

Aspergillus and other moulds in the air of Kuwait

Z.U. Khan, M.A.Y. Khan, Rachel Chandy & P.N. Sharma*

Department of Microbiology and *Health Sciences Computer Center, Faculty of Medicine, Kuwait University, Kuwait

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Abstract

A one-year survey was carried out to study the aerial prevalence of *Aspergillus* species and other moulds in the outdoor and indoor environments of Kuwait. Petri plates containing rose-Bengal medium were exposed for 20 minutes twice a month using a six-stage Andersen air sampler at the pre-determined sites. The exposed plates were incubated at $28\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ up to 5 days and colonies were enumerated and identified by colonial and microscopic morphology. The data revealed that *Aspergillus* species were the predominant component (27.7%) of the outdoor aerospora of Kuwait and *A. fumigatus* alone accounted for 21.3% of the total aspergilli. In contrast, *Cladosporium* species formed the major component of the indoor aerospora (22.8%), followed by *Aspergillus* species (20.9%), *Penicillium* species (14.6%), and *Bipolaris* species (10.6%). A comparison of the fungi recorded in the outdoor and in the indoor air revealed that *Aspergillus*, *Alternaria* and *Fusarium* were significantly higher in the outdoor environment, whereas *Cladosporium*, *Penicillium*, and *Bipolaris* were significantly higher in the indoor environment. The relative prevalence of *Aspergillus* species and other moulds in the outdoor and indoor air of Kuwait was as follows: *A. fumigatus* 5.9 and 9.8%, *A. flavus* 4.9 and 3.9%, other aspergilli 16.8 and 7.0%, *Alternaria* species 19.8 and 7.9%, *Cladosporium* species 13.7 and 22.8%, *Penicillium* species 7.6 and 14.6%, and other moulds 31.2 and 34.1%, respectively. During the study, 25 different genera were identified, indicating a wide diversity in the spectrum of local fungal aerospora. The study provides useful information on the prevalence of allergenic fungi in the outdoor and indoor environments of Kuwait.

Key words: aerial moulds, aerial prevalence, aeromycology, aspergilli in Kuwait, allergenic fungi

Introduction

Aspergillus species and other moulds are widely distributed in the outdoor and indoor environments and are capable of causing a variety of hypersensitive respiratory disorders, predominantly extrinsic asthma and allergic rhinitis [1, 2]. In addition, *Aspergillus* and some other fungi are also known to cause allergic fungal sinusitis [3, 4] and allergic bronchopulmonary mycosis [5–7]. As the prevalence of bronchial asthma and other hypersensitive lung disorders has steadily increased in the last decade [8, 9], there is now renewed interest in the study of aeroallergens. In this paper we present results of a one-year survey on aerial prevalence of fungi in Kuwait.

Materials and methods

Exposure sites and meteorological information

The air sampling was conducted at the two sites at fortnightly intervals for a period one year (January to December, 1995). The outdoor sampling was done at the terrace of the library, Faculty of Medicine, Kuwait University, about 12 ft above ground level. For indoor sampling, plates were exposed in the corridor of the 5th floor of the Faculty of Medicine about 60 ft above ground level. The building has central heating and cooling facilities. Throughout the study period, sampling was carried out between 12 p.m. and 1 p.m..

The State of Kuwait is located in the north-eastern part of the Arabian Peninsula. Broadly, it has two distinct seasons: summer and winter. The summer season

extends from May to September. It is characterized by dry weather with little or no rainfall, and low humidity (<30%). The temperature ranges from 35 °C to 50 °C. The highest temperatures are in the months of June and July. The winter season extends from November to February and it is marked by rainfall (average 24 mm) and high humidity (85%). The temperature normally varies between 16 to 24 °C (Courtesy Weather Record Department, Kuwait Port). The wind blows from two main directions, the north-west and to lesser extent, the south-east. The months of June and July are marked by dust- and sandstorms.

Sampling method and viable counts

Air sampling was performed with an Andersen 6-stage viable impactor (Graseby-Andersen, Atlanta Ga.) at a rate of 28.3 liters per minute [10]. Six glass plates, covered with an aluminum lid were sterilized by autoclaving at 12 °C for 15 minutes. Twenty-seven ml of medium containing rose Bengal Agar Base (Difco laboratories) [11] cooled to 60 °C, was dispensed in each plate. The plates were incubated at room temperature to ensure there was no aerial contamination after removing the covers. Before starting the pump, the sampling assembly was tightly closed. The sampler was run for 20 minutes. The exposed plates were removed from the assembly, covered with the lid and numbered stagewise. All the plates were kept inverted to prevent condensation drip and incubated in the dark for 3–7 days at 28 °C. The colonies which showed poor growth or did not sporulate were transferred to Sabouraud glucose agar for further study. The identification was based on colonial and microscopic morphology. For the identification of *Aspergillus* species, the Manual of *Aspergilli* by Fennell and Raper [12] was consulted, and for other fungi reference was made to standard fungal identification keys. Volumetric enumeration of fungal aerospora was done by the formula recommended by the manufacturer and the counts were expressed as colony forming units (CFU) per cubic meter of air sampled.

Statistical analysis

Statistical Package for Social Science (SPSS) version 7.5 on PC Windows was used for data management and statistical analysis. The Z-normal test was applied to test the differences between outdoor and indoor counts of fungi of corresponding genera. A probability level of ≤ 0.05 was considered as statistically significant.

Results

Aerial prevalence of fungi

Outdoor: The results of total count, per cent prevalence and frequency of occurrence of fungi in outdoor and indoor air are presented in Table 1. A total of 1215 CFUs representing 25 different genera were recorded during the one year study period extending from January to December 1995 (Table 1). Data on fortnightly prevalence are given in Figure 1.

The higher aerial prevalences were recorded in January and July; the lowest was in August. Most of the fungi occurred throughout the year and no seasonal patterns have emerged. The fungi representing the major components of the outdoor aerospora included *Aspergillus* (27.7%), *Alternaria* (19.8%), *Cladosporium* (13.7%), *Penicillium* (7.6%), *Drechslera* (4.9%), *Fusarium* (4.8%), and *Bipolaris* (4.4%) (Table 1). Within the genus *Aspergillus*, the relative distribution of the different species was as follows: *A. nidulans* – 26.4%, *A. fumigatus* – 21.4%, *A. niger* – 21.4%, *A. flavus* – 17.8%, *A. terreus* – 6.5% and other aspergilli – 6.5%. The highest counts of *Aspergillus* was recorded in the month of July and the lowest during March and April (Figure 2).

Indoor: A total of 254 CFUs were recorded during the study period (Table 1). Fungi belonging to 18 different genera were identified. Their monthly prevalence is depicted in Figure 1. The maximum counts were recorded in July and the lowest in August. The fungi representing the major component of the indoor aerospora included *Cladosporium* (22.8%), *Aspergillus* (20.9%), *Penicillium* (14.6%), *Bipolaris* (10.6%) and *Alternaria* (7.9%) (Table 1). Within the genus *Aspergillus*, the relative distribution of the species was as follows: *A. fumigatus* – 47.1%, *A. flavus* – 18.9%, *A. niger* 22.6%, *A. nidulans* – 3.8%, *A. terreus* and *A. tamaraii* – 1.9% each and other aspergilli – 3.8% (Figure 2). The highest counts of *Aspergillus* was recorded in July. A comparison of the fungi recorded in the outdoor and in the indoor air revealed that *Aspergillus*, *Alternaria* and *Fusarium* were significantly higher in the outdoor environment, whereas *Cladosporium*, *Penicillium*, and *Bipolaris* were significantly higher in the indoor environment (Table 1). Statistically significant differences were also observed between fortnightly total CFU counts and *Aspergillus* CFU counts. In case of outdoor counts, a significantly ($p < 0.001$) low *Aspergillus* counts was observed as

Table 1. Total count, per cent prevalence and frequency of occurrence of fungi in outdoor and indoor air of Kuwait

Genus	Aerial prevalence of fungi					
	Outdoor			Indoor		
	Total count	% prevalence	Frequency	Total count	% prevalence	Frequency
<i>Aspergillus</i> *	337	27.7	24	53	20.9	18
<i>Alternaria</i> †	241	19.8	22	20	7.9	12
<i>Cladosporium</i> †	167	13.7	18	58	22.8	16
<i>Penicillium</i> †	92	7.6	23	37	14.6	17
<i>Drechslera</i>	59	4.9	13	8	3.1	4
<i>Fusarium</i> *	58	4.8	20	4	1.6	4
<i>Bipolaris</i> †	53	4.4	15	27	10.6	11
<i>Ulocladium</i>	25	2.1	11	4	1.6	3
<i>Paecilomyces</i>	16	1.3	5	10	3.9	6
<i>Botrytis</i>	10	0.8	5	1	0.4	1
<i>Curvularia</i>	10	0.8	6	1	0.4	1
<i>Aureobasidium</i>	8	0.7	2	1	0.4	1
<i>Trichoderma</i>	7	0.6	3	0	0	–
<i>Phoma</i>	6	0.5	4	0	0	–
<i>Rhizopus</i>	6	0.5	4	2	0.8	2
<i>Chaetomium</i>	4	0.3	2	1	0.4	1
<i>Mucor</i>	3	0.2	1	4	1.6	1
<i>Syncephalastrum</i>	3	0.2	1	0	0	–
<i>Nigrospora</i>	2	0.2	2	0	0	–
<i>Stachybotrys</i>	2	0.2	2	1	0.4	1
<i>Verticillium</i>	2	0.2	1	0	0	–
<i>Stemphylium</i>	1	0.1	1	2	0.8	2
<i>Scopulariopsis</i>	1	0.1	1	0	0.4	–
<i>Epicoccum</i>	1	0.1	1	3	1.2	1
<i>Arthrographis</i>	1	0.1	1	0	0	–
Unidentified	100	8.2	22	17	6.7	7
Total	1215			254		

* $p < 0.05$, † $p < 0.001$.

compared to total CFU counts during mid January to April, and significantly elevated ($p < 0.01$) proportion of *Aspergillus* counts occurred in July, November and December. A similar pattern was noticed in indoor samples but the difference did not reach a statistically significant level.

Discussion

The study reports the occurrence of a wide spectrum of airborne fungal propagules in outdoor and indoor environments of Kuwait. The environment sampled is distinct, in that most of the areas of Kuwait are desert comprising sandy, gypsiferous, saline and alluvial soils [13]. They are scarce in vegetation cover and

there is a paucity of organic (humus) content in the soil. None of these characteristics appear to provide optimal ecological environments for the natural occurrence of fungi. Moustafa and Al-Mussallam [14], however, reported recovery of as many as 44 genera of fungi from 40 composite soil samples originating from salt marshes, salt depressions and coastal sands. The most frequently isolated genera included *Aspergillus*, *Alternaria*, *Penicillium*, *Fusarium* and *Drechslera*. In another study, Mustafa [15] isolated 14 species of *Aspergillus* from saline soil of Kuwait which constituted 25% of the total fungi recorded. These findings suggest that saline soils of Kuwait can serve as a natural habitat for a large number of diverse fungi and thus contribute to the aerial prevalence of these fungi. In addition, local vegetation, though scanty, appears to

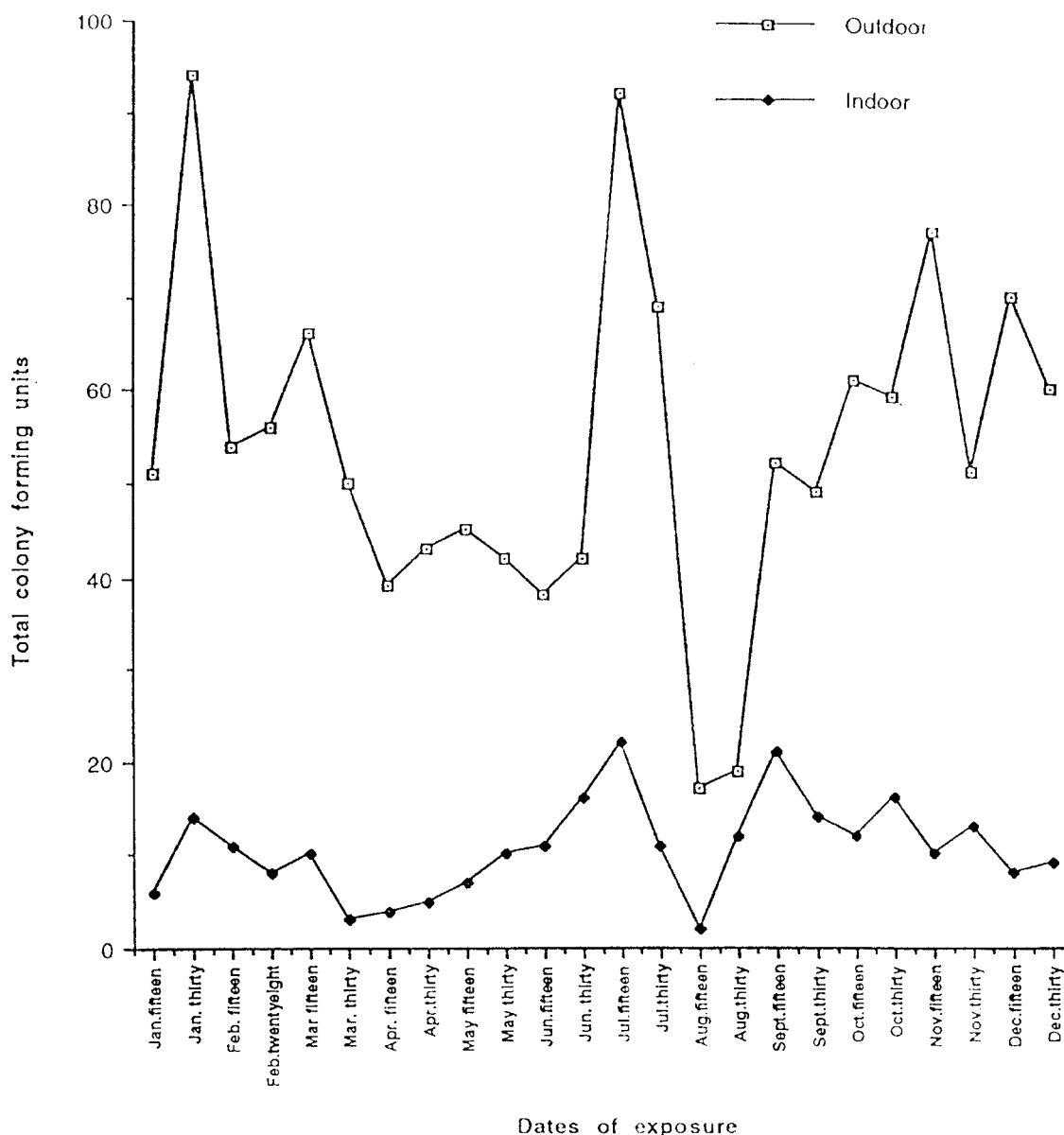


Figure 1. Comparative aerial prevalence of moulds in the outdoor and indoor air of Kuwait (Jan.–Dec., 1995).

provide another important habitat for the growth of these fungi. Moustafa and Kamal [16] suggested that much of the aerospora of Kuwait had its origin in the neighboring countries of Iraq and Iran where cereals and grasses are grown. They believed that the vegetation over there provides a suitable natural substrate for the production of a variety of fungal spores which then could be carried into Kuwait on winds which normally blow in a north-westerly direction [17]. Davies [18], on the other hand, considered that most of

the aerospora of Kuwait are indigenous except *Cladosporium* and *Ustilago* which may come from the north-west region.

Since the primary source of indoor propagules in this study was the infiltration of outside air, it broadly reflected the spore composition of the outdoor air. However, some differences in the relative concentrations of outdoor and indoor fungi were observed, which may be attributed to the differences in the location of sampling points, that is, 12 ft versus

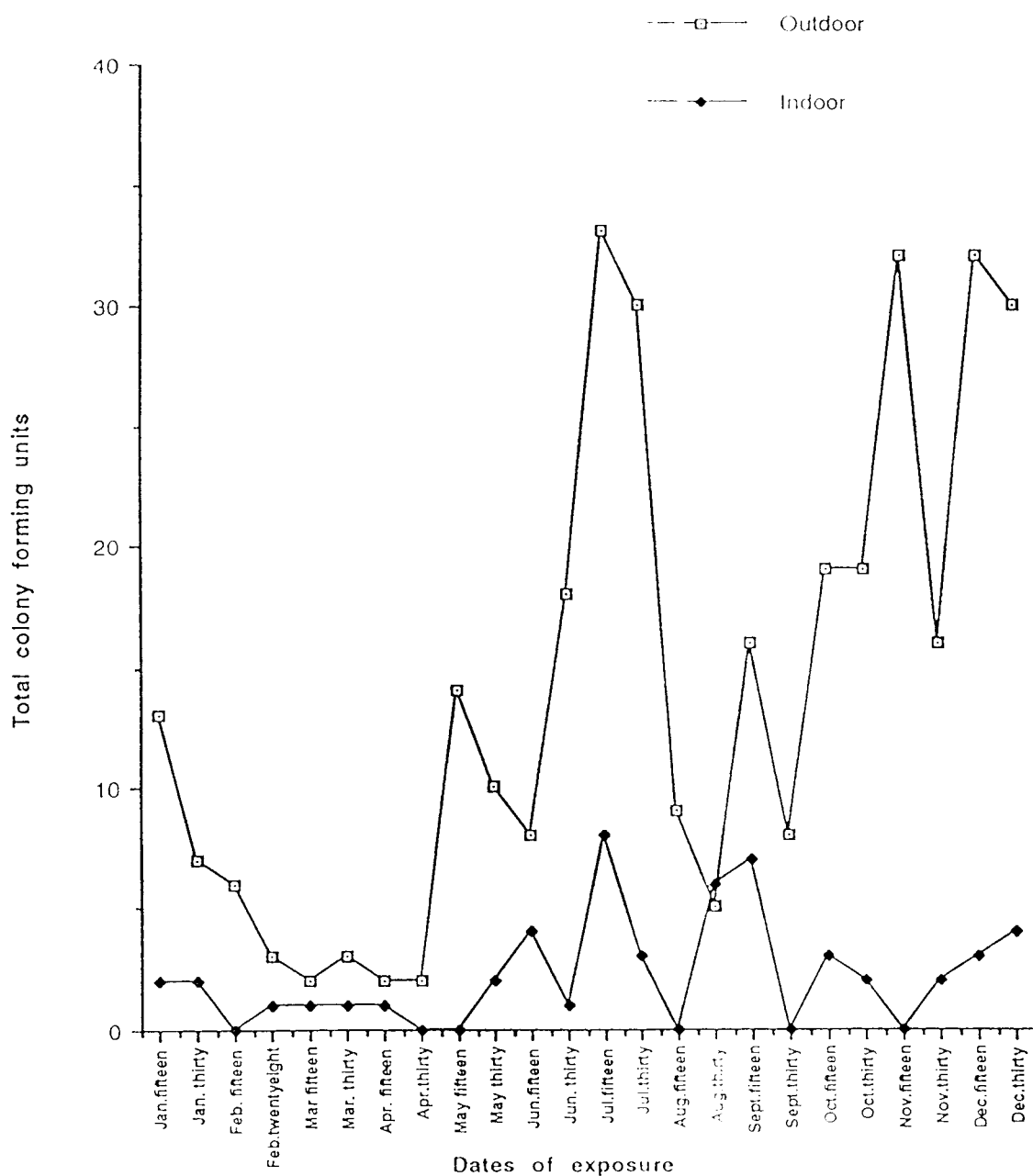


Figure 2. Comparative aerial prevalence of aspergilli in the outdoor and indoor air.

60 ft above the ground level, respectively. Samples of aerospora at 12 ft above ground level perhaps are more reflective of soil-associated fungi, such as *Aspergillus* species, whereas sampling at 60 ft above ground level is likely to be influenced by higher level air masses, probably coming in from *Cladosporium* and *Bipolaris*-generating crop fields of Iraq/Iran or from

relatively distant salt marshes. In addition, the outdoor site had more general wind exposure than the indoor site, besides the presence of lawns and trees in the vicinity. In contrast, at the indoor site, the fresh air intake from outside environment was less than 25% and the incoming air passes through the filters (Type P-116 GG, Air-Maze Incom International, USA) before it is

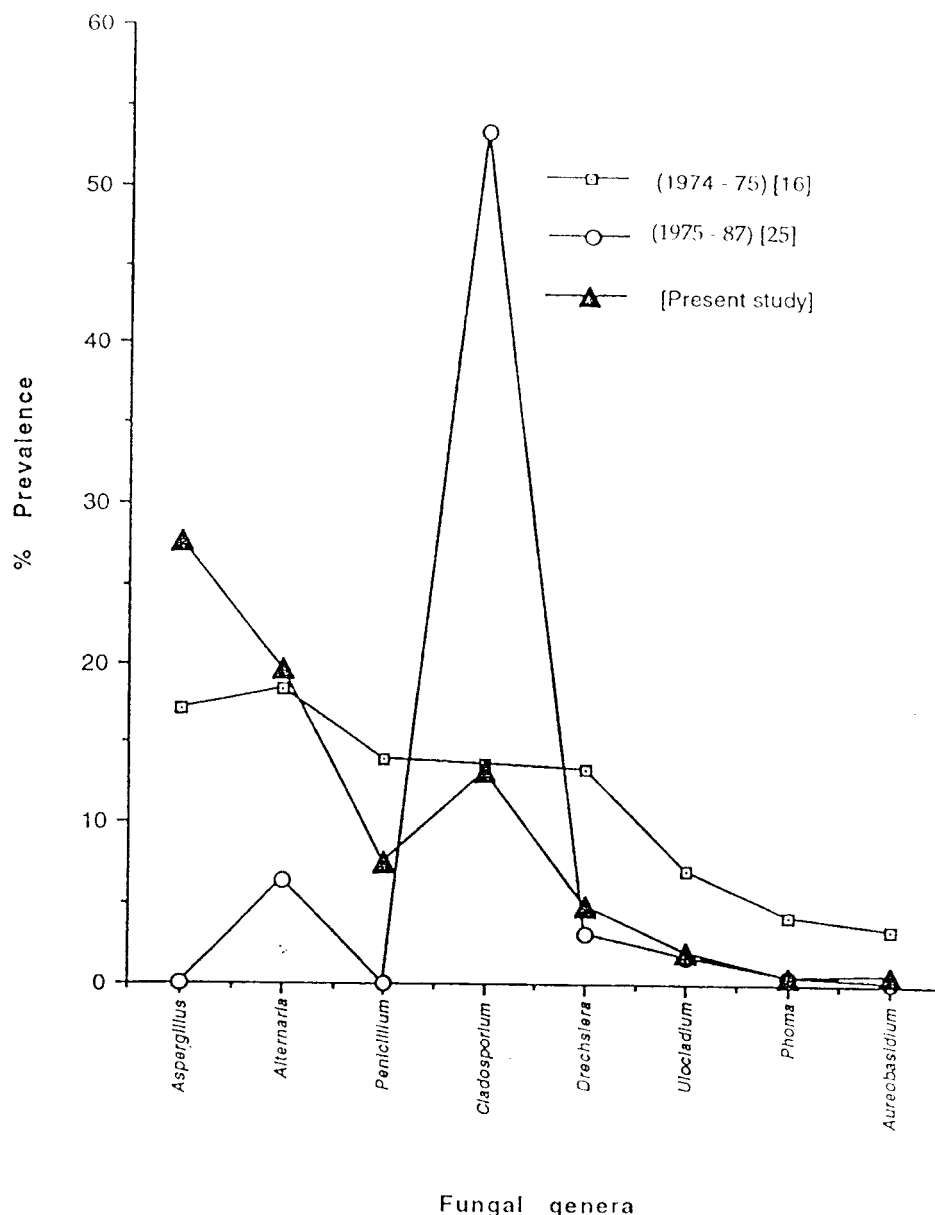


Figure 3. Comparison of three studies on aerial prevalence of fungi in Kuwait.

released for indoor circulation. All these factors might have contributed to the relative differences observed in the number and spectrum of fungi recorded in the outdoor and indoor environments during the study.

In general, the density of outdoor and indoor airborne fungal spores was low. It never exceeded 14 CFU/M³ for the outdoor air and 3 CFU/M³ for the indoor air. This may be attributed to the reasons stated above. Furthermore, Kuwait has a typical arid climate with very long periods of hot and dry summer (tem-

perature >45 °C) and cool rainy winter, which may also contribute to low aerial prevalence of some fungi. However, when one considers the relative prevalence of the fungi recovered, it was not much different from those reported by other investigators from Kuwait [16–19] (Figure 3) and other countries of the region [20–24]. In 1974–75, Moustafa and Kamel [16] reported a wide variety of fungi in the air of Kuwait representing 55 genera and 116 species. The predominant aerial fungi were *Alternaria*, *Aspergillus*,

Penicillium and *Cladosporium* constituting 13 to 18% of the fungal aerospora. In the present study, prevalence of these fungi varied between 7.6 to 27.7% and *Aspergillus* was the predominant component of the outdoor fungi. In contrast, *Cladosporium* was the most common in the indoor environment, followed by *Aspergillus*, *Penicillium* and *Alternaria*. In fact, these four genera formed the major component of the fungal aerospora of Kuwait. In two comprehensive studies from Kuwait [17, 25], one extending over 12 years period between 1975–87, Halwagy, employing Hirst volumetric spore trap sampler, reported much higher spore counts for *Cladosporium* (53.2%), whereas the prevalence of other genera did not exceed 7%. *Aspergillus*/*Penicillium* spore-types were not listed in the 25 genera of hyphomycetes identified [25]. This difference in the prevalence of fungal aerospora may be attributed to the sampling method used [17].

Most of the commonly encountered species of *Aspergillus* in this study are both geographically widely distributed and capable of primary colonization on a variety of substrates [26]. They mainly include *A. nidulans*, *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus*, all belong to the most broadly cosmopolitan group of *Aspergillus* species. There is also some evidence in support of the view that a high proportion of the *Aspergillus* species are restricted in respect to geographic distribution, ecological specificity or both. Christensen and Tuthill [27] while comparing the occurrence of *Aspergillus* species between temperate and tropical/subtropical regions, found that approximately 58 species of the genus appear to be restricted to tropical and subtropical soils. However, none of these species belonged to the cosmopolitan group of aspergilli encountered in the current study. Further, in an attempt to verify that *Aspergillus* is predominantly a tropical and subtropical genus, these authors examined 16 soil surveys, worldwide, at locations within 33° of the equator. The number of *Aspergillus* species recorded in these surveys ranged from 10 to 37, while the total number of fungal species reported ranged from 27 to 283. Based on these observations, they concluded that aspergilli are most abundant, both in absolute and relative numbers, in the tropics/subtropics, particularly in cultivated soils, saline soils and subtropical deciduous soils. The aerial preponderance of *Aspergillus* in the present study therefore, is consistent with these findings.

With the gradual increase in the plantation cover in Kuwait, particularly near the Capital area, the vegetable mass of the soil is continuously expanding. This

may not only lead to a greater number of fungal propagules in the atmosphere but may also change their relative aerial concentrations. Thus, fungi like *Aspergillus* and other moulds associated with plants or decaying vegetable matter [28] may be encountered with greater frequency in future. In 1984, Micallef-Ellul and Al-Ali [29] reported an extremely low incidence (0.8%) of skin reactivity to mould extracts including *A. fumigatus* (0.6%) in Kuwait as determined by skin prick tests performed on 756 (569 Kuwaiti and 187 expatriates) asthmatics. It would be of interest to know whether there has been an increase in mould allergy over the past 15 years, particularly in the pediatric population, which has shown a significant increase in asthmatic attack rates [30].

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Address for Offprints: Z.U. Khan, Professor of Medical Mycology, Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait
Phone: 965-531-2300; Fax: 965-531-8454