

Monitoring and assessment of airborne fungi in Kolkata, India, by viable and non-viable air sampling methods

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Abstract The composition and variability of airborne fungal spores were studied using two complementary sampling methods in an outdoor environment in Kolkata suburb for 2 years, from November 2002 to October 2004. For monitoring the total fungal spore burden in the air, Burkard 7-day volumetric sampler was used, whereas Andersen two-stage viable sampler was used for isolating the cultivable airborne fungi. Among the 37 fungal spore types identified in the air samples, the predominant ones were *Cladosporium*, unidentified ascospores, unidentified basidiospores, *Aspergilli*/Penicilli, *Nigrospora*, *Periconia*, *Chaetomium*, *Drechslera*, *Alternaria*, *Coprinus*, *Ganoderma*, *Pithomyces*, and rust spores. Only six fungal spore types (*Alternaria*, *Aspergilli*/Penicilli, *Cladosporium*, *Curvularia*, *Drechslera*, and *Nigrospora*) were recovered in common by the two samplers. For *Aspergilli*/Penicilli, *Drechslera*, and *Nigrospora*, the spore concentration was underestimated in the non-viable sampling method (Burkard sampler). In general, higher spore count was recorded in winter. The highest fungal species variability was observed in

early monsoon (June). Relative humidity could significantly predict the seasonal periodicity of the maximum number of airborne spores. The total airborne fungi concentration recorded in the study ($15\text{--}16 \times 10^3$ spores m^{-3} of air) was lower than the proposed threshold limit value for clinical significance, suggesting apparently no or less airborne-fungi-exposure-related health risk in the sampling area. *Cladosporium cladosporioides* was recorded beyond the proposed threshold limit value in January 2003 and March 2004; *Aspergillus fumigatus* and *Aspergillus nidulans* in winter that might have posed considerable health risk to sensitized individuals.

Keywords Airborne fungal spores · Climatic factors · Collection efficiency · Cultivable fungi · Diurnal periodicity · Exposure related health risk · Outdoor environment · Seasonal fluctuation · Threshold limit value · Viable and non-viable sampling methods

Introduction

Fungal spores, asexual and sexual reproductive units of fungi, become airborne as a part of the spore dispersal mechanism and eventually contribute significantly to bioaerosol load in both indoor and outdoor environments. The air quality, more precisely the occurrence and concentration of fungal propagules (spore and fragment of hyphae) in the atmosphere, has been implicated in various plant, animal, and human diseases. For the past few decades, airborne fungi have been receiving immense attention due to their potentiality to

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act as the reservoir(s) of allergen(s) and/or toxin(s). Upon inhalation, fungal propagules may trigger mast cell bound IgE-mediated allergic response (type I hypersensitivity) in atopic individuals who may subsequently develop symptoms of allergic respiratory disorders, such as allergic rhinitis and/or allergic asthma. In addition to the pathogenic activities, the airborne fungi are also implicated in the deterioration of food commodities, stored products, and various organic matters such as wood, paper, leather, textiles, etc.

Various air-sampling methods are in use to monitor the occurrence of fungal propagules in the atmosphere, such as non-viable sampling method (capture of total airborne fungal spores, fixation, microscopic identification, and enumeration) and viable sampling (isolation of cultivable airborne fungi in growth medium for the purpose of identification and enumeration). The non-viable sampling method enables the microscopic identification of fungal spores deposited on an inert surface, and thus it allows access to the total airborne fungi. But certain fungi belonging to *Aspergilli*/*Penicilli* group which lack the production of visually distinguishable spores may not be accurately identified by the method. On the other hand, the viable sampling method permits identification of the predominant viable fungal propagules in air by the way of isolating them in artificial growth media. But this method excludes the biotrophic parasitic fungi that do not usually grow on routine culture media. Thus, monitoring the air quality adopting either of these methods may not be able to represent the comprehensive scenario in terms of the actual composition and variability of airborne fungi. In fact, these two methods are complementary in nature.

Adequate information is available on the monitoring and assessment of airborne fungi by individual air samplers, but a comprehensive as well as comparative representation of data on airborne fungi monitored by synchronous non-viable and viable sampling is largely lacking. Only a few reports (Adhikari et al. 2004; Pyrri and Kapsanaki-Gotsi 2007) have been as yet published on the volumetric assessment of airborne fungi by concurrent viable and non-viable sampling. Our study was focused on the sampling of airborne fungal spores in an agricultural farm on the outskirts of Kolkata Metropolitan area, the capital city of West Bengal, India. Chakraborty et al. (2000, 2001) assessed the concentration of airborne fungi of Kolkata by using the non-viable volumetric air

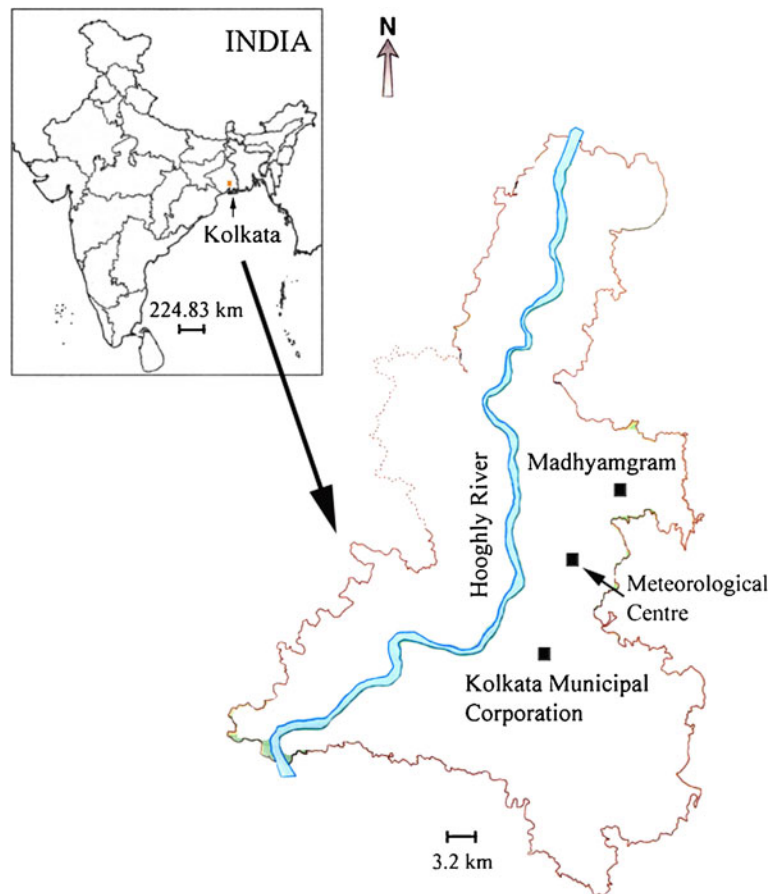
sampling method, but it lacks the volumetric assessment of cultivable fungi. This study was aimed to enumerate the total airborne fungi in Kolkata by simultaneous use of non-viable and viable volumetric samplers and compare the results thereof. Our study was one of a kind that first implemented synchronous non-viable and viable volumetric sampling methods in order to get a comprehensive scenario of total airborne fungal propagules in Kolkata. In literature, the threshold limit value (TLV) for clinical significance was mentioned for airborne fungi; TLV was described as the minimal concentration of the airborne fungi following exposure to which respiratory symptoms, such as nasal and ocular symptoms, might have developed in sensitized individuals. The following TLVs for clinical significance were indicated in previous studies: 20×10^3 fungal spores m^{-3} for total airborne fungi, 10^4 CFU m^{-3} for airborne cultivable fungi, and 500 CFU m^{-3} for single fungal species (Eduard et al. 2001; Heida et al. 1995). This study would consider the threshold limit values while interpreting the findings with respect to human health deterioration. The in-depth survey was further aimed to provide some facts based on that some plausible strategies could be framed to prevent the exposure of the agricultural farm workers to the airborne fungi.

Materials and methods

Sampling site and vegetation

An agricultural farm in Madhyamgram, a suburb situated in the northern fringe of Kolkata metropolis ($22^\circ 82' \text{ N}$, $88^\circ 20' \text{ E}$) (Fig. 1) was selected as the sampling site. Kolkata, the capital city of the state West Bengal, India, comprises of $1,380 \text{ km}^2$ and has a population density of $33,000/\text{km}^2$. The city is close to the Tropic of Cancer and is situated on the banks of the Hooghly river at 20 ft above sea level. The climate is warm and humid throughout the year. Four seasons, namely summer (March to May), monsoon (June to September), post-monsoon (October to November), and winter (December to February), have been distinctly recognized in West Bengal. The temperature fluctuates between 24 and 38°C during summer and 12 – 27°C in winter. Average rainfall is about $1,582 \text{ mm}$ per annum (June–September).

Fig. 1 Location of Madhyamgram (sampling site) and Regional Meteorological Centre at Dum Dum in Kolkata (formerly Calcutta) metropolis (Kolkata is located in the eastern part of India)



The agricultural farm has a sparse vegetation of margosa tree (*Azadirachta indica*), royal Poinciana tree (*Delonix regia*), shield tree (*Petlophorum pterocarpum*), and orchards of mango (*Mangifera indica*), banana (*Musa paradisiaca*), coconut (*Cocos nucifera*), betel nut (*Areca catechu*), and papaya (*Carica papaya*). A number of herbs and shrubs, namely *Amaranthus viridis*, *Parthenium hysterophorus*, *Cyperus* sp., *Justicia simplex*, *Chenopodium album*, and *Catharanthus roseus*, grow abundantly in the farm.

Spore sampling and identification

Air sampling was conducted for two consecutive years from November 2002 to October 2004. A Burkard 7-day non-viable volumetric air sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire WD3 1PJ, UK) and an Andersen two-stage viable sampler (manufactured by Thermo Andersen, Smyrna, 300082-5211, USA) were concur-

rently used for monitoring and assessment of airborne fungal spores.

The Burkard air sampler (air suction rate=10 l/min) was installed on a 2.0-m-high concrete base amidst a square plot (10 m×10 m) in the farm and operated uninterruptedly for two consecutive years (November 2002–October 2004). The total airborne spores captured by the air sampler were deposited on a strip of petroleum wax-coated Melinex tape, and the tape was changed once a week. The exposed tape was cut into 48-mm-long segments, and the segments were mounted on microscopic slides. Subsequent identification (by high resolution light microscope) and enumeration of individual fungal spore (number of spores per cubic meter of air per day) were performed as described by Das and Gupta Bhattacharya (2008). For determining diurnal periodicity, only those sampling days were selected, when the daily mean concentration of each spore type exceeded the

average mean concentration (total count/number of days in which the specific spore type was recorded) in absence of rainfall. The spore concentration of each hour of such days was divided by respective daily mean concentration and the obtained data were plotted against time (in hour) to get the diurnal periodicity pattern of relevant spore.

Apart from spore monitoring by Burkard air sampler, a portable Andersen two-stage viable sampler (manufactured by Thermo Andersen) was simultaneously used for monitoring the cultivable fungal spores in the air. Each stage of the sampler contains 200 pores, each having a diameter of 1.5 mm in the first stage and 0.4 mm in the second stage. The Andersen sampler was usually placed at the same height and sampling plot as that of the Burkard sampler and operated thrice in a week for 3 min each between 12:15 and 12:45 h. Based on the outcomes of a preliminary study conducted at the same sampling site [i.e., most fungi detected by Andersen sampler were found to attain the daily peak concentration around 12:00 noon or later, and air sampling for longer duration (>3 min) caused overlapping of fungal colonies in plates that might have led to improper enumeration of colonies], the sampling time and duration were standardized. The viable spores captured by the sampler (air suction rate=28.3 l/min) were isolated on 2% malt extract agar medium supplemented with streptomycin sulfate (40 µg/ml) following incubation for 3 days at $27\pm 2^{\circ}\text{C}$. The sporulating fungal colonies were identified using standard reference manuals (Subramanian 1971; Onions et al. 1981). *Aspergillus* and *Fusarium* colonies were further subcultured on Czapek–Dox agar medium to facilitate the identification up to species level. The fungal colonies that did not sporulate even after prolonged incubation were referred to as non-sporulating colonies or sterile mycelia. The concentration of cultivable fungi was expressed as colony-forming units per cubic meter of air sampled (CFU m^{-3}).

Meteorological data

The meteorological data were provided by Dum Dum Regional Meteorological Centre (~3.5 km away from the sampling area). Four climatic factors such as temperature ($^{\circ}\text{C}$), relative humidity (%), rainfall for 24 h (mm), and wind speed (km/h) were taken into consideration in this study.

Statistical analyses

The relationship between seasonal fluctuation in airborne fungal spore concentration (dependent variable) and meteorological factors (predictors) was studied by linear regression analysis at <0.05 significance level (P) using SPSS statistical software, version 10.0 (SPSS Inc., Chicago, IL, USA, 1999). Further, paired two-tailed Student's t test was performed ($P<0.05$) to determine whether there is any significant difference between the mean fungal spore counts assessed by non-viable (Burkard) and viable (Andersen) samplers. Student's t test was performed for six common spore types, *Alternaria*, *Aspergilli*/Penicilli, *Cladosporium*, *Curvularia*, *Drechslera*, and *Nigrospora*, sampled by the two air samplers.

Results and discussion

In the study, an attempt was made to determine the composition and variability of airborne fungal spores in a comprehensive manner by synchronous use of non-viable and viable sampling methods. The study was of a kind that first volumetrically assessed the cultivable airborne fungal spore in the region and determined its relationship with the meteorological factors.

Non-viable sampling of total airborne spore

The total airborne fungi monitored by the Burkard non-viable spore trap showed a perennial occurrence pattern and attained the main peak in early winter (December) and a subsidiary peak in early monsoon (June) during the 2-year monitoring period (2002–2004) (Fig. 2a). The relative humidity and temperature in moderate range, very low wind speed, and minimal rainfall during early winter (December) might have facilitated the release and dispersion of dry spore mass; these factors presumably contributed towards achieving the main peak during this month. Similar seasonal periodicity pattern of airborne fungi was documented in a rural agricultural area of India (Adhikari et al. 2004) and also in an urban area of Athens (Bartzokas 1975; Pyrrri and Kapsanaki-Gotsi 2007). *Cladosporium* spp. (a dry spore) was identified as the key contributor towards attaining the main peak

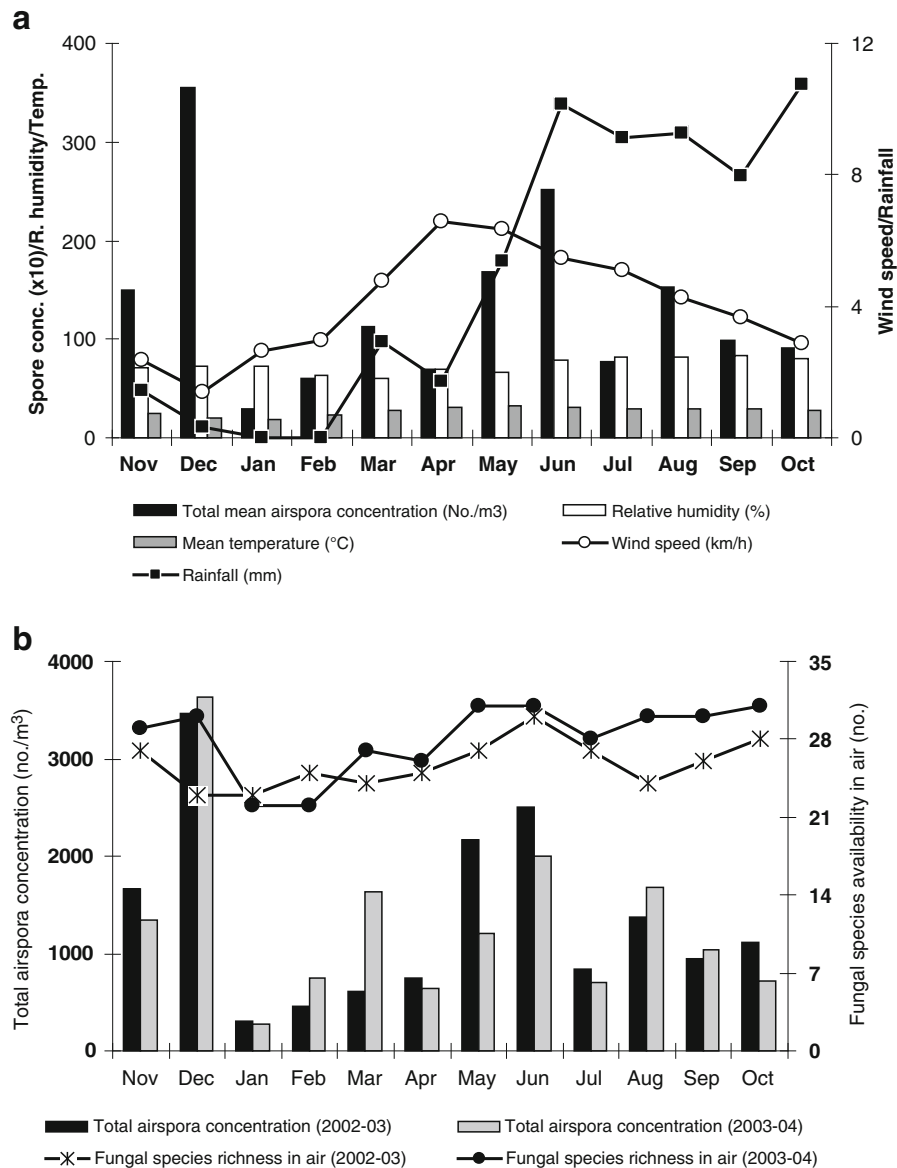


Fig. 2 **a** Airborne fungal spore concentration (monthly mean value) as recorded by Burkard 7-day volumetric air sampler over two sampling years with respect to the meteorological factors. **b** Seasonal periodicity pattern of airborne fungi during

the 2-year sampling period (2002–2004) with respect to the fungal species richness in the air. **c** Diurnal periodicity patterns of certain airborne fungi as recorded by Burkard air sampler

in December, whereas ascospores and basidiospores (wet or humid spore) were the major components of airborne fungi which peaked in June (subsidiary peak). The spore count fluctuated between 305.72 and 3,470.06 spores m^{-3} and 278.72–3,628.09 spores m^{-3} in the first and second sampling years, respectively. Among the 37 fungal spore types (belonging to 33 fungal genera) identified by the Burkard sampler, the predominant ones (mean annual contribution over

two sampling years >1%) were *Cladosporium* spp., unidentified ascospores, unidentified basidiospores, *Aspegilli*/Penicilli, *Nigrospora* spp., *Periconia* spp., *Chaetomium* spp., *Drechslera* spp., *Alternaria* spp., *Coprinus* spp., *Ganoderma* spp., *Pithomyces* spp., and rust spores (Table 1). The remainder was detected in low frequency (<1%). Asexual spores, especially conidia, were the major contributor (62.77%) to the total spore load in the air, followed

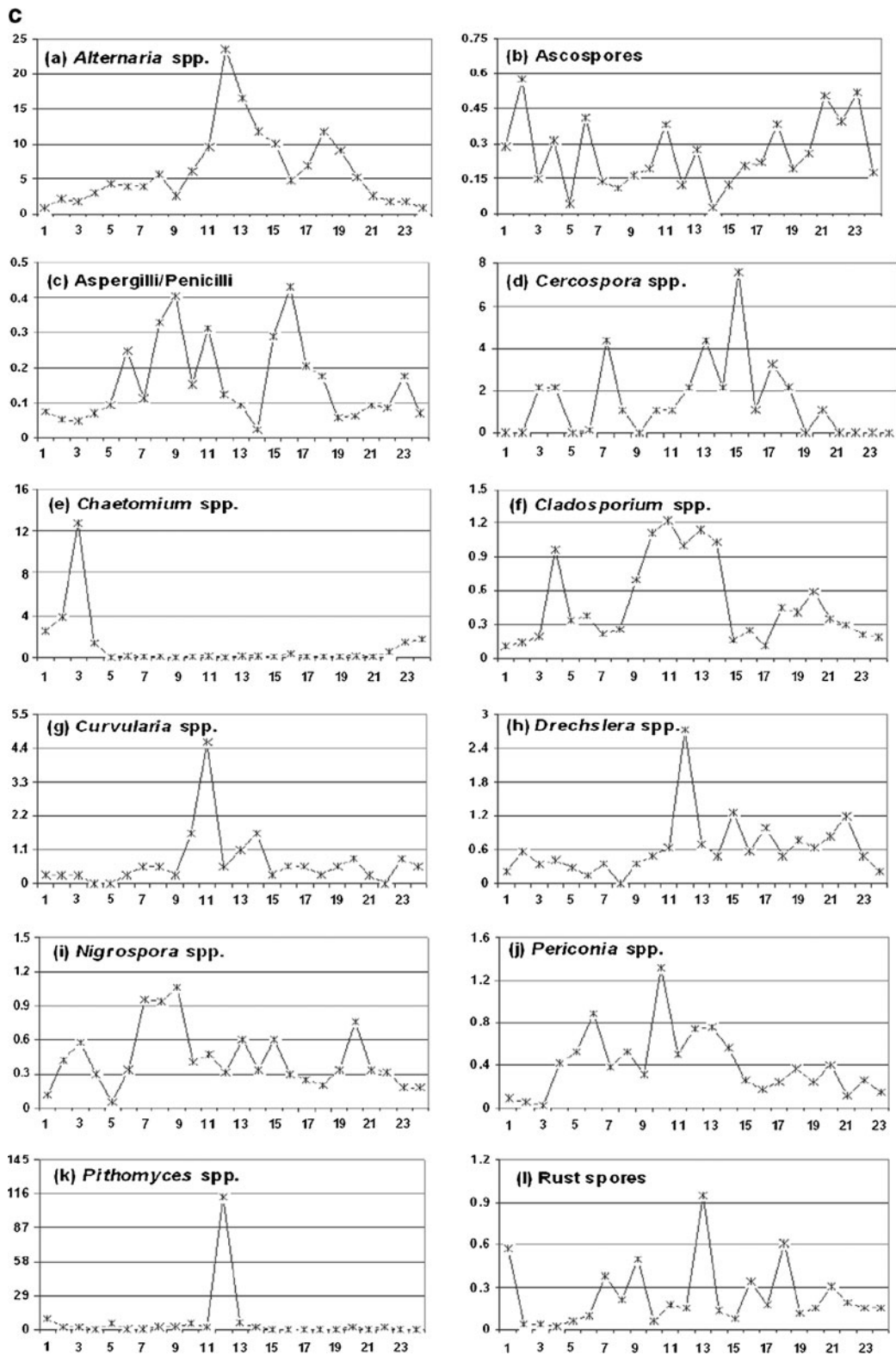


Fig. 2 (continued)

Table 1 Airborne fungal spore types and their individual contribution to total airborne fungi load in the atmosphere of Kolkata, India

Category	Airborne fungal spore type	Contribution to total airborne fungi load (mean contribution) (%)	Total contribution (%)
Asexual spore (conidia)	<i>Alternaria</i> spp.	2.65	62.77
	<i>Aspergilli</i> /Penicilli	5.34	
	<i>Beltrania</i> sp.	0.02	
	<i>Bispora</i> sp.	0.15	
	<i>Cercospora</i> sp.	0.24	
	<i>Cladosporium</i> sp.	39.25	
	<i>Corynespora</i> sp.	0.25	
	<i>Curvularia</i> spp.	0.95	
	<i>Deightoniella</i> sp.	0.04	
	<i>Drechslera</i> sp.	2.71	
	<i>Dwayabeeja</i> sp.	0.15	
	<i>Epicoccum</i> sp.	0.15	
	<i>Lasiodiplodia</i> sp.	0.16	
	<i>Memnoniella</i> sp.	0.13	
	<i>Nigrospora</i> sp.	4.31	
	<i>Periconia</i> sp.	3.95	
	<i>Periconiella</i> sp.	0.45	
	<i>Pestalotia</i> sp.	0.14	
	<i>Phaeotrichoconis</i> sp.	0.05	
	<i>Pithomyces</i> sp.	1.13	
	<i>Spegazzinia</i> sp.	0.1	
	<i>Tetraploa</i> sp.	0.2	
	<i>Torula</i> sp.	0.05	
	<i>Trichoconis</i> sp.	0.2	
Sexual spore (ascospore)	<i>Chaetomium</i> sp.	3.19	23.82
	<i>Leptosphaeria</i> sp.	0.32	
	<i>Podospora</i> sp.	0.13	
	<i>Pleospora</i> sp.	0.16	
	<i>Sporidesmium</i> sp.	0.11	
	<i>Sporormia</i> sp.	0.05	
	Unidentified ascospores	19.63	
	Xylariaceae ascospores	0.23	
Sexual spore (basidiospore)	<i>Coprinus</i> sp.	2.23	13.41
	<i>Ganoderm</i> sp.	1.83	
	Rust spores	1.03	
	Smut spores	0.68	
	Unidentified basidiospores	7.64	

by sexual spores, such as ascospores (23.82%) and basidiospores (13.41%) (Table 1). The predominance of conidia or “dry spores”, produced by 24 out of 33 airborne fungal genera recorded in the study, was consistent with previous reports (Adhikari et al. 2004; Chakraborty et al. 2001; Segvić Klarić and

Pepeljnjak 2006). The composition and species variability of airborne fungi observed in the study corroborated with earlier reports in India (Adhikari et al. 2004; Vittal and Krishnamoorthy 1981) and other countries (Al-Suwaine et al. 1999; Kasprzyk et al. 2004; Pyrri and Kapsanaki-Gotsi 2007).

In this study, *Cladosporium* spp. was recorded as the most prevalent airborne fungal genus. Similar aerobiological monitoring in rice crop fields in West Bengal (Chakraborty et al. 2001) and in other parts of India (Sreeramulu and Ramalingam 1966; Vittal and Krishnamoorthy 1981) also reported *Cladosporium* as the most dominant airborne fungus. *Cladosporium*, being a saprophyte, thrives on decaying organic materials. In agricultural fields, abundance of decomposing leaves and plant debris are noted, which in turn provides ideal growth substrata for *Cladosporium*. Moreover, they produce dry spores (3–5 µm in size), which could be easily spread in the air by wind. These factors perhaps could explain the dominance of *Cladosporium* over other fungal genera found in outdoor environments worldwide (Kasprzyk et al. 2004; Hasnain et al. 2004; Gioulekas et al. 2004; Bouziane et al. 2005).

During early monsoon (June), the maximum airborne fungal species variability (mean value 30.5) was observed while the least species count (mean value 22.5) was recorded in mid-winter (January) (Fig. 2b). Achieving the peak species variability in June could be attributed to moderate temperature and availability of water that might have facilitated in the germination of airborne fungal spores.

Most of the airborne spores (e.g., *Alternaria*, unidentified basidiospores, *Curvularia*, *Drechslera*, *Pleospora*, unidentified ascospores, and *Ganoderma*) attained the peak concentration in monsoon, followed by post-monsoon (e.g., *Aspergilli*/Penicilli, *Corynes-*

pora, rust spores, smut spores, and *Spegazzinia*) and summer (e.g., *Epicoccum*, *Periconiella*, *Cercospora*, and *Nigrospora*), respectively. The spore levels of *Cladosporium*, *Periconia*, and *Pithomyces* peaked towards early winter season (December). The rest did not exhibit any distinct seasonal oscillation pattern. The seasonal fluctuation trends of spores except for *Cercospora*, *Coprinus*, *Pithomyces*, *Torula*, *Tetraploa*, *Trichoconis*, and *Sporidesmium* observed in the study were in agreement with earlier survey reports from a similar outdoor environment (Vittal and Krishnamoorthy 1981; Chakraborty et al. 2003). The deviation in the seasonal oscillation trend could be attributed to the variation in local environmental variables, edaphic factors, and availability of fungal growth substrates as well as changes in vegetation surrounding the sampling site due to anthropogenic activities such as crop cultivation and harvest as evidenced earlier by Atluri et al. (1988a).

The diurnal periodicity patterns of the fungal spores as observed in this study have been categorized following the guiding principles of Gregory (1973) (Table 2). *Alternaria*, *Aspergilli*/Penicilli, *Cercospora*, *Cladosporium*, *Curvularia*, *Drechslera*, *Pithomyces*, and rust spores showed day pattern while *Chaetomium* and unidentified ascospores exhibited night pattern. Considering the peak time, the day pattern of diurnal rhythm was further divided into three subgroups: forenoon peak (10:01–12:00 h), noon peak (12:01–14:00 h), and afternoon peak (14:01–18:00 h). The forenoon peak was recorded in

Table 2 Diurnal periodicity patterns of 12 fungal spore types recorded by Burkard sampler

Fungal spore type	Peak hours (IST)	Diurnal periodicity patterns
<i>Alternaria</i> spp.	12:00 h	Middle day pattern; forenoon peak (10:01–12:00 h)
Ascospores	2:00 h, 23:00 h	Night-time double maxima; midnight peaks (20:01–03:00 h)
<i>Aspergilli</i> /Penicilli	9:00 h, 16:00 h	Day-time double maxima; morning peak (7:01–10:00 h) and afternoon peak (14:01–18:00 h)
<i>Cercospora</i> spp.	15:00 h	Day pattern; afternoon peak (14:01–18:00 h)
<i>Chaetomium</i> spp.	3:00 h	Night pattern; midnight peak (20:01–03:00 h)
<i>Cladosporium</i> spp.	11:00 h	Day pattern; forenoon peak (10:01–12:00 h)
<i>Curvularia</i> spp.	11:00 h	Day pattern; forenoon peak (10:01–12:00 h)
<i>Drechslera</i> spp.	12:00 h	Middle day pattern; forenoon peak (10:01–12:00 h)
<i>Nigrospora</i> spp.	9:00 h	Post-dawn pattern; morning peak (7:01–10:00 h)
<i>Periconia</i> spp.	10:00 h	Post-dawn pattern; morning peak (7:01–10:00 h)
<i>Pithomyces</i> spp.	12:00 h	Middle day pattern; forenoon peak (10:01–12:00 h)
Rust spores	13:00 h	Day pattern; noon peak (12:01–14:00 h)

Alternaria, *Cladosporium*, *Curvularia*, *Drechslera*, and *Pithomyces*; noon peak in rust spores; and afternoon peak in *Cercospora* (Fig. 2c). Among them, *Alternaria* and *Drechslera* revealed specific middle day pattern as they reached the peak concentration at 12:00 h in particular. Aspergilli/Penicilli displayed daytime double maxima pattern attaining two peaks during daytime (9:00 h and 16:00 h). Unidentified ascospores showed nighttime double maxima pattern with two midnight peaks (2:00 h and 23:00 h) while *Chaetomium* achieved a single peak at night (3:00 h). Apart from this, post-dawn pattern (7:01–10:00 h) was recorded in *Nigrospora* and *Periconia* spores. The diurnal rhythms were more or less similar to that recorded by earlier workers in India (Sreeramulu and Ramalingam 1966; Atluri et al. 1988b; Chakraborty et al. 2003), although a minor variation in peak hour was noticed in some spores, namely ascospores, *Cercospora*, *Chaetomium*, *Curvularia*, *Nigrospora*, *Periconia*, and rust spores. This might be pertaining to the spore release pattern controlled mostly by the local climatic factors (Kramer 1979; Hirst 1953).

Sampling of cultivable airborne fungi

The total cultivable airborne fungi attained two peaks in the first sampling year, the primary one (1,944.80 CFU m⁻³) in January and the secondary one (1,214.20 CFU m⁻³) in July. Similarly, in the second sampling year, two peaks were observed, the first one (1,287.53 CFU m⁻³) in March, followed by the second one (1,089.85 CFU m⁻³) in July (Fig. 3a). The highest species variability among the cultivable spores was observed in early winter (December) while in mid-monsoon (August) the species variability was considerably less (Fig. 3b). Total cultivable spore concentration varied from 417.22 to 1,944.80 CFU m⁻³ in the first and 314.93–1,287.53 CFU m⁻³ in the second sampling year.

Among 13 cultivable fungal genera (comprising 26 species) recorded by viable sampler, the predominant fungal genus was *Aspergillus* spp. (Fig. 3c), though *Cladosporium cladosporioides* occupied the first position when the contribution of individual species was taken into account. Other prevalent genera included *Curvularia* spp., *Alternaria* spp., *Penicillium* spp., *Nigrospora sphaerica*, *Trichoderma harzianum*, *Fusarium* spp., *Drechslera hawaiiensis*, and *Rhizopus nigricans*. Sterile mycelia (non-sporulating colonies),

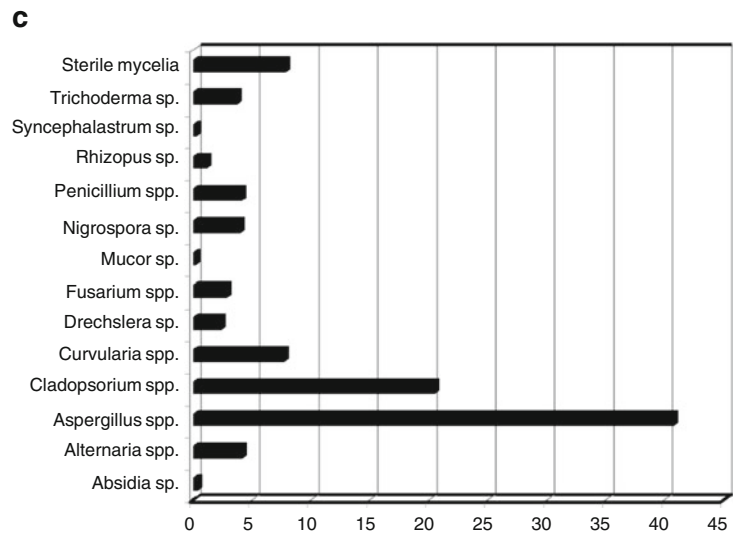
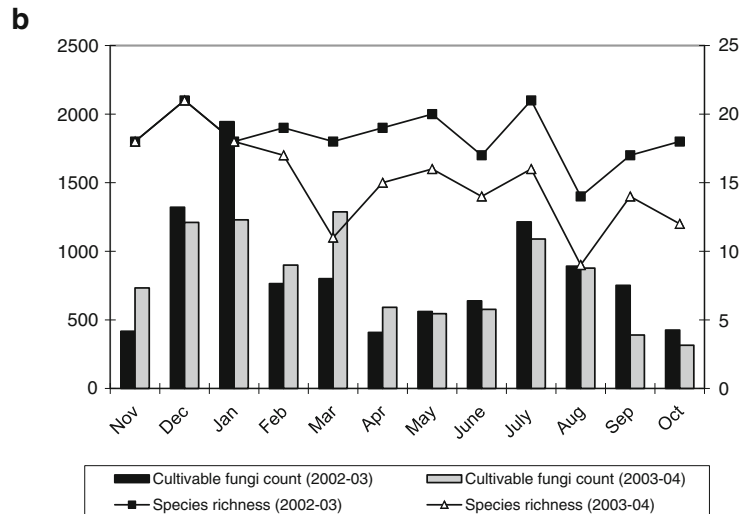
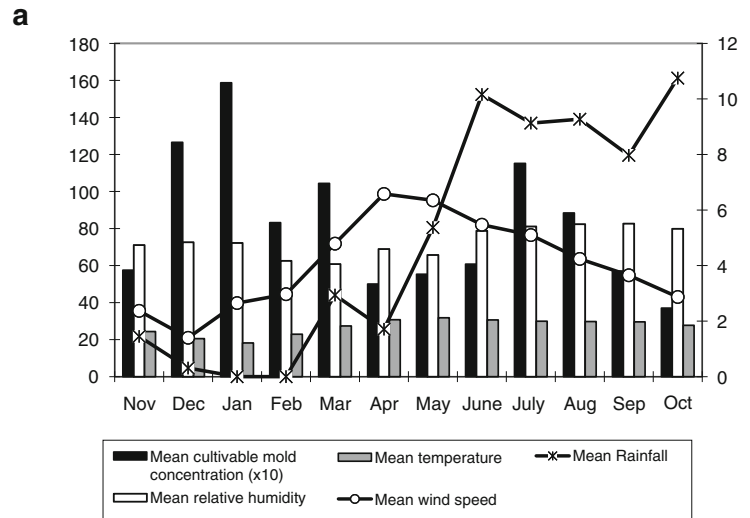
which may represent the vegetative phase of either Ascomycota or Basidiomycota, contributed a significant fraction to total viable spore count. The remainder such as *Absidia corymbifera*, *Mucor hiemalis*, and *Syncephalastrum racemosum* was observed in considerably low concentration throughout the year (annual contribution <1% of total viable spore count). Eight species of *Aspergillus* were isolated of which *Aspergillus niger* occupied the first position in order of dominance, followed by *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus ochraceus*, *Aspergillus japonicus*, *Aspergillus ustus*, and *Aspergillus sydowi* (Fig. 3d).

Several cultivable fungal spores (e.g., *Curvularia lunata* and *Curvularia pallescens*; *Aspergillus japonicus*, *A. niger*, *A. sydowi*, and *A. ustus*; *Drechslera hawaiiensis*, *Mucor hiemalis*, *Rhizopus nigricans*, and *Syncephalastrum racemosum*) and sterile mycelia attained the peak concentration in monsoon (June–September). During post-monsoon, only *Penicillium* spp. (*Penicillium citrinum* and *Penicillium oxalicum*) achieved an elevated concentration level. A handful of cultivable spores (e.g., *Absidia corymbifera*, *A. flavus*, *A. fumigatus*, *A. nidulans*, and *A. ochraceus*; *Cladosporium cladosporioides* and *T. harzianum*) attained peaks in winter while a few cultivable spores [e.g., *Cladosporium herbarum*, *Fusarium* spp. (*Fusarium lateritium* and *Fusarium solani*), *Alternaria* spp. (*Alternaria alternata* and *Alternaria tenuissima*), and *Nigrospora sphaerica*] achieved high concentration in summer.

Comparison of the spore count data recorded by two air samplers

Our study indicated that there were certain qualitative and quantitative variations in the sampling data collected by the two samplers. Thus, the accuracy of the spore sampling data to a greater extent depends on the air sampling method applied. Only six fungal genera, such as *Alternaria*, *Aspergillus*, *Penicillium* (*Aspergilli*/*Penicilli* as a single group for Burkard Spore trap), *Cladosporium*, *Curvularia*, *Drechslera*, and *Nigrospora* were recovered by both the sampling methods. The rare genera were recovered and identified by one or the other method, depending on the viability and diagnostic features of the genera. The ascospores and basidiospores (mentioned as “group”) that constitute a significant part of the airborne fungi were recorded only by the non-viable sampling

Fig. 3 a The concentration of airborne cultivable mold (monthly mean value) as recorded by Andersen viable sampler over two sampling years with respect to the meteorological factors. *Y* axis: spore count (CFU m^{-3})/relative humidity (%) / temperature ($^{\circ}\text{C}$). Secondary *Y* axis: wind speed (km/h) / rainfall (mm). **b** Seasonal variation of the total cultivable mold in two consecutive sampling years (2002–2004) in relation to fungal species richness in the air. *Y* axis: spore count (CFU m^{-3}). Secondary *Y* axis: species richness (number). **c** Annual contribution (%) of individual cultivable mold to total atmospheric load of cultivable mold. *X* axis: percent (%) contribution of spore. **d** Annual contribution (%) of different species of *Aspergillus* to total cultivable airborne mold



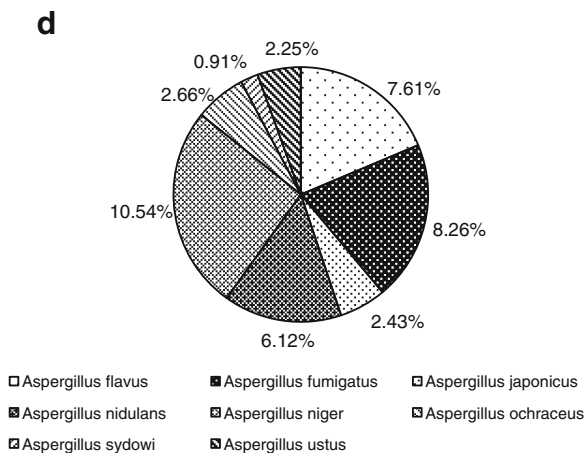


Fig. 3 (continued)

method since they rarely produce the teleomorph in culture.

Statistical analyses and comparison of collection efficiency of samplers

In order to evaluate the collection efficiency of the samplers, the spore concentration recorded by the two samplers were analyzed by Student's *t* test (Table 3). For three spore types, *Aspergilli*/*Penicilli*, *Drechslera*, and *Nigrospora*, a statistically significant difference was observed between the spore concentrations recorded by the two samplers; the difference could be attributed to the difference in the sampling efficiency thereof. For *Aspergilli*/*Penicilli*, *Drechslera* sp., and *Nigrospora* sp., the spore concentration was underestimated by non-viable sampling method.

In this study, the ratio of total airborne spores (captured by Burkard sampler) to cultivable mold

(captured by Andersen sampler) was obtained in the range of 0.18 to 4.15. This finding was in agreement with the observation of Adhikari et al. (2004) where the authors found the same ratio in the range of 0.29 and 7.61. The spores of Ascomycetes and Basidiomycetes members are visible in light microscope, but they are unable to grow in laboratory culture media. For this reason, decrease in the concentration of cultivable mold compared to total fungal spores is generally anticipated while analyzed by light microscopy. The finding showed some inconsistency as the concentration of cultivable mold sometimes exceeded that of total aerospora. Such disparity might be attributed to the difference in collection efficiency of the two air samplers used in this study. Aizenberg et al. (2000) showed that lower collection efficiency of Burkard sampler below the d_{50} cut-off diameter (2.52 μm) as well as above the cut-off diameter was caused due to the bounce of airborne spores. The collection efficiency of Andersen sampler for the smaller fungal spores is expected to be higher than that of Burkard sampler as the d_{50} for the second stage of the Andersen sampler is 0.95 μm (aerodynamic diameter < Burkard's d_{50}). These factors could explain the inconsistency in the aerobiological data recorded by the two air samplers.

Relationship between climatic factors and occurrence of spore in the air

The effect of meteorological factors on the occurrence of fungi in the air was analyzed by linear regression analyses (Table 4). Relative humidity and rainfall seemed to have a distinct influence on the seasonal fluctuation of spores. A number of wet or humid spores (mainly ascospores and basidiospores), namely *Ganoderma*, *Podospora*, *Chaetomium*, rust spores, and smut spores, were significantly predicted either by precipitation or moisture content of ambient air, i.e., relative humidity (Table 4). This observation was consistent with earlier findings (Bartzokas 1975; Hasnain 1993). An increase in airborne ascospore (*Chaetomium* and *Podospora*) concentration was recorded in response to higher rainfall that was in agreement with the observation made by Davies (1969). This phenomenon could be well explained by the importance of water in fungal spore discharge and dispersion. The occurrence of *Bispora*, *Epicoccum*, *Lasiodiplodia*, *Periconiella*, and *Torula* in air, even though they were dry spores,

Table 3 Results of Student's *t* test

Spore count by Burkard/Andersen sampler	Probability
Total spore count	0.0966
<i>Alternaria</i>	0.9327
<i>Aspergilli</i> / <i>Penicilli</i>	0.0085*
<i>Cladosporium</i>	0.1782
<i>Curvularia</i>	0.0796
<i>Drechslera</i>	0.00073*
<i>Nigrospora</i>	0.0015*

* $P < 0.01$

Table 4 Results of linear regression analyses performed using concentration of various airborne fungal spores as dependent variables and meteorological factors as predictors (only statistically significant *B* values were shown)

Airborne fungi	Climatic factors	<i>B</i> (95% CI)	<i>P</i> value
Sampled by Burkard sampler			
<i>Alternaria</i> spp.	Wind speed	18.15 (3.21 to 33.09)	0.022*
<i>Chaetomium</i> spp.	Rainfall	9.67 (0.63 to 18.71)	0.038*
<i>Ganoderma</i> spp.	Relative humidity	3.55 (0.07 to 7.03)	0.046*
<i>Pithomyces</i> spp.	Wind speed	−5.95 (−11.00 to −0.89)	0.025*
<i>Bispora</i> spp.	Relative humidity	0.18 (0.003 to 0.37)	0.047*
<i>Epicoccum</i> spp.	Relative humidity	−0.21 (−0.41 to −0.01)	0.044*
<i>Lasiodiplodia</i> spp.	Rainfall	0.06 (0.01 to 0.12)	0.021*
<i>Periconiella</i> spp.	Rainfall	−0.86 (−1.40 to −0.03)	0.006**
<i>Podospora</i> spp.	Rainfall	0.27 (0.05 to 0.48)	0.019*
Rust spores	Rainfall	−2.22 (−3.46 to −0.99)	0.002**
Rust spores	Relative humidity	−0.92 (−1.82 to −0.02)	0.046*
Smut spores	Rainfall	−1.12 (−1.72 to −0.53)	0.002**
Smut spores	Relative humidity	−0.47 (−0.91 to −0.03)	0.038*
<i>Torula</i> spp.	Relative humidity	0.59 (0.10 to 1.09)	0.023*
Sampled by Andersen sampler			
Mean cultivable mold	Mean temperature	−5.55 (−9.91 to −1.19)	0.018*
<i>Aspergillus nidulans</i>	Mean temperature	−20.15 (−35.28 to −5.01)	0.014*
<i>Aspergillus ochraceus</i>	Mean temperature	−8.56 (−15.63 to −1.49)	0.022*
<i>Cladosporium cladosporioides</i>	Mean temperature	−25.95 (−50.05 to −1.84)	0.037*
<i>Cladosporium herbarum</i>	Relative humidity	−4.98 (−7.72 to −2.24)	0.002*
<i>Nigrospora sphaerica</i>	Mean temperature	4.63 (0.07 to 9.18)	0.047*
<i>Trichoderma harzianum</i>	Relative humidity	−3.86 (−7.14 to −0.58)	0.025*

B coefficient of linear regression, *CI* confidence intervals

P*<0.05, *P*<0.01

was also significantly associated with either rainfall or relative humidity. This striking observation could be attributed to the micro-environmental conditions responsible for spore release and dispersal.

Wind velocity had a lesser impact on the concentration of spores as it could significantly predict the seasonal periodicity of only two spore types (*Alternaria* and *Pithomyces*). Wind is an important climatic factor for dispersion of spores. The occurrence in the air and dispersion of large-sized conidia namely *Alternaria* and *Pithomyces* were significantly predicted by wind velocity. This observation in the present study corroborated with previous reports (Lacey 1991; Marchiso and Airaudi 2001). Several factors including the shape, size, and nature of spores are important for their buoyancy in the air. The typical obclavate shape of *Alternaria* can be considered as adaptive features that aid buoyancy and so enable them to float in air for long periods. Temperature was identified as the key climatic factor significantly affecting the occurrence of total airborne cultivable mold and several individual mold species including *A. nidulans*, *A. ochraceus*, *C. cladosporioides*, and *N.*

sphaerica. *C. herbarum* and *T. harzianum* were found to be predicted by relative humidity.

Interpretation of the study outcomes and proposition of effective strategies for preventing mold exposure related human health deterioration

The seasonal periodicity of airborne spores can provide ample information regarding the occurrence of fungi including the pathogenic ones in the air in a particular region. This information could be utilized in identifying the fungal pathogen and avoid due loss or suffering by designing proper preventive measures. The daily periodicity pattern can offer useful information regarding sporulation and spore dispersal of airborne fungi. This information may be taken into account by farm workers in order to avoid the peak hours for acute exposure to fungal aerosol aiming at minimizing the risk of mold sensitization among workers.

Purchio et al. (1984) suggested the use of the relative frequency of non-sporulating fungi as a

microbiological indicator of atmospheric pollution. The occurrence of non-sporulating fungi in high frequency in the atmosphere of present survey area that was supposed to be contaminated with automobile exhausts and smokes from small industry supported the suggestion of Purchio et al. (1984).

The spores produced by *Alternaria*, *Beltrania*, *Curvularia*, *Epicoccum*, *Drechslera*, *Leptosphaeria*, *Pithomyces*, *Pleospora*, rusts, *Sporidesmium*, *Spegazzinia*, *Tetraploa*, Xylariaceae, and hyphal fragments are more than 10 µm in size whereas Basidiomycete, *Cladosporium*, *Coprinus*, smuts, and *Torula* produce spores in the size range of 3–10 µm. The spores produced by *Aspergilli*/*Penicilli* are smallest in size, i.e., less than 3 µm. Larger spores (spore size >10 µm) are deposited in the nasopharynx and are associated with nasal and ocular symptoms (allergic rhinitis) (Luo 1991). Conversely, smaller spores (size <10 µm, especially those of <5 µm) manage penetration into the smaller airways of the lung and eventually cause allergic asthma (Pepys 1969). Nasal and ocular symptoms were reported to develop following exposure to $20\text{--}500 \times 10^3$ fungal spores m^{-3} whereas exposure to higher spore concentration ($>500\text{--}17,000 \times 10^3$ fungal spores m^{-3}) induces development of cough in sensitized agricultural workers (Eduard et al. 2001). The total airborne fungi concentration recorded in the study ($15\text{--}16 \times 10^3$ fungal spores m^{-3}) did not exceed the threshold limit value (TLV) for clinical significance ($20\text{--}500 \times 10^3$ fungal spores m^{-3}), indicating that there would apparently be no exposure-related health risk for the farm workers during the sampling period. Similarly, the total airborne cultivable mold count was never observed beyond the threshold limit value (10^4 CFU m^{-3}) indicated earlier (Heida et al. 1995). Thus, the airborne cultivable mold, though many of them were reported as inhalant allergens in earlier reports (Adhikari et al. 2004; Burge and Rogers 2000; Chakraborty et al. 2001; Kurup et al. 2000; Latge and Paris 1991), did presumably not pose any harm to the sensitive individuals exposed to it. But potential risk of developing exposure-related respiratory symptoms was seemingly prevailed in the farm during the sampling period if the TLV for individual fungal species was taken into consideration (for single fungal species, a TLV of 500 CFU m^{-3} was suggested as a threat to human health as indicated by Heida et al. 1995). The airborne spore count of *A. fumigatus* attained values higher than TLV in December of both

the sampling years, whereas *A. nidulans* spore level was recorded beyond the borderline value during January of the first sampling year, which may trigger allergic reactions in sensitized individuals. *C. cladosporioides* spores exceeded its borderline value in January 2003 and March 2004, suggesting a risk of developing respiratory symptoms in individuals sensitized to *Cladosporium* spores.

Precisely, the results of the study ensure that both viable and non-viable sampling methods are complementary to each other, and such synchronous sampling approach could enable researchers to overcome the demerits of using each sampling method alone. The data represented in the article would likely be a more complete set of information on airborne spores. The outcomes of the study along with the insightful explanation could aptly provide strong basis for strategizing effective preventive measures against airborne-fungi-exposure-related health risk among agricultural farm workers.

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