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Cladosporium and respiratory allergy: Diagnostic implications in Saudi Arabia

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Abstract

An allergological study to evaluate allergenicity to *Cladosporium*, Burkard 7-Day Volumetric Spore Trap and Personal Volumetric air sampler (viable mode) were employed to conduct air sampling for 12 months in three regions of Saudi Arabia. The study was extended for a continuous 3rd year at one site. Skin prick testing (SPT) was also conducted on 605 allergic individuals using commercial extracts of *C. herbarum*. *Cladosporium* emerged to be the most prevalent genus in the outdoor environment constituting up to 25% of all fungal spores in the dry region and 37.1 and 41.2% in two coastal cities respectively. Amongst the species *C. sphaerospermum*, *C. macrocarpum*, *C. cladosporioides* and *C. herbarum* were noted. Maximum hourly concentrations up to 14×10^3 m^{-3} were recorded in coastal region during winter months. Morning concentrations were higher at both city sites compared to afternoon concentration. SPT result revealed an overall 19.67% positive reactions with majority showing mild reactions.

Key words: air-borne fungi, *Cladosporium*, fungal allergens, respiratory allergy, Saudi Arabia

Introduction

Statistics of pediatric allergic populations for Saudi Arabia have been reported recently which revealed up to 9.2% of children nationally diagnosed with asthma [1, 2]. Allergic rhinitis, both seasonal and perennial, with nasal obstruction, sneezing, and watery discharge is even more common in the Kingdom [3], with children nationally diagnosed as positive up to 18%. The incidence of allergy in Saudi Arabia appears greater than in most other countries except New Zealand and Australia [4].

Extrinsic factors or allergens play an important role in the development of bronchial asthma and other allergic diseases while climatic factors and geography may influence the overall levels of airborne spores and pollen 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 generally have high humidity and may provide sufficient moisture for conidial development in the environment. However, airborne spores of saprophytic micro-fungi, mainly representing the dry-airspora with such widely encountered genera of aeroallergens as *Alternaria* (ALT), *Cladosporium* (CLD), and *Ulocladium* (ULD), may originate from both indoor and outdoor sources.

The role of fungi in the sensitization and elicitation of allergic symptoms in atopic subjects is well established [5]. The occurrence of various species of fungi in the outdoor and indoor environments from many parts of the world has been reported [6]. Lately, occurrence of some fungi in the environment of Saudi Arabia has also been reported [7, 8].

While skin prick testing (SPT) may help to identify IgE mediated reactions to these allergens, details of the range of potential allergens present in any environment (under study) provides essential background information to enable the relevant skin test range to be selected.

Materials and methods

Burkard Volumetric (7-day recording) Spore Trap and (2) Personal Volumetric (viable mode) Air Sampler (Both from Burkard Manufacturing Co., Rickman worth England), were used to analyze airborne CLD from different locations.

Burkard volumetric spore trap

Burkard Volumetric (7-day recording) Spore Traps were installed on the flat roof of hospital buildings about 5 M above ground level. Air was drawn through the 2 × 14 mm orifice at 10 L/min to impact onto adhesive-coated, transparent tape. The clockwise mechanism of the trap moves the tape past the orifice at 2 mm/h to give a 48 mm band 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^2. Most identifications were conducted at a magnification of ×1500. Spores were converted to concentration per cubic meter of air by a factor obtained by applying the following formula: concentration propagules $m^{-3} = N_T A_E / n \times a \times V_a$, described earlier [9]. Maximum concentrations of CLD spores per cubic meter of air were determined. Percentages of individual types were calculated against the total spores counted.

Sampling locations

In total four sites for air sampling were selected; two in Riyadh, the capital city, in the Central Province situated in the middle of the desert; in Jeddah, in the Western Province, an ancient coastal city by the Red sea; and in Al-Khobar, in the Eastern province, another coastal but comparatively new business city. In Riyadh, the two sites were located at King Faisal Specialist Hospital and Research Centre (KFSH&RC) and at King Khalid University Hospital (KKUH). The two sites are 8 km apart represent micro-climatic environments. In Jeddah (JED), the site was at King Abdulaziz University Hospital and in Al-Khobar (AKH), the site was at King Fahad University Hospital. Sampling at KFSH&RC, JED & AKH site was conducted for twelve months period each (1986–1988) and at KKUH, it was extended for continuous three years(1987–1990).

Personal volumetric air sampler

Two portable personal volumetric air sampler (Burkard Manufacturing Co. Ltd., Rickman worth, UK) were also used to sample air from two locations. The sampler allows insertion of a petri plate with sterile culture media on which spores are impacted by suction. The air suction rate was maintained at 10 L min^{-1}. 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 samples 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. The two sites chosen for Personal Volumetric Sampling were also located in the capital city of Riyadh. The sites were: (1) Al-Batha and (2) Al-Ulia. The distance between the two sites is 7 km by road. Al-Batha is a developed and congested area consisting of mainly commercial old commercial and residential buildings. Al-Ulia, a modern, and less developed area, consisting of new commercial and residential buildings. The samplings were conducted at both sites twice a week for a period of 12 months (May 1990–April 1991). Morning samplings were done at 9.00 at Al-Batha site and 10.00 a.m. at Al-Ulia site. Afternoon samplings were conducted at 3.00 p.m. 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.

Skin prick test (SPT)

Skin Prick Test was conducted using standard technique at Allergy & Asthma Clinic of Investigators at KKUH. Positive histamine control and negative saline control were incorporated. Results were recorded into three categories. Mild (weal size: 3 mm) moderate (weal size > 3-5 mm) and severe (weal size: > 5 mm) against negative control of zero.

Results

Cladosporium was the prevalent spore type trapped out of doors at all four sites and in all three years at KKUH. The data further revealed that airborne spores

of CLD were present in the air throughout the year with greater numbers during winter months of the year.

Airborne composition

Cladosporium spp was by far the dominant individual spore type at all locations. Airborne composition of CLD for 3 sites and all 3 years data for KKUH is presented in Table 1. Between 20 and 40% (range 3-67%) of air spora in Riyadh was comprised of CLD spores alone while values for Jeddah and Al-Khobar are even higher, i.e., 37.5 and 41.2% respectively. Approximately 50 fungal spores could be recognised on slides with hundreds of organisms prevalent in the air and yet the composition of CLD is outstanding. Just for comparison, the value for ALT, another potent allergenic category constituted up to 6.6% of the total air spora. The above are the mean values for the whole year period. The monthly variations exhibit much higher levels as presented in the table along with the minimum and maximum range. Mean percentage and range for 12-month period for each sites are presented in Table 1.

Seasonal and regional diversity

Monthly variation for the different sites are presented in Figure 1. Monthly mean concentration of CLD at the two Riyadh sites (KFSH&RC and KKUH) showed similar trend, which differed from those at JED and AKH.

Diel periodicities

Up to five different diurnal periodicities have been described for allergenic propagules. Three have daytime maxima. "Diurnal" with day or afternoon maxima and the others late evening or early morning maxima. CLD usually gives midday maxima but climate conditions may sometimes cause a two peaked (bimodal) pattern. These observations were based on studies in Western countries where the mid day temperature normally remain around 20–25 °C. The diel periodicities for CLD, presented in Figure 2, for all locations, did not display any clear pattern except AKH site where it displayed afternoon maxima confirming diurnal pattern of CLD. This aspect needs further elaboration and investigation in line with climatological conditions, which prevails in the region. Data for diel periodicities are based on accumulated monthly values m^{-3} for each month for each site for complete 12-month period. Information of diel periodicities is important in relation to exposure time when the concentration of a given allergen is likely to be higher outdoor during 24 h period.

Maximum concentrations

Maximum concentrations of CLD are presented in Figure 3. It shows that the peak concentration of CLD exceeded 3×10^{-3} m^{-3} level on many occasions and reached unto 14×10^{3} m^{-3} on one occasion.

Maximum concentrations at KKUH sites exceeded 3×10^{3} only in November but remained between 1×10^{3} and 2×10^{3} on many occasions.

Personnel volumetric air sampling result

Results for morning and afternoon sampling both at Al-Batha and Al-Ulia sites are presented in Figures 4a and 4b and the seasonal comparison in Figures 5a and 5b. A comparison of morning and afternoon concentrations shows morning almost always higher than afternoon concentration.

SPT results

SPT results on 605 individuals using commercial *Cladosporium* extract is presented in Table 2. The result shows an overall percentage of 19.67 positive reactivity, mostly mild.

Discussion

CLD is one of the only few genera of fungi that have been studied in relation to allergic diseases. However, the genus CLD (a member of fungi imperfecti) comprises several species that occur on a wide range of hosts. Those most studied are *C. herbarum*, and *C. cladosporoides*). Consequently, the most readily available commercial antigen for diagnostic and treatment purpose are from the same species (Table 3). Even in the mixed extract of CLD, these two species are present.

There is little information on the threshold or clinically significant concentration of airborne CLD spores necessary to bring about allergic symptoms. Frankland and Davies [10] suggested that 3×10^{3} spores of CLD m^{-3} of air was clinically significant for sensitization of susceptible individuals and/or provoking symptoms in already sensitized subjects. Our maximum contraction reached 16×10^{3} spores m^{-3} of air. However, individual sensitivities may differ as that

Table 1. Mean Percentage and range of airborne *Cladosporium* spp in the total air spora for the 12 months period for each site.

	KFSH&RC 1986–1987	JED 1987–1988	AKH 1987–1988	KKUH 1987–1988	KKUH 1988–1989	KKUH 1989–1990
Mean	24.7%	37.5%	41.2%	25.0%	20.1%	21.4%
Range	8.5–40	10.8–67	18.6–66	10.6–45	5.7–33	3–35

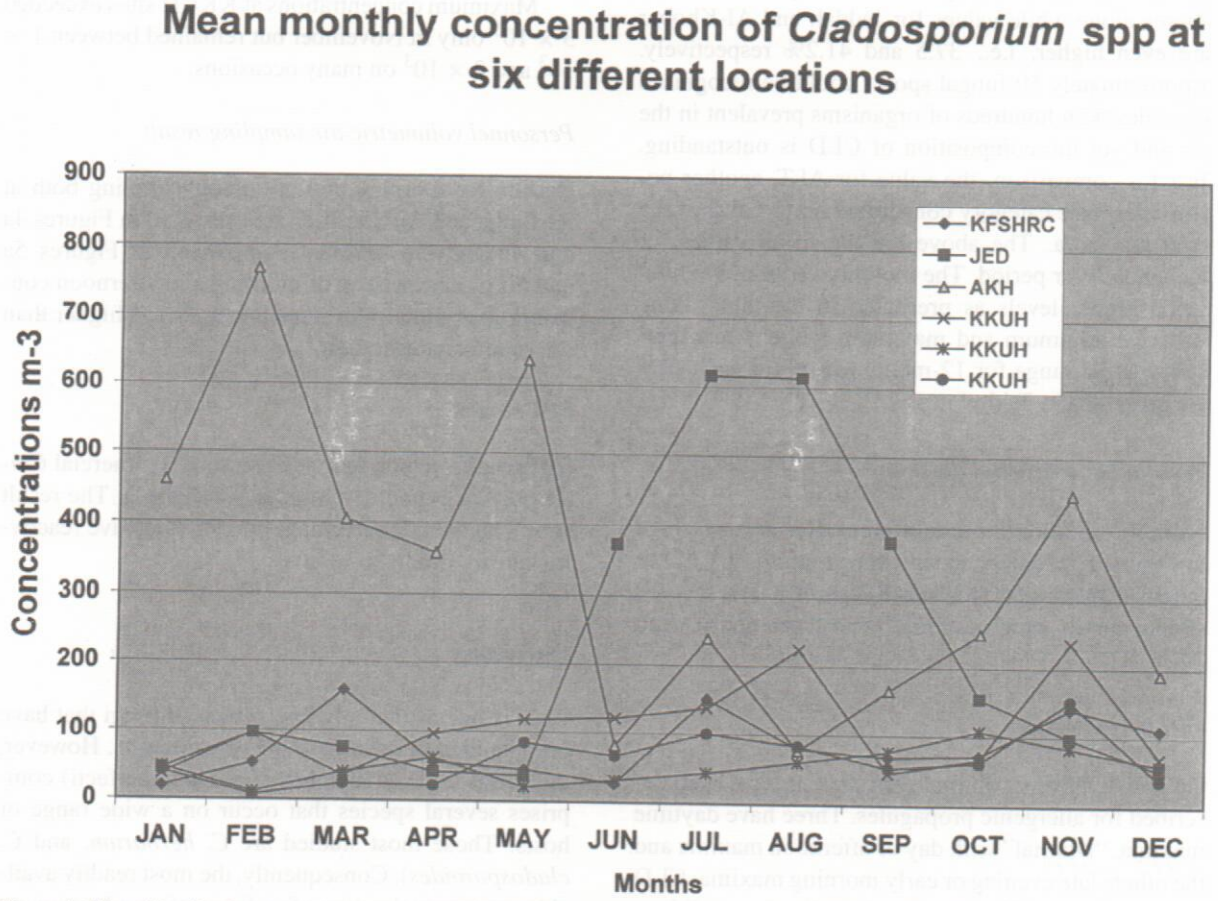

Figure 1. Mean Monthly Concentration of *Cladosporium* spp at six different locations viz., KFSH&RC (King Faisal Specialist Hospital and Research Centre, Riyadh), JED (Jeddah) and AKH (Alkhobar) and KKUH (King Khalid University Hospital, Riyadh).

Table 2. Overall result of skin prick test (SPT) conducted in 5 geographically different regions of Saudi Arabia.

Extract	Total No. of individual	Total No. of males	No. of males positive	Total No. of females	No. of females positive	Total No. positive	% Mild positive	% Moderate positive	% Severe positive	Total % positive
Cladosporium herbarum	605	362	77	243	42	119	16.67	2.00	1.00	19.67

SPT weal and erythema = Mild = 3 mm, Moderate = > 3 mm < 5 mm, Severe = > 5 mm, Negative = No reaction.(Weal and erythema were at least 3 mm higher compared to negative saline control.)

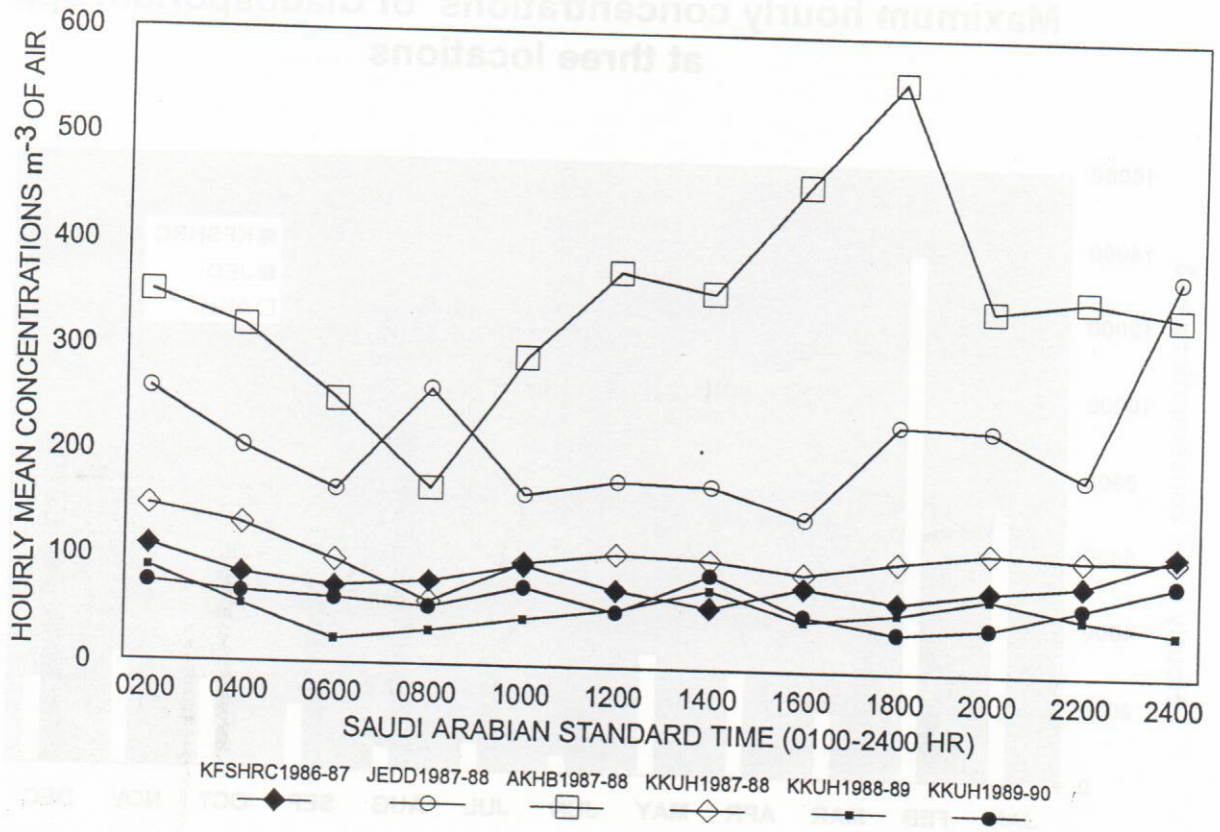

Figure 2. Diel periodicities of *Cladosporium* spp at three locations.

Table 3. List of *Cladosporium* species being used for commercial antigen preparation by some leading manufacturers

Name of extract	Manufacturer	Code No.	Mode
Cladosporium herbarum	Greer Laboratories, Inc. USA	M9	Solution
*Hormodendrum hordei***	Greer Laboratories, Inc. USA	M13	Solution
(*Cladosporium sphaerosperumum*)			
Cladosporium cladosporioides	Allergy Laboratories, Inc. USA	5-140	Solution
Cladosporium herbarum	Allergy Laboratories, Inc. USA	5-140	Solution
*Hormodendrum***	Allergy Laboratories, Inc. USA	5-140	Solution
Cladosporium cladosporioides	Greer Laboratories, Inc. USA	–	Powder
*Cladosporium fulvum****	Greer Laboratories, Inc. USA	–	Powder
Cladosporium herbarum	Greer Laboratories, Inc. USA	–	Powder
*Hormodendrum cladosporioides***	Hollister-Stir, Inc. USA	5129 QA	Solution
*Hormodendrum hordei***	Hollister-Stir, Inc. USA	5394 QA	Solution
Cladosporium herbarum	ALK Laboratories, Denmark	417	Solution
Cladosporium herbarium	Allergo-Pharma, Germany	405	Solution

* Based on available information through brochure by the manufacturer since 1991.
** *Cladosporium* is still being referred to as *Hormodendrum* by various manufacturers, which is now a synonym of the former.
*** Current terminology *Fulvia fulvum*.

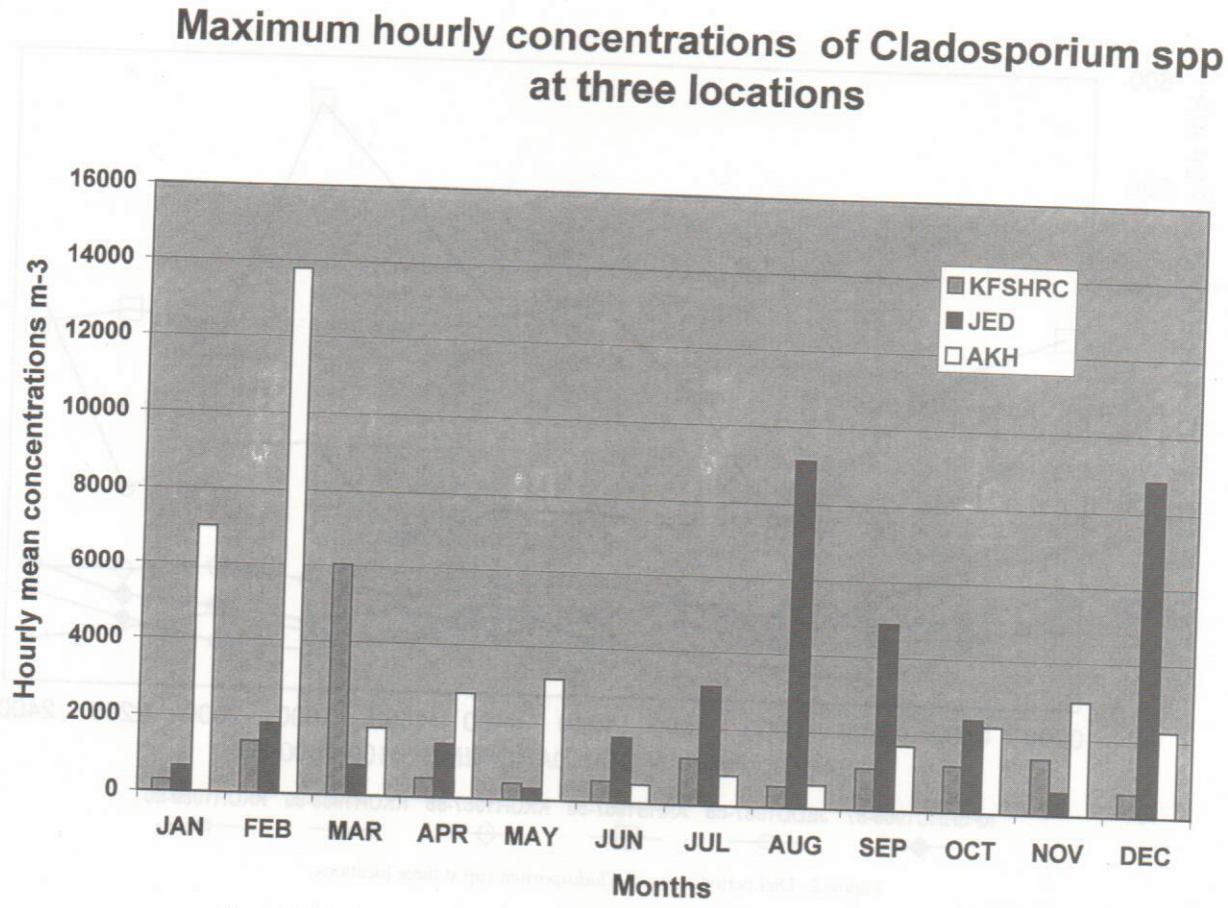

Figure 3. Maximum hourly concentrations m^{-3} of *Cladosporium* spp at three locations.

the clinically significant concentration could be higher or lower than this depending upon such factors as age of the patients and the degree of sensitivity. Again, the concentrations necessary for sensitization and for the subsequent elicitation of symptoms may differ.

The suggestion that CLD is only a weak allergen [11] is usually based on SPT result alone. The quality of allergen depends upon the material used for extraction, whether sporogenous mycelium or spores from culture isolate period of growth medium, method of preparation protein content also need to be considered. It is very difficult to obtain spores of *Cladosporium* without 20–25% mycelium. Also, separate batches of extract produced by the same strains of a fungal species grown under same cultural conditions and duration may show batch to batch variability in potency. This variability in potency has been well documented by Salvaggio and Aukrust [12].

At least 10 different species of CLD have been found in outdoor and indoor environment in Saudi Arabia. Of these, *C. sphaerospermum*,*C. herbarum*, *C. cladosporioides* and *C. macrocarpum* have been identified and at least six more are being identified.

Of 62 species of CLD [13, 14], only 3–4 are used to prepare diagnostic antigens. Since no fewer than ten species occur in air samples in Saudi Arabia, detection of sensitivity to many species relies on cross-reactivity with another extracts of other species. However, such cross-reactivity has seldom been demonstrated. This does not appear to be a realistic approach to the diagnosis of allergies caused by *Cladosporium* species. Characterization of CLD allergen has been limited to *C. herbarum* in which two major allergens, *Cla h* I and *Cla h* II, previously known as, respectively, Ag32 and Ag54 have been recognized [15, 16].

Selection of diagnostic method may play a key role in the evaluation of CLD related response in allergic individual. SPT is convenient to perform and does not involve the extra effort or risk of provocation challenges. However, a study conducted by Collins-

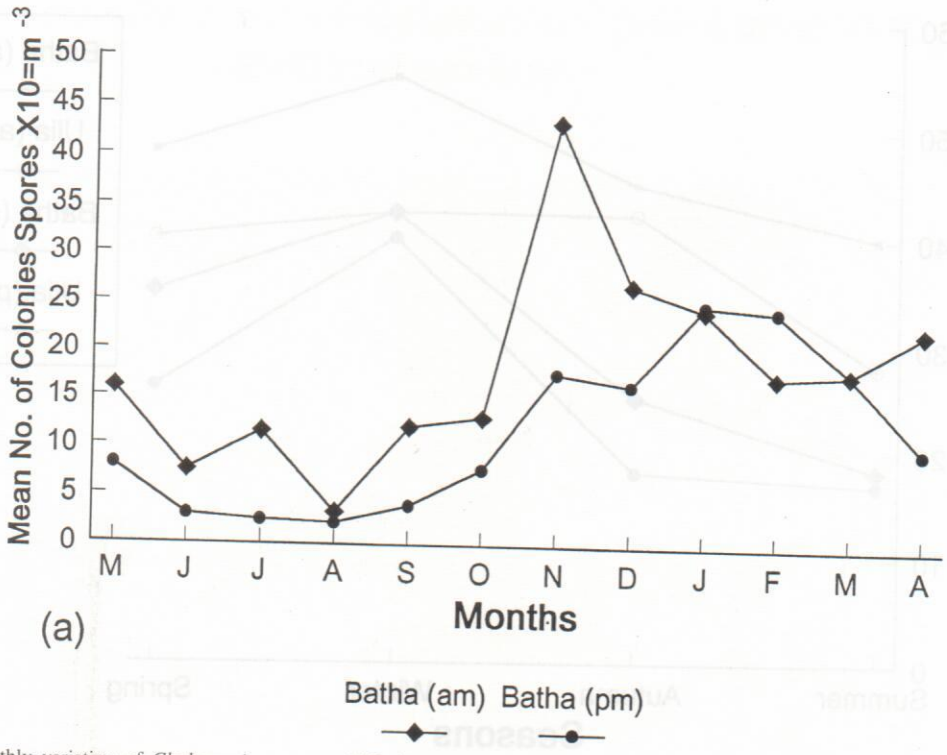

Figure 4a. Monthly variation of *Cladosporium* spp at Al-Batha site, both morning and afternoon concentration, using Burkard Personal Volumetric (culture) sampler.

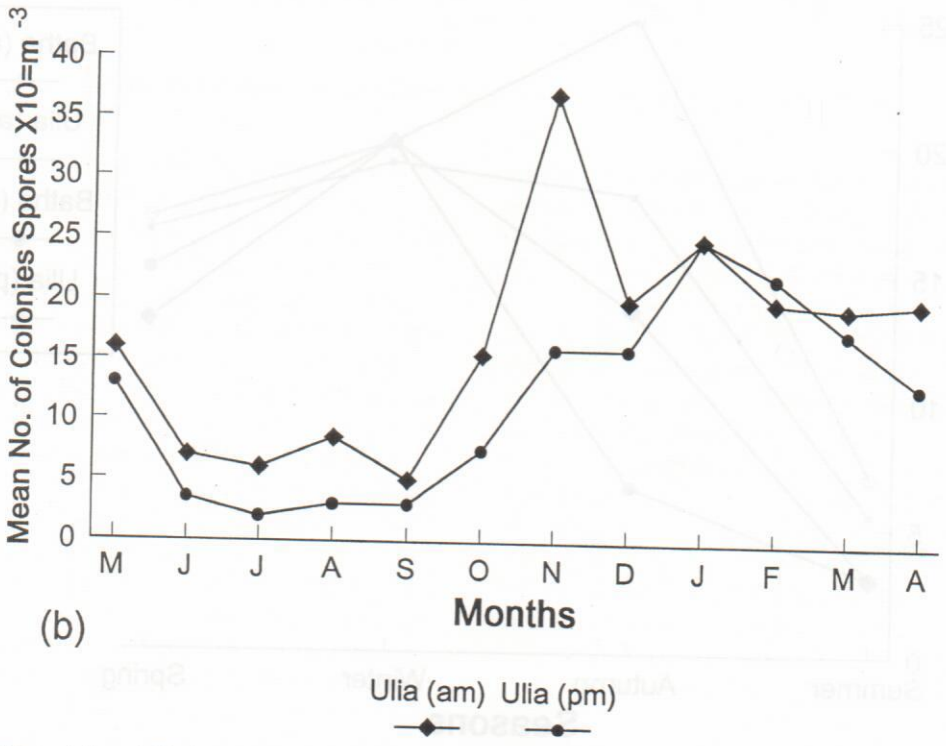

Figure 4b. Monthly variation of *Cladosporium* spp at Al-Ulia site, both morning and afternoon concentration, using Burkard Personal Volumetric (culture) Sampler.

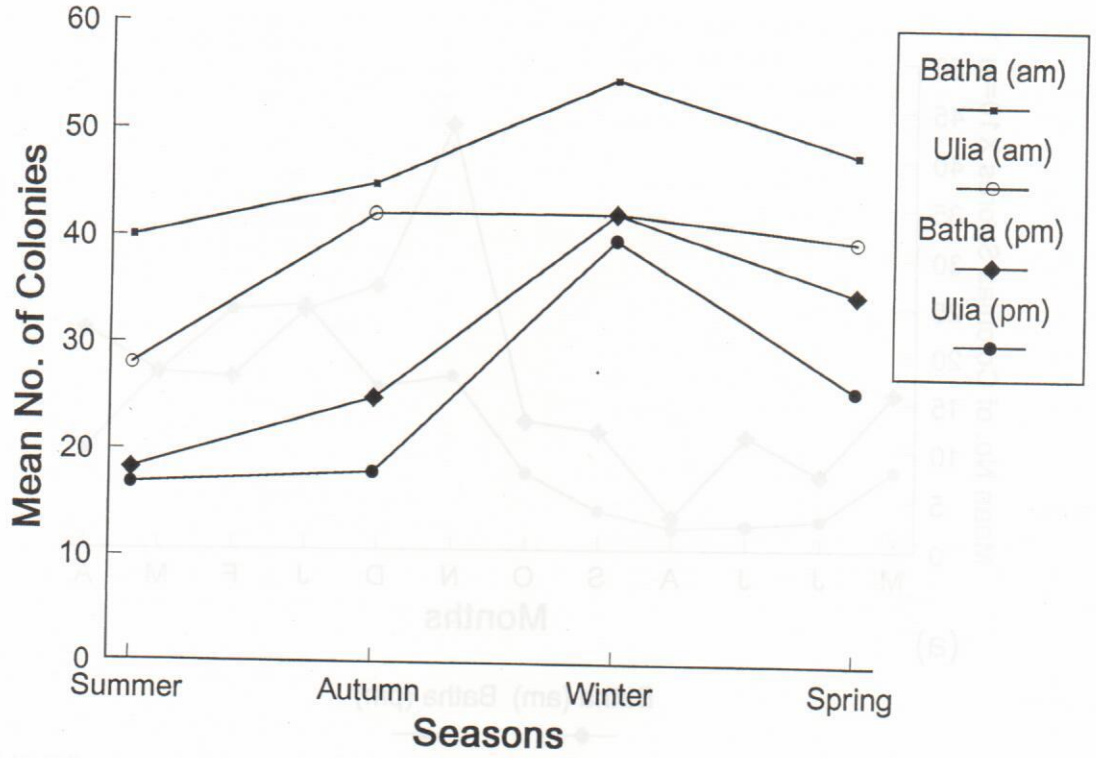

Figure 5a. Seasonal variation of total spores at Al-Batha site (morning and afternoon), using Burkard Personal Volumetric (culture) Sampler.

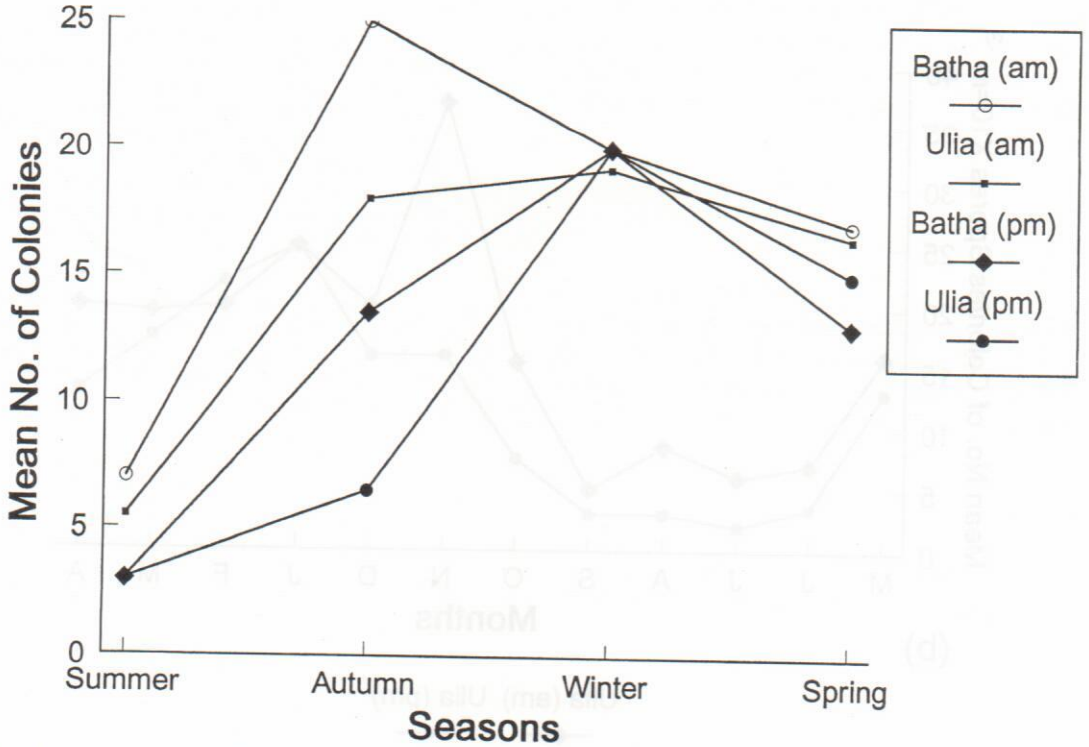

Figure 5b. Seasonal variation of *Cladosporium* spores at Al-Ulia site (morning, afternoon), using Burkard Personal Volumetric (culture) Sampler.

Williams et al. [17] using scratch, intradermal and nasal provocation tests on 150 children found significant differences in positive reactivities ranging from 1.3% to 56% for various fungal antigen. For *Cladosporium* (as *Hormodendrum*) scratch tests gave 6.6% positive, intradermal 39% and for nasal provocation 24.6%. However, for safety and convenience, SPT is more preferable and the same was used in this study.

In view of the fact that there are a number of CLD species, some of them are prevalent in the air, there is a lack of appropriate diagnostic extract(s) including lack of characterized or purified CLD allergen(s). In addition, there are differences in diagnostic results with different methods. Thus, for a precise result, a careful selection of extract for the diagnosis of *Cladosporium* sensitivity be made. We suggest that, in addition to giving utmost care to the species, the characterized or the best available quality of extract should be used to avoid "false negative" results.

Conclusion

Since availability of commercial extracts of *Cladosporium* is limited to a few species only, inclusion of diagnostic extracts from different species for SPT is suggested. As *Cladosporium* is also known to contain weak antigens, preparation using pure culture and confirmation by *in vitro* diagnostic or provocations tests are recommended.

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