured in the houses were classified and evaluated in five groups, i.e. *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp. and others. The subgroups of these 5 groups were as follows: *Alternaria* spp.: *A. alternata* and *A. radicina*; *Aspergillus* spp.: *A. niger, A. versicolor, A. fumigatus, A. auricomus, A. flavus* and *A. nidulans*; *Cladosporium* spp.: *C. herbarum* and *C. cladosporoides*; *Penicillium* spp.: *P. charlesii, P. brevicompactum, P. jensenii, P. farinosum* and *P. humuli.* Others: *Drechslera* spp., *Fusarium* spp., *Gliocladium roseum, Mucor* spp., *Mucor circinelloides, Mycelia sterilia, Phoma* spp., *Rhizopus nigricans, Spheropsedales* spp., *Trichoderma aureoviride, Trichoderma harzianum, Ulocladium atrum, Phytophtora* spp., *Monila* spp., *Acremonium* spp., *Embellisia chlamydospora and Helminthosporium* spp.

Statistics

SPSS 10.0 for Windows was used for the statistical analysis. Statistical analyses were performed by chi-square tests. P values lower than 0.05 were accepted as significant.

RESULTS

Twenty-one different genera of fungi were detected in the 10 houses. A total of 323 fungal colonies were identified. The seasonal distribution of the 323 fungal colonies is shown in Fig. 1. The highest number

of colonies was seen in summer and the lowest in winter. The correlation of the colony numbers with the seasons was statistically significant ($p < 0.001$).

Out of the above mentioned five groups, most fungal colonies were *Cladosporium* spp. with 103 colonies (31.9%). Second were *Aspergillus* spp. with 60 colonies (18.6%), followed by *Penicillium* spp. with 50 colonies (15.5%), and *Alternaria* spp. with 42 colonies (13.0%). The total number of other fungal colonies was 68 colonies (21.0%).

Aspergillus spp. colonies were significantly higher in summer regarding the correlation of fungal species with the seasons ($p = 0.012$). The correlation of other fungal species with the seasons was not statistically significant.

Regarding the indoor distribution, fungal colonies were significantly higher in the kitchens, second in the living rooms and lowest in bedrooms ($p < 0.001$). The distribution of fungal species and colony numbers according to places are shown in Fig. 2. The correlation of fungal species to temperature and humidity is shown in Fig. 3.

DISCUSSION

Fungi that are found worldwide reproduce rapidly. The distribution of aeroallergens changes from country to country and even within regions of the same countries. Plant cover, climatic factors and geographical character change the distribution of aeroallergens. Most fungi commonly considered allergenic such as *Alternaria, Cladosporium,* and *Epicoccum* have a seasonal spore releasing pattern.[11]

The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and up to 6% in the general population.[12,13] The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis. These diseases can result from exposure to spores, vegetative cells, or metabolites of the fungi. More than 80 genera of the major fungal groups have been associated with symptoms of respiratory tract allergy.[14]

Indoor fungi are a mixture of those which have entered from outdoors and those which readily grow and multiply indoors.[11,15] *Aspergillus* and *Penicillium* are less common outdoors and are usually considered the major indoor fungi. Recently, *Alternaria* has been

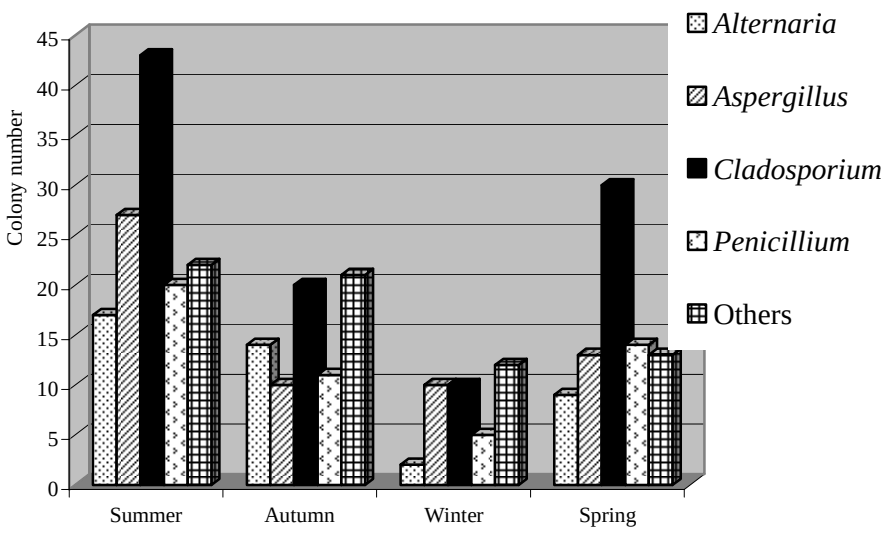

Fig. 1 Seasonal changes in the fungal flora in the indoor air.

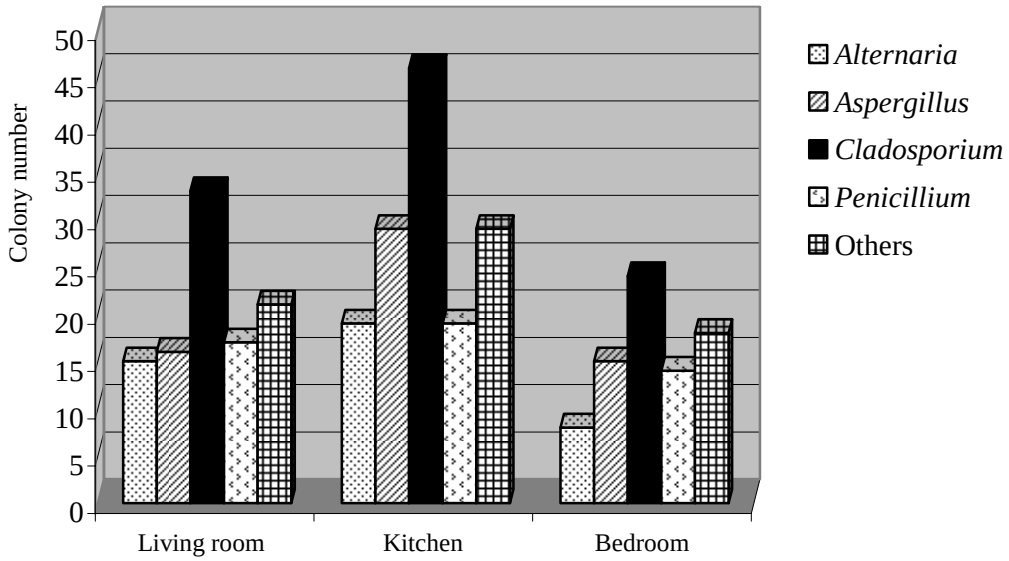

Fig. 2 Distribution of fungal species and colony numbers according to the places.

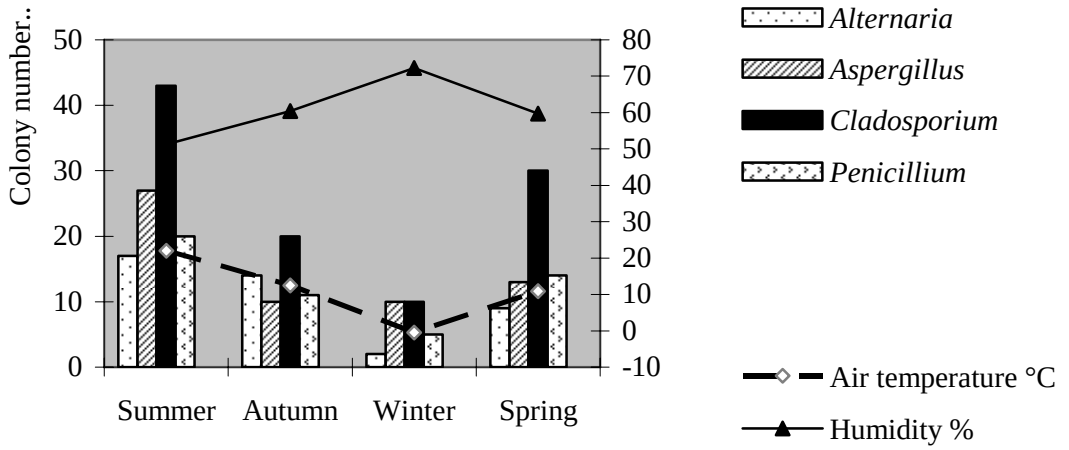

Fig. 3 Indoor fungus distribution with air temperature and humidity during the study period.

reported in house dust samples in the absence of environmental mold spores.[16] Alternaria mold species are a major allergen and have been found in both indoor and outdoor environmental samples and correlate with severe or fatal asthma.[17]

The indoor air fungal flora may differ from that of the outdoor air both quantitatively and qualitatively. The ratio of indoor/ outdoor concentration of spores is usually less than one and is of concern when this ratio reverses. The intramural sources of fungi alter the composition of indoor airborne fungi compared to outdoor air.[18] The fungal spore counts of outdoor and indoor air vary considerably depending on various environmental and other factors.[19]

In our investigation, the predominant genera in our region were *Cladosporium*, *Aspergillus*, *Penicillium and Alternaria.* Several studies have reported that *Cladosporium* is the most abundant genus identified from both indoor and outdoor samples.[20,21,22,23] *Cladosporium* spp. was also the most abundant genus identified in all seasons (31.9%). *Cladosporium* colonies varied from summer and spring to autumn and winter from higher to lower numbers respectively. In a study from Taiwan the average total fungal concentrations were generally high, with the highest level occurring in winter with a significant difference to the other seasons.[24] *Penicillium* genus was found to be most abundant in summer, followed by spring, autumn and winter respectively in our study. A study conducted in southeastern Australian homes showed a high number of airborne fungal spores, featuring *Cladosporium*, *Penicillium*, and yeasts, both indoors and outdoors in winter and late spring.[25] Yankova and Peneva[26] have noted similar seasonal relationships when investigating airborne fungal spores in Sofia, where the numbers of *Cladosporium* were greatest in July and August.

Our study showed *Aspergillus* genus was abundant and significantly higher in summer. *Aspergillus* is a genus associated with mycotoxin-producing, allergic bronchopulmonary disease and other opportunistic infections.[27] In another study, the genus *Aspergillus* showed the highest concentration in winter and another peak concentration in summer.[24]

Other European studies that have reported such variation in *Cladosporium* include those of Herrero *et al.*[28] done in Palencia and Fernandez *et al.*[29] done in Leon, both in Spain. The former noted in addition that the spores of *Aspergillus* were prevalent in winter and those of *Penicillium* in the first-half of the year. *Alternaria* genus is mostly found in summer and in lower concentrations in autumn, spring and winter respectively. *Alternaria* shows a peak concentration in spring and stays at generally low concentrations in other seasons in Taiwan.[24] In addition, several studies have reported that *Alternaria* is a fungus producing important allergens associated with asthma and other allergic diseases.[30]

The spores of *Alternaria* and *Cladosporium* species have been found to be at maximal levels in the months of April, May, June, September and October in a study done in the city of Cagliari, Italy.[31] In another study from Milwaukee, USA, the spores of *Alternaria* species have been detected at high levels in the atmosphere in the months of September and October, however, the spores of the *Cladosporium* species have been found at high levels in the months of June and July.[32]

All fungal species were found significantly higher in kitchens than in living rooms and bedrooms in our study. Unlu *et al.*[33] showed high fungal concentrations in the kitchens of asthmatic patients. Fungal growth favors damp homes with high humidity levels and cold surfaces onto which moisture can condense. Therefore damp basements or humid bathrooms within an otherwise dry house can generate and spread mould spores throughout the house.[17] Studies in Melbourne, Australia, found that mold levels were decreased in rooms with decreased dampness, which were frequently vacuumed, had no pets, and had smooth non carpeted floors.[34]

Indoor mold exposure occurs through infiltration of spores from outdoors and through growth of mold indoors. Abatement strategies need to consider both sources of contamination. The mainstay of mold control is to decrease humidity through air conditioning, cooling, and closing of doors. This can reduce spore infiltration. Air filters may remove particles from the air, with the most effective being high efficiency particle air filters. Electrostatic, electronic, and negative ionizers have shown a modest effect. In addition, regular maintenance, inspection, and cleaning of heating, ventilation, and air-conditioning systems is necessary because spores can circulate throughout the building.[17]

It is important to be aware of the fungal flora in places where asthmatic and sensitive people live, because prevention from allergens is the most important therapy in people who are sensitive to allergens. A person who is highly sensitive to mold should use particle masks when involved in activities such as cleaning that can disperse spores in the air. People with severe allergy should avoid handling, vacuuming, and cleaning areas of fungal contamination.

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