Assessment of allergenicity to fungal allergens of Rohtak city, Haryana, India

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ABSTRACT

Fungal spores are known as one of the important bioparticles causing allergic manifestation in human beings. Hence, knowledge of season and prevalence of the airborne allergens to which the patients are exposed is a prerequisite for proper diagnosis and treatment of allergic disorders in hypersensitive individuals. Keeping this in view, aerial survey was performed in the atmosphere of Rohtak city for 2 consecutive years (March 2008–February 2010), using a volumetric petri plate sampler. A total of 45 fungal spore types were recorded during the survey period. In the present study, February–April and July–November were identified as the peak seasons for Rohtak city. Cladosporium *was the main contributor to the total fungal load with 25.14% followed by* Alternaria *(18.05%),* Aspergillus niger *(7.66%),* Curvularia *(5.31%), and* Epicoccum *(5.29%). Fifteen dominant viable fungal spore types were represented in the form of a fungal calendar. An attempt has also been made to assess the allergenicity of some of the fungal types recorded from the atmosphere of Rohtak city. The magnitude of variations observed in markedly positive skin reactions (2+ and above) varied from 17.3 to 2.3%.* Penicillium oxalicum *showed a markedly positive reaction in maximum number of patients (26; 17.3%) followed by* Rhizopus nigricans *(23; 15.3%). ELISA was performed with the sera of patients showing markedly positive skin reactions and the sera were classified into four groups based on percent binding. The majority of the sera showed 0–15% binding to different antigenic extracts, while sera showing >60% binding were least in number. Greater than 30% binding was observed against antigens of* Rhizopus nigricans, Epicoccum purpurascens, Penicillium oxalicum, Curvularia lunata, Aspergillus flavus, Candida albicans *and* Neurospora sitophila. *The concordance between positive skin reaction and serum-specific IgE antibodies ranged from 16.7 to 69.2%.*

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The biodiversity of fungi is immense in both an outdoor and indoor environment. They are prevalent in all climates and in every geographical area and contribute a major part of suspended bioparticulate matter of the air. They are the causative agents of hypersensitivity in susceptible individuals, associated with conditions such as bronchial asthma, allergic rhinitis, allergic bronchopulmonary mycoses, hypersensitivity pneumonitis, and atopic dermatitis.[1–8] It has been estimated that nearly 20–30% of the world population suffers from allergic ailments.[9–11] A preliminary study performed about 30 years ago in India reported that 9.2% of the Indian population suffer from fungal allergy.[12] During the last 30–40 years, numbers of allergic patients have shown an exponential increase and the graph continues to rise. The escalating trends are caused by increased exposure to sensitizing allergens and lesser stimulation of our immune system during critical periods of its development.[13] In addition, urbanization, industrialization, environmental pollution, and change in lifestyle are some other factors contributing toward increase in these disorders.[14–16]

India with divergent geoclimatic zones is a hub of biodiversity. The prevailing climatic conditions favor the growth and sporulation of fungi. As a result, airborne concentration of fungal propagule tends to be high. Information on airborne fungi and prevalence of sensitization to fungal allergens is of paramount importance for diagnosis and therapeutic management of allergic diseases. A great deal of work has been performed in different ecogeographic regions of the country with regard to fungal allergens.[8,12,17–25] However, not much information is available from Haryana, a northern state of India, because it has not been explored with regard to dominant aeroallergens.

The present study was therefore undertaken to conduct an aeromycological survey in a suburban area (Rohtak) of Haryana, India, with an aim of achieving the following goals: (i) to understand the aerial spectrum of fungal diversity of Rohtak, (ii) to prepare a fungal spore calendar of the sampling site, (iii) to determine the prevalence of skin reactivity to fungal antigenic extracts among local patients suffering from nasobronchial allergy, and (iv) to compare skin-prick tests (SPTs) and specific-serum IgE tests used in the

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diagnosis of fungal sensitization. The fungal spore calendar constructed in our study provided information on the intensity of dominant spores in different months of the year and was be useful in understanding the fungal spore load in the atmosphere of Rohtak city. The information obtained on prevalence of skin test reactivity to fungal antigens in the local patients will assist in making people aware about the avoidance measures to control consequent health hazards. The study gains its importance because it is first of its kind in the region and is of great value to the local clinicians in the management of allergic ailments.

MATERIALS AND METHODS

Aerial Investigation

The present study was undertaken in Rohtak city, which is located in the southeastern part of Haryana state. It lies between 28°40′/29°05′ N latitudes and 76°13′/76°51′ E longitudes and has an area of 100.57 km^2 according to the Surveyor General of India. The city harbors a population of 373,133 people (2011 census) with a population density of 466 persons/km^2.

Aerial surveys were performed for 2 consecutive years (March 2008–February 2010) at human height (1.8 m) using a personal volumetric petri plate sampler. The sampler is portable, compact, battery-power operated, 10 cm in height, and 8 cm in diameter with a flow rate of 10 L/min. The sampler was operated between 10:00 A.M. to 12:00 P.M. daily for 10 minutes to collect samples from a fixed site situated in the center of the city. Fungal spores were collected directly onto a 90-mm Petri dish containing Sabouraud's agar medium through a sieve plate having 100 holes of 1-mm diameter each. The exposed Petri plates were incubated at 27 ± 2°C for 2–3 days for the development of fungal colonies.

Fungal colonies were identified to the lowest taxonomic rank possible based on their morphological characteristics such as color, size, shape, and other features of mycelia and spores. Different atlases and published literature were used for authentic identification.[26–28] Fungal colony counts obtained were expressed as colony forming units (CFU) per cubic meter of air sampled.

Skin-Prick Test

SPT was performed on 150 patients with respiratory allergy attending the Department of Pulmonary and Critical care, Post Graduate Institute of Medical Sciences, University of Health Sciences, Rohtak. All of the patients with respiratory discomforts were clinically investigated and their medical history was noted. The patients were advised not to take antihistamines and tranquilizers (for at least 48 hours) and other sympathomimetic drugs (for at least 18–12 hours) before the skin test. It was also ensured that the patients had not received corticosteroids for the last 3 weeks. Prior informed written consent was obtained from all of the subjects. Patients <15 and >60 years of age were excluded from the skin test.

A total of 17 fungi predominant in the atmosphere of Rohtak city were used for SPT based on their availability and reported allergenicity. The study was approved by the Ethics Committee of the concerned institute. The consent of each patient was also obtained before skin testing. Sterilized antigenic extracts in 50% glycerinated buffer were procured from All Cure Pharmaceutical Co. (Bahadurgarh, Haryana, India). Fifty percent glycerinated phosphate buffer (1:10) and histamine dichloride (1 mg/mL) were also obtained as negative and positive controls, respectively. A drop of sterile antigenic extract was placed on the precleaned volar surface of the forearm of the subjects. A prick was made through the drop into the skin with the help of a 26G needle and excess antigen was wiped out with the help of a tissue paper. The skin response was measured after 20 minutes with respect to the wheal and/or erythema produced. The results of SPT were examined and classified into four grades as per criteria given by Shivpuri[1]: wheal size same as negative control = negative; wheal size twice the size of negative control = 1+; wheal size >3–5 mm = 2+, and >5 mm = 3+. To ensure that each antigenic extract was devoid of nonspecific irritants, a skin test was also performed on 20 healthy nonatopic volunteers.

Serum Samples

Venous blood was collected from patients showing markedly positive skin reaction (≥2+) to different antigenic extracts. Blood was allowed to clot for ~1 hour at room temperature. The expressed serum was separated and centrifuged at 2000 × *g* for 5 minutes for sedimentation of erythrocytes. The sera obtained were stored at −20°C for immunoassay. Serum samples were also collected from healthy volunteers and treated as control.

Enzyme-Linked Immunosorbent Assay

The presence of the specific IgE antibodies in the sera of patients against different fungal antigenic extracts was determined as per the procedure outlined by Sepulveda *et al*.[29] ELISA titer plates (NUNC 96-well microtiter plate; Tarsons, U.K.) were coated with 100 μL of antigenic extract (200 μg/mL) and incubated overnight at 4°C. Plates were then washed three times with 0.05 M of PBS (0.0 5 M of potassium dihydrogen phosphate, 0.05 M of dipotassium hydrogen phosphate, and 0.8% sodium chloride, pH 7.4) at 15-minute intervals. To block the unbound sites, 100 μL of 1% bovine serum albumin in PBS was added to each well. After incubation for 2 hours at 37°C, washing was

repeated as mentioned previously. Patient's sera diluted to 1:10 with PBS containing 0.05% bovine serum albumin was then added to each well and incubated overnight at 4°C. Washing was repeated with PBS containing 0.1% Tween-20 PBS and incubated with enzyme-labeled (alkaline phosphatase) anti-human IgE (1:1000) for 4 hours at 37°C and enzyme assay was determined using *p*-nitrophenol phosphate (1 mg/mL) in glycine buffer containing 0.001 M of magnesium chloride and zinc chloride. Reaction was terminated at the end of 45 minutes with 50 μL of 5 N of sodium hydroxide solution. Optical density was taken at 405 nm. Serum from normal volunteers was also assayed against different antigenic extracts.

RESULTS

Aerial Fungal Spectrum

In the present investigation, 45 fungal spore types belonging to 30 genera and 17 species were identified. The annual counts of fungal types obtained during the 2-year survey and their average percent contribution are given in Table 1.

Cladosporium was the main contributor to the total fungal load with 25.14% followed by *Alternaria* (18.05%), *Aspergillus niger* (7.66%), *Curvularia* (5.31%), and *Epicoccum* (5.29%). Other important contributors were *Aspergillus flavus* (4.96%), *Candida albicans* (3.78%), *Ulocladium* (3.72%) and *Fusarium* (3.02%). Types other than these contributed <3% to the total air spora. The total viable spore count was higher (3428 CFU/m^3) in the 1st year when compared with spore catch (2300 CFU/m^3) recorded in the 2nd year of survey (Fig. 1).

Seasonal Dynamics

The fungal spore types were encountered from the atmosphere of Rohtak city throughout the survey period. However, two peaks were evident: (i) February–April (spring) and (ii) July–November (autumn; Fig. 2). *Candida albicans*, *Nigrospora*, *Penicillium citrinum*, *Epicoccum* and *Ulocladium* contributed significantly during the first season whereas species of *Aspergillus*, *Curvularia*, *Fusarium*, and *Penicillium* were the major contributors in autumn.

Based on the average values of viable fungal spores obtained during the 2-year investigation, a fungal calendar was constructed for 15 dominant types (Fig. 3). *Cladosporium* was recorded throughout the investigation period in varying concentrations with an increase from November to February. Lowest catch was observed in the month of July. *Alternaria* showed a season from February to May with a peak in the month of April. The major season for *A. niger*, *A. flavus*, and *Curvularia* was from July to October. January 2009 was the month that recorded lowest catch of *A. niger.* A very short and distinct season from March to April was observed for *Epicoccum*. Poor catch was exhibited from November to May by *Curvularia* and *Epicoccum* spores.

Monsoon (July–August) was the favorable season for the spores of *C. albicans.* The peak season for *Ulocladium* was from February to May with highest catch in the month of May. *Fusarium* spp. exhibited in the early winter season, *i.e.*, October–November. However, no definite seasonal pattern was observed for *Nigrospora* spp. and *Rhizopus* spp. and their spores were encountered throughout the survey period. *Penicillium oxalicum* was prevalent from July to November with a peak in August. *P. citrinum*, on the other hand, showed a peak from January to March. *Aureobasidium* was recorded in all of the months with a higher catch from December to April.

Bioassay

The overall results of SPT showing 1+ to 4+ reactions to 17 antigenic extracts are given in Table 2. The magnitude of variations observed in markedly positive skin reactions (2+ and above) varied from 17.3 to 2.3%. *P. oxalicum* showed a markedly positive reaction in maximum number of patients (26; 17.3%) followed by *Rhizopus nigricans* (23; 15.3%). Antigenic extracts of both *Epicoccum purpurascens* and *Fusarium solani* elicited skin reactions in 12% of the patients whereas *A. flavus* caused positivity in 11.3% of patients. *Curvularia lunata* and *Neurospora sitophila* also showed positive response (10.7%), whereas 9.3% of patients showed positivity to *Trichoderma*, *Helminthosporium*, and *Nigrospora oryzae.* Antigenic extract of other fungi elicited 2+ and above reactions in 6.7–8.7% of the patients. Four antigenic extracts, *viz.*, *Aspergillus fumigatus*, *C. albicans*, *Cladosporium herbarum*, and *Mucor mucedo* showed positivity in 8% of cases. Of all of the antigenic extracts tested, minimum allergenicity (3.3%) was shown to *Alternaria solani.*

The percentage of patients showing highly positive response (3+ and above) varied from 1.3 to 8.7%. Maximum sensitization (8.7%) was shown by *R. nigricans* followed by *P. oxalicum* (7.3%). *N. sitophila*, *E. purpurascens*, and *Trichoderma viride* exhibited skin response in 4.0% of cases. Four antigens, *viz.*, *A. flavus*, *A. fumigatus*, *Fusarium solani*, and *N. oryzae* showed positive reaction in 3.3% of cases whereas 2.7% of the cases showed positive reaction to the antigenic extract of *A. niger*.

Immunoassay

Of 26 patients showing markedly positive skin reactions (2+ and above) to any one of the 17 antigenic extracts used, only 20 consented for *in vitro* test. ELISA was thus performed with the sera of these patients for the presence of specific IgE antibodies against the antigens to which they showed skin positivity. Sera of five healthy volunteers were also screened. The mean

Table 1 **Total airborne fungal types identified (their CFU/m^3 and average percent contribution)**

S. No.	Fungal Types	CFU/m^3 (2008–2009)	CFU/m^3 (2009–2010)	Average Contribution (%)
1	*Absidia* spp.	3	1	0.06
2	*Acremonium* spp.	137	4	2.08
3	*Alternaria* spp.	572	480	18.77
4	*Aspergillus flavus*	135	137	4.0
5	*Aspergillus flavipes*	15	0	0.21
4	*Aspergillus fumigatus*	39	29	1.19
7	*Aspergillus giganteus*	1	0	0.01
8	*Aspergillus nidulans*	35	0	0.51
9	*Aspergillus niger*	210	233	8.12
10	*Aspergillus sydowi*	30	0	0.43
11	*Aspergillus terreus*	5	7	0.22
12	*Aspergillus ustus*	14	9	0.39
13	*Aspergillus versicolor*	12	29	0.80
14	*Aureobasidium* spp.	38	24	1.07
15	*Candida albicans*	151	120	4.80
16	*Chaetomium* spp.	2	3	2.63
17	*Choanephora* spp.	1	0	0.01
18	*Circinella* spp.	4	0	0.05
19	*Cladosporium* spp.	978	505	25.23
20	*Curvularia* spp.	246	78	5.28
21	*Drechslera* spp.	3	2	0.08
22	*Epicoccum* spp.	139	152	5.32
23	*Fusarium* spp.	132	51	3.03
24	*Geotrichum* spp.	23	2	0.37
25	*Microsporum* spp.	5	4	0.15
26	*Mucor* spp.	22	3	0.38
27	*Neurospora* spp.	26	17	0.74
28	*Nigrospora* spp.	64	89	2.86
29	*Paecilomyces* spp.	10	4	0.23
30	*Penicillium chrysogenum*	12	19	0.58
31	*Penicillium citrinum*	65	22	1.42
32	*Penicillium digitatum*	7	3	0.16
33	*Penicillium frequentens*	15	0	0.21
34	*Penicillium funiculosum*	7	2	0.14
35	*Penicillium oxalicum*	62	36	1.68
36	*Penicillium restrictum*	19	3	0.34
37	*Rhizopus* spp.	21	104	2.56
38	*Scopulariopsis* spp.	8	6	0.24
39	Sterile mycelium	12	5	0.28
40	*Syncephalastrum* spp.	19	3	0.34
41	*Trichoderma* spp.	15	1	0.23
42	*Trichophyton* spp.	1	2	0.05
43	*Trichosporon* spp.	5	2	0.11
44	*Ulocladium* spp.	100	107	3.78
45	*Verticillium* spp.	10	2	0.18

Total annual count = 3428 CFU/m^3 (2008–2009); 2300 CFU/m^3 (2009–2010). S. No. = serial number.

optical density values obtained against each antigen for patients and normal sera are shown in Table 2. The sera was classified into four groups based on percent binding, *viz.*, 0–15%, 15–30%, 30–60%, and >60% (Table 3). The majority of the sera showed 0–15% binding to different antigenic extracts, and sera showing >60% binding were least in number. Greater than 30% binding was observed against antigenic extract of *R. nigri-*

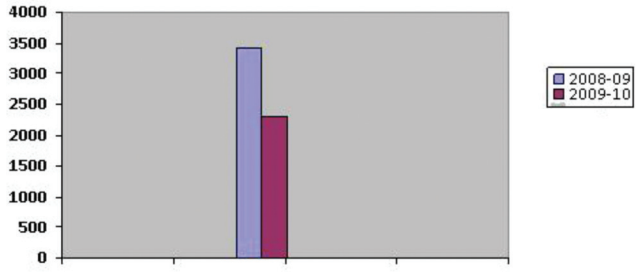

Figure 1. *Year-to-year variations of total fungal spore types.*

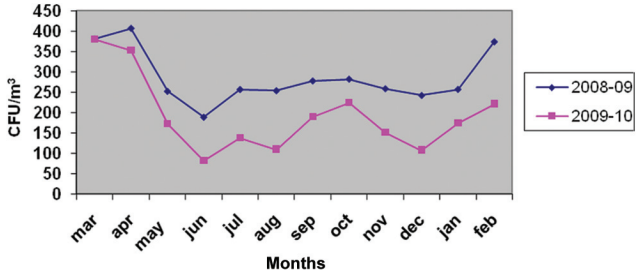

Figure 2. *Seasonal variations of total viable fungal spore types.*

cans, *E. purpurascens*, *P. oxalicum*, *C. lunata*, *A. flavus*, *C. albicans*, and *N. sitophila.* However, none of the sera tested showed >30% binding to the antigenic extract of *M. mucedo* and *T. viride.*

Correlation between Skin Test Positivity and ELISA Results

To study concordance between the two methods, percent correlation was calculated between the results obtained by SPT (2+ and above) and ELISA (>15% binding). The concordance between bioassay and immunoassay varied from 16.7% (*T. viride*) to 69.2% (*C. lunata*). More than 50% concordance was observed against antigenic extracts of *R. nigricans*, *N. sitophila*, *E. purpurascens*, *F. solani*, *A. fumigatus*, and *A. flavus* (Table 4).

DISCUSSION

Aerial spectrum of Rohtak city exhibited rich fungal diversity with 20 genera belonging to class Deuteromycetes, 6 to Zygomycetes, 3 to Ascomycetes, and 1 to mycelia sterilia. The dominance of Deuteromycetes in the airborne fungal flora has also been reported from other geographical regions of the country such as Orissa, Rajasthan, and Uttar Pradesh.[28,30,31] In the present study, 10 species of *Aspergillus* were recorded. However, from Delhi a nearby station only six species of *Aspergillus* have been reported: *A. flavus*, *A. fumigatus*, *Aspergillus nidulans*, *Aspergillus ochraceus*, *Aspergillus sydowi*, and *Aspergillus versicolor*.[32] In our study, four additional species were observed: *Aspergillus flavipes*, *Aspergillus giganteus*, *A. niger*, *Aspergillus terreus*, and *Aspergillus ustus.* However, *A. ochraceus*, which was reported from Delhi, was not encountered by us.

Total fungal colonies recorded were high during the 1st year of investigation when compared with the 2nd year. *Cladosporium* spp., *Curvularia* spp., *C, albicans*, and *Fusarium* spp. contributed the maximum to the 1st year catch. *A. niger*, *Epicoccum* spp., *Ulocladium* spp., and *Nigrospora* spp. were significant contributors during the 2nd year of survey. However, *Alternaria* spp. and *A. flavus* were encountered in both of the years. Low spore catch in our results during the 2nd year may be attributed to rapid industrialization and urbanization of the city, which led to a decrease in the vegetative cover and, hence, less availability of decaying matter especially for the growth of saprophytic fungi. Year-to-year variations in aerial fugal spectrum have also been reported from other ecozones of the country.[8,33]

Spring and autumn are the two seasons for airborne fungi of Rohtak city. Rich vegetation amounting to high organic matter along with suitable climatic conditions corresponds to high prevalence of fungi during these periods. The existence of two peaks of high fungal density was also reported from Delhi.[34–39]

Fungal spore calendars are important because they provide important information on the seasonal dynamics of airborne fungi. These calendars are of immense help to the clinicians in management of respiratory ailments. In the present investigation, fungal calendars were constructed for 15 dominant viable fungal spore types. Similarly, fungal calendars from different parts of India are also available.[8,18,39,40]

Cladosporium spp. was the major contributor to the air spora followed by *Alternaria* spp., *A. niger*, and *Curvularia* spp. The dominance of *Cladosporium* spp. has also been reported in the air spora of different geographical regions of the world including India.[30,34,39,41–46] Thus, *Cladosporium* can be regarded as a "universal dominant" fungus.[47] In compliance with our observation, Al*ternaria* spp. has been reported as the second dominant fungi from parts of India such as Pune, Dehradun, and Nagpur.[48–50]

Poor incidence of fungal spores during monsoon period, *i.e.*, July and August could be attributed to their washing off by rains, thus minimizing their presence in the atmosphere. Moreover, high relative humidity leads to absorption of water by the spores, making them heavier and less transportable by air.[51] Similar observations have also been made from Delhi, Bikaner, Modinagar, and Allahabad.[23,30,52–53]

In our study, SPT was performed on 150 patients suffering from nasobronchial allergy to assess the allergenicity of 17 fungal types because these only were available commercially. Highest positivity (17.3%) was shown to the antigenic extract of *P. oxalicum* followed by *R. nigricans* (15.3%). However, low positivity to *P oxalicum* has been reported from Delhi and Bangalore.[18,22] In compliance to our study, Singh reported *P. oxalicum* to show high positivity in susceptible individ-

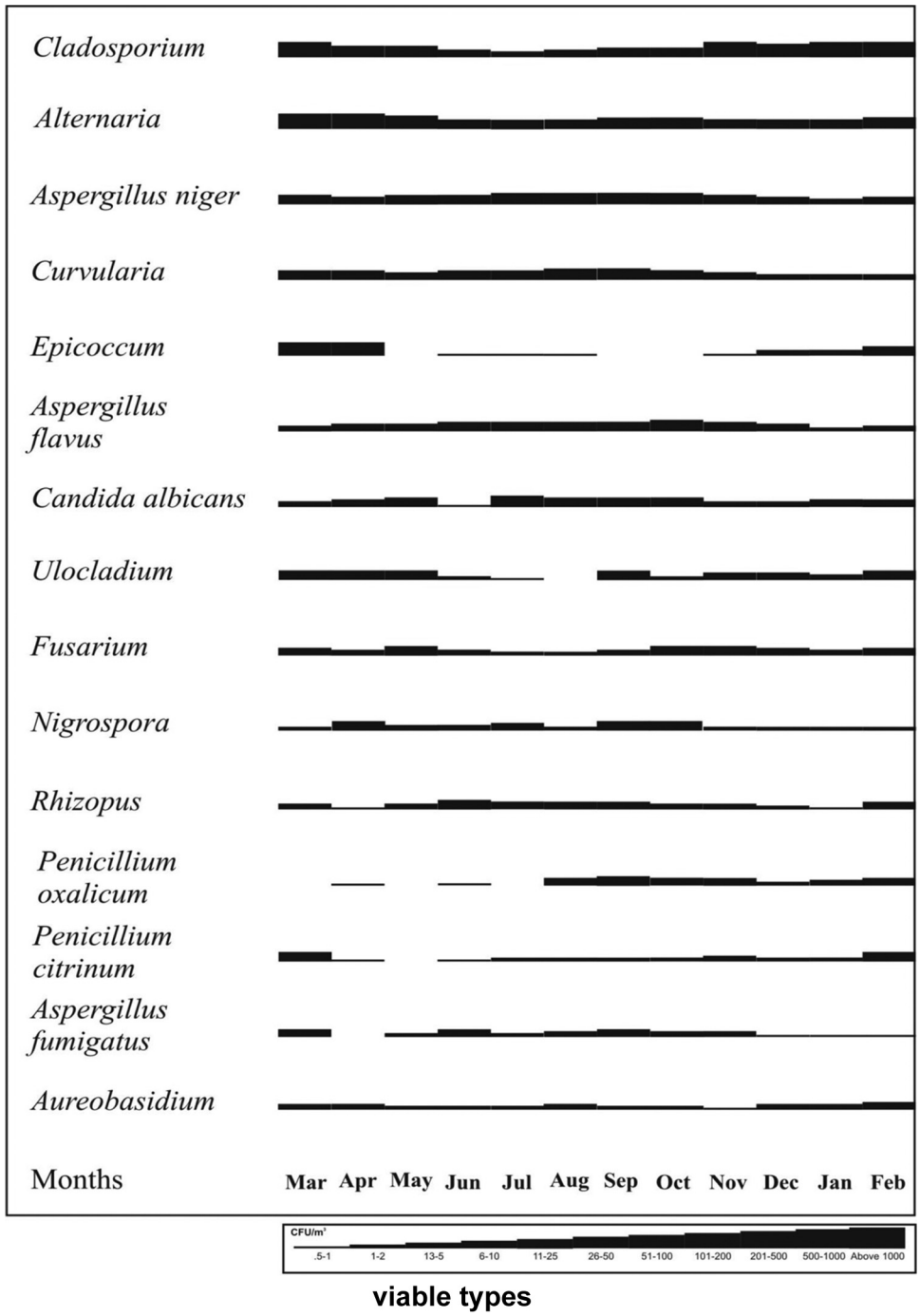

Figure 3. *Fungal calendar of dominant types prevalent in the atmosphere of Rohtak city.*

uals from Delhi.[23] *R. nigricans* is also reported to be an important allergen from Calcutta.[54] Donthi *et al.* from Hyderabad also reported high sensitivity (36.75%) to *Rhizopus* among patients of their region.[55] However, from Andhra Pradesh, only 5.7% of patients showed 2+ and above reaction to the antigenic extract of *R. nigricans*.[56] With respect to other antigenic extracts, 10% of the patients exhibited markedly positive skin reactions to the antigenic extract of *E. purpurascens*, *F. solani*, *C. lunata*, *N. sitophila*, and *A. flavus*. From Andhra Pradesh also these fungi are reported to be important allergens showing markedly positive skin reaction in 6–11% of the patients tested.[56] Among all 17 antigenic extracts tested, the least allergenicity (4.7%) was shown against antigenic extract of *Alternaria tenuis.*

The correlation between skin positivity to individual fungal extract and presence of specific IgE to the respective fungi in the sera of patients varied from 16.7 (*T. viride*) to 69.2% (*C. lunata*) with an average of 48.4%. More than 50% concordance was observed for antigenic extract of *R. oryzae*, *N. sitophila*, *E. purpurascens*, *F. solani*, *A. fumigatus*, and *A. flavus.* Variability

Table 2 **SPT reactions (on 150 patients) to 17 fungal antigens**

Antigens	1+ to 4+		2+ to 4+		3+ and above	
	n	%	*n*	%	*n*	%
Penicillium oxalicum	68	45.3	26	17.3	11	7.3
Epicoccum purpurascens	63	42	18	12	8	5.3
Rhizopus nigricans	62	41.3	23	15.3	14	9.3
Curvularia lunata	51	34	16	10.7	3	2
Mucor mucedo	50	33.3	13	8.7	3	2
Neurospora sitophila	45	30	16	10.7	4	2.7
Trichoderma viride	45	30	13	8.7	5	3.3
Fusarium solani	44	29.3	18	12	5	3.3
Cladosporium herbarum	42	28	12	8	2	1.3
Candida albicans	41	27.3	13	8.7	3	2
Nigrospora oryzae	38	25.3	12	8	5	3.3
Helminthosporium sativum	35	23.3	14	9.3	3	2
Aspergillus flavus	36	24	16	10.7	4	2.7
A. Fumigates	28	18.7	12	8	5	3.3
A. Versicolor	28	18.7	10	6.7	2	1.3
A. Niger	23	15.3	10	6.7	4	2.7
Alternaria tenuis	23	15.3	7	4.7	0	0

SPT = skin-prick test.

Table 3 **Percent binding of allergen-specific IgE antibodies against 17 fungal antigens in the sera of patients showing markedly positive skin reactions**

Antigenic Extracts	No. of Sera Tested	Percent Binding 15–30%		Percent Binding 15–30%		Percent Binding 30–60%		Percent Binding >60%	
		n	%	*n*	%	*n*	%	*n*	%
Alternaria tenuis	3	2	66.7	0	0	1	33.3	0	0
Aspergillus flavus	14	6	42.9	3	21.4	4	28.6	1	7.1
Aspergillus fumigates	10	4	40.0	2	20.0	3	30.0	1	10.0
Aspergillus niger	9	5	55.6	2	22.2	2	22.2	0	0
Aspergillus versicolor	6	3	50.0	2	33.3	1	16.7	0	0
Candida albicans	12	5	41.7	2	16.7	3	25.0	2	16.7
Cladosporium herbarum	13	8	61.5	2	15.4	2	15.4	1	7.7
Curvularia lunata	13	4	30.8	3	23.1	4	30.8	2	15.4
Epicoccum purpurascens	17	6	35.3	4	23.5	3	17.6	4	23.5
Fusarium solani	17	7	41.1	6	35.3	4	23.5	0	0
Helminthosporium sativum	8	5	62.5	2	25.0	1	12.5	0	0
Mucor mucedo	10	6	60.0	4	40.0	0	0	0	0
Neurospora sitophila	11	4	36.4	2	18.2	2	18.2	3	27.3
Nigrospora oryzae	10	7	70.0	2	20.0	1	10.0	0	0
Penicillium oxalicum	20	13	65.0	1	5.0	4	20.0	2	10.0
Rhizopus nigricans	18	6	33.3	5	27.8	2	11.1	5	27.8
Trichoderma viride	12	10	83.3	2	16.6	0	0	0	0

in the concordance between the results of bioassay and immunoassay has been reported from different parts of the world. From a study in Delhi, a correlation between skin positivity to different fungal extracts and specific IgE to the respective fungi in the sera was recorded in the range of 33–100%.[57] However, in a study performed in the united Kingdom, the concordance rates for individual fungi varied

Table 4 **Correlation of ELISA with SPT**

Antigens	No. of SPT$^+$ Sera Tested for ELISA	No. of ELISA$^+$ Sera (>15% binding)	Correlation between SPT and ELISA (%)
Alternaria tenuis	3	1	33.3
Aspergillus flavus	14	8	57.1
Aspergillus fumigates	10	6	60.0
Aspergillus niger	9	4	44.4
Aspergillus versicolor	6	3	50.0
Candida albicans	12	7	58.3
Cladosporium herbarum	13	5	38.5
Curvularia lunata	13	9	69.2
Epicoccum purpurascens	17	11	64.7
Fusarium solani	17	10	58.8
Helminthosporium sativum	8	3	37.5
Mucor mucedo	10	4	40.0
Neurospora sitophila	11	7	63.6
Nigrospora oryzae	10	3	30.0
Penicillium oxalicum	20	7	35.0
Rhizopus nigricans	18	12	66.7
Trichoderma viride	12	2	16.7

SPT = skin-prick test.

from 14 to 56% with an average of only 40%. Highest concordance (56%) was reported for *Alternaria*; and only 29% was observed for *Penicillium*, which is in compliance with our study. O'Driscoll *et al.*[58] reported 54% concordance for *Aspergillus* and a weak concordance (35%) for *Cladosporium*.

Our results show that the most predominant fungi may not necessarily be the most potent allergens. For example, *Cladosporium* was the major contributor to the total fungal load (25%) but it showed skin reactivity in only 8% of cases and concordance obtained with ELISA was 38.5%. On the other hand, *P. oxalicum* contributed <2% to the total catch but was found to be the most important allergen from the Rohtak city. Similarly, *Rhizopus* contributed only 2.6% to the aerial catch but showed skin positivity in 15.5% of the patients studied. Similar observations have also been made from Netherlands.[59,60]

STRENGHTS AND LIMITATIONS OF PRESENT STUDY

The present study aimed at identifying the fungal allergens predominant in the atmosphere of Rohtak city. The findings are significant because identification of dominant fungal allergens along with their seasonal variations presented in the form of fungal calendar will help the clinicians in effective and efficient management of allergic ailments of local inhabitants. For the first time, routine SPTs for fungal allergens have been initiated in Post Graduate Institute of Medical Sciences, University of Health Sciences, Rohtak; therefore, the study is of a great benefit to the local patients who had to go to far places for the diagnosis and treatment of their respiratory disorders. The information obtained will also help the allergy sufferers to manage their routine activities so that they can minimize the exposure to various allergens. Moreover, there is paramount scope of using the findings by public authorities in predicting potential fungal types as a source of respiratory allergy in susceptible individuals. This will also prevent unnecessary exposure of patients to the fungal allergens that are not prevalent in the atmosphere of their city. The study is a pioneer and groundbreaking effort in the city, especially in the field of aerobiology and allergy.

Although efforts have been made to understand the aeromycoflora of Rohtak city and identify dominant fungal allergens for the city, this work has a few limitations such as sampling could not be performed at different sites of the city because of limitation of funds and practical inconvenience. Apart from this, the allergenicity of all of the fungal types identified from the atmosphere of study area could not be studied because all of the fungal antigens are not commercially available for skin testing. Based on our observations, we recommend that a comprehensive study over a greater number of years involving more sampling sites is required to understand the relationship between symptoms of respiratory allergies and prevalence of airborne fungi.

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