

ASTHMA AND ENVIRONMENTAL DETERMINANTS

Quantification of Airborne *Aspergillus* Allergens: Redefining The Approach

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Background. Airborne *Aspergillus* species are significant environmental components involved in the pathogenesis and persistence of allergic respiratory diseases. The detection and quantification of airborne allergens is important to elucidate the clinical implications of environmental exposure of patients suffering with allergic asthma and/or allergic rhinitis. **Objective.** The authors report a simple volumetric approach to measure atmospheric concentration of four common airborne species of *Aspergillus*—*A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii*. **Methods.** As particulate aeroallergens may also exist in amorphous form in addition to morphologically identifiable fungal spores/hyphae, a volumetric technique using membrane filters was developed for simultaneous quantification of (a) viable *Aspergillus* counts, i.e., colony-forming units (cfu)/m³, and (b) actual *Aspergillus* allergen content (ng/m³) in the air. Further, immunochemically quantified airborne *Aspergillus* allergens were compared with their corresponding colony counts. **Results.** The average monthly aerial counts of the four *Aspergillus* species recorded during the sampling year were *A. flavus*: 0.25–15.2 cfu/m³; *A. fumigatus*: 1.25–15.6 cfu/m³; *A. niger*: 0.75–16.0 cfu/m³; and *A. tamarii*: 0.5–11.8 cfu/m³ of air. Aerial *Aspergillus* allergen(s) concentration varied from species to species: *A. flavus*: 26.8–680.8 ng; *A. fumigatus*: 18.0–380.4 ng; *A. niger*: 28.2–1879.0 ng; and *A. tamarii*: 9.2–238.3 ng/m³ of air. Seasonal distribution of airborne colony counts of the four species didn't correlate with their respective allergen content. **Conclusion.** *Aspergillus* allergens were present in the air of Delhi area throughout the year with seasonal variations. The authors feel that by using the immunochemical technique it will be possible to measure actual exposure of patients to various airborne *Aspergillus* allergens.

Keywords *Aspergillus*; colony counts; fungi; immunochemical quantification; inhalant allergens

INTRODUCTION

Environmental factors play an important contributory role in the expression of immunoglobulin E (IgE)-mediated allergic diseases such as bronchial asthma and allergic rhinitis, the most important etiologic factor being allergen exposure (1–3). The common inhalant allergens include pollen, fungal spores and associated fragments, house dust mite and house dust, animal danders, insect emanations and detritus, etc. The significance of fungal spores and mycelial fragments in the etiology of allergic respiratory disorders has been extensively studied and conclusively established (4–9). Fungal sensitization has been reported to be associated with hospital admissions for asthma, life-threatening exacerbations of asthma, and even death from asthma (9–12).

Aspergillus species have been reported to be one of the most predominant constituents of aeromycoflora worldwide (4–8, 13–16). Besides conidiospores (2–5 μ m), its hyphal fragments, mostly conidiophores, are also present in the air throughout the year (17). Once inhaled, they either reach terminal airways or are deposited in large clusters in the upper respiratory tract and cause sensitization, resulting in allergic symptoms. The reported frequency of sensitization with *Aspergillus* species in patients with allergic diseases has varied from 15.3% to 38% worldwide (13, 18–22). As

such, the knowledge of seasonal patterns of occurrence and concentrations of various *Aspergillus* species in a given geographical area becomes very important for proper diagnosis and management of *Aspergillus* hypersensitive patients.

Exposure to fungi including *Aspergillus* species has been traditionally assessed by gravimetric and volumetric surveys with the results being reported as spore/colony counts (23, 24). However, fungal allergens may also be present in some nonidentifiable submicronic particles such as fungal hyphae and conidial fragments smaller than intact fungal spores. On the other hand, due to the effect of various climatic factors such as high temperature, allergenic activity of many viable intact spores may have been lost (25, 26). In view of the above considerations, spore counts or viable colony counts may not be relevant markers of actual airborne allergen content for a given fungal species in the air. A more direct way to assess the role of fungal (*Aspergillus*) allergen exposure as a determinant of allergic respiratory diseases is to measure actual allergen concentration in air samples (24–27).

Thus, the present study was undertaken for the immunochemical measurement of actual total allergen(s) content of four common *Aspergillus* species, viz. *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii*, in the air and comparison with their corresponding viable colony counts.

METHODS

Aeromycological survey was conducted from May 2007 to April 2008 in Delhi metropolitan area, India (latitude 28°4'N and longitude 77°09'E) to study the monthly prevalence of

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the four species of *Aspergillus*. A simple volumetric technique using membrane filters was developed for simultaneous quantification of (a) viable *Aspergillus* counts, i.e., colony-forming units (cfu)/m³, and (b) actual *Aspergillus* allergen content (ng/m³) in the air.

Air Sampling

A volumetric suction sampler (APM 823; Envirotech Limited, India) was installed on the terrace of seven-storey building of Vallabhbhai Patel Chest Institute, Delhi, India, at a height of 20 m above the ground. It was operated for consecutive 24 hours every week and in total 52 weekly air samples were collected during 1 year. The sampler was operated at a flow rate of 40 L/min. A volume totalizer was attached in the system to record the volume of the air sampled at any given time to eliminate the error of fluctuations in the flow rate due to loading of filters during sampling. The airborne particles were collected on a polytetrafluoroethylene (PTFE) membrane filters (47 mm diameter; 0.45 µm pore size) mounted on the air sampler in a filter holder assembly.

Quantification of Airborne *Aspergillus* Viable Counts

The exposed filters were processed under aseptic conditions. The thin filter membranes with collected particles were carefully removed from their support and soaked in 10 ml of sterile extraction buffer (0.1 M NH₄HCO₃ solution, pH 7.8). The collected particles were removed from the membrane by gentle stirring for 30 minutes resulting in a suspension of airborne particles. This suspension was further used for the simultaneous quantification of airborne *Aspergillus* counts and allergen content. An aliquot of 0.1 ml of the above suspension was plated on culture plates containing Rose Bengal agar medium (in duplicates). The plates were then incubated at 28°C ± 2°C for 3–5 days. The total fungal and *Aspergillus* colonies along with the four species—*A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii*—were identified in each plate and counted. The average of duplicate plates was taken for further analysis and expressed as colony-forming units per m³ (cfu/m³) of air calculated as per the formula given below:

$$\text{cfu/m}^3 \text{ of air} = \left(\frac{\text{No. of colonies in 0.1 ml}}{0.1 \text{ ml}} \right) \times \frac{10 \text{ ml}}{v} \quad [1]$$

where v = volume of air sampled (m³).

The elution of particles in the remaining suspension (9.8 ml) was continued for 72 hours at 4°C with intermittent shakings. The suspension was further processed for immunochemical quantification of various airborne *Aspergillus* allergens described later.

Allergen Extracts and Sera Pools

Allergen Extracts. Crude allergen extracts of the four species of *Aspergillus*, viz. *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* were purchased from a commercial manufacturer (All Cure Pharma, Bahadurgarh, Haryana, India).

Study Subjects. A total number of 300 patients (14–60 years) suffering with bronchial asthma and/or allergic rhinitis, diagnosed as per the Global initiative for asthma (GINA) and Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines were included in the present study (2, 28). These

patients were selected from the patients attending the Out Patients Department of Viswanathan Chest Hospital, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India. Twenty nonallergic, healthy volunteers (NHVs) with no personal or family history of any allergic disorder were also included as negative controls. The protocol of the study was approved by the Institutional Ethics Committee of Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India. Informed consent was obtained from each patient and healthy subject.

Preparation of Sera Pools. Skin prick tests (SPTs) were performed on patients with *Aspergillus* extracts, with 50% glycerinated phosphate-buffered saline (GPBS) and histamine (5 mg/ml in GPBS) as negative and positive controls, respectively (29). Enzyme allergosorbent test (EAST) was performed to estimate *Aspergillus* allergen-specific IgE in patients' sera (30). The sera from patients, who showed highly positive SPT as well as EAST results to a given species of *Aspergillus* were selected ($n = 19$ –23). Four separate pools of patients' sera—PPS (*Afl*), PPS (*Afu*), PPS (*Ani*), and PPS (*Ata*) were prepared using sera of patients hypersensitive to *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii*, respectively. This was done to ensure the presence of IgE molecules against all the allergenic proteins of each extract in each PPS. Another pool of sera from 11 nonallergic healthy subjects (PHS), showing uniformly negative skin test and EAST reactions to each *Aspergillus* extract, was also prepared to serve as a negative control.

Immunochemical Quantification of Airborne *Aspergillus* Allergens

Processing of Air Sample Eluates. The remaining suspensions (after volumetric quantification of *Aspergillus* counts) were centrifuged at 4°C for 10 minutes. The supernatants were dialyzed, lyophilized, and reconstituted in 1.0 ml of PBS.

Inhibition of *Aspergillus* EAST With Air Sample Eluates. For quantifying the airborne *Aspergillus* allergens, air sample eluates ($n = 52$) were used as liquid phase inhibitors (50 µl) in each *Aspergillus* EAST. In brief, wells of the microtiter plate were coated with *Aspergillus* extract (1 µg/100 µl/well) in carbonate buffer (pH 9.5) at 4°C for 16 hours. The wells were washed with phosphate-buffered saline–bovine serum albumin–Tween 20 (PBS-BSA-T) and unoccupied binding sites were blocked by PBS-BSA. Inhibition of IgE binding was attempted by preincubating 50 µl of PPS (appropriately diluted with PBS-BSA-T) with 50 µl air sample eluate at 4°C for 16 hours. The preincubated mixture was then added to different wells of a microtiter plate coated with homologous *Aspergillus* extract. *Aspergillus* extract-specific PPS (100 µl) without any inhibitor and PHS were used as positive and negative controls, respectively. After incubation and washing of the plate with PBS-BSA-T, 100 µl of alkaline phosphatase-conjugated monoclonal anti-human IgE (Sigma Chemical, St. Louis, MO, USA) in Tris-buffered saline (TBS; 1:1000 v/v) was added to each well. The plate was then incubated at room temperature for 3 hours. Thereafter, the wells were washed with PBS containing 0.05% Tween 20 (PBS-T), followed by addition of 100 µl of substrate solution

(1 mg/ml *p*-nitrophenyl phosphate in 0.1 M diethanolamine, pH 10.3) in each well. After 30 minutes, the reaction was stopped by adding 100 μ l of sodium hydroxide (0.75 N). The color developed (optical density; OD) was read by an automated microplate reader at 405 nm. EAST inhibition was calculated as follows:

$$\text{Inhibition(\%)} = \left(1 - \frac{\text{OD of PPS with inhibitor}}{\text{OD of PPS without inhibitor}}\right) \times 100$$

Inhibition of *Aspergillus* EAST With Homologous Extract. In addition, to generate a dose-related reference inhibition curve, inhibition of each *Aspergillus* EAST was also conducted using increasing amounts of corresponding homologous *Aspergillus* extract as liquid-phase inhibitor. Inhibition of IgE binding was attempted by preincubating specific PPS (appropriately diluted with PBS-BSA-T) with increasing amounts of homologous *Aspergillus* extract (1, 10, 100, 1000, 10000 ng of crude extract) at 4°C for 16 hours. The preincubated mixture (100 μ l) was then added to different wells of a microtiter plate coated with homologous *Aspergillus* extract. After incubation at 4°C for 16 hours and washing of the plate with PBS-BSA-T, 100 μ l of alkaline phosphatase-conjugated monoclonal anti-human IgE in TBS (1:1000 v/v) was added for 3 hours. Thereafter, the wells were washed with PBS-T followed by addition of 100 μ l of substrate solution. The reaction was stopped after 30 minutes by adding 100 μ l of 0.75 N sodium hydroxide. The OD (405 nm) was recorded and inhibition induced by homologous extract was calculated.

A dose-response curve was plotted (amount of allergen extract versus % inhibition). On interpolating the values of % inhibition induced by each air sample eluate in reference inhibition curve, the amount of allergen extract in 50 μ l of air sample eluate was determined. The amount of *Aspergillus* allergen (ng/m³ of air) was calculated as given below:

- (i) v = Total air sampled in 24 hours (in m³)
- (ii) The eluate of 24-hour air samples was lyophilized and reconstituted in 1000 μ l
- (iii) Amount of eluate used per well in EAST inhibition assay = 50 μ l

Amount of allergen/m³ of air

$$= \left(\frac{\text{Allergen in } 50\mu\text{l of air sample}}{v} \right) \times 20$$

In each assay, specific PPS without any inhibitor and PHS were used as positive and negative controls, respectively. Besides, three unrelated heterologous allergen extracts, pollen: *Prosopis juliflora*, insect: mosquito (*Culex quinquefasciatus*), and horse dander, failed to produce significant inhibition in each *Aspergillus* EAST even when an amount of 10 μ g was used as liquid phase inhibitor, establishing the specificity of inhibition assays.

Specificity of Immunochemical Quantification of *Aspergillus* Allergens. Specificity of air samples induced inhibition in different *Aspergillus* EAST was evaluated by performing dose related inhibition of each *Aspergillus* EAST by

a pool of air samples. For this purpose, equal volumes of 6 to 11 air samples showing high allergen content of various *Aspergillus* species were pooled separately to make four separate pools of air samples. Separate inhibition assays of each *Aspergillus* EAST were conducted with increasing amounts (1.56 to 100 μ l) of these specific pools of air sample eluates.

As a positive control, dose-related inhibition of each *Aspergillus* EAST was also conducted with homologous *Aspergillus* extract. Slopes of inhibition curves obtained with (a) specific air sample pool and (b) homologous extract in each *Aspergillus* EAST were compared. As a negative control, extract of a nonexposed filter membrane was also used in each *Aspergillus* EAST.

Statistical Analysis

Mean and standard error of the mean (SEM) were determined for data analysis. Spearman correlations (GraphPad Prism version 4.00 for Windows; GraphPad Software, San Diego, CA, USA; www.graphpad.com) were determined to quantify the relationship (if any) between immunochemically quantified allergen(s) and colony counts of the four *Aspergillus* species in the air of Delhi metropolitan area, India.

RESULTS

Airborne *Aspergillus* Viable Counts (cfu/m³ of air)

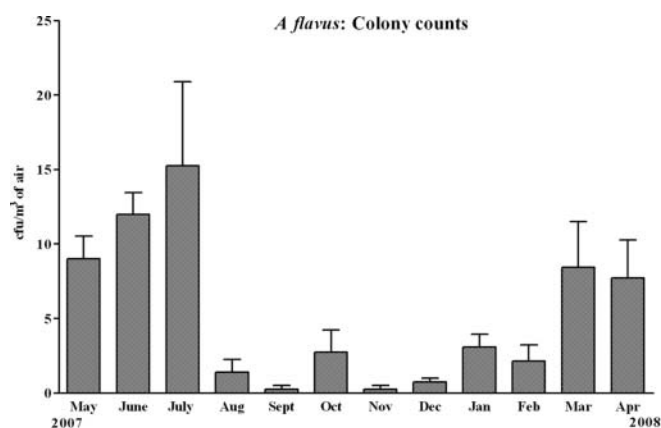
Average daily airborne fungal count was found to be 78.2 cfu/m³ of air; ranging from 33.0 cfu/m³ in December to 180.4 cfu/m³ in March. *Aspergillus* species constituted 30.5% of the daily viable fungal counts. Average daily *Aspergillus* counts ranged from 4.2 cfu/m³ in December to 54.0 cfu/m³ in March.

Monthly distribution of average daily colony counts of *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* in the air during the sampling duration of 1 year are presented in Figures 1A to 4A. The peak periods and range of colony counts of the four *Aspergillus* species during 1 year are presented in Table 1. Of the four *Aspergillus* species, the most prevalent was *A. niger*, accounting for 26.4% of the average daily *Aspergillus* counts. The prevalence of *A. tamarii* among the four species of *Aspergillus* was relatively low, accounting for 16.6% of the average daily *Aspergillus* counts.

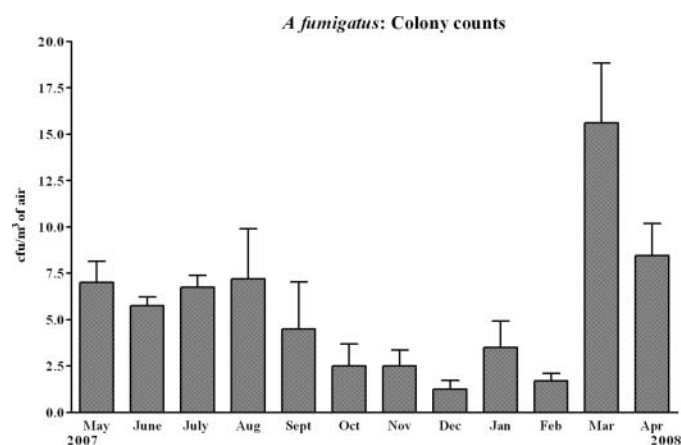
Airborne *Aspergillus* Allergen Content (ng/m³ of air)

Immunochemically quantified airborne *Aspergillus* allergen in Delhi metropolitan area showed seasonal variations (Figures 1B to 4B). The peak periods and range of allergen content of the four *Aspergillus* species during 1 year are presented in Table 1. Of the four *Aspergillus* species, *A. niger* and *A. tamarii* showed the highest and lowest aerial allergen concentration, respectively.

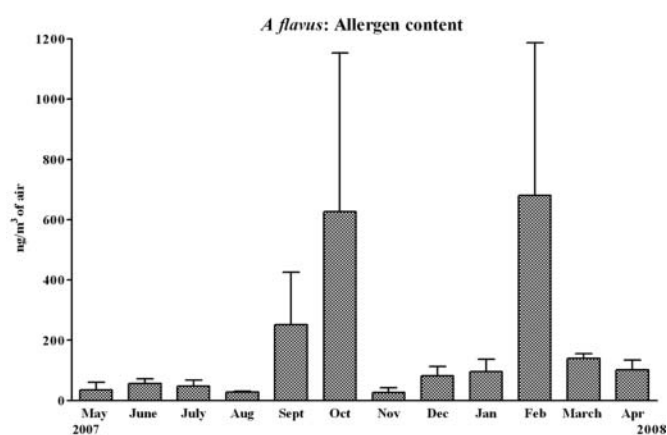
The four pools of air sample eluates produced dose-related inhibition in the corresponding *Aspergillus* EAST (Figures 5 and 6). In each *Aspergillus* EAST, the slopes of these inhibition lines were not statistically different from the inhibition lines produced by respective homologous extract ($p > .05$). Furthermore, extract of a nonexposed filter membrane used as a liquid phase inhibitor



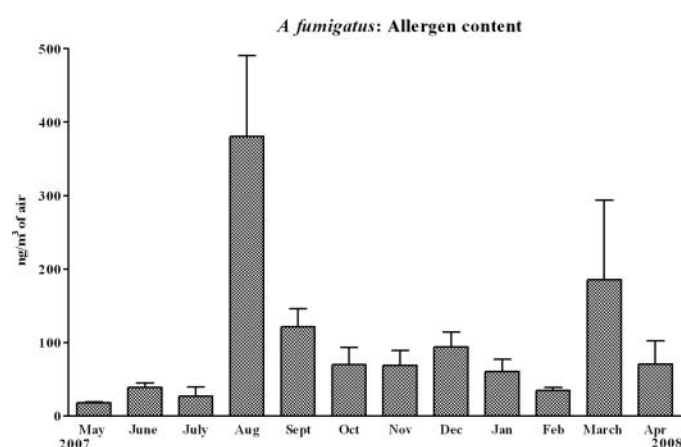
A



A



B



B

FIGURE 1.—Monthly variations in airborne colony counts (cfu/m³) of *A. flavus* and its immunochemically quantified allergen content (ng/m³) in Delhi, India. Bars represent monthly mean $\pm$ SEM.

FIGURE 2.—Monthly variations in airborne colony counts (cfu/m³) of *A. fumigatus* and its immunochemically quantified allergen content (ng/m³) in Delhi, India. Bars represent monthly mean $\pm$ SEM.

in all the four *Aspergillus*, EAST did not show any inhibition (Figures 5B and 6B). These results gave evidence for the specificity of inhibition produced by air sample eluates.

Comparison of Airborne Aspergillus Viable Counts and Allergen Content

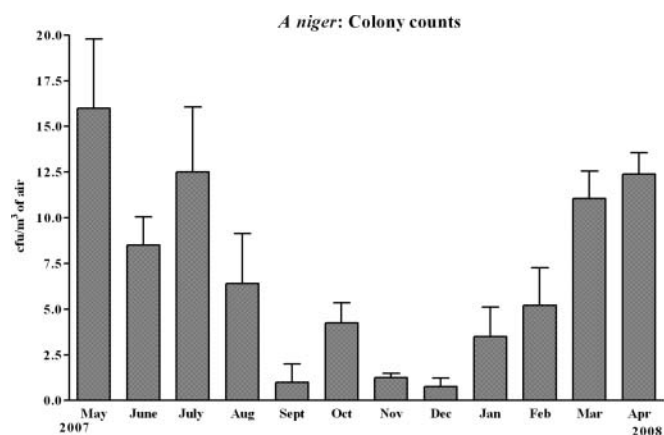
The highest viable colony counts of *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* were observed in July, March, May,

TABLE 1.—Airborne *Aspergillus* counts and allergen content in Delhi metropolitan area (May 2007–April 2008).

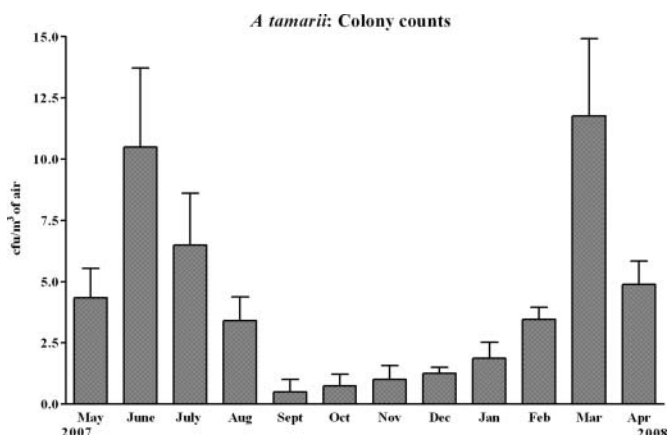
Species	Airborne prevalence	Highest	Lowest	Mean	Months of peak prevalence	Correlation coefficient*
<i>A. flavus</i>	Colony counts (cfu/m ³)	July 15.2	November 0.25	5.1	March–July	.0129 (p = .9313#)
	Allergen content (ng/m ³)	February 680.8	November 26.8	181.4	February, October	
<i>A. fumigatus</i>	Colony counts (cfu/m ³)	March 15.6	December 1.25	5.7	March–August	-.1338 (p = .3698#)
	Allergen content (ng/m ³)	August 380.4	May 18.0	97.5	August	
<i>A. niger</i>	Colony counts (cfu/m ³)	May 16.0	December 0.75	6.7	March–July	.2386 (p = .1063#)
	Allergen content (ng/m ³)	February 1879.0	August 28.2	798.7	February–April, October	
<i>A. tamarii</i>	Colony counts (cfu/m ³)	March 11.8	September 0.5	4.2	March–July	.2046 (p = .1677#)
	Allergen content (ng/m ³)	August 9.2	February 238.3	76.7	February, October	

*Spearman correlation coefficient.

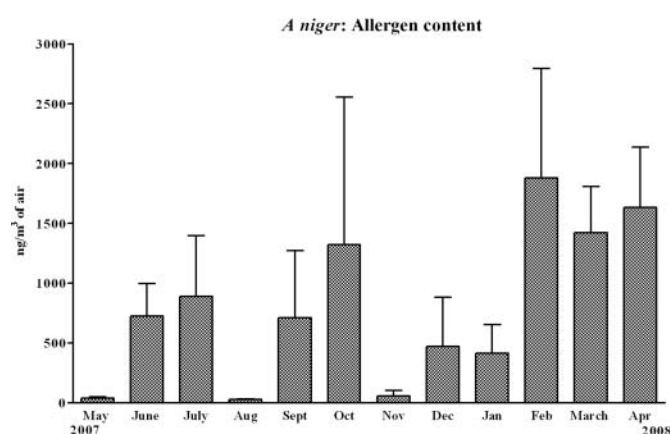
#NS = not significant.



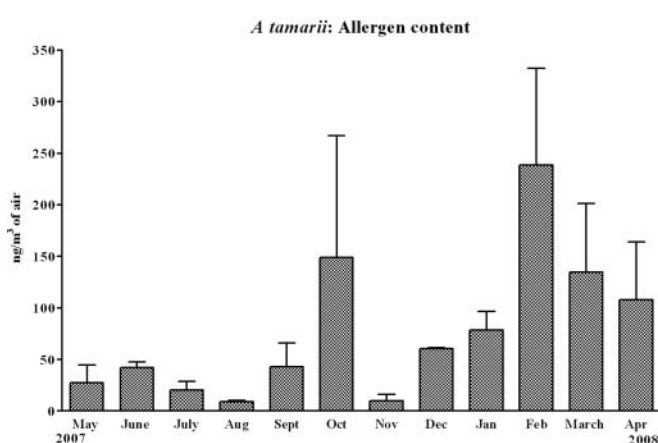
A



A



B



B

FIGURE 3.—Monthly variations in airborne colony counts (cfu/m³) of *A. niger* and its immunochemically quantified allergen content (ng/m³) in Delhi, India. Bars represent monthly mean $\pm$ SEM.

FIGURE 4.—Monthly variations in airborne colony counts (cfu/m³) of *A. tamarii* and its immunochemically quantified allergen content (ng/m³) in Delhi, India. Bars represent monthly mean $\pm$ SEM.

and May, respectively (Table 1). The highest allergen content of *A. flavus* and *A. niger* was observed in February and that of *A. fumigatus* and *A. tamarii* in August (Table 1). *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* viable counts were statistically compared with their corresponding airborne allergen concentration, Spearman correlation coefficients (r) were not significant ($p > .05$) in any comparison (Table 1).

DISCUSSION

The actual clinical significance of any bioparticle depends on its allergenic potential as well as its presence in the environment of the allergic patients (genetically predisposed). The onset and severity of symptoms of the allergic patients is related to the appearance and concentration of specific airborne allergens. Therefore, aeromycological studies giving information about fungal allergens of a given geographical area and their seasonal patterns are an important supplement for the diagnosis and treatment of allergic diseases (5, 24, 31). This information helps the clinicians in selecting proper allergens for diagnostic purposes, providing guidance on allergen avoidance and on scheduling medication for allergic patients (24).

In the present study, a volumetric filtration sampler was used for the quantification of viable *Aspergillus* counts (cfu/m³) in the air. In one of our earlier studies, the volumetric collection method has been compared with a Burkard spore trap for evaluation of its efficiency and reliability (32). Simultaneous air sampling was conducted with a standard Burkard spore trap at the same site. A significant correlation was obtained between daily mean concentrations of major airborne spore types obtained by both the samplers (32).

Various aeromycological surveys conducted in different parts of India from time to time have reported *Aspergillus* to be the most prevalent constituent, with concentration ranging from 5.8% to 33% of total airborne fungi (4, 5, 33–39). During our 1-year survey period, colony counts (cfu/m³ of air) of *Aspergillus* species constituted a predominant component of total viable airborne fungi (30.5%).

For studying allergenic significance of *Aspergillus* species, a majority of the air surveys worldwide have usually enumerated them up to genus level only (4, 5, 33, 40–42). However, some *Aspergillus* species have been consistently encountered in the air surveys conducted in Delhi metropolitan area, such as *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* (4, 13).

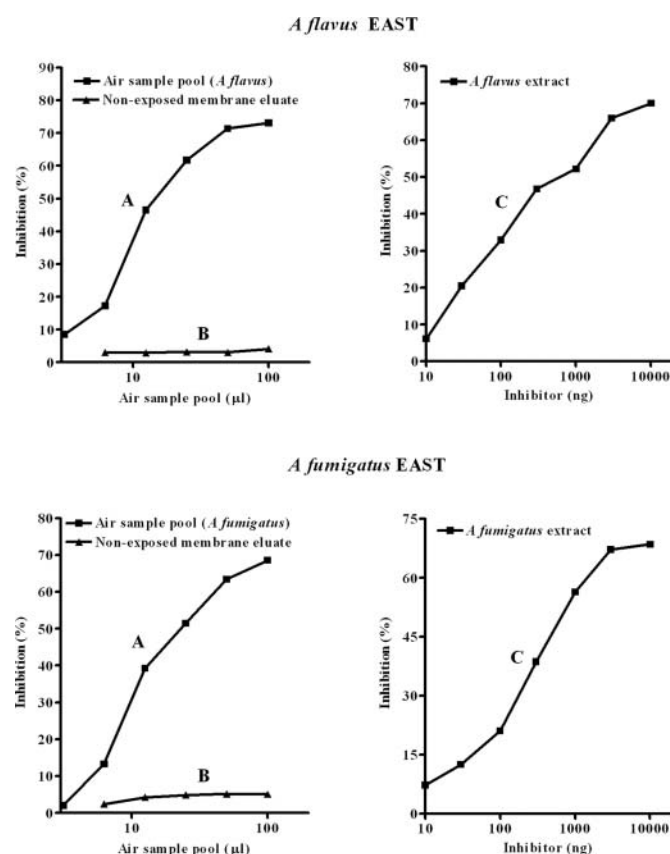


FIGURE 5.—Inhibition of *A. flavus* and *A. fumigatus* EAST with (A) specific pools of air sample eluates; (B) eluate of a nonexposed filter membrane (negative control); and (C) corresponding homologous *Aspergillus* extract as liquid-phase inhibitors. Slopes of the inhibition curves induced by air sample pools were not significantly different from the inhibition curves produced by corresponding homologous extracts (ANOVA, $p > .05$).

In the present air survey, these four common species of *Aspergillus* were identified and quantified. The relative frequency of aerial counts of *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* with respect to the total airborne fungi was 6.5%, 7.2%, 8.6%, and 4.2%, respectively. Some investigators have also reported variable airborne concentration of *A. flavus*, *A. fumigatus*, and *A. niger* in different places in India and abroad (34, 36, 38, 43, 44).

In the present study, the most prevalent species was *A. niger* (26.4%), followed by *A. fumigatus* (22.2%), *A. flavus* (20.2%), and *A. tamarii* (16.6%), with respect to total *Aspergillus* counts. The relative contribution of these four species to total airborne *Aspergillus* concentration has been reported to be variable in different studies (34, 36, 43, 44). *A. fumigatus* was also the most common species in Madrid, Spain, accounting for 54% of the *Aspergillus* isolates recorded (44). *A. flavus* and *A. niger* constituted only 25% and 11%, respectively, of the total *Aspergillus* isolates. The variations in relative frequency of occurrence of *Aspergillus* spp., taken together and the four individual species separately, may have been due to changes in geographical conditions, climatic conditions, local vegetation, urbanization, anthropogenic pollution, etc.

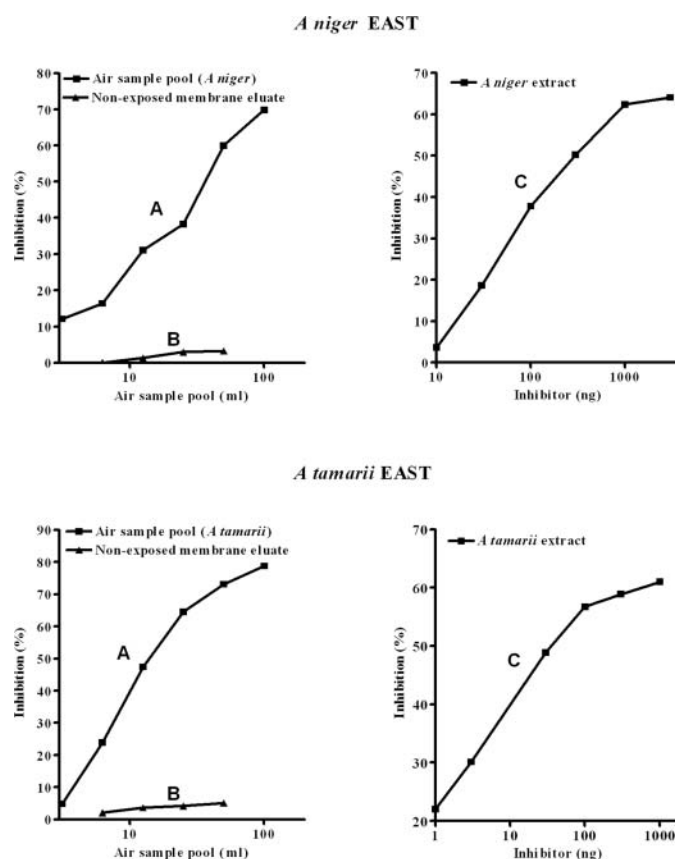


FIGURE 6.—Inhibition of *A. niger* and *A. tamarii* EAST with (A) specific pools of air sample eluates; (B) eluate of a nonexposed filter membrane; and (C) corresponding homologous *Aspergillus* extract as liquid-phase inhibitors. Slopes of the inhibition curves induced by air sample pools were not significantly different from the inhibition curves produced by corresponding homologous extracts (ANOVA, $p > .05$).

Despite being simple and convenient, morphologically quantified airborne colony counts have their own limitations. Culture techniques omit nonviable spores that may nevertheless be allergenic (25, 26). Studies have shown that airborne allergens are present not only in intact fungal spores but also in nonidentifiable fungal hyphae and other airborne particles (45). Intact fungal spores may also lose their allergenicity due to environmental factors such as heat, desiccation, etc. Thus, morphologically quantified airborne fungi may not adequately reflect the total airborne fungal allergen load and total allergen concentration in the air may provide more useful information than airborne particle counts alone (24–26).

The detection of such submicronic fungal aeroallergens requires sensitive immunoassays (24). In the present study, an enzyme immunoassay using allergen specific IgE antibodies (PPS from *Aspergillus*-hypersensitive patients) was developed to measure actual allergen concentration of the four species of *Aspergillus* in the air irrespective of their morphological features. In this study the same air samples were used for quantification of viable counts and total allergen content. There were marked seasonal variations in the immunochemically quantified airborne *A. flavus*, *A. fumigatus*, *A. niger*, and *A. tamarii* allergen content. Average daily

concentration of *A. niger* allergens was found to be highest in the air of Delhi metropolitan area, followed by *A. fumigatus*, *A. flavus*, and *A. tamarii* allergens. To the best of our knowledge, quantification of total airborne allergen content of these four *Aspergillus* species is being reported here for the first time.

The nonexposed filter membrane eluates failed to produce any inhibition in each *Aspergillus* EAST, establishing the specificity of the immunochemical assay for airborne *Aspergillus* allergens. Furthermore, the slopes of inhibition lines produced by pooled filter membrane eluates were not statistically different from the inhibition lines produced by specific homologous extract.

An important point of consideration was that the seasonal distribution of airborne colony counts of the four species didn't correlate with respective airborne *Aspergillus* allergen content. During some sampling months, allergen content was high with low viable counts and vice versa. *A. flavus* colony counts were highest in July (15.2 cfu/m³), whereas maximum allergen content was recorded in February (680.8 ng/m³). The peak of *A. fumigatus* allergen content was observed in August, whereas colony counts were highest in March. The lowest *A. niger* allergen content was recorded in August, whereas lowest colony counts were observed in December. The allergen concentration of *A. tamarii* was maximum in February and October, whereas colony counts in these months were significantly low.

The high allergen content in samples with low colony counts could be due to the presence of (a) fungal allergens in some nonviable fungal particles, and (b) spores and hyphae of heterologous species having cross-reacting *Aspergillus* allergens. The presence of cross-reactivity in various *Aspergillus* species has been well documented (7, 8, 13–16). On the other hand, high *Aspergillus* colony count with corresponding low allergen content could have been due to the denaturation of its allergenic components due to exposure to adverse weather conditions such as high temperature, desiccation, etc.

Overall, these results showed that *Aspergillus* allergens are present in the air of Delhi area throughout the year with seasonal variations. Using the immunochemical technique, it is possible to measure actual exposure of patients to various airborne *Aspergillus* allergens. Symptoms of allergic patients may be better correlated with the airborne total allergen content as compared to total spore/colony counts. Hence, we recommend that immunochemical quantification of various airborne *Aspergillus* allergens should be undertaken in lieu of conventional morphologic estimations.

ACKNOWLEDGMENTS

The authors thankfully acknowledge the financial support (Senior Research Fellowship) from Council of Scientific and Industrial Research, India, to one of the authors (M.V.). The authors also thank Mrs. Indu Bisht and Mr. Prahlad Rai for their help in collecting air samples.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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