

## Allergenic implication of airborne *Ulocladium* in Saudi Arabia

SYED M. HASNAIN, ABDULRAHMAN S. AL-FRAYH, ABDULRAHMAN AL-SUWAINI, MOHAMMED O. GAD-EL-RAB, HARB A. HARFI and SULTAN AL-SEDAIRY

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As part of a national programme on the role of inhalant allergens in respiratory allergic disorders particularly bronchial asthma in the Kingdom of Saudi Arabia, a qualitative, volumetric and immunological study of outdoor aeroallergens was conducted. Among various pollen grains and fungal spores identified, *Ulocladium* spp. emerged to be one of the major spore categories in the outdoor air spora. *Ulocladium* data using Burkard volumetric sampler from three different sites for 12 months each and for 3 continuous years from one site are presented. In addition, data for 12 months for two other sites in Riyadh City using personal volumetric sampler are also presented. The peak mean weekly concentration of *Ulocladium* was highest in the populated coastal (Jeddah) with > 120 spores m<sup>-3</sup> of air. The data displayed a trend at all sites in seasonal variations showing winter with higher concentration and summer with lower concentration. The maximum concentrations for all sites fluctuated and remained under 300 spores m<sup>-3</sup> except for Jeddah reaching over 1200 m<sup>-3</sup> in late winter. The percentage of *Ulocladium* in the air also varied at all sites with highest composition being 7% of the total air spora using Burkard trap and 6% using personal volumetric sampler. However, at all sites *Ulocladium* was found to be among the most frequently encountered genera. Extracts of *Ulocladium* were prepared from local isolates and tested on 100 allergic patients attending allergy clinic at investigators hospital. 13% positive SPT result with various fungal extracts including 5% by *Ulocladium* were recorded in these patients. The study suggests a possible role of *Ulocladium* as an allergen in the region and necessitates further investigation of the genus by inclusion of *Ulocladium* antigens in all *in vivo* and *in vitro* allergy diagnostic tests in the Kingdom.

Syed M. Hasnain and Sultan T. Al-Sedairy, Allergy and Immunology Section, Department of Biological & Medical Research, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211; Abdulrahman S. Al-Frayh and Mohammed O. Gab-El-Rab, Department of Pediatrics and Pathology, College of Medicine, King Saud University, P.O. Box 7805, Riyadh 11472; Harb A. Harfi, Department of Pediatrics/Medicine, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.

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Inhaled particles, such as pollen grains, animal danders, and fungal spores, can provoke allergic rhinitis and bronchial asthma in sensitized subjects and can significantly influence the prevalence of such diseases in selected communities (Gregg 1977). Statistics of pediatric allergic populations for Saudi Arabia have just been reported (Al-Frayh et al. 1989) which revealed up to 9.2% diagnosed asthmatic children nationally. Allergic rhinitis, both seasonal and perennial, with nasal obstruction, sneezing, and watery discharge is even more common in the Kingdom, with up to 18% diagnosed children nationally (Al-Frayh 1990). A comparison of the incidence with other countries shows Saudi figure comparatively higher than most countries but below New Zealand and Australia (Review 1986).

The role of various fungi in the sensitization and elicitation of allergic symptoms in atopic subjects is well established (Burge 1985). The occurrence of various species of fungi in the outdoor and indoor environment from many parts of the world have also been reported (Burge 1985). Lately, occurrence of fungi including *Ulocladium* in the environment of Saudi Arabia were also reported (Al-Frayh et

al. 1988, Hasnain et al. 1989). Proper diagnosis and the treatment of allergic diseases by means of immunotherapy, depend much on the correct identification of the extrinsic causative factor(s) both *in vivo/in vitro* and patients' ambient environment.

This paper presents allergological data on a fungal genus *Ulocladium* for the first time from Saudi Arabia and suggests its potential role as inhalant allergen in different regions of the country. The ubiquitous presence of *Ulocladium* rarely reported from our climate in the past emphasizes the importance of intense local studies on this genus in the Kingdom of Saudi Arabia.

### MATERIALS AND METHODS

#### Burkard Volumetric Spore Trap

Burkard volumetric 7-day spore traps (Burkard Manufacturing Co. Ltd., UK) were installed on the roof tops of different sites mentioned below. Air at a speed of 10 l min<sup>-1</sup> was drawn through the 2 × 14 mm orifice onto adhesive-coated, transparent tape. The clockwise mechanism of the trap moves 2 mm hr<sup>-1</sup> and gives a 48

mm band deposit of airborne particles over a 24-hour period. Each 24 hour segment was mounted with gelvatol-Phenol mixture onto a glass slide. Identification and counting were undertaken with five random fields for each alternate hour, i.e., a total of 60 fields for each 24-hour period were scanned at a magnification of 600 with field area = 0.152053 mm<sup>2</sup>. Most identifications were conducted at a magnification of  $\times 1500$ .

The trap operates on the principle of "impaction through suction" with 2-mm movement of the drum each hour. Spores were converted to cubic meter of air by a factor obtained by applying the following formula: concentration propagules m<sup>-3</sup> =  $N_T A_E / n \times a \times V_a$ , 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<sup>2</sup> (= 0.15205 mm<sup>2</sup>);  $V_a$  is the air volume sampled, m<sup>-3</sup> (= 0.6<sup>3</sup>); and  $A_E$  is the total effective area, mm<sup>2</sup> (i.e., long axis of orifice  $\times$  distance tape moves in 1 hour, = 14  $\times$  2 = 28 mm<sup>2</sup>).

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.

### Sampling Sites

Several appropriate sites for air sampling covering a total of 1400 km area were chosen. These sites were selected in Riyadh, the capital city situated in the middle of the desert, in the Central Province; in Jeddah, ancient coastal city by the Red sea, in the Western province; and in Al-Khobar, another coastal but comparatively new business city in the Eastern province of Saudi Arabia.

1. Riyadh: two sites located at King Faisal Specialist Hospital and Research Centre (KFSH&RC) and King Khalid University Hospital (KKUH) area. The two sites are 4–8 km apart from the main city centre and represent a somewhat microclimatic environments with plants and grasses being managed on day to day basis and irrigated everyday. The trap was operated on the roof of hospital buildings about 5 meter above ground level (a.g.l.).
2. Jeddah (JEDD), the site was roof of a building situated in the compound of King Abdulaziz University Hospital about the same height as in Riyadh sites.
3. Al-Khobar (AKHB), the site was roof of King Fahd University Hospital about 5 m a.g.l. The distance between Riyadh and Al-Khobar is about 400 km towards the East and from Riyadh to Jeddah is about 1000 km towards the West.

For convenience of readers, the 4 sites with 2 extended years at KKUH will be considered a total of 6 different sites for the presentation of data.

### Personal Volumetric Sampler

Two portable personal volumetric air sampler (Burkard Manufacturing Co. Ltd., UK) were used to sample air from the above two locations. The sampler allows insertion of a petri plate with culture media on which spores are impacted by suction. The air suction rate was maintained at 10 l min<sup>-1</sup>. Both samplers were used at a time with two different media (Sabouraud's Dextrose agar and Czapek Dox agar). Each sampling (exposure) was for 10 minutes duration. The two samplers were located 20 cm above the ground. The mean number of colonies from the two media were taken for the presentation of results.

In each of the culture media 1% streptomycin (antibiotic) was added in order to inhibit the growth of bacteria. Media were prepared and sterilized by autoclaving at 121°C for 15 minutes and dispensed into disposable petri dishes.

### Sampling Sites

The sites chosen for personal volumetric sampling were located in the capital city of Riyadh. It is approximately 1000 km from the red sea port of Jeddah and about 400 kms from Arabian Gulf. The weather in Riyadh is known to be rather hot and dry. Temperature in summer normally range between 40°C and 50°C during day time. The sites were:

- Site A: Al-Batha
- Site B: Al-Ulia

The distance between the two sites is 7 km by road. Al-Batha is a developed area located in the South of Riyadh, consisting of mainly commercial old buildings with crowded roads and streets. Al-Ulia is located in the north of Riyadh, a modern, and less developed area, consisting of new villas, planned streets with plants and trees.

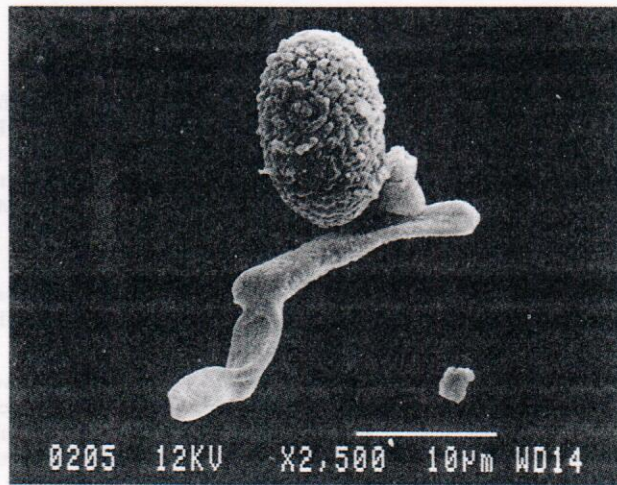
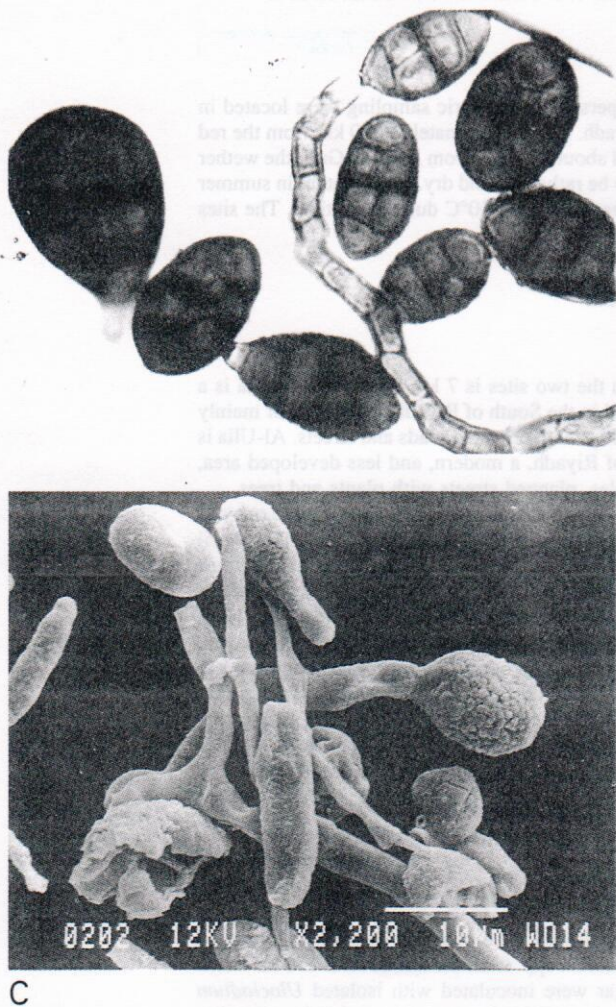
The samplings were conducted at both sites twice a week on each Saturdays and Tuesdays for a period of 12 months (May 1990–April 1991). The days were chosen to allow the beginning of the week after a quiet day (Friday, being a holiday in Saudi Arabia) and the middle part of the week when all activities are considered to be in full motion. Morning samplings were done at 9.00 at Al-Batha site and 10.00 at Al-Ulia site. Afternoon samplings were conducted at 3.00 pm at Al-Batha and 4.00 p.m. at Al-Ulia site. Weekly and monthly means were calculated for this data. With the assumption that at least one spore resulted in one colony, the number of colonies can be multiplied by a factor of 10 to obtain number of spores per cubic meter of air.

### Preparation of Antigenic extract

*Ulocladium* antigenic extracts were prepared by mass culturing of locally isolated *Ulocladium* spp. from the air. Several 250 ml flasks with Czapek Dox agar were inoculated with isolated *Ulocladium* and allowed to grow for about two weeks. Mycelial mats were harvested from the culture and washed with 95% ethanol. Pellicles were dried and defatted with Diethyl ether for 24 hours. After separation and drying of spores, material was ground and weighed. Extraction was carried out in buffered saline (PH-8) in 250 ml flask at room temperature for 72 hour on rotary shaker (Philips 1967). All material for extraction was standardized on weight per volume (w/v 1:20) ratio. Several lots of extracts from different isolates of *Ulocladium* spp. were prepared. For convenience of testing, these extracts were pooled together as multiple screening mixtures (MSM). Under sterile conditions 2 ml aliquots of each extract were dispensed into small vials. These mixture were shaken for 30 minutes on a rotary shaker, left overnight at 4°C, then dialysed against 15% buffered saline for 24 to 48 hours. All extracts and MSM were sterilized by Millisporer filtration using final 0.22  $\mu$ m filters and transferred aseptically to sterile vials. All extracts were tested for sterility on blood and nutrient agar. 50% glycerine was added to the half of aqueous extracts and other half was lyophilized and stored at -20°C. In order to ensure the strength of lyophilized extracts each mixture was reconstituted at half the original volume before skin testing.

### Skin Prick Test (SPT)

One hundred patients suffering from bronchial asthma and allergic rhinitis were skin prick tested using locally prepared fungal antigen. The SPT was conducted as a routine procedure being used in the Allergy Clinic at investigators Hospitals which also included negative saline and 1% positive histamine control tests (Al-Frayh et al. 1992).



B

Fig. 1. (A) Photomicrograph of conidia of *Ulocladium chartarum* ( $\times 1000$ ). (B) Scanning electron photomicrograph of a conidium of *Ulocladium atrum* isolated from Riyadh city atmosphere ( $\times 2500$ ). (C) Scanning electron photomicrograph of conidia of *Ulocladium atrum* with hyphae isolated from Riyadh environment ( $\times 2200$ ).

RESULTS

Analysis of results of Buckard trap data from all four sites including extended period (a total of 3 years) at KKHU, revealed that *Ulocladium* (Fig. 1) is one of the most preva-

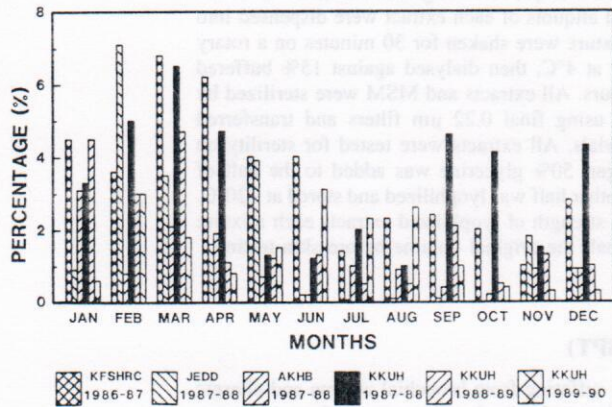


Fig. 2. Percentage of *Ulocladium* spores in the outdoor air at different sites. (Each of the 3 extra years at KKHU is considered one site for the purpose of comparison).

lent individual categories of airborne fungal conidia almost at all sites as well as in the indoor environment including some patients' home (Hasnain et al., personal communication). The percent catch (Fig. 2) of *Ulocladium* out of the total air spora ranged from zero (only in October in Jeddah) to 7.1% in Jeddah in February, from 1–6.8% at KFSH&RC in March, from 0.2–3.1% at KKHU in January, and from 1–6.5% at KKHU in March 1988, 0.5–4.7% at KKHU again

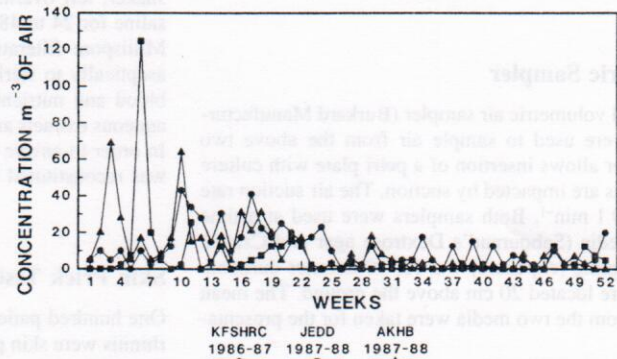


Fig. 3. A comparison of mean weekly concentrations of *Ulocladium* spores at three different sites (KFSH&RC, JEDD and AKHB).

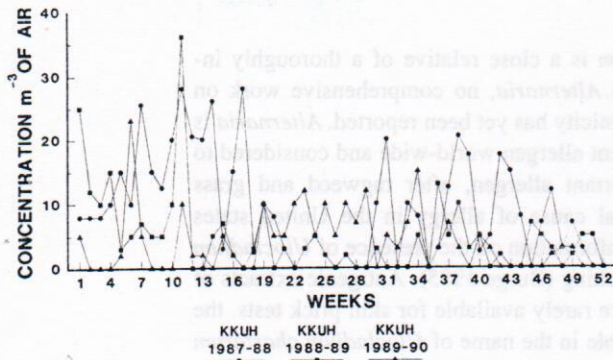


Fig. 4. A comparison of mean monthly concentrations of *Ulocladium* spores at KKUH site for three continuous years.

in March 1989, and 0.3–3% at KKUH in February 1990. The corresponding value for total pollen at each site was below 1%. However, just for an idea, the three other fungal categories viz. *Cladosporium*, *Aspergillus* and *Penicillium* constituted more at all sites.

**Seasonal Variation**

The mean weekly (Figs. 3 and 4) and mean monthly (Fig. 5) concentrations of *Ulocladium* indicate airborne presence of conidia throughout the year with increased activities during the period January to April at almost all sites, and a distinct peak in Jeddah in February and in Al-Khobar in January and March.

The maximum concentration recorded was 1220 m<sup>-3</sup> of air in Jeddah (February), 305 m<sup>-3</sup> in Al-Khobar (March), 244 m<sup>-3</sup> at KKUH in Riyadh (January & March) and 183 m<sup>-3</sup> at KFSH&RC in Riyadh (March to May) (Fig. 6).

**Viable Personal Sampling**

Data for airborne *Ulocladium* spp. using personal volumetric sampling for both Al-Batha and Al-Ulia for 12 months period are presented in Figs. 7–9.

*Ulocladium* constituted 4.5% at Al-Batha and 6% at Al-Ulia of the total air spora of the respective sites during the full year period and remained one of the major components

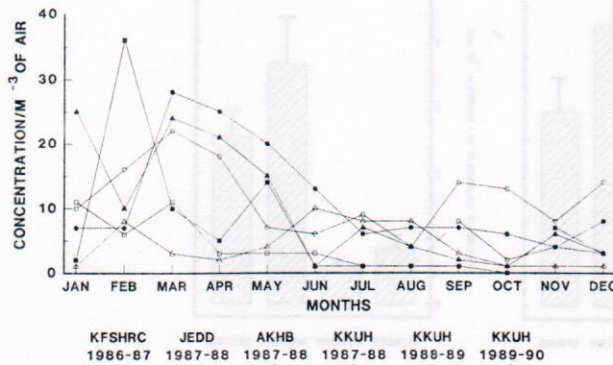


Fig. 5. A comparison of mean weekly concentrations of *Ulocladium* spores at different sites.

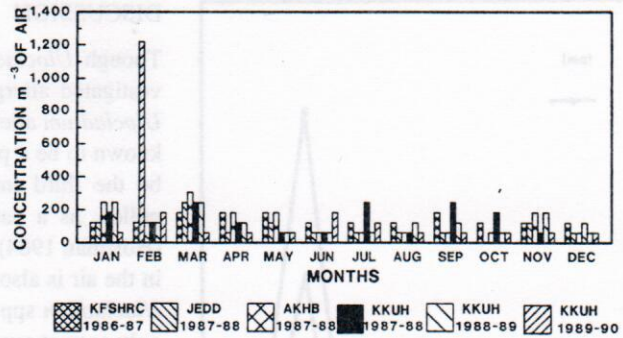


Fig. 6. Maximum concentrations of airborne *Ulocladium* spores at different sites.

along with *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*.

***Ulocladium* spp. at Al-Batha site**

Mean monthly variation of *Ulocladium* spp. is presented in Fig. 7. The figure shows that the *Ulocladium* spores were present in the air almost throughout the year at Al-Batha site, both in the morning and in the afternoon. However, from June to November the mean monthly concentration were <20 spores m<sup>-3</sup> of air. The concentrations rose for both morning and afternoon during winter with peaks of 40 spores

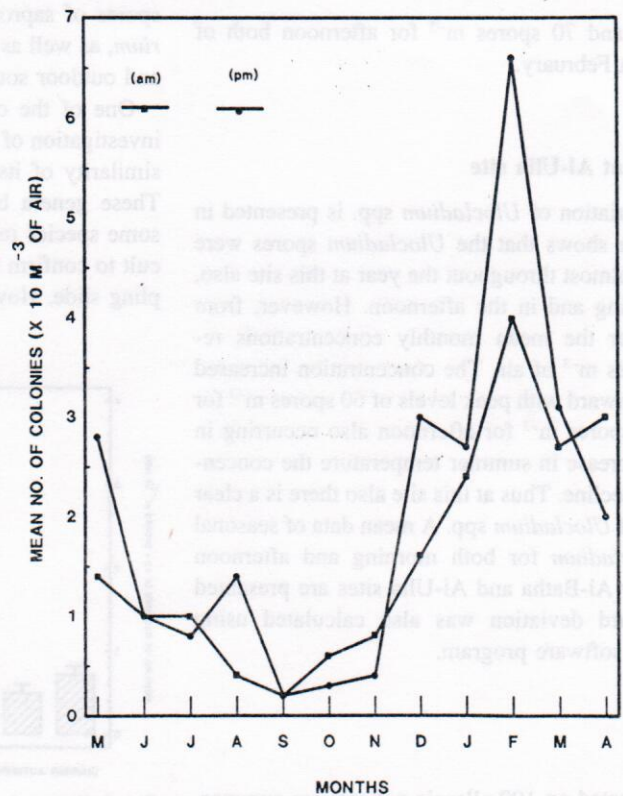


Fig. 7. Mean monthly variation of *Ulocladium* spp. at Al-Batha site showing both morning and afternoon concentrations.

Table I. Immediate skin test reactions to local fungal allergens extracts in 100 consecutive patients attending Allergy Clinic at King Khalid University Hospital, Riyadh.

Patient No.	Diagnosis	Sex	Reactions to fungal allergen	Multiple Sensitivities
1	Asthma	Male	<i>Alternaria</i> 3 mm <i>Ulocladium</i> 3 mm	Cat fur wool Cotton flock, cockroach
2	Asthma	Male	<i>Ulocladium</i> 3 mm	Cockroach Cotton flock
3	Rhinitis	Female	<i>Aspergillus niger</i> 3 mm	Cockroach 6 mm Cotton flock 6 mm
4	Asthma	Asthma	<i>Aspergillus niger</i> 4 mm	Cat fur 8 mm <i>D. farinae</i> 3 mm Cockroach 3 mm <i>Chenopodium album</i> 6 mm
5	Rhinitis	Male	<i>Alternaria</i> A 5 mm <i>Alternaria</i> B 4 mm <i>Ulocladium</i> A 8 mm <i>Ulocladium</i> B 5 mm	Cockroach 3 mm Cotton flock 3 mm <i>Lolium perrene</i> 5 mm <i>Poa pratense</i> 6 mm
6	Asthma	Female	<i>Aspergillus mix</i> 3 mm	None
7	Asthma Rhinitis	Female	<i>Aspergillus niger</i> 3 mm	<i>D. farinae</i> 6 mm Cockroach 3 mm Cotton flock 4 mm Cat fur 8 mm <i>Chenopodium album</i> 5 mm <i>D. farinae</i> 6 mm
8	Asthma Rhinitis	Male	<i>Alternaria</i> A 6 mm <i>Ulocladium</i> A 7 mm	<i>Candida</i> 3 mm
9	Asthma Rhinitis	Female	<i>Alternaria</i> A 3 mm <i>Aspergillus niger</i> 3 mm	Cat fur 9 mm
10	Rhinitis	Male	<i>Cladosporium mix</i> 4 mm	Cotton flock 6 mm Cat fur 7 mm Wool 5 mm Grass mix 4 mm <i>Chenopodium album</i> 5 mm Cockroach 5 mm Cotton flock 3 mm Cat fur 10 mm Wool 4 mm <i>Chenopodium album</i> 4 mm
11	Rhinitis	Male	<i>Alternaria</i> A 3 mm	House dust mix 3 mm Cockroach 7 mm Cat fur 8 mm Grass mix 4 mm
12	Asthma Rhinitis	Male	<i>Aspergillus mix</i> 4 mm	House dust mix 3 mm <i>D. farinae</i> 3 mm Cotton flock 3 mm <i>Candida</i> 3 mm
13	Rhinitis	Male	<i>Cladosporium</i> 3 mm	

mons 1967), which are of much help in the identification of the species of these genera.

The data presented from different sites using both viable and non-viable volumetric methods very clearly demonstrate that *Ulocladium* spp. have a seasonal trend in their occurrence; winter being the season with highest concentrations followed by spring. Summer and autumn being seasons with comparatively low concentrations. There appears to be no volumetric data on airborne *Ulocladium* from other countries for comparison.

Climatic factors influence the overall levels of airborne microorganisms causing such diseases. Humid climates offer more opportunities for growth of various groups of allergen producing organisms, especially house dust mites and fungi.

Coastal areas such as JEDD and AKBH have high humidity (>80%) compared to KFSH&RC (<50%) but horticultural practices throughout the year in Riyadh also provide sufficient moisture for fungal growth. Temperature also play an important role in the growth of fungi. However, it should be noted that winter temperature in Saudi Arabia is not as low as in Europe and North America and even during the winter, the day-time temperature remains between 10–20°C with some fluctuations with time. The day-time temperature in summer is extreme high 40–50°C which may have an impact on growth and sporulation of this genus during these seasons.

Maximum concentration level of 1200 spores of *Ulocladium* in a cubic meter of air recorded at JEDD site in winter

is also not comparable as no record of such level is available for this purpose. On the other hand, threshold of *Ulocladium* spores to sensitize and induce allergic reaction is also not known. Our data shows that a peak of 1200 *Ulocladium* spores  $m^{-3}$  occurred in mid winter in JEDD while at other places the maximum concentrations remained between 200–300 spores  $m^{-3}$  at most occasions. Though the interpretation of these values as regard to allergenic threshold is difficult, yet base on information for *Alternaria* (Frankland 1965), one can assume that these values are likely to have some implications in respiratory allergic diseases.

While skin testing will identify only those inhaled allergens to which the patients is sensitive, details of the range of potential allergens present in any environment provide essential background information in order to select relevant skin test material. A total of 13% of the patients reacted positively (Table I) to fungal allergens including 5% to *Ulocladium*. As such *Ulocladium* ranked second in its reactivity with one of the strongest reactions in two patients (7 and 8 mm weal). the reactions to other locally prepared fungi are presented for comparative reasons. The table shows that *Alternaria* attained 6 points with positive reactions in 5 patients, *Ulocladium* 5 points with positive reaction in 4 patients. *Aspergillus niger* reacted positively in 4 patients, *Aspergillus mix* in 2 patients and *Cladosporium* also in 2 patients. positive reactions to *Ulocladium* were all in male patients, for which, no explanation is possible at this stage.

The purpose of this communication is to assist physicians working in the field of respiratory allergic diseases by identifying potential allergens in their selection of antigens and possible inclusion *in vivo* and *in vitro* diagnostic profile and to related symptoms during peak occurrence of *Ulocladium*.

#### CONCLUSIONS AND IMPLICATIONS

1. The genus *Ulocladium* emerged to be one of the five most prevalent fungi in the outdoor environment of Saudi Arabia.
2. The genus displayed a seasonal pattern with higher concentration during winter and spring and a distinct peak concentration of 1200 spores  $m^{-3}$  of air at one site. Maximum concentration for other sites were between 200–300  $m^{-3}$  of air.
3. Preliminary 13% positive reactions to local fungi including 5% to *Ulocladium* crude extracts in patients suffering from asthma and allergic rhinitis indicate possible involvement of *Ulocladium* in the sensitization and elicitation of symptoms particularly during the period of its peak concentrations.
4. Though the cross reactivities between the species of *Alternaria* and *Stemphyllium* are known to exist, cross reactivities of these allergens with the species of *Ulocladium* require investigation with carefully selected *Ulocladium* antigens.
5. Allergenicity to *Ulocladium* is likely to be more prevalent or to be suspected during January to April when the spores are prevalent in the air. Though the IgE mediated skin reactions to specific antigen in sensitized individuals may occur any time, symptoms appear only as a result of second or subsequent exposure. Hence SPT profile or other diagnostic tests should include *Ulocladium* antigens for diagnosis of patients having symptoms during above period of time.

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