Research article

Airborne fungal spore concentration in some selected indoor and outdoor sites: Threats of respiratory problems

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Abstract: Airborne fungi are among the most important aeroallergens and main components of causing different respiratory disorders and also agricultural crop diseases. The main objectives of the present study were to determine the concentration of indoor and outdoor fungal spores in the air. The open plate method was adopted for the analysis qualitative estimation of airborne fungal spores. Sabouraud Dextrose Agar (SDA), Czapek-Dox Agar (CDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were used to detect the airborne fungi. The plates were exposed in indoor and outdoor environments of the Library, Canteen, Newly Constructed Building and Class Room. A total of 132 colonies were found comprising of 18 genera. The dominant fungal genera were *Alternaria* sp. (9.85%), *Aspergillus* sp (43.18%), *Cladosporium* sp (7.58%), *Curvularia* sp (3.03%), *Fusarium* sp (6.06%), *Penicillium* sp (18.18%) and *Trichoderma* sp (3.79%). The present results revealed *Aspergillus* sp, *Penicillium* sp and *Cladosporium* sp were found to be dominant in indoor and outdoor sites. Malt Extract Agar (MEA) showed the best suitable media for the isolation of fungi. The highest concentration of fungi observed in July and August. The meteorological parameters also recorded during the sampling period. This study provided some information regarding the indoor and outdoor airborne fungal composition in this region.

Keywords: Respiratory disorder - Open plate method - Dominant - Allergenic - Crop diseases.

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INTRODUCTION

In indoor environments people require fresh air because people spent most of the time inside the dwelling (Lingnel 2008, Ayanbimpe *et al.* 2010). According to Chadeganipour *et al.* (2010), all atmospheric air, whether indoor or outdoor, contains certain varieties of some fungal spores. Generally, outdoor air is the dominant source of indoor fungi (Shelton *et al.* 2002). Pasanen *et al.* (1991) found that the temperature of 21–30°C and relative humidity of 75–92% could induce rapid fungal germination and growth. Occurrence of airborne fungal spores in the air is affected by many factors such as geographical locations, weather patterns, meteorological parameters etc. Fungal spore concentration in outdoor environments consistently differs from indoor environments. In India many aerobiological studies have been conducted for airborne fungal spores (Khandelwal 2008) but mainly from outdoor environments (Almina *et al.* 2019) but indoor environments are equally important because people spend most of their time indoor, punctuated by physicians (Portnoy *et al.* 2005).

The environmental differences between urban and rural areas reveal extremes of exposure and provide us with a unique insight into the potential causes of an increased prevalence of allergy (Nicolaou *et al.* 2005). In urban areas, high levels of vehicle emissions have been associated with an increased prevalence of respiratory allergy (Nicolai *et al.* 2003). In rural areas, on the other hand, exposure has been shown to protect against sensitization to common aeroallergens, asthma and allergic diseases (Pekkarinen *et al.* 2007). Nowadays fungal allergy is very common to people mainly those who spend most of the time indoor but it is difficult for diagnosis from the other type allergy due to fungi which are many and antigenically variable than the other

allergens. The main objectives of the present study were to determine the concentration of indoor and outdoor fungal spores in the air.

MATERIALS AND METHODS

Study sites

Tripura is a state in Northeast India. It extends from 22° 56' N to 24° 32' N and 91° 09' E to 92° 20' E. Tripura is a landlocked state in Northeast India, where the seven contiguous states. Tripura is collectively known as seven sister states with (Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram and Nagaland). The investigation was carried out in Library (23° 45'37" N, 091° 16'59" E), Canteen (23° 45'41" N, 091° 15'47" E), Newly Constructed Building (23° 26'02" N, 091° 15'22" E), M.Sc. Class Room (23°45'80" N, 091° 15'20" E) of Tripura University, Suryamaninagar, Tripura, Northeast India between May and August, 2014 (Fig. 1).

Figure 1. Map showing the study sites.

Isolation and identification of airborne fungi

The assessment of airborne fungal concentration was performed by sedimentation plate technique. Sabouraud Dextrose Agar (SDA), Czapek-Dox Agar (CDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) was prepared. The plates were exposed in indoor and outdoor environments of the Library, Canteen, Newly Constructed Building and Class Room. These plates were exposed for 5 minutes during the time in between hour 11 am to 5 pm after which they are sealed, labeled and transported to the laboratory. The culture plates were incubated at room temperatures (25°C) until growth appeared. Isolates were identified based on the standard texts and keys (Ellis 1971, Domsch *et al.* 1980, Watanabe 2002).

Meteorological data

Meteorological parameters were obtained from India Meteorological Department, Agartala, Tripura.

RESULT

The dominant species were the members of genera of *Aspergillus* sp. (43.18%), *Penicillium* sp. (18.18%), *Alternaria* sp. (9.85%), *Cladosporium* sp. (7.58%), *Fusarium* sp. (6.06%) and *Trichoderma* sp. (3.79%), followed by others (Table 1; Fig. 2).

In indoor environments, *Aspergillus* sp. (53.13%) was the dominant genus followed by *Penicillium* sp. (10.94%) and *Alternaria* sp. (9.38%), *Fusarium* sp. (7.81%) *Curvularia* sp. (6.25%) and *Cladosporium* sp. (4.69%). In outdoor environments *Aspergillus* sp. (33.82%) followed by *Penicillium* sp. (25.00%), *Alternaria* sp. (10.29%), *Cladosporium* sp. (10.29%) *Trichoderma* sp. (7.35%) (Table1). The rate of isolation of fungi from outdoor (51.51%) was higher than indoors (48.48%). Also, several sterile mycelium colonies were isolated as well.

Table 1. Percentage of fungal isolate from indoor and outdoor environments of Tripura.

Fungal genera	CR		L		C		NCB		I		O		Total Isolate	Grand Total %
	I	O	I	O	I	O	I	O	Total Isolate	Total %	Total Isolate	Total %		
<i>Acremonium</i> sp	0	0	0	0	0	0	0	1	0	0.00	1	1.47	1	0.76
<i>Alternaria</i> sp	3	4	3	3	0	0	0	0	6	9.38	7	10.29	13	9.85
<i>Aspergillus</i> sp	5	5	7	6	10	5	12	7	34	53.13	23	33.82	57	43.18
<i>Cladosporium</i> sp	0	0	0	0	1	4	2	3	3	4.69	7	10.29	10	7.58
<i>Curvularia</i> sp	3	0	1	0	0	0	0	0	4	6.25	0	0.00	4	3.03
<i>Fusarium</i> sp	4	1	0	0	1	2	0	0	5	7.81	3	4.41	8	6.06
<i>Monilia</i> sp	0	1	0	0	0	0	0	0	0	0.00	1	1.47	1	0.76
<i>Nigrospora</i> sp	0	0	0	0	0	0	1	0	1	1.56	0	0.00	1	0.76
<i>Geotrichum</i> sp	0	0	0	0	1	0	0	0	1	1.56	0	0.00	1	0.76
<i>Penicillium</i> sp	1	9	2	4	0	0	4	4	7	10.94	17	25.00	24	18.18
<i>Phoma</i> sp	0	0	0	0	1	0	0	0	1	1.56	0	0.00	1	0.76
<i>Pythium</i> sp	0	1	0	0	0	0	0	0	0	0.00	1	1.47	1	0.76
<i>Rhizopus</i> sp	0	1	0	0	0	0	0	0	0	0.00	1	1.47	1	0.76
<i>Torula</i> sp	1	0	0	0	0	0	0	0	1	1.56	0	0.00	1	0.76
<i>Trichoderma</i> sp	0	2	0	0	0	3	0	0	0	0.00	5	7.35	5	3.79
Unidentified sp. 1	0	0	0	0	1	0	0	0	1	1.56	0	0.00	1	0.76
Unidentified sp. 2	0	0	0	0	0	0	0	1	0	0.00	1	1.47	1	0.76
Unidentified sp. 3	0	0	0	0	0	0	0	1	0	0.00	1	1.47	1	0.76
Total Isolates	17	24	13	13	15	14	19	17	64	-	68	-	132	-

Note: I= Indoor; O= Outdoor; CR- Classroom; L- Library; C- Canteen; NCB- Newly Constructed Building.

Figure 2. Air-fungal species from outdoor and indoor environments of Tripura: A–C, *Aspergillus* sp.; D, *Geotrichum* sp.; E, *Cladosporium* sp.; F, *Torula* sp.; G, *Curvularia* sp.; H, *Alternaria* sp.; I, *Penicillium* sp.; J, *Phoma* sp.; K, *Acremonium* sp.; L, Unidentified sp. 1; M, *Pythium* sp.; N, *Nigrospora* sp.; O, *Fusarium* sp.; P, *Rhizopus* sp.; Q, *Trichoderma* sp.; R, Unidentified sp. 2; S, *Monilia* sp.

In this present study it was found that the number of fungal isolate was maximum in outdoor (68) compare than indoor (64) in table. *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., *Fusarium* sp. were isolated both from indoor and outdoor environments. *Phoma* sp., *Torula* sp., *Nigrospora* sp., *Geotrichum* sp. and Unidentified sp. (1) were isolated from indoor-environments. *Monilia* sp., *Rhizopus* sp., *Pythium* sp., *Acremonium* sp., Unidentified sp. (2), Unidentified sp. (3) and *Trichoderma* sp. were isolated from outdoor environments.

The no. of isolated fungal genera highest in M.Sc. second semester classroom (indoor-outdoor=41) and lowest in Library (indoor-outdoor=26) shows in figure 3. A total of 132 colonies were isolated. Out of 132 colonies, 64 were isolated from indoor environments and 68 were isolated from outdoor environments. Out of 64 isolated colonies from indoor conditions 4 colonies isolated from CDA, 14 from SDA, 16 from PDA and 30 from MEA were found. And out of 68 isolated colonies from outdoor conditions, 12 from CDA, 17 from SDA, 17 from PDA and 22 from MEA found. The various species of fungi were isolated from the outdoor and indoor environments. After data analysis it was found that in both the environments the highest isolated recorded from MEA media (Fig. 4).

Figure 3. Number of isolated fungal genera from four selected sites.

Figure 4. Fungal growth in different media.

Figure 5. Relation between fungal concentration with meteorological parameters.

Meteorological data table 2 and their relation with the fungal colony were depicted in figure 5. Dominant fungal genera from indoor and outdoor, namely *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Penicillium* sp. shows in figure 6 and the total percentage of dominant fungal genera from four sampled sites *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. show in figure 7.

Table 2. Average percentage of meteorological data during sampling period.

Month	R/F (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Max. R/H (%)	Min. R/H (%)
May	9.98	34.91	26.87	90.83	59.61
June	16.14	32.69	24.93	96.4	76.46
July	4.67	32	24.57	93.22	66.61
August	12.27	30.84	24.18	91.83	72.41

Figure 6. Percentage of dominant fungi from indoor and outdoor environments.

Figure 7. Total percentage of dominant fungal genera from four sampled site.

DISCUSSION

This study described culturable fungi obtained from indoor air and outdoor air of M.Sc. second semester classroom (Botany), Library, Canteen, and Newly Constructed building. *Aspergillus* sp. (43.18%), *Penicillium* sp. (18.18%), *Alternaria* sp. (9.85%) and *Cladosporium* sp. (7.58%) were the dominant indoor and outdoor fungi in this study. Other fungal isolates include *Fusarium* sp. (6.06%), *Trichoderma* sp. (3.79%), *Curvularia* sp. (3.03%), *Phoma* sp. (0.76%), *Monilia* sp. (0.69%), *Pythium* sp. (0.76%), *Acremonium* sp. (0.76%), *Rhizopus* sp. (0.76%), *Torula* sp. (0.76%), *Nigrospora* sp. (0.76%), *Geotrichum* sp. (0.76%). Chadeganipour *et al.* 2010, Ayanbimpe *et al.* (2010) who also isolated similar fungi in indoor and outdoor environments. Studies carried out in North, South and Central America has shown that *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Alternaria* sp. were the prevalent indoor and outdoor airborne fungi (Shelton *et al.* 2002). In India similar result also found by Jothish & Nayar (2013) and Premila (2013).

The present results revealed on an indoor and outdoor fungal genus diversity with the predominant genera at all sampling points being *Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp. Similar findings are reported by other authors, both in Cuba (Aira *et al.* 2002, Rojas *et al.* 2002, Labarrere *et al.* 2003, Borrego *et al.* 2010) and elsewhere (Medrela-Kuder 2003, Hedayati *et al.* 2005, Codina *et al.* 2008).

In India Jothish & Nayar (2004) also found *Aspergillus* sp., *Penicillium* sp. was the dominant and most abundant spore type inside the sawmill environment while *Cladosporium* sp. was dominant in the outdoor. Our result revealed *Aspergillus* sp. and *Penicillium* sp. was in both indoor and outdoor environments. The percentage of *Cladosporium* sp. is higher in outdoor (10.29%) than indoor (4.69%) which is similar to Jothish & Nayar (2004).

In this study *Torula* sp. and *Phoma* sp. isolated from indoor environments and *Monilia* sp. isolated from outdoor environments. This result also similar to Durugbo *et al.* (2013) in south-western Nigeria.

In the present study *Nigrospora* sp., *Trichoderma* sp., *Fusarium* sp., *Acremonium* sp., *Pythium* sp. and *Rhizopus* sp. isolated from indoor and outdoor environments. Previously, *Acremonium* sp and *Rhizopus* sp were also reported by Abu-Dieyeh *et al.* (2010) in Jordan.

Among the four different types of culture media, MEA showed best-suited media for isolation of airborne fungi. It's maybe due to higher amount of carbohydrates particularly maltose, protein and other medium which corroborates with earlier findings (Muthu & Shanmugasundaram 2015).

In this study, the highest concentration of fungal colony observed in July and August. It may be due to the presence of favourable temperature and low rainfall in these two months. Our result showed proximity with the finding of Taia *et al.* (2019) where they found in July the highest spore concentration.

CONCLUSION

This study provided some information regarding the indoor and outdoor airborne fungal composition and suggesting a further investigation on airborne fungi in the indoor and outdoor environment of this site. This study showed that there were abundant and diverse mycoflora in the atmosphere. *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Trichoderma*, *Fusarium* and *Curvularia* constituted the dominant components in the airspora. Much literature revealed that some of these spore types are hazardous because they are allergenic. Hence, it is essential that proper and periodic maintenance of working environments involving frequent cleaning, disposal of accumulated wastes, setting up of modern infrastructure facilities, application of fumigants/fungicides and use of dust masks while working can certainly help to improve the air quality and reduce the allergic incidence to people who work in such environments.

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