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INCIDENCE OF AIRBORNE FUNGI IN RAJSHAHI METROPOLITAN CITY IN RELATION TO SEASONAL FLUCTUATIONS

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Abstract: Using PDA, Saboraud's, Czapek's and Richard's media and settling plate technique, a total of 16, 593 fungal colonies belonging to 28 genera were identified during November 2006 and October 2007 in the atmosphere of the Rajshahi Metropolitan City. Seasonal fluctuations in the incidence of airborne fungi and their relation to some meteorological parameters were noticed. The incidence varied significantly by seasons. Maximum incidence of air borne fungi was recorded from November to January, where as the minimum was found from August to October. The most prevalent fungal genera were *Aspergillus, Candida, Penicillium, Rhizopus, Fusarium, Alternaria, Curvularia* and *Cladosporium* in respect of seasons. *Aspergillus* was the most dominting fungi contributed 25.6, 26.3, 24.4 and 26.3% in PDA, Sabouraud's, Czapek's and Richard's media, respectively during the twelve months of observation. The percentage contributions of the most predominant 14 genera were 83.1, 84.1, 83.9 and 885.6 collected in PDA, Sabouraud's, Czapek's and Richard's media, respectively. Rest of the 14 genera were covering about 8.3, 8.0, 7.1 and 84.2% of the total aero-fungi and sterile mycelium and unidentified fungi were contributed 7.8, 7.4, 8.4 and 8.1%, respectively.

Key words: Airborne fungi, incidence, culture media, season, fluctuation.

mvims‡¶c: ivRkvnx †g‡UvcwjUb bM‡ii evqgÛ‡j b‡f¤i 2006 †_‡K A‡±vei 2007 mvj chš †mUwjs †cU c×wZ‡Z PDA, Sabourauds, Czapek's and Richard's Lv`¨ gva¨‡g e¨envi K‡i †gvU 16,593 QÎvK K‡jvbx msMn Kiv nq Ges G‡`i g‡a¨ 28wU MY mbv³KiY Kiv hvq| FZ‡f‡` evqewnZ QÎv‡Ki Awa‡K¨ ZviZg¨ Ges AvenvIqv Dcv`v‡bi mv‡_ G‡`i m¤úK j¶¨ Kiv nq| evqewnZ QÎv‡Ki AwaK¨ FZi mv‡_ ZvrchcYfv‡e cwiewZZ n‡Z †`Lv hvq| me‡P‡q †ewk cvPhZv †`Lv hvq c_g FZi (b‡f¤i-Rvbqwi) PDA Lv`¨ gva¨‡g Ges me‡P‡q Kg PZ_ FZi (AMv÷-A‡±vei) Richard's Lv`¨ gva¨‡g| me‡P‡q cvPhcY QÎvK MY n‡jv *Aspergillus, Candida, Panicillium, Rhizopus, Fusarium, Alternaria, Curvularia* and *Cladosporium* hv FZ Abhvqx msMnxZ n‡qwQj| evi gvm ch‡e¶‡Y me‡P‡q cvavb¨ we¯viKvix QÎvK n‡jv *Aspergillus* hv 25.6, 26.3, 24.4 Ges 26.3% msMnxZ n‡qwQj h_vµ‡g PDA, Sabourauds, Czapek's and Richard's Lv`¨ gva¨‡g| me‡P‡q †ekx cvavb¨ we¯viKvix 18wU M‡Yi kZKiv Ae`vb n‡jv 83.1, 84.1, 83.9 Ges 85.6 hv h_vµ‡g PDA, Sabourauds, Czapek's and Richard's Lv`¨ gva¨‡g msMnxZ n‡qwQj| †gvU QÎv‡Ki g‡a¨ cieZx 14wU MY 8.3, 8.0, 7.1 Ges 84.2%, Ges eÜ¨v gvB‡mwjqvg I Ambv³KZ QÎvK 7.8, 7.4, 8.4 Ges 8.1% Ae`vb iv‡L|

Introduction

Airborne microbe is a component of our environment and is of potential economic and health implications (Gregory, 1961; Hashimoto, 1986). There are essentially no fungus-free environments in our daily lives (Chao *et al*., 2002). Moreover, viable microbes in extramural air may be responsible for many illness during certain seasons of the year and may have serious consequence (Cantane *et al.*, 1984; Maestreet *et al.*, 1986). Exposure to fungi has been reported to cause several types of human health problems, primarily irritations, infections, allergies, and toxic effects, and it has been suggested that toxigenic fungi are the cause of additional adverse health effects (Etzel *et al.*, 1994; Hodgson *et al.*, 1998; Epstein and Fan, 2001). A field guide published by the American Industrial Hygiene Association recommends that the presence of some toxigenic fungi requires urgent risk management decisions (Dillon *et al.*, 1996). To systematically evaluate the relationship between airborne fungi and adverse health effects, the fungal types and their relative frequencies in air need to be known (Shelton *et al.*, 2002).

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Air is the most common medium for the dispersal of fungal spores and hyphal fragments (Marchisio *et al.*, 1992). The composition and concentrations of the airborne fungal spores are largely determined by geographic location, meteorological factors, vegetation, and human activities (Lacey, 1981; Lyon *et al.*, 1984). The concentrations of fungal spores in the atmosphere at any particular moment are influenced by the processes involved in their production, release, and deposition (Lyon *et al.*, 1984). The influence of meteorological factors on the concentrations of airborne spores appears to be additive, not independent (Munk, 1981). Keeping the above views in mind, the present investigation was undertaken to study the incidence of atmospheric fungi in the Rajshahi Metropolitan City and to find out the possible relations between the obtained data and the prevailing climatic conditions. The incidence of airborne fungi and their fluctuations by seasons were also recorded.

Materials and Methods

Sample site: The sampling was conducted in outdoor atmosphere at different places of Rajshahi Metropolitan

City, especially Rajshahi University Campus and satellite town (Upashahar) areas from November 2006 to October 2007.

Sample collection: The air samples were collected per week followed by settling plate culture method (Brown, 1953) using Potato Dextrose Agar (PDA), Richard's, Czapek's and Saboraud's media. To avoid bacterial contamination, 50% lactic acid was added to the media. The Petri dishes (9 cm-diameters) containing 20 ml selected media were exposed at 1 m height from ground level in every cases for 15 min. Then the plates were taken into the laboratory and incubated at 30^{o} C for 5-7 days. Meteorological data were collected from Government Meteorological Information Centre, Rajshahi.

Microbial examination: The fungal colonies were enumerated after their growth on the plates. Identification of fungal colonies was made by visual and microscopic examinations. Identification up to generic level was done with the help of standard mycological books and manuals (Gilman, 1957; Booth, 1971; Subramanian, 1971; Ellis, 1971; Alexopoulos and Mims, 1979). Sub-cultures that failed to sporulate at the end of one month was designated as sterile mycelium. Details regarding the qualitative nature of the mycoflora, their incidence, abundance and percentage contribution were recorded. The percentage contribution of each genus was calculated on the basis of the number of colonies of the genus against total number of colonies of all recorded genera during the entire twelve months' sampling period. Seasonal variations in fungi were also carried out.

Statistical analyses: The experiment was conducted by using a randomised design with five replications. Using a statistical package (MSTAT-C) all data were analysed by F-tests (analysis of variance) and the comparison of means were done separately. Correlation was determined among the season and different fungi. Results of all analyses were judged for their significance at 5% level.

Results and Discussion

A total of 16,593 colonies were recorded in PDA, Sabouraud's, Czapek's and Richard's media. Out of these 15,279 colonies were identified, 1,171 were sterile mycelium and 143 were unidentified. The isolated fungi were assigned to 28 genera belonging to Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. The range of temperature, average RH (relative humidity) and rainfall were gathered from Meteorological Information Centre (Table 1). Among the four media tested, PDA was the most favourable for growth and development of identified fungi and showed the highest count. The data showed clear variations on different climatic factors in different seasons (Table 1). The prevalence of airborne mycoflora was intimately related with prevalent climatic conditions including temperature and RH (Wright *et al.*, 1969; Webb, 1959).

Season-wise fluctuations in the incidence of airborne fungi: Seasonal counts of fungal colonies in four culture media were determined. Total number of fungal colonies were counted as 1566, 1440, 1171 and 1091 in 1^{st} season ; 969, 1045, 956, and 849 in 2^{nd} season ; 1054, 1034, 960 and 869 in 3^{rd} season ; and 1073, 1002, 735 and 700 in 4^{th} season in PDA, Sabouraud's, Czapek's and Richard's media, respectively. Statistical analysis revealed that positive and significant correlation ($r=0.767$; $P<0.05$) was present in different seasons and colony number of different fungi. Analysis of variance showed significant ($F_{3, 89} =3.977$; $P<0.05$) results in different seasons and in different fungi.

The incidence of airborne fungi varied from season to season. The maximum incidence of air borne fungi were exhibited in the atmosphere of the 1^{st} season(November to January) in PDA medium and the minimum in 4^{th} season (August to October) in Richard's medium. Chao *et al.* (2002) reported that total airborne fungal concentrations varied significantly by season and noted highest in summer and lowest in winter. Shelton *et al.* (2002) observed the highest fungal population in the fall and summer and lowest in the winter and spring. Uddin (2005) reported that temperature and relative humidity is probably not so significant for aeromycoflora of jute fields but the incidence of aeromycoflora is inversely proportional to the total rainfall. Chakraborty *et al.* (2003) reported that there were no significant correlation between total air spora and the meteorological parameters and local cropping practices may have some regulating role on spore concentration in the air.

Season-wise airborne fungal genera: The genera *Aspergillus*, *Candida*, *Penicillium*, *Rhizopus*, *Fusarium*, *Alternaria*, *Curvularia* and *Cladosporium* were the most frequently occurring fungi in all the seasons as recorded in PDA, Sabouraud's, Czapek's and Richard's media. Among the genera, *Aspergillus* was the most predominant and showed highest incidence in 3^{rd} and 4^{th} seasons (May to October) in PDA medium where high temperature and heavy rainfall were recorded (Table 1). *Candida* is the 2^{nd} dominating fungi and showed the highest incidence in 1^{st} season (November to January) in PDA medium where low temperature and minimum

rainfall were recorded. The 3rd dominating fungi was *Penicillium* sp. showed the highest incidence in 3rd season (May to July) in Czapek's medium whereas *Rhizopus* and *Colletotrichum* showed maximum incidence in 2nd season (February to April). The highest incidence of common fungi as *Fusarium, Alternaria, Curvularia, Cladosporium, Monilia, Helminthosporium, Geotrichum, Trichoderma* and *Gleocladiopsis* were recorded in 1st season whereas *Oidiodendron* was recorded maximun in 3rd season and *Mucor* in 2nd season. Maximum sterile mycelium were exhibited in 3rd and 4th seasons (May to October) of the total count as recorded in PDA, Sabouraud's, Richard's and Czapeks media (Table 2). Begum *et al.* (2007) reported that *Penicillium, Sporobolomyces, Aspergillus, Neurospora, Cladosporium, Alternaria, Curvularia, Fusarium, Gloeosporium* and *Candida* were the most frequent genera of Rajshahi Metrpolitan City. Uddin (2005) reported that *Penicillium* and *Aspergillus* are the most dominant fungi followed by *Curvularia* and *Cladosporium*. Chakraborty *et al.* (2003) studied aeromycoflora in an agricultural farm at a suburban area of Calcutta and reported that the most abundant types were basidiospores (21.85%) followed by *Cladosporium, Penicillium, Nigrospora, Aspergillus* and ascospore. Albuquerque *et al.* (2004) studied airborne fungi of Brazil and reported that *Aspergillus, Penicillium, Curvularia, Cladosporium*, mycelial sterila, *Fusarium, Rhizopus, Dreschleria, Alternaria,* and *Absidia* were predominant fungi**.** Shelton *et al.* (2002) stated that *Cladosporium, Penicillium* and non sporulating fungi are prevelance fungi in the air of U.S.A. Maestre *et al.* (1986) observed that frequently isolated air borne fungi were *Aspergillus* followed by *Penicillium, Candida, Alternaria, Cephalosporium* and *Rhizopus.* Paramasivam and Gnanarethinum (1986) noted that *Aspergillus, Penicillium, Cladosporium* and *Drechslera* were frequent genera contributed 80% of the sporal load. The present results support the above findings.

Percentage contribution of airborne fungi: Among all the identified genera the highest percentage covering genus is *Aspergillus* contributed 25.6, 26.3, 24.4 and 26.3% in PDA, Saborauds, Czapek's and Richard's media recorded as total colony number 4261 in different seasons and in different media. The next dominant genus was recorded in this investigation is *Candida* and contributed about 20.6, 23.8, 26.7 and 26.6 % of the total counts as in PDA, Sabourauds, Czapeck's and Richards media showing colony number 4009 in different seasons and in different media. In order of rank third position is *Penicilllium* contributed 7.0, 8.5, 11.7 and 11.8% of the total count as in PDA, Sabourauds, Czapek's and Richard's media in different seasons and different media. Next in order is *Rhizopus*. constituting flora in November, 2001 to October, 2002 and contributed 6.4%, 4.2%, 1.7% and 2.9% in PDA, Sabouraud's, Czapek's and Richards's media, respectively. *Fusarium, Alterneria, Curvularia, Cladosporium, Monilia* and *Helminthosporium* belongs to the following status covering 3.8, 2.6, 3.7, 2.8, 3.0 and 2.6% in PDA ; 6.2, 3.5, 2.7, 2.6, 0.8 and 2.8% in Sabouraud's; 1.7,3.9,1.7, 5.2,2.2 and 0.8 % in Czapek's and 3.4, 2.4, 2.5, 2.5, 1.1 and 1.5% in Richard's medium among the total air borne fungi. *Papularia* contributed 2.4% of the aeromycoflora in PDA. The next of 16 genera viz. *Geotrichum, Colletotrichum, Trichoderma, Oidiodendron, Gleocladiopsis, Mucor, Epicoccum, Botrytis, Cephalosporium, Leptographium, Gleocladium, Pullularia, Wardomyces, Fusaiella, Streptomyces, Xylohypha* and *Trichophyton* had a little contribution. Sterile mycellium were contributed 7.0, 6.7, 7.2 and 7.2 % and unidentified fungi were 0.8, 0.7, 1.2 and 0.9% of the total count of aerofungi as recorded in PDA, Sabouraud's, Czapek's and Richard's media, respectively. The variations among the most five dominant air borne fungi are shown in Fig. 1 to 4.

The prevalence of atmospheric fungi: Prevalence of 14 genera *viz. Aspergillus, Candida, Penicillium, Rhizopus, Fusarium, Alternaria Carvularia, Cladosporium, Monilia, Helmonthosporium, Papularia, Geotrichum, Colletotrichum* and *Trichoderma* were recorded 83.1, 84.1, 83.9 and 885.6% in PDA, Sabouraud's, Czapek's and Rhichard's media, respectively during total twelve months observation from November, 2006 to October, 2007 (Fig. 5). Rest of the 14 genera were recorded 8.3, 8.0, 7.1 and 84.2% in the above mentioned media, respectively. Sterile mycelium and unidentified fungi were recorded as 7.8, 7.4, 8.4 and 8.1%, respectively.

The relationships between climate and fungi are complex, and should be examined from numerous perspectives (Sneller, 1984). The effect of the meteorological factors varied among seasons (Li and Kendrick, 1995). Many environmental factors are interrelated and it is often difficult to know which the most significant (Skre, 1981). The averages of the meteorological factors are generally more important than maximum and minimum values (Li and Kendrick, 1995). A better understanding of the relative importance of these factors and their relationships would be of help in determining the relationships of air borne spores to allergies caused by airborne fungal spores (Lyon *et al.*, 1984).

Table 1. Monthly variations in total fungal colony counts with respect to temperature, average relative humidity and rainfall

Months	Temperature (ºC)			Average relative humidity (%)	Rainfall (mm)	Total colonies on			
	Maximum	Minimum	Mean			PDA medium	Sabouraud's medium	Czapek's medium	Richard's medium
November	28.7	17.8	24.2	81	10.8	446	500	276	358
December	26.3	12.6	19.4	79	0	472	445	428	392
January	24.5	9.4	16.9	76	0	645	495	467	352
February	26.4	14.6	20.5	79	26.4	303	296	351	325
March	31.1	17.1	24.1	67	28.8	361	379	344	202
April	35.4	23.7	29.5	69	13.8	305	270	261	318
May	36.1	24.9	30.5	75	125.8	349	275	336	321
June	33.8	25.6	29.7	84	312.1	397	363	355	263
July	31.5	26.6	29.0	88	362.4	311	396	275	283
August	33.2	26.6	29.9	86	235.7	315	318	190	188
September	32.8	26.1	29.4	87	306.9	366	302	248	196
October	31.9	23.3	27.6	85	75.4	386	382	297	316

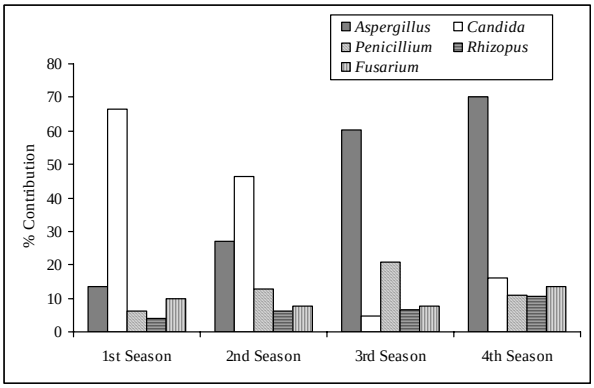

Fig. 1. Percentage contribution of five dominant airborne fungi trapped in the air of Rajshahi Mertropolitan City in different seasons in PDA medium.

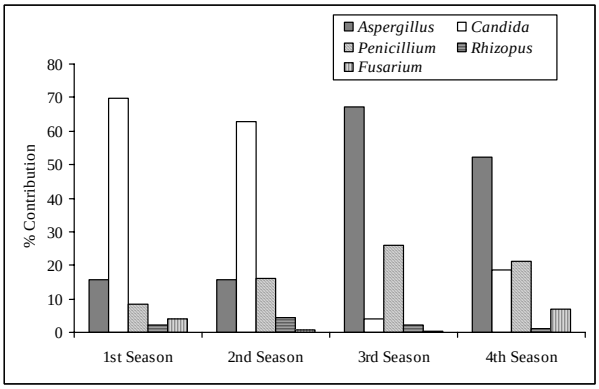

Fig. 3. Percentage contribution of five dominant airborne fungi trapped in the air of Rajshahi Mertropolitan City in different seasons in Czapek's medium.

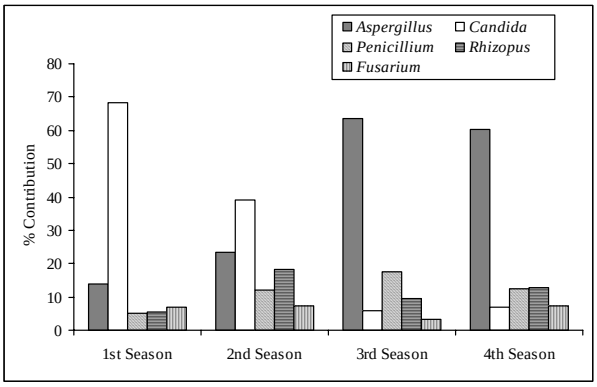

Fig. 2. Percentage contribution of five dominant airborne fungi trapped in the air of Rajshahi Mertropolitan City in different seasons in Saboraud's medium.

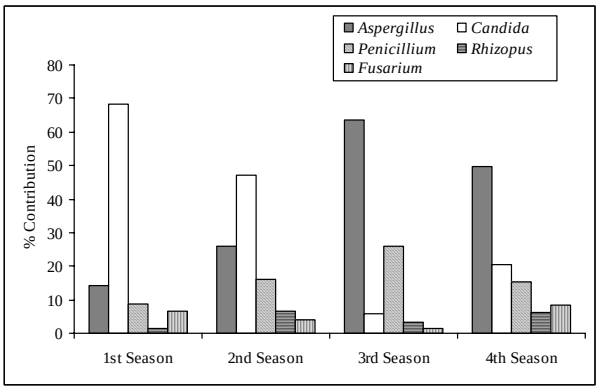

Fig. 4. Percentage contribution of five dominant airborne fungi trapped in the air of Rajshahi Mertropolitan City in different seasons in Richard's medium.

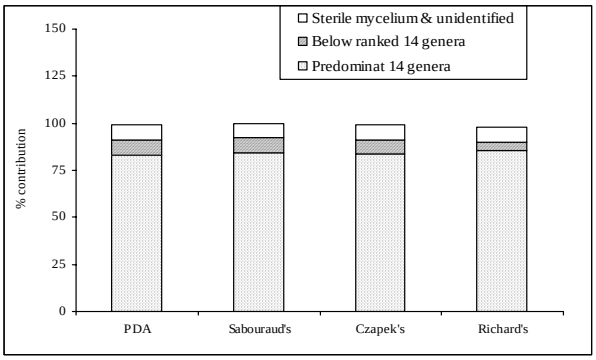

Fig. 5. Prevalence of airborne fungi in the air of Rajshahi Metropolitan City in different media from November 2006 to October 2007.

Conclusion

Climate plays important role in the initiation and exacerbation of mould induced allergic symptoms in man. In order to understand fully the influences of airborne fungi on human allergies, indoor studies should be carried out in parallel to out door studies for distinguishing their roles in triggering allergies. The present work provides important information on airborne fungal incidence in outdoor environment, which is helpful in making public awareness as well as successful hygienic sanitation programmes.

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