

SEASONAL PERIODICITIES OF FUNGAL ALLERGENS IN THE ATMOSPHERE OF RIYADH

Syed M. Hasnain, PhD; Abdulrahman Al-Frayh, MD, Facharzt;
Richard Thorogood, MIBiol; Harb A. Harfi, MD, FAAACI, FACA;
J. Douglas Wilson, PhD, FRACP, FRCPA

أجريت دراسات جوية حيوية لتقسيم الأبواغ الفطرية المؤرجة في جو الرياض باستخدام اساليب «بوركارد» للتسجيل بالمقياس الحجمي لمدة ٧ أيام بطريقة مصيدة الأبواغ وأسلوب صفيحة الزرع، وذلك من شهر نوفمبر/تشرين الثاني ١٩٨٦م لغاية أكتوبر/تشرين الأول ١٩٨٧م، تم تسجيل اثنتين وثلاثين فئة جنسية وشكلية. وكانت غالبية هذه الأبواغ الفطرية تنتمي إلى أبواغ الهواء الجاف للفطريات الغبيرية التي تنشأ من النفايات، وصناديق القمامة، والفاكهة والخضر المخزونة، وماشابه ذلك. وقد شكلت الطوقيات البوغية ما متوسطه نسبه ٢٤.٨٪ من مجموع الأبواغ الهوائية على مدار الشهر الاثني عشر وتراوحت شهرياً بين ٨.٥٪ و ٤٠٪. كما بلغت نسبة كل من السخام (فطر عش الغراب وأنواع أخرى) ١٣.٤٪ (تراوحت بين ١.٦٪ و ٢٥.٦٪)، والفطور من نوع *Alternaria* ٥.٦٪ (تراوحت بين ١.٩٪ و ٩.٦٪)، والفطور من نوع *Ulocladium* ٣.٤٪ (تراوحت بين ١.٠٪ و ٦.٨٪) ومن نوع كل من *Drechslera* و *Helminthosporium* ١.٨٪ (تراوحت بين ٠.٧٪ و ٤.٢٪). أما الأبواغ القاعدية فقد شكّلت نسبتها ٣.١٪ (تراوحت بين ١.٥٪ و ٥.٧٪) وتم تسجيل نسب التركيز الساعية (في الساعة) لجميع هذه الفئات وشكلت الطوقيات البوغية أعلى نسبة بينها. كما أظهرت الدوريات الموسمية زيادة في نسبة التركيز خلال الأشهر الدافئة وانخفاضاً في فصل الشتاء.

Aerobiological studies to evaluate allergenic fungal spores in the atmosphere of Riyadh were conducted using Burkard volumetric 7-day recording spore trap and culture plate techniques from November 1986 through October 1987. Thirty-two generic and morphologic categories were recorded. The majority of these fungal spores belonged to dry-air spora of conidial fungi, originating from garbage, rubbish bins, stored fruits and vegetables, and the like. *Cladosporium* spp. constituted 24.8% (mean value) of total air spora over the 12-month period, ranging monthly from 8.5% to 40%; smuts (*Ustilago* and other species) 13.4% (range, 1.6% to 25.6%); *Alternaria* spp. 5.6% (range, 1.9% to 9.6%); *Ulocladium* spp. 3.4% (range, 1.0% to 6.8%); *Chaetomium* 1.8% (range, 0.7% to 4.2%); *Drechslera* and *Helminthosporium* 1.8% (range, 0 to 5.4%). Basidiospores constituted 3.1% (range, 1.5% to 5.7%). Peak hourly concentrations for all categories were recorded, *Cladosporium* having the highest concentration. Seasonal periodicities showed an increase in concentration in the warmer months and a decline in the winter.

From the Department of Biological and Medical Research (Dr. Hasnain, Mr. Thorogood, and Dr. Wilson) and Department of Medicine (Dr. Harfi), King Faisal Specialist Hospital and Research Centre, and the Department of Pediatrics, College of Medicine, King Saud University (Dr. Al-Frayh), Riyadh.

Address reprint requests and correspondence to Dr. Hasnain: Department of Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.

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The role of some fungal spores and pollen grains present in the air is well known in the etiology of respiratory allergic diseases such as asthma and allergic rhinitis. Before symptoms and exposure to such allergens can be related, however, information on the qualitative nature and distribution patterns is required.

In a previous communication¹ we described the airborne fungal allergens identified in the atmos-

phere of Riyadh, using both viable and visual methods of trapping. This article describes the nature and distribution of airborne fungal spores based on 12 months of aerobiological studies in Riyadh from November 1986 through October 1987.

Methods

The Burkard volumetric 7-day recording spore trap and gravity culture plate technique with general purpose culture media were used to analyze the air spora. A detailed description of both techniques has already been presented in our preliminary communication.¹

The Burkard volumetric spore trap was operated on the flat roof of the clinical building of King Faisal Specialist Hospital and Research Centre (KFSH&RC) about 6 meters above ground level. The site is approximately 3 km from the main city center with clinical buildings of equal height in the east and northeast, shaded parking area in the south, and a large garden and parkland area in the west. The compound is surrounded by various indigenous and imported plant species. The whole area is irrigated abundantly each day.

The trap operates on the principle of "impaction through suction" with 2-mm movement of the drum each hour. A suction rate of 10 liters per minute was maintained. Tapes were mounted with gelvatol-phenol mixture. Spores were counted in 60 fields per 24-hour period beginning from 1 AM which involved handling of three slides for each day's count. Spores were converted to cubic meter of air by a factor obtained by applying the following formula: concentration propagules $m^{-3} = N_T \times A_E / n \times a \times V_a$, where N_T is the total number of spores counted in "n" areas ($= x$); n is the number of field areas counted ($= 5$ field areas); a is a field area, $mm^2 (= 0.15205 mm^2)$; V_a is the air volume sampled, $m^{-3} (= 0.6 m^3)$; and A_E is the total effective area, mm^2 (i.e., long axis of orifice \times distance tape moves in 1 hour, $= 14 \times 2 = 28 mm^2$).

Each alternate hour's counts were added to calculate daily, weekly, and monthly means and, based on the above equation, were converted to spores per cubic meter. Maximum or peak hourly concentrations of various spores per cubic meter of air were determined. Percentages of individual

types were calculated against the total spores counted.

Results

A total of 32 different generic and morphologic categories of fungal spores were recorded during the 12-month period. The majority of these spores came from dry-air spora belonging to the dematiaceous hypomycetes group of fungi imperfecti and originating from a wide range of sources, both indoors and outdoors, including dead and decaying vegetation, garbage, rubbish bins, stored plants, fruits, and vegetables.

The mean monthly concentrations of total spores ranged from 150 spores per cubic meter in January to 500 spores per cubic meter in May, displaying a seasonal trend to rise in concentration during warmer months. A decline in spore concentrations was noted in the coldest months.

The genera which were present consistently almost 12 months with higher individual percentages were termed as "major components." These included *Cladosporium*, smuts, *Alternaria*, *Ulocladium*, *Chaetomium*, *Drechslera*, and *Helminthosporium* as well as basidiospores. The distribution of these generic categories is summarized in Table 1. The seasonal distribution is presented in Figure 1. Peak hourly concentrations of some categories are presented in Figure 2.

Major Components

Conidia of several species of *Cladosporium* including *C. herbarum*, *C. cladosporioides*, *C. macrocarpum*, and *C. sphaerospermum* were observed. They constituted 24.8% of all spores during the 12-month period and ranged from 8.5% in June to 40% in December (39.4% in March). Peak hourly concentrations of *Cladosporium* exceeded 6000 spores per cubic meter in March.

Smuts, a common name for Ustilaginales fungi, responsible for many phytopathogenic diseases in crop plants, constituted 13.4% of the total spores during the year. This group also included *Tilletia caries* and *Sphacelotheca*-type smut chlamydo-spores, the commonest one being *Ustilago nuda* (on wheat). The seasonal maximum concentrations was recorded in May. Peak hourly concentrations of smuts reached 976 spores per cubic meter in April.

TABLE 1. Percentage of the major spore types in the total air spora, based on each month's catch using Burkard volumetric spore trap.

Month	<i>Cladosporium</i>	Smuts	<i>Alternaria</i>	<i>Ulocladium</i>	<i>Drechslera</i> and <i>Helminthosporium</i>	<i>Chaetomium</i>	Basidiospores	Damaged spores	Others (minor components)	Total
Nov 86	33.8	9.2	4.4	1.0	1.3	3.1	2.0	18.7	26.5	100
Dec 86	40.0	1.6	6.0	2.8	0.7	3.0	2.5	22.7	20.7	100
Jan 87	12.5	11.4	6.4	4.5	2.3	4.2	2.3	24.2	32.2	100
Feb 87	27.6	6.2	6.6	3.6	1.3	1.6	4.9	19.4	28.8	100
Mar 87	39.4	9.5	7.8	6.8	0.4	1.4	4.2	9.3	21.2	100
Apr 87	14.0	12.0	9.6	6.2	1.8	1.6	3.7	27.0	24.1	100
May 87	9.2	25.6	7.4	4.0	1.4	1.3	2.6	35.0	13.5	100
Jun 87	8.5	23.5	3.8	4.0	0.2	0.8	2.0	53.0	4.2	100
Jul 87	37.0	24.0	1.9	1.4	0.3	1.1	3.3	22.2	8.8	100
Aug 87	26.2	17.2	5.1	2.3	1.8	0.7	1.5	33.4	11.8	100
Sep 87	23.5	14.8	2.2	2.4	5.4	1.1	5.7	29.3	15.6	100
Oct 87	24.8	6.0	5.2	2.0	5.4	2.2	3.0	33.9	17.5	100
Total % score	296.5	161.0	66.4	41.0	22.3	22.1	37.7	328.1	224.9	1200
Mean % for whole year	24.8	13.4	5.6	3.4	1.8	1.9	3.1	27.3	18.7	100

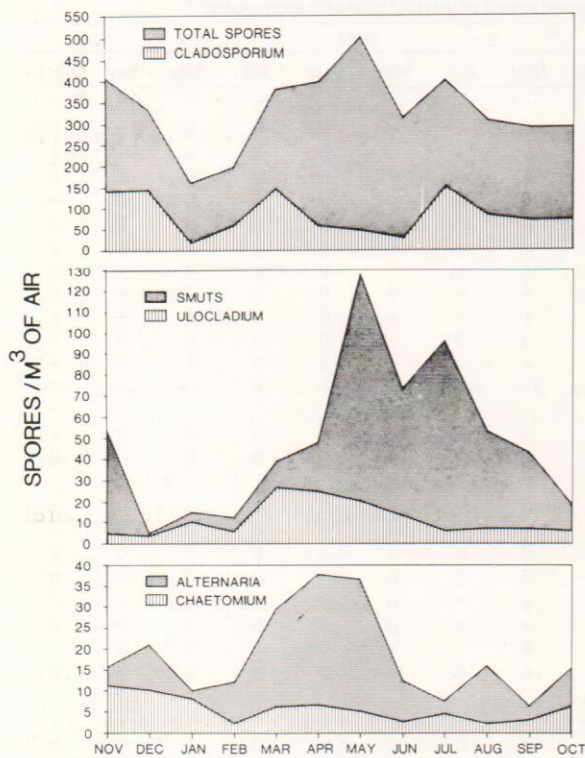


FIGURE 1. Seasonal periodicities of major components of Riyadh atmosphere (KFSH&RC site), November 1986 through October 1987. (Concentration scales of spores per cubic meter of air are different.)

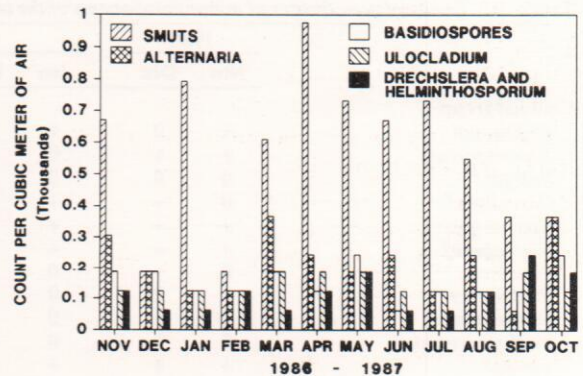


FIGURE 2. Peak hourly concentration levels per cubic meter of air of some spore categories, recorded using Burkard volumetric spore trap from November 1986 through October 1987.

Ulocladium, a rarely reported genus from the air, sometimes mistaken as *Alternaria*, was encountered throughout the period. *Ulocladium* constituted 3.4% of all spores during the year and ranged from 1.0% in November to 6.8% in March. The peak hourly concentration of *Ulocladium* was 183 spores per cubic meter recorded in March, April, May, and September. In April and September, the value was recorded several times.

Species of *Alternaria*, a defined allergenic genus in the United States, were present in all months.

Mean monthly concentration was about 40 spores per cubic meter in April and May. *Alternaria* constituted 5.6% for the year and ranged from 1.9% in July to 9.6% in April. The peak hourly concentration was recorded as 366 spores per cubic meter in March and October.

Drechslera and *Helminthosporium*, two different genera but somewhat similar in their conidial morphology, were grouped together. They constituted 1.8% for the year and ranged from 0.4% in March to 5.4% in September and October. The peak hourly concentration reached 244 spores per cubic meter in September.

Chaetomium is an ascomycetous fungus, and ascospores (spores) were recorded in all 12 months. *Chaetomium* constituted 1.8% for the year and ranged from 0.7% in August to 4.2% in January. Peak hourly concentration of *Chaetomium* reached 183 spores per cubic meter.

Basidiospores, the majority of which are constituents of damp and wet environments, were

also recorded. They were mainly colored, except on rare occasions when hyaline basidiospores were encountered. Basidiospores of *Merulius lacrymans* (an allergenic species), *Gymnopilus*, *Coprinus*, and *Hypholoma* types were noted. This group constituted 3.1% during the 12-month period and ranged from 1.5% in August to 4.9% in February and 5.7% in September. The peak hourly concentration reached 244 spores per cubic meter both in May and October. A lesser concentration of these spores ranging from 122 to 243 per cubic meter was recorded throughout the year, except in June when maximum recording was 61 spores per cubic meter.

Minor Components

Spore categories showing sporadic occurrence with mean monthly concentrations up to 100 per cubic meter were termed "minor components" (Table 2). These included *Arthrinium*, *Asperisporium*, *Curvularia*, *Epicoccum*, *Nigrospora*,

TABLE 2. Seasonal periodicities of minor components of the air spora which displayed sporadic appearance.*

	1986		1987									
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Conidial group												
<i>Arthrinium</i>	+	0	+	0	+	+	+	0	0	0	0	0
<i>Asperisporium</i>	+	+	0	0	+	+	0	0	0	0	0	+
<i>Botrytis</i> †	0	0	0	+	0	0	0	0	0	0	0	0
<i>Curvularia</i> †	0	+	+	0	0	0	0	0	0	0	0	+
<i>Epicoccum</i> †	+	+	+	0	+	0	0	0	0	0	0	+
<i>Nigrospora</i> †	+	+	+	+	+	+	+	+	0	+	+	+
<i>Periconia</i>	0	+	0	+	+	+	+	+	+	0	+	0
<i>Pithomyces</i>	+	0	0	0	0	+	+	0	+	0	+	0
<i>Stemphylium</i> †	0	+	0	+	+	+	+	0	0	+	+	+
<i>Trichothecium</i> †	0	0	0	0	0	0	+	0	0	0	0	+
<i>Torula</i> †	+	+	+	+	+	+	+	+	+	+	0	+
Spores‡ < 10 µm	++++	++	++	+++	++	+++	+	+	+	++	++	++
Basidiospore group												
<i>Ganoderma</i> †	0	0	0	0	0	0	0	0	0	0	0	+
Rust spores† (uredospores)	0	+	0	+	+	+	0	0	0	0	0	0
Ascospore group												
Ascospores, 3 septate	+	+	+	+	0	+	0	0	+	+	0	0
Ascospores, nonseptate	+	+	+	+	+	+	+	0	0	0	0	+
<i>Leptosphaeria</i>	0	0	0	0	0	0	+	+	0	0	0	+
<i>Leptosphaerulina</i>	0	+	0	0	0	0	0	0	0	0	0	0
<i>Pleospora</i>	+	+	0	0	0	+	0	0	+	0	+	0
<i>Sporormiella</i>	0	+	0	+	+	+	0	+	0	+	0	0
<i>Venturia</i>	+	0	+	+	+	+	+	0	+	0	0	+
<i>Xylaria</i> †- <i>Hypoxylon</i>	+	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous group												
Hyphal fragments	+++	+++	+++	++	+++	+++	++++	+++	+++	+++	+++	+++
Algae	0	0	0	0	0	0	+	0	0	+	0	0

*Frequency or occurrence is based on mean monthly concentration per cubic meter of air where + = 1-5; ++ = 6-25; +++ = 26-50; ++++ = 51-100 spores.

†Allergenic genera.

‡Includes *Aspergillus*, *Penicillium*, and other indistinguishable spores, mainly hyaline and greenish, having a size < 10 µm in diameter.

Periconia, *Pithomyces* (facial eczema spores), *Stemphylium*, *Trichothecium*, *Torula*, *Leptosphaeria*, *Pleospora*, *Sporormiella*, and *Venturia*, and other sporadic categories.

Spores from two important contaminating genera, *Aspergillus* and *Penicillium*, obscured by the dust deposit, were grouped under spores $< 10 \mu\text{m}$ along with other hyaline conidia.

Sharp fluctuations in hourly and daily values of many genera were recorded. For example, on 24 April, the mean daily concentration of total spores recorded was 269 spores per cubic meter. On 25 April, the level rose to 772 spores per cubic meter, coming down almost to the previous peak of 290 spores per cubic meter on 26 April. Similarly, *Cladosporium* mean daily value on 28 March was 39 spores per cubic meter, rising to 900 spores per cubic meter on 29 March. *Alternaria*, which showed a concentration of 86 spores per cubic meter on 25 April, declined to 25 spores per cubic meter on 26 April. Sharp fluctuations were also noted in other genera. Changes in daily concentrations are usually dictated by the sharp hourly changes in spore levels and are attributable to changes in meteorologic factors such as wind direction.

On 25 April, a variety of spores were recorded with the highest mean daily concentration. Thus, it was the most polluted day among the days slides were scanned.

Discussion

The present study is part of a comprehensive aerobiological survey of fungal spores in the Saudi environment. The study revealed that, in spite of low natural humidity ($< 40\%$), a large number of fungal genera of class Deuteromycotina (fungi imperfecti), mostly of dry nature, originating from common sources such as garbage and rubbish bins, are present in the atmosphere of Riyadh. The majority of allergenic spores identified worldwide to date belong to the same class of fungi.²

The mean monthly concentration (which absorbs the peaks) of various genera ranged from 150 to 500 spores per cubic meter, and the maximum concentration for only one category, *Cladosporium*, for 1 hour exceeded 6039 spores per cubic meter. Clinical experience in the West

has demonstrated that the abundance of environmental fungal agents represents sufficient exposure for a large number of individuals to become sensitized.³

There has been no systematic study on the threshold of fungal spores capable of inducing symptoms in susceptible individuals. The value determined on the basis of relative cases differs among workers.^{4,5} Threshold figures based on tenuous extrapolations from gravity slide data (which are nonvolumetric) in many cases appeared to underestimate true threshold exposure requirements.⁶ However, once sensitization has taken place in sensitive individuals, as a result of massive exposure, even for a shorter period, a much smaller concentration of spores may induce symptoms.⁷ It can be expected that the mean monthly concentration of any generic category presented, together with the maximum value (usually several times higher), may cause an allergic attack in sensitized individuals.

Seasonal periodicities of major components based on mean monthly values as presented exhibit a tendency to rise and peak in the summer season. Three peaks in *Cladosporium* may relate to the availability of various growth sources in the area. This genus has more than 30 species growing on over 100 different substrates. Species of *Drechslera* (and *Helminthosporium*) may be thermotolerant (thriving above 40°C). *Chaetomium*, on the other hand, is an ascomycetous fungus, which generally prefers high moisture content with low temperature. Basidiospore periodicity remains unexplained. Although there is no well-defined season for fungi,⁸ as the number of spores and pollen counted are influenced by the sampling technique, locality, surroundings, height above ground level, meteorologic factors, and irrigation, as well as spore discharge rhythm (active or passive), both seasonal distribution and circadian pattern for one type or more may vary.

In Riyadh the growth opportunities are greater for conidial fungi than for basidiomycetous ones; irrigation and surroundings of the present site offer a "microclimatic" situation, evident by the year-round presence of both basidiospores and ascospores. This indicates that growth sources of many different genera are present with the availability of artificial humidity. As the spore clouds dilute from the source area, a regional quantita-

tive variation is expected to emerge when the data from two other sites, currently under investigation in the city, are ready for comparison.

Clinical experience indicates that many patients react weakly or negatively in skin-prick testing with commercial fungal extracts, and the information on allergenicity to an allergen or allergens is usually based on the result of such tests rather than on the direct relationship between the occurrence in the environment of the organism concerned and the onset of symptoms in the allergic patient.⁹ Certain problems are associated with the preparation of a reliable fungal extract^{8,10} from an appropriate species or strain, and the potency of any prepared extracts varies from company to company or from batch to batch.¹¹ Even within the testing procedure itself, there appears to be a significant variation. In a comparative study of scratch, intradermal, and provocation tests,¹² *Alternaria* gave 56% positive reaction in intradermal test compared to 4.5% in scratch test. Similarly, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Helminthosporium* gave 39%, 40%, 38%, and 50% positive reactions, respectively, in intradermal tests, compared to 6.6%, 3.6%, 1.3%, and 2.0% in scratch tests. The value for nasal provocation test with the same antigen was also about 10-fold higher than the scratch test. Bruce¹³ emphasized that to confirm the bronchial manifestation of allergic individuals, the skin test should be replaced by the bronchial provocation test.

Further, on skin testing with extracts of four different species of *Cladosporium*, atopic persons in the U.S. Midwest showed variable reactivity patterns suggesting allergenic differences among the species.⁶ Our studies indicate five or six different species of *Cladosporium* were present throughout the 12-month period. Availability and testing of commercial extract are normally limited to two species only, i.e., *C. herbarum* and *C. cladosporioides*.

Unlike basidiospores and ascospores, which are characteristic of damp and wet climates such as Auckland¹⁴ and New Orleans,¹⁵ presence of dry-nature fungi identified in Riyadh is in agreement with similar surveys in the temperate region. Davies¹⁶ used a similar method (Hirst spore trap) in Kuwait and identified *Cladosporium* and *Ustilago* as two major spores in the Kuwait atmos-

phere. *Cladosporium* alone constituted 66% of the total fungal spores. This is comparable with our data where *Cladosporium* (up to 40%) and *Ustilago* (up to 13.4%) were the top two spore categories. The only available study of indoor allergens in the Eastern Province¹⁷ using the culture plate method documented *Aspergillus*, *Cladosporium*, *Penicillium*, *Mucor*, *Alternaria*, and unidentified colonies of 10 or more among a total of 44 cultures, which confirms our findings in both visual and viable identification. We have already documented¹ *Rhizopus*, *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium* through culture plate studies.

Presence of innumerable thick dust particles obscuring the identity of hyaline and smaller spores as well as the efficiency of this trap which varies with wind (air intake in the trap is not equal to the wind speed owing to speed fluctuation) and particle size¹⁸ and further limitation imposed by the height, as the ground level is considered to be more contaminated, the data presented may underestimate the actual concentration to which an individual is exposed.

While the main emphasis in this paper is on outdoor allergens, indoor allergens must not be forgotten. Spores derived from indoor growth can be a source of perennial allergic symptoms. Food storage areas, soiled upholstery, and garbage containers are favored sites for domestic mold growth. Cattle hair and other organic substances (e.g., wool) are readily colonized by fungi. Cellulose splitting organisms may dominate the mycoflora of cotton, kapok, and conventional wallpaper. A number of known allergenic species of fungi including *Aureobasidium*, *Geotrichum*, *Rhizopus*, *Mucor*, *Aspergillus*, *Penicillium*, *Drechslera*, and *Ulocladium*, have already been isolated from homes of asthmatic patients in Riyadh (S.M. Hasnain, personal communication). This emphasizes the importance of some difference in the indoor mycoflora. A possible link of such different indoor fungi and incidence of asthma and perennial allergic rhinitis needs to be investigated thoroughly.

Complexities of pleomorphism (asexual and sexual forms of fungus) and synonymism (taxonomical change of name) also need to be clarified. Identification of *Drechslera* spp., which are very similar to *Helminthosporium* spp., resulting in

some synonymic changes, requires the selection of an appropriate extract for testing and treatment. The similarity of these two genera has led in the past to the preparation of extract of *Drechslera* labeled as *Helminthosporium*.¹⁹ The same situation arises for many other pleomorphic fungi.

The clinical importance of high levels of some spore categories in outdoor air, especially several different species of *Cladosporium* and *Alternaria*, as well as the ubiquitous presence of the rarely reported genus, *Ulocladium*, from the air, necessitate an extensive in vivo and in vitro investigation of asthmatic patients to confirm the importance of these allergenic spores and that of *Ulocladium* in the respiratory allergic problems in Saudi Arabia.

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