

## Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations

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### Abstract

The information on airborne allergenic fungal flora in rural agricultural areas is largely lacking. Adequate information is not available to the bioaerosol researchers regarding the choice of single versus multiple sampling stations for the monitoring of both viable and non-viable airborne fungi. There is no long-term study estimating the ratios of viable and non-viable fungi in the air and earlier studies did not focus on the fractions of airborne allergenic fungi with respect to the total airborne fungal load. To fill these knowledge gaps, volumetric paired assessments of airborne viable and non-viable fungi were performed in five outdoor sampling stations during two consecutive years in a rural agricultural area of India. Samples were collected at 10-day intervals by the Burkard Personal Slide Sampler and the Andersen Two-Stage Viable Sampler. The data on the concentrations of total and individual fungal types from five stations and 2 different years were analyzed and compared by statistical methods. The allergenicity of the prevalent airborne viable fungi was estimated by the skin-prick tests of > 100 rural allergy patients using the antigenic fungal extracts from isolates collected with the Andersen sampler. The ranges of total fungal spore concentration were 82–2365 spores per cubic meter of air (spores/m<sup>3</sup>) in the first sampling year and 156–2022 spores/m<sup>3</sup> in the second sampling year. The concentration ranges of viable fungi were 72–1796 colony-forming units per cubic meter of air (CFU/m<sup>3</sup>) in the first sampling year and 155–1256 CFU/m<sup>3</sup> in the second sampling year. No statistically significant difference was observed between the total spore data of the 2 years, however, the data between five stations showed a significant difference ( $P < 0.0001$ ). No statistically significant difference existed between stations and years with respect to the concentration of viable fungi. When the data of individual allergenic fungal concentrations were compared between stations and years, no statistically significant difference was observed in all cases except for *Aspergillus japonicus* and *Rhizopus nigricans*, which showed significant difference in case of stations and years, respectively. The ratios between the total fungal spores collected by the Burkard sampler and the viable fungi collected by the Andersen sampler from all sampling stations ranged between 0.29 and 7.61. The antigenic extracts

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of eight prevalent viable airborne fungi (*A. flavus*, *A. japonicus*, *A. fumigatus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia pallescens*, *Fusarium roseum*, and *R. nigricans*) demonstrated >60% positive reactions in the skin prick test. These selected allergenic fungi collectively represented 31.7–63.2% of the total airborne viable fungi in different stations. The study concluded that: (i) a rich fungal airspora existed in the rural study area, (ii) to achieve representative information on the total airborne fungal spores of an area, the monitoring in multiple sampling stations is preferable over a single sampling station; for viable fungi, however, one station can be considered, (iii) the percentage of airborne fungal viability is higher in rural agricultural areas, and (iv) approximately 52% of the viable airborne fungi in the rural study area were allergenic.

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## 1. Introduction

The association between airborne fungi and symptoms of respiratory allergy and asthma is now well established (Malling, 1986; Strachan, 1988; Garrett et al., 1998). More than 80 genera of fungi have been reported to be associated with respiratory tract allergy (Latgé and Paris, 1991; Horner et al., 1995) and more than 100 species of fungi are involved with serious human and animal infections and many other species cause serious plant diseases (Cvetnić and Pepelnjak, 1997). Sensitization to fungal allergens is sometimes associated with life-threatening asthma (Black et al., 2000) and death (Targonski et al., 1995).

An important concern for the past studies on the airborne fungi around the world is, most of the reports dealt with the urban and sub-urban areas. Detailed long-term aerobiological studies in rural and agricultural areas are largely lacking. However, the rural agriculture based areas are reported to carry higher airborne load of fungal allergens and farmers are reported to get longer exposure of outdoor airborne fungi compared to the people of all other professions. Airborne fungi in the working environments of farmers have been widely recognized as an important agent for causing occupational lung diseases (Śpiwak et al., 2001). Palmas and Cosentino (1990) observed the largest fungal spore concentrations of most genera in rural areas and Gravesen (1972) noted a higher number of fungal colonies in rural homes as opposed to urban. Hamilton (1957) found that the types of major airborne fungal spores in a country site were similar like a nearby city site, but the spore concentration was higher at the countryside. Ham-

ilton and Eggleston (1997) indicated that a high mold count in rural homes might possible due to the decaying organic matter in the fields. Mitakakis et al. (2001) found that higher concentration of airborne *Alternaria* spore during maturation and harvest of wheat and cotton crops affected the airborne load of this spore in two nearby rural towns of southeast Australia. Abdel-Hafez et al. (1990) found some potentially pathogenic fungi in great numbers in combine harvester wheat and sorghum dusts as well as in the atmosphere of their hay sites. The present study was conducted in the southern part of the Gangetic West Bengal in India, which has a more humid climate and dense vegetation flora compared to the other parts of India. The information on the airborne allergenic fungi in this area would be useful to elucidate geographical patterns of asthma and hay fever among the rural people in India.

The concentration of airborne fungi can differ from place to place based on the local environmental variables, fungal growth substrates, and human activity. The daily mold alerts in different countries for the allergists and allergy patients, and most of the earlier reports on airborne fungi in India and other countries of the world were mostly based on the stationary sampling at one sampling station. However, there have been concerns about the degree to which these reports could be utilized as a whole for the surrounding area, because multiple local sources for fungal growth exist in outdoors. Few studies compared the concentrations of airborne fungal spores at two contrasting ecological sites (Long and Kramer, 1972), various sites within a 60 km radius (Brown and Jackson, 1978) and 10 km radius (Kramer and Eversmeyer,

1984), but all of these studies were performed for short periods in urban areas and none dealt with airborne viable fungi. It appears that so far there is no long-term multi-station study on the airborne allergenic fungi in rural areas based on both viable and non-viable sampling methods, which can set a recommendation regarding this important issue. In the present study, airborne fungi in five sampling stations were analyzed in a rural area of approximately 30 km<sup>2</sup> in diameter.

There are reports on the allergenic potentials of different airborne fungal species, however, to the best of our knowledge the fraction of the allergenic fungi in the air remained unfocussed by the earlier workers.

The ratio between the airborne viable and non-viable fungal spores is a controversial topic. Several researchers reported higher concentration of airborne non-viable spores compared to viable types in the indoor environments (Karlsson and Malmberg, 1989; Eduard et al., 1990). However, it appears that this type of data is not available from the outdoors based on the long-term side-by-side use of the viable and non-viable sampling techniques. The ratios of viable and non-viable fungal spores may provide information on the relationship between the dynamics of airborne fungi and associated environmental variables. Both the viable and non-viable fungal spores can act as potent aeroallergens, however, the pathogenicity of fungi depends largely on the spore viability. Thus, a change in the ratio of non-viable and viable fungi may be directly related with the adverse human health effects and may be relevant to the investigations on different plant and animal diseases caused by fungi. In the present study the Burkard sampler collected both airborne non-viable and viable fungi while the Andersen sampler provides data on the viable fungal types.

The objectives of the present study were: (i) to fill the knowledge gaps regarding the information on airborne fungi from rural agricultural areas of India, (ii) to investigate the differences between the data of airborne total fungal spores, viable fungal spores, and allergenic fungal spores from different stations and to check whether the multiple-station sampling is preferable over the single station sampling, or vice versa, for the reporting

of airborne mycoflora, (iii) to investigate the proportions of dominant airborne allergenic fungi in relation to the viable fungi of the air, which showed positive allergenic sensitivity in >60% allergy patients, and (iv) to investigate the ratios between the total airborne fungal spores collected by the Burkard sampler with sum total of the viable colony forming units collected by the Andersen sampler in all outdoor sampling stations.

## 2. Materials and methods

### 2.1. Description of the sampling sites

All five sampling stations were situated in a rural block of the Howrah district, in the state of West Bengal in India, named Domjur (latitude: 88°10', longitude: 22°40'). One central sampling station was selected at 'Makardah'. The approximate distances of north (Domjur), south (Salap), east (Jhalua), and west (Khati) sampling stations from the central sampling station were: 3.5 km, 3.0 km, 2.5 km, and 3.5 km, respectively. The characteristic features of the different sampling stations that might play some role on fungal growth, spore release, and dispersal are presented in Table 1. The seasons in the study area can broadly be divided into three: summer (March through June), rainy season (July through October), and winter (November through February) based on the distinct temperature, relative humidity, and rainfall levels. Major crop in the study area was rice, however, sporadic farming of wheat, potato, mustard, peas, beans, and other vegetables were there. In addition, several banana orchards were present close to the central sampling station.

### 2.2. Collection of samples and sample analysis

Samples were collected during 2 successive years from October, 1996 to September, 1998 in all five stations using the Burkard sampler (Burkard Manufacturing Co. Ltd., Hertfordshire WD3 1PJ, UK) for total airborne fungal spores and Andersen sampler (Andersen, 1958; manufactured by ThermoAndersen, Smyrna, 300082-5211, USA) for viable airborne fungal spores. Samples were collected three times a month, at 10-day intervals

Table 1

Characteristic features of five sampling stations

| Stations | Central                                                                                   | North                                                                                                                                                                                            | South                                                                                                    | East                                                                                                                        | West                                                                                                                                               |
|----------|-------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Features | Large rice fields, swamps and ponds, infrequent tree population, and a garden of flowers. | Vegetable market, garbage dumping place, small-scale industries (coconut fiber processing, rice husking, bakery, flourmill, and brick manufacturing), a drainage canal, and a congested traffic. | Sawmills, poultries, cattle sheds, shops of grocers, confectionery, tea stalls, and a congested traffic. | Dense vegetation of trees, small farmlands of vegetables, a small cow shed, small scale galvanizing and welding industries. | Surrounded by agricultural fields and grasslands, close to a national highway with heavy traffic, and sparse vegetation of trees on the roadsides. |

between 09:30 h and 11:00 h throughout the year. Thus, everyday five samples were collected and all together 360 samples were collected during the 2 sampling years. Except the west sampling station, in all other cases, both Burkard and Andersen samplers were placed on the rooftops in between 9 and 12 m heights. Due to the unavailability of any roof top with electric connection in the west sampling station, the samples were collected at approximately 6 m height from the level of the nearby agricultural field. Burkard sampler was operated for 9 min; however, Andersen sampler was operated for 3 min to avoid the overloading of fungal colonies. The duration of sampling and sampling time of the day were selected after the standardization based on a short pilot study.

The Burkard sampler collected air (10 l/min) through a rectangular orifice at the top and particles were impacted on a glycerin jelly coated microslide in the form of a band. The exposed slides were then mounted and examined at 400X and 1000X magnification of a Nikon high-resolution light microscope (Labophot 2, Nikon Corp., Japan) to identify and count fungal spore types up to genus level based on the morphological features. The counts were then converted into the number of spores per cubic meter of air (spores/m<sup>3</sup>) and finally the average monthly concentration was calculated from three observations of each month.

Each stage of the Andersen sampler has 200 holes, 1.5 mm diameter in the first stage and 0.4 mm in the second stage. The  $d_{50}$  (aerodynamic

diameter, above which the sampler can approach 100% collection efficiency) for the first and second stages of the Andersen sampler are 8.0  $\mu\text{m}$  and 0.95  $\mu\text{m}$ , respectively. The Andersen sampler drew air (28.3 l/min) through the orifice at the top and impinged airborne fungal spores successively onto two Petri plates containing 27 ml of solid Sabourauds Rose Bengal Agar media. After exposure, the Petri plates were incubated in a B.O.D. incubator at  $27 \pm 2$  °C temperature for 2–7 days for proper growth of fungal colonies to render identification. The colonies were identified up to species level based on the colony characters and the morphological characteristics of spores and spore bearing structures. Few colonies did not generate spores even after incubation of up to 7 days and classified into ‘non-sporulating colonies’ group. The number of colonies recorded from both Petri plates were then combined and converted into the number of colony forming units per cubic meter of air (CFU/m<sup>3</sup>). Average monthly concentration was calculated from the three observations.

### 2.3. Skin prick test of the allergy patients using the antigenic extracts of the dominant airborne viable fungal types

Mass cultures of the selected fungi were prepared in sterile Sabouraud’s liquid broth medium in conical flasks. Flasks were incubated for approximately 3–4 weeks at  $27 \pm 2$  °C temperature in a B.O.D. incubator until proper sporulation occurred.

The medium was then decanted and the sporulating masses were rinsed with sterile distilled water. The resulting pellets were kept overnight in absolute ethanol for killing the fungi, then dried, powdered in a grinder, and defatted by using diethyl ether. Antigenic extracts were prepared from the defatted powder in 1:10 w/v 0.05 M ammonium bicarbonate buffer (pH 8.1). The extraction was performed by continuous stirring for 24 h using a magnetic stirrer at 4 °C. The resulting suspension was centrifuged at 21000 rev./min for 30 min at 4 °C. The supernatant was dialyzed (molecular cut off: 10 000) against PBS for overnight, centrifuged and filtered through a membrane filter (Millipore Corp., Bedford, MA, USA) of 0.22 µm porosity. The final suspension was stored at –20 °C until used for the skin prick tests.

For the skin prick test, adult subjects showing the symptoms of type-I respiratory allergy were selected mainly from the rural study area and other adjacent districts. 10–20 µl drops of the antigenic extracts (containing 100 µg protein/ml) were placed on the ventral side of the subjects' forearm and each site was pricked using a sterile hypodermic needle (#26). The intensity of reactions with respect to the wheal or erythema responses were measured after 20 min and classified into following categories (Terr, 1994): negative reaction (no wheal or erythema), 1+ (no wheal, erythema diameter <20 mm), 2+ (no wheal, erythema diameter >20 mm), 3+ (both wheal and erythema), and 4+ (wheal with pseudopods, erythema). Histamine di phosphate (1 mg/ml) and buffered saline dilutant were used as positive and negative controls, respectively. Ten normal subjects, who had not symptoms of type-I respiratory allergy, were also tested as control using the selected fungal antigenic extracts.

#### 2.4. Recording of meteorological parameters

Temperature (°C) and relative humidity (%) were recorded in each sampling station during sample collection using a Wet and Dry Bulb Hygrometer (G.H. Zeal Ltd., London, UK). Since there was no statistically significant difference in temperature and relative humidity between stations, the data from all stations were combined

and overall monthly average was calculated for each sampling year. The rainfall data were obtained from the nearest meteorological office at Alipore.

#### 2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to study the differences between the data of five sampling stations and the data between 2 sampling years. *P* values of <0.05 were considered as significant.

### 3. Results

Considering all sampling stations, the range of total airborne spore concentration was 82–2365 spores/m<sup>3</sup> in the first sampling year and 156–2022 spores/m<sup>3</sup> in the second sampling year. The range of viable fungal concentration during the same time period was 72–1796 CFU/m<sup>3</sup> in the first year and 155–1256 CFU/m<sup>3</sup> in the second year. All together 40 fungal spore types were observed in the samples collected by the Burkard sampler and the Andersen sampler collected 42 species of viable fungi from all sampling stations (Table 2). The members of mitosporic fungi dominated in both total fungal spore and viable fungi concentrations compared to other fungal groups.

#### 3.1. Total concentration of fungal spores in five sampling stations (measured by the Burkard sampler)

The total fungal spore concentrations in five sampling stations during the first and second sampling year are presented in Fig. 1a,b, respectively. Noticeably higher concentration was observed at the central sampling station compared to the other stations. The prevalent spore types (overall contribution in all stations during 2 sampling years: > 1.5%) and their corresponding overall contribution percentages in all sampling stations during the 2 sampling years are presented in Table 3. As shown in the table, the maximum spore concentration was contributed by *Cladosporium* followed by *Nigrospora*, basidiospores and the members of *Aspergillus*/*Penicillium* spores. Spores of *Aspergillus* and

Airborne fungal populations collected by Burkard sampler and Andersen sampler

[illegible]

Table 2 (Continued)

| Deuteromycotina<br>(Mitosporic fungi) |   |   | Ascomycotina   |   |   | Basidiomycotina |   |   | Zygomycotina   |   |   |
|---------------------------------------|---|---|----------------|---|---|-----------------|---|---|----------------|---|---|
| Genera/Species                        | B | A | Genera/Species | B | A | Genera          | B | A | Genera/Species | B | A |
| <i>Pithomyces</i>                     | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Rhizoctonia solani</i>             |   | × |                |   |   |                 |   |   |                |   |   |
| <i>Rosellinia</i>                     | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Spegazzinia</i>                    | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Sporidesmium</i>                   | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Tetracoccusporium</i>              | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Tetraploa</i>                      | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Thermomyces</i>                    | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Torula</i>                         | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Trichochochis</i>                  | × |   |                |   |   |                 |   |   |                |   |   |
| <i>Trichoderma harzianum</i>          |   | × |                |   |   |                 |   |   |                |   |   |
| <i>Trichoderma viride</i>             |   | × |                |   |   |                 |   |   |                |   |   |
| <i>Trichothecium roseum</i>           |   | × |                |   |   |                 |   |   |                |   |   |

B, Fungal spores collected by Burkard sampler; A, Viable fungi collected by Andersen sampler.

*Penicillium* were grouped together because they were morphologically indistinguishable by light microscopy. The concentration of *Aspergillus*/*Penicillium* spores was lower in 'Central' and 'North' sampling stations compared to other stations. A statistically significant difference was observed ( $P < 0.0001$ ) between the data of stations, however, no statistically significant difference ( $P = 0.79$ ) was observed between the data of 2 years, when the spore concentrations of different stations during the 2 sampling years were compared by two-way ANOVA.

### 3.2. Viable fungi concentration in five sampling stations (measured by the Andersen sampler)

The total concentrations of viable fungi for the first and second sampling years are presented in Fig. 2a,b, respectively. No statistically significant difference was found between the data from different stations ( $P = 0.764$ ) and also between the data of 2 years ( $P = 0.071$ ), when the viable fungi concentrations of the different stations as well as two different years were compared by two-way ANOVA.

### 3.3. Ratios between total airborne spores and sum total of viable fungi in five sampling stations

The ratios between the concentrations of total fungal spores (spores/m<sup>3</sup>) and viable fungal col-

ony forming units (CFU/m<sup>3</sup>) in five sampling stations during the 2 sampling years are presented in Table 4. Relatively higher values of the ratios were observed in 'Central' station and lower values were found in the 'North' sampling station compared to the other three stations.

### 3.4. Results of skin prick test of allergy patients to test the allergenic potentials of eight dominant airborne fungi

Antigenic extracts of eight fungi demonstrated positive reactions in >60% allergy patients selected from the rural study area and adjacent districts. These selected fungi collectively represented 52% of the total airborne viable fungi recorded during both sampling years. Results of skin prick test in >100 allergy patients using antigenic extracts of these fungi are presented in Table 5. *A. fumigatus* demonstrated positive reactions in maximum number of patients (95.1%) and *C. cladosporioides* demonstrated positive reactions in least number (63.3%) of patients. Extracts of *A. fumigatus* and *R. nigricans* showed highest intensities of allergic reactions (i.e. 4+ reaction) in one patient and two patients, respectively.

### 3.5. Detailed aerobiological analysis of the selected eight airborne allergenic fungi

The overall percentage contributions of the viable fungi, which were prevalent (overall contribu-

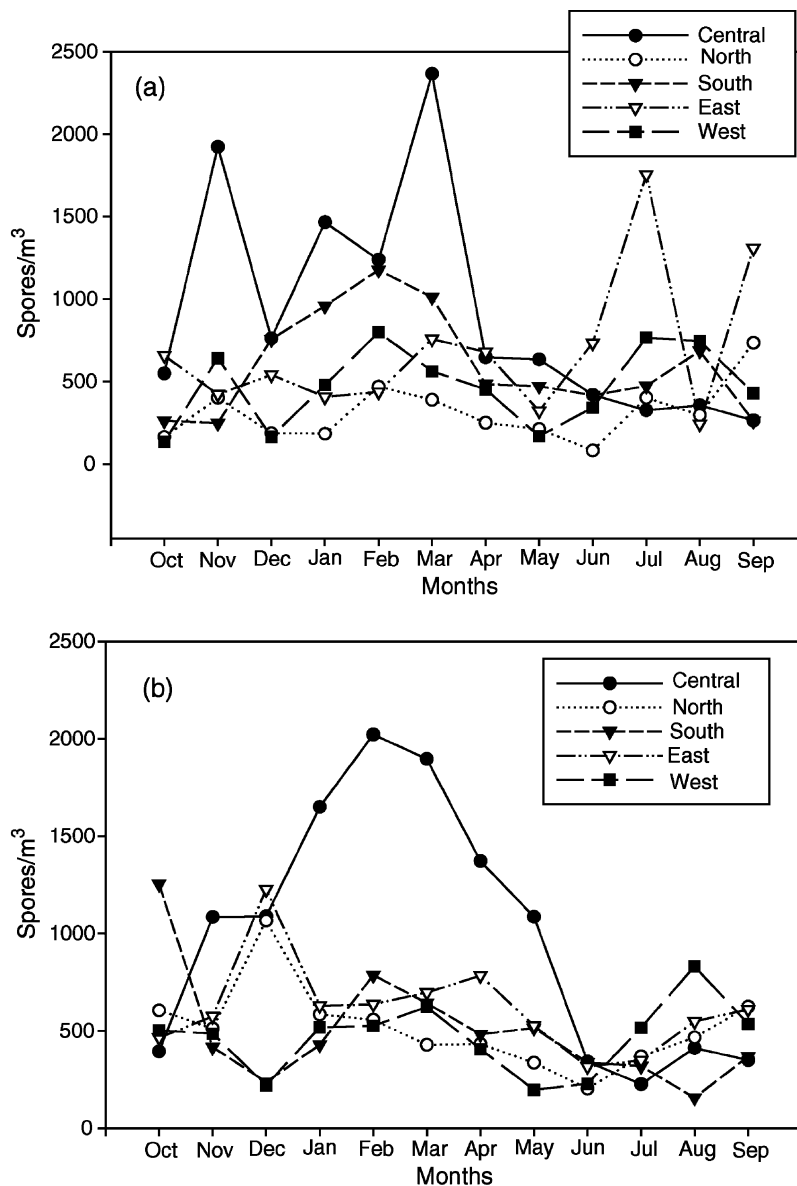


Fig. 1. (a) Total concentrations of airborne fungal spores in five sampling stations during the year 1996–1997, as collected by the Burkard sampler. (b) Total concentrations of airborne fungal spores in five sampling stations during the year 1997–1998, as collected by the Burkard sampler.

tion in all stations during 2 sampling years: > 1.5%) in the air and were also significant with respect to allergenicity (showed positive responses in >60% allergy patients in skin prick tests), are presented in Table 6. As presented in the table, the

maximum contribution was made by the CFUs of *C. cladosporioides* followed by *A. flavus*, *A. japonicus*, *A. fumigatus*, *A. alternata*, *C. pallescens*, *F. roseum*, and *R. nigricans*. The monthly variation patterns of the airborne concentrations of the

Table 3

Overall percentage contributions of prevalent airborne fungal spore types to the total airborne spore load in five sampling stations during 2 sampling years

| Fungal spore types                           | Overall contribution (%) in different stations during two sampling years |        |        |        |        |        |        |        |        |        | Mean by fungal type |
|----------------------------------------------|--------------------------------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------------------|
|                                              | Central                                                                  |        | North  |        | South  |        | East   |        | West   |        |                     |
|                                              | Year-1                                                                   | Year-2 | Year-1 | Year-2 | Year-1 | Year-2 | Year-1 | Year-2 | Year-1 | Year-2 |                     |
| 1. <i>Alternaria</i>                         | 2.99                                                                     | 9.49   | 2.09   | 0.48   | 1.91   | 1.85   | 2.48   | 0.65   | 2.07   | 2.39   | 2.64                |
| 2. Ascospores                                | 3.61                                                                     | 3.17   | 2.81   | 3.75   | 1.13   | 3.16   | 3.97   | 5.83   | 5.54   | 5.49   | 3.84                |
| 3. <i>Aspergillus/</i><br><i>Penicillium</i> | 0.03                                                                     | 0.31   | 2.49   | 0.48   | 12.71  | 15.55  | 14.46  | 17.84  | 16.44  | 17.60  | 9.79                |
| 4. Basidiospores                             | 13.04                                                                    | 6.75   | 4.51   | 5.67   | 2.97   | 5.16   | 9.20   | 13.76  | 7.35   | 9.68   | 7.80                |
| 5. <i>Cladosporium</i>                       | 39.34                                                                    | 41.68  | 18.12  | 11.32  | 36.51  | 15.89  | 32.17  | 20.27  | 22.48  | 10.18  | 24.79               |
| 6. <i>Curvularia</i>                         | 2.17                                                                     | 2.23   | 1.85   | 3.13   | 0.52   | 1.68   | 2.99   | 1.38   | 1.49   | 2.16   | 1.96                |
| 7. <i>Drechslera</i>                         | 4.53                                                                     | 2.37   | 9.12   | 3.25   | 1.37   | 3.50   | 9.27   | 3.62   | 3.32   | 3.36   | 4.37                |
| 8. <i>Epicoccum</i>                          | 4.86                                                                     | 4.63   | 6.26   | 3.94   | 12.70  | 12.47  | 0.43   | 0.67   | 7.29   | 1.34   | 5.45                |
| 9. <i>Ganoderma</i>                          | 1.55                                                                     | 2.13   | 6.06   | 3.59   | 0.37   | 0.60   | 3.46   | 4.24   | 1.81   | 1.82   | 2.56                |
| 10. <i>Nigrospora</i>                        | 7.60                                                                     | 8.35   | 18.57  | 21.66  | 7.83   | 13.65  | 5.29   | 11.72  | 9.37   | 14.11  | 11.81               |
| 11. <i>Periconia</i>                         | 8.55                                                                     | 6.86   | 8.19   | 3.77   | 9.28   | 11.95  | 6.44   | 6.94   | 2.55   | 3.54   | 6.80                |
| 12. Smut spores                              | 0.78                                                                     | 2.45   | 2.70   | 3.94   | 1.31   | 3.14   | 1.21   | 2.56   | 10.94  | 13.72  | 4.27                |

selected fungi in five sampling stations during the 2 sampling years are presented in Fig. 3a (*A. alternata*, *A. flavus*, *A. fumigatus*, and *A. japonicus*) and 3b (*C. cladosporioides*, *C. pallescens*, *F. roseum*, and *R. nigricans*). As shown in the figures, some noticeable differences were observed in the monthly variation patterns between different stations as well as between two years in case of all fungal types. However, when the individual fungal data from different sampling stations were compared by two-way ANOVA, a statistically significant difference was observed only for *A. japonicus* ( $P < 0.05$ ). A statistically significant difference was detected only for *R. nigricans* ( $P < 0.05$ ) when the airborne concentrations for the individual fungal types in the 2 years were compared by two-way ANOVA.

### 3.6. Fractions of prevalent airborne allergenic fungi with respect to the total airborne viable fungi in different sampling stations

It was observed that, 31.71–63.26% of the viable airborne fungi in different stations were represented by the selected eight allergenic fungi (Table 6). Maximum overall percentage contribution was found at the ‘East’ sampling station

(63.26% in the first year and 61.46% in the second year).

### 3.7. Meteorological data

Monthly averages of the overall temperature and relative humidity in all five sampling stations and the monthly rainfall data in the area during the 2 sampling years are presented in Table 7. As presented in the table, the temperature levels was higher in the months between April and July and relative humidity levels was higher in the months between July and October compared to the rest other months of both the sampling years. Maximum rainfall was observed in the months from July to September.

## 4. Discussion

The volumetric assessments of total airborne viable and non-viable fungi, and fungi of allergenic significance based on this 2-year study will be useful while addressing the respiratory allergy, different health hazards or diseases, and occupational health problems caused by airborne fungi among the rural people in India as well as other countries of similar climate. It appears that there are not adequate reports on the quantitative anal-

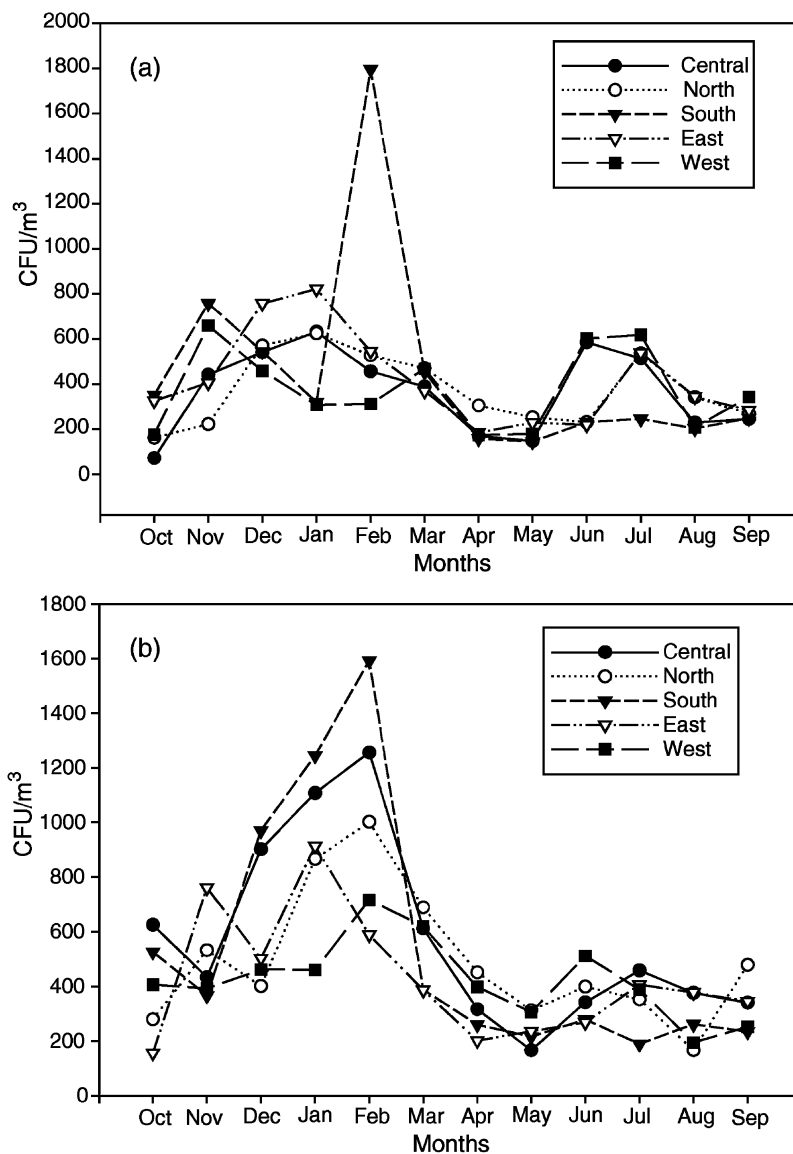


Fig. 2. (a) Total concentrations of airborne viable fungi in five sampling stations during the year 1996–1997, as collected by the Andersen sampler. (b) Total concentrations of airborne viable fungi in five sampling stations during the year 1997–1998, as collected by the Andersen sampler.

ysis of airborne viable fungi from the outdoors in India, particularly from the rural areas. Except the study of Gupta et al. (1993) from city of Delhi, to the best of our knowledge, no study in India reported the viable airborne allergenic fungi from outdoors using volumetric sampling methods. The

present study is anticipated to fill this knowledge gap. Several studies in India, however, investigated total fungal spores (analyzed by light microscopy) in outdoor environments by using some volumetric samplers, like the Rotorod Sampler (Nayak et al., 1998; Chakraborty et al., 2000; Khandelwal, 2001;

Table 4

The ratios between the concentrations of total fungi collected by the Burkard Sampler and the total viable fungal spores collected by the Andersen Sampler in five sampling stations collectively during the two sampling years

| Months             | Ratios between the total spore concentrations and total viable spore concentrations in different sampling stations |                 |                 |                 |                 |
|--------------------|--------------------------------------------------------------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                    | Central                                                                                                            | North           | South           | East            | West            |
| October            | 7.61                                                                                                               | 1.00            | 0.74            | 2.01            | 0.76            |
| November           | 4.35                                                                                                               | 1.81            | 0.32            | 1.03            | 0.97            |
| December           | 1.40                                                                                                               | 0.32            | 1.38            | 0.71            | 0.36            |
| January            | 2.31                                                                                                               | 0.29            | 3.00            | 0.49            | 1.55            |
| February           | 2.71                                                                                                               | 0.88            | 0.65            | 0.80            | 2.55            |
| March              | 6.07                                                                                                               | 0.82            | 2.22            | 2.05            | 1.20            |
| April              | 3.81                                                                                                               | 0.81            | 3.06            | 3.66            | 2.58            |
| May                | 4.28                                                                                                               | 0.84            | 3.25            | 1.41            | 0.93            |
| June               | 0.71                                                                                                               | 0.35            | 1.80            | 3.35            | 0.56            |
| July               | 0.63                                                                                                               | 0.75            | 1.93            | 3.25            | 1.23            |
| August             | 1.55                                                                                                               | 0.86            | 3.37            | 0.70            | 3.58            |
| September          | 1.07                                                                                                               | 2.74            | 1.05            | 4.60            | 1.25            |
| Average $\pm$ S.D. | 3.04 $\pm$ 2.22                                                                                                    | 0.96 $\pm$ 0.68 | 1.90 $\pm$ 1.08 | 2.00 $\pm$ 1.39 | 1.46 $\pm$ 0.94 |

Kakde et al., 2001) and the Burkard sampler (Vittal and Krishnamoorthi, 1988; Gupta et al., 1993; Chakraborty et al., 2001). However, Chakraborty et al. (2001) studied the airborne fungal spores in an agricultural farm and all other studies were restricted only to urban areas. While com-

paring the concentrations of total airborne fungal spores with the previous studies performed in India, it was found that the present study recorded higher spore concentration (82–2365 spores/m<sup>3</sup> in the first sampling year and 156–2022 spores/m<sup>3</sup> in the second sampling year) compared to the

Table 5

Allergenic potentials of dominant airborne viable fungi demonstrated in the skin prick tests of allergy patients

| Fungal types              | Allergy patients (P) and normal subjects (N) tested |     | Negative reactions (%) | Grades of positive reactions and % of occurrence |       |      |      |
|---------------------------|-----------------------------------------------------|-----|------------------------|--------------------------------------------------|-------|------|------|
|                           |                                                     |     |                        | 1+                                               | 2+    | 3+   | 4+   |
| <i>A. flavus</i>          | P                                                   | 128 | 27.30                  | 64.00                                            | 8.59  | –    | –    |
|                           | N                                                   | 10  | 100.00                 | –                                                | –     | –    | –    |
| <i>A. japonicus</i>       | P                                                   | 128 | 19.53                  | 68.75                                            | 10.93 | 0.78 | –    |
|                           | N                                                   | 10  | 90.00                  | 10.00                                            | –     | –    | –    |
| <i>A. alternata</i>       | P                                                   | 100 | 33.00                  | 48.00                                            | 19.00 | –    | –    |
|                           | N                                                   | 10  | 100.00                 | –                                                | –     | –    | –    |
| <i>A. fumigatus</i>       | P                                                   | 102 | 4.90                   | 67.67                                            | 19.60 | 6.86 | 0.98 |
|                           | N                                                   | 10  | 90.00                  | 10.00                                            | –     | –    | –    |
| <i>C. cladosporioides</i> | P                                                   | 117 | 36.70                  | 51.20                                            | 10.20 | 1.70 | –    |
|                           | N                                                   | 10  | 100.00                 | –                                                | –     | –    | –    |
| <i>C. pallescens</i>      | P                                                   | 100 | 34.00                  | 45.00                                            | 20.00 | 1.00 | –    |
|                           | N                                                   | 10  | 100.00                 | –                                                | –     | –    | –    |
| <i>F. roseum</i>          | P                                                   | 102 | 8.82                   | 64.70                                            | 19.64 | 4.92 | –    |
|                           | N                                                   | 10  | 100.00                 | –                                                | –     | –    | –    |
| <i>R. nigricans</i>       | P                                                   | 102 | 8.82                   | 60.78                                            | 24.50 | 3.92 | 1.96 |
|                           | N                                                   | 10  | 90.00                  | 10.00                                            | –     | –    | –    |

Table 6

Overall percentage contributions of dominant airborne viable fungal types to the total airborne viable spore load in five sampling station during two sampling years

| Fungal CFU types             | Overall contribution (%) in different stations during two sampling years |        |        |        |        |        |        |        |        |        | Mean by fungal type |
|------------------------------|--------------------------------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------------------|
|                              | Central                                                                  |        | North  |        | South  |        | East   |        | West   |        |                     |
|                              | Year-1                                                                   | Year-2 | Year-1 | Year-2 | Year-1 | Year-2 | Year-1 | Year-2 | Year-1 | Year-2 |                     |
| 1. <i>A. flavus</i>          | 7.16                                                                     | 7.84   | 11.83  | 9.64   | 7.70   | 6.43   | 6.31   | 7.72   | 8.85   | 11.72  | 8.52                |
| 2. <i>A. japonicus</i>       | 5.01                                                                     | 3.20   | 10.01  | 7.17   | 2.40   | 2.00   | 3.87   | 3.50   | 11.98  | 10.50  | 5.96                |
| 3. <i>A. fumigatus</i>       | 6.98                                                                     | 5.61   | 5.76   | 5.61   | 3.08   | 2.57   | 9.90   | 11.46  | 4.88   | 3.60   | 5.94                |
| 4. <i>A. alternata</i>       | 8.09                                                                     | 4.83   | 5.47   | 6.91   | 2.03   | 1.70   | 7.65   | 13.89  | 2.77   | 4.29   | 5.76                |
| 5. <i>C. cladosporioides</i> | 23.10                                                                    | 6.20   | 20.25  | 9.90   | 34.99  | 29.25  | 29.14  | 19.77  | 15.26  | 12.21  | 20.00               |
| 6. <i>C. pallescens</i>      | 1.31                                                                     | 1.51   | 3.25   | 3.74   | 2.32   | 1.94   | 2.18   | 2.66   | 1.99   | 1.74   | 2.26                |
| 7. <i>F. roseum</i>          | 1.78                                                                     | 0.85   | 1.50   | 2.53   | 2.02   | 1.73   | 2.52   | 1.55   | 3.17   | 4.25   | 2.19                |
| 8. <i>R. nigricans</i>       | 1.31                                                                     | 1.67   | 1.44   | 2.48   | 0.23   | 0.19   | 1.69   | 0.91   | 2.36   | 2.84   | 1.51                |

previous studies conducted in urban and industrial areas. Spore concentration recorded by Khandelwal (2001) in Lucknow city during different months of the year ranged between 70 and 267 spores/m<sup>3</sup> and Nayak et al. (1998) observed a total spore concentration range of approximately 300–900 spores/m<sup>3</sup> in an industrial area of India. Gupta et al. (1993) reported a lower total spore concentration (monthly spore concentrations <100 spores/m<sup>3</sup>) from the city of Delhi. Chakraborty et al. (2000) recorded a higher spore concentration (3728–6140 spores/m<sup>3</sup>) in a semi-rural town of India; however, the samples were collected at 1 m heights from the ground level. The airborne fungal concentration from other parts of the world revealed a mixed scenario. Few studies showed lower concentration of total airborne fungal spores from the city environments. For example, Herrero et al. (1996) found a total spore concentration of <200 spores/m<sup>3</sup> from Palencia, Spain; Waisel et al. (1997) reported <400 spores/m<sup>3</sup> from the three cities of Israel; and Mitakakis and Guest (2001) reported average daily total spore concentration of 1207 spores/m<sup>3</sup> from Melbourne, Australia. However, Henríquez et al. (2001) reported monthly average concentration range of 1500–4500 spores/m<sup>3</sup> from Santiago, Chile and Emberlin et al. (1995) found <1000 spores/m<sup>3</sup> until March, but increased levels of up to several thousands spores per cubic meter of air during the months of April through June in London city.

While comparing the viable fungi concentration of the present study with a previous study performed at the urban environment of the city of Delhi, India (Gupta et al., 1993), a much higher concentration of viable fungi concentration was recorded in the present study (72–1796 CFU/m<sup>3</sup> in the first sampling year and 155–1256 CFU/m<sup>3</sup> in the second sampling year) compared to the previous study (monthly concentrations of viable fungi <50 CFU/m<sup>3</sup>). While comparing the viable fungi concentration of the present investigations with the other studies from the urban environments of the world, a mixed scenario was observed similar to the reports of total fungal spores. Few studies reported lower viable fungi concentrations from the urban environments. For example, Di Giorgio et al. (1996) reported a low concentration of viable fungi (63 CFU/m<sup>3</sup>) from the city of Marseilles, Al-Suwaine et al. (1999) reported a concentration of <800 CFU/m<sup>3</sup> from Riyadh city, Saudi Arabia, and Rosas et al. (1997) reported a mean concentration of 143 CFU/m<sup>3</sup> from Mexico City. However, few studies from the urban city environments reported higher viable fungi concentrations too. For example, Takahasi (1997) reported a concentration of 13–2750 CFU/m<sup>3</sup> from the city of Yokohama, Japan. These contrasting observations on the total and viable fungal spore concentrations can be attributed to different climatic conditions, vegetation, and different fungal growth substrates in different countries of the world as

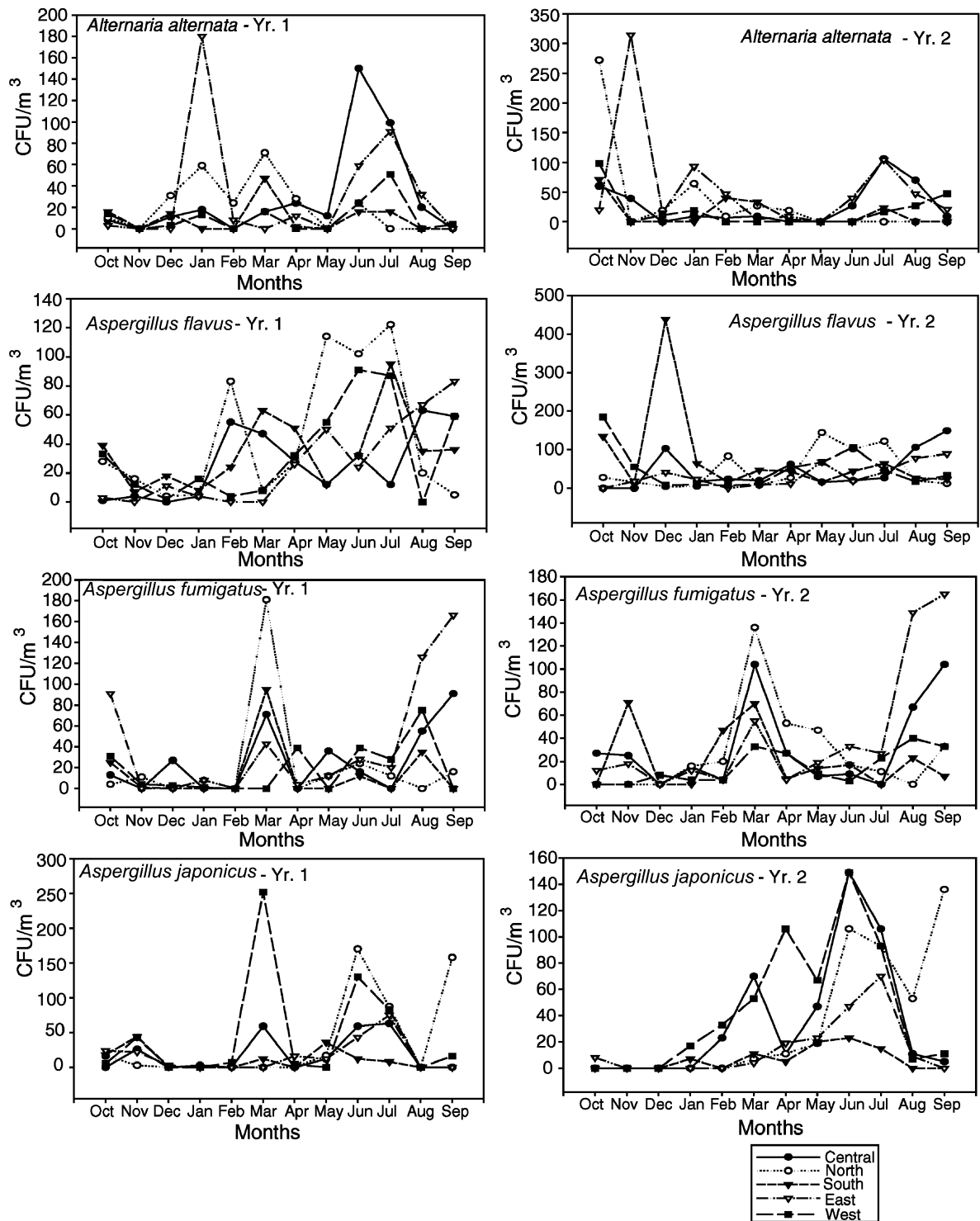


Fig. 3. (a) Monthly variation patterns of the airborne concentrations of selected allergenic fungi (*A. alternata*, *A. flavus*, *A. fumigatus*, and *A. japonicus*) during 2 sampling years.

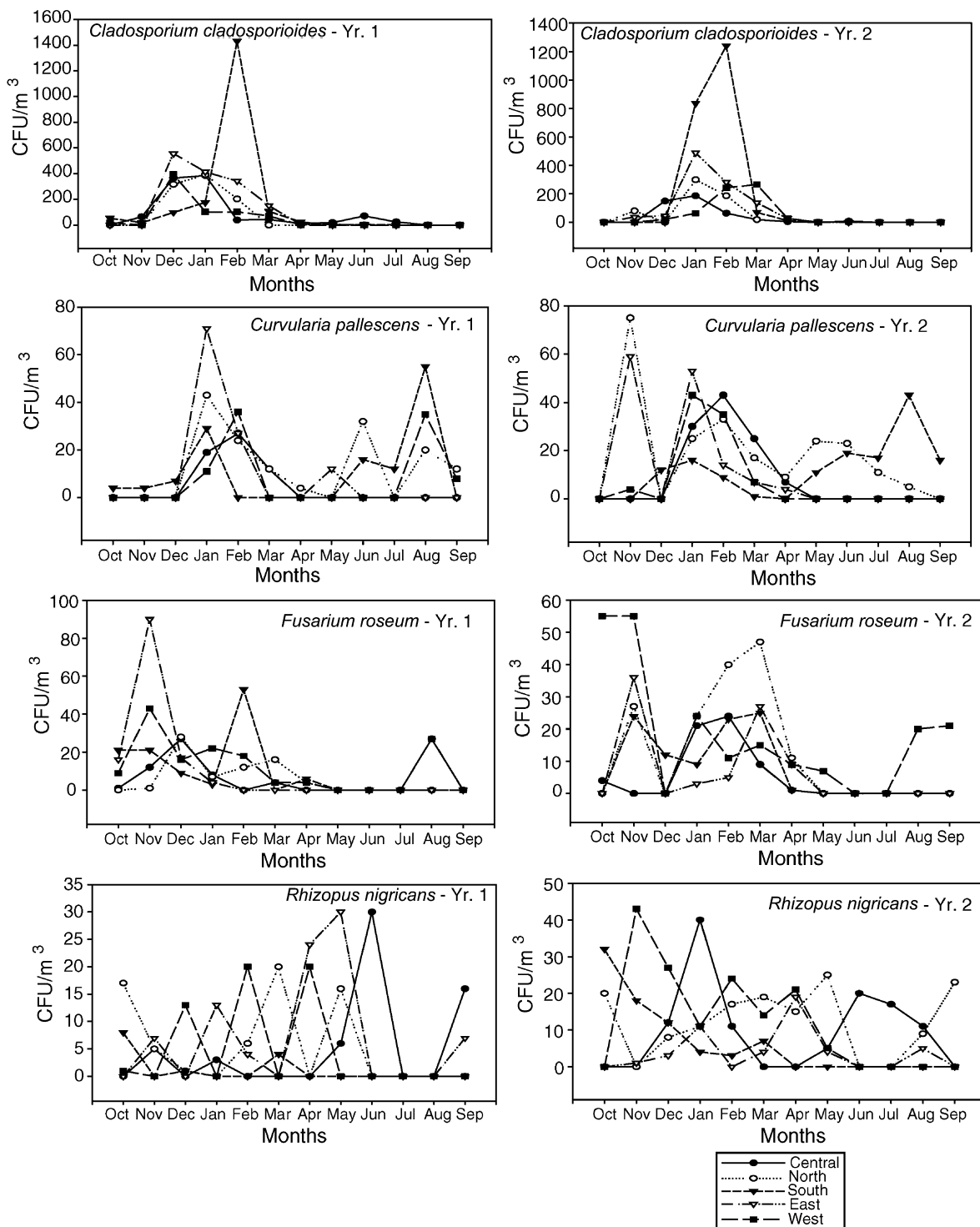


Fig. 3. (b) Monthly variation patterns of the airborne concentrations of selected allergenic fungi (*C. cladosporioides*, *C. pallescens*, *F. roseum* and *R. nigricans*) during 2 sampling years.

Table 7

Overall monthly average temperature, relative humidity, and rainfall in the study area during 2 sampling years

| Months           |        | October | November | December | January | February | March | April | May  | June | July | August | September |
|------------------|--------|---------|----------|----------|---------|----------|-------|-------|------|------|------|--------|-----------|
| Temperature (°C) | Year.1 | 31.8    | 30.4     | 28.3     | 27.3    | 27.5     | 31.1  | 32.9  | 35.1 | 34.2 | 34.3 | 32.7   | 31.6      |
|                  | Year.2 | 32.6    | 31.6     | 27.0     | 24.8    | 26.3     | 30.1  | 33.8  | 34.4 | 35.2 | 34.2 | 32.5   | 30.8      |
| R.H. (%)         | Year.1 | 75.6    | 67.9     | 63.2     | 49.0    | 54.8     | 60.1  | 62.3  | 64.6 | 67.4 | 84.2 | 88.7   | 90.2      |
|                  | Year.2 | 62.1    | 69.0     | 59.4     | 53.2    | 48.9     | 64.8  | 61.4  | 59.5 | 72.6 | 84.8 | 78.1   | 84.6      |
| Rainfall (mm)    | Year.1 | 4.82    | 0        | 0        | 2.3     | 0.72     | 2.62  | 3.84  | 4.42 | 3.18 | 20.3 | 18.7   | 24.5      |
|                  | Year.2 | 2.32    | 0        | 0.6      | 1.8     | 2.5      | 4.2   | 4.8   | 6.1  | 5.4  | 24.8 | 26.3   | 31.2      |

well as the different sampling methods used by the researchers.

While comparing the total spore data between stations and years, statistically significant difference was observed between stations, however, no statistically significant difference was found between the data of 2 years. Few earlier studies supported this observation. Lacey (1962) studied the air spora of an exposed site with that of a valley near a stream 600 m away, and found 2.5 times higher spore counts from the air near the stream. Palmas and Cosentino (1990) compared the total airborne fungal spores between a rural and urban site 50 km away from each other and found higher fungal spore concentration in the rural area. Gupta et al. (1993) found a statistically significant ( $P < 0.05$ ) quantitative variation in total spore counts at five different sites in Delhi metropolis, India during a 2-year aerobiological survey. Several earlier reports based on short-term air sampling experiments, however, demonstrated some contrasting observations. For example, Kramer and Eversmeyer (1984) found no significant difference in mean spore concentrations between sites during a 30-day period and 100-day period of air sampling experiments performed at eight sampling locations within a 10-km radius. However, during the 30-day sampling period, they found significant difference in 29% of the comparative data. Brown and Jackson (1978) found that, total numbers for most spore types were similar in eight sampling stations within a 60 km radius during a 6-month air sampling study. However, these reports were based on urban areas, where the chance of variability in local fungal growth substrates is lower compared to rural areas. Thus, these contrasting observations can be attributed to the variations of local fungal spore sources and the environmental factors responsible for spore release and dispersal, which might be different in rural and urban environments as well as in different country's climatic conditions.

However, when the concentration of viable fungi from the five sampling stations were considered, the present study did not demonstrate any statistically significant difference. When the concentration of individual viable allergenic fungi were also considered, no significant difference was there in

most cases. As a possible explanation of these observations, the aerodynamic sizes of the fungal spores can be considered. Members of *Aspergillus*, *Penicillium* and *Cladosporium* were the predominant types among the viable fungi collected by the Andersen sampler. The aerodynamic diameters of the spores of these fungi range between 2 and 5  $\mu\text{m}$ . Due to this small aerodynamic size, the spores might be uniformly distributed in the whole study area. More variability was observed in the concentrations of the large smuts, basidiospores, and ascospores, which contributed a large proportion to the total airborne spores collected by the Burkard sampler.

The proportion of common viable allergenic fungi in all rural sampling stations, in most cases, exceeded 50% of the viable airborne fungi. This observation can be a matter of concern in the study area for the rural people suffering from respiratory allergy. *C. cladosporioides* contributed maximum concentrations followed by *A. flavus*, *A. japonicus*, *A. fumigatus*, and *A. alternata*. The monthly variation patterns of these fungi should be published to the rural public for taking advance precaution measures.

For total airborne fungal spores no clear seasonal periodicity was observed, however, lower concentration levels were observed in the months of extreme summer. For viable airborne fungi, on the other hand, higher concentration levels were observed in the months of winter followed by the late rainy season months.

While analyzing the samples collected from the air of Swedish farms and cow barns, Karlsson and Malmberg (1989) found that the average count of the viable colony forming units of molds and actinomycetes was one-sixth of the total count obtained with scanning electron microscopy and fluorescence microscopy. In several occupational indoor environments (e.g. woodchip handling, straw handling, grain elevator, pig house, and saw mill), Eduard et al. (1990) found that 3–98% fungal spores were viable while comparing the airborne viable fungi analyzed by culture and airborne total fungal spores analyzed by light microscopy, scanning electron microscopy, and epifluorescence microscopy. In the present study, while comparing the concentration of total fungal

spores collected by the Burkard sampler with the total concentration of viable fungal colony forming units collected by the Andersen sampler, we achieved some contrasting results. As presented in Table 5, the concentration of total airborne viable fungi collected by the Andersen sampler was sometimes greater than the concentration of total airborne fungal spores collected by the Burkard sampler. This contrasting result will be little surprising to the researchers dealing with airborne fungi because spores of several airborne fungal genera (mainly from the Basidiomycetes and Ascomycetes) are visible in light microscope but unable to grow in the laboratory culture media. For that reason, decrease in the concentration of viable fungi compared to total fungal spores analyzed by microscopic methods is generally anticipated. Our contrasting results compared to the studies of Karlsson and Malmberg (1989) and Eduard et al. (1990) can be attributed to four points. First, both of the previous studies were performed using filtration, which may largely be responsible for the viability loss of airborne fungal spores by desiccation stress (Lacey and Venette, 1995; Wathes, 1995). Crook and Sherwood-Higham (1997) recommended the use of filtration when the analytical method does not rely on the viability of collected microorganisms. Second, the sampler performance in outdoors compared to indoors might be different, because Macher (1997) indicated that wind speed, wind direction, turbulence, air temperature, pressure, and moisture content might affect bioaerosol sampler performance, and these factors are sometimes largely different between outdoors and indoors. Eduard et al. (1990) collected all samples from indoor occupational environments and it appears that a part of the samples collected by Karlsson and Malmberg (1989) were also from indoors. Third, the viability of airborne fungal spores between indoors and outdoors might be different because of the difference in air temperature, moisture content and other environmental factors. Fourth, the present contrasting observation can also be partially attributed to the difference in collection efficiency of the two selected samplers for airborne fungi. Aizenberg et al. (2000) showed lower collection efficiency of fungal spores by the Burkard sampler below the

$d_{50}$  cut off diameter ( $2.52\ \mu\text{m}$ ) as well as above the cut off diameter because of the bounce of airborne spores. Andersen sampler's collection efficiency for the smaller airborne fungal spores (aerodynamic diameter  $<$  Burkard's  $d_{50}$ , i.e.  $2.52\ \mu\text{m}$ ) is anticipated to be higher than the Burkard sampler because the  $d_{50}$  for the second stage of the Andersen sampler is  $0.95\ \mu\text{m}$ .

## 5. Conclusions

This study has shown that a rich fungal airspora existed in the rural study area and the concentrations of total airborne fungal spores collected by the Burkard sampler and viable airborne fungi collected by the Andersen sampler were higher in this area compared to the other urban places of India as well as few other urban places from different countries of the world. This study indicated that, to achieve representative information on the total airborne fungal spores of a rural area, the monitoring in multiple sampling stations is preferable over a single sampling station. For viable fungi, however, one station can be considered. Approximately 52% of the viable airborne fungi in the investigated rural area were found to be allergenic, which demonstrated positive skin prick test reactions in  $>60\%$  allergy patients. The ratios between the total and viable airborne fungal spores ranged between 0.29 and 7.61. The viability of airborne fungal spores in the rural outdoor sampling stations were obviously higher compared to the previously reported airborne spore viability in indoor environments.

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