

AEROMYCOFLORA OF THE DHAKA UNIVERSITY CAMPUS

JUGLUL AHMED, KS HOSSAIN¹ AND MA BASHAR

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Aeromycoflora, Dhaka University campus

Abstract

In an average, 2106 fungal colony forming units were settled within ten minutes on one square meter area at noon from the air of Dhaka University campus during February 2011 to January 2012. Among the identified fungi, *Aspergillus* was one of the most dominating genus in all the stations over the study period. The second was *Penicillium* followed by *Cladosporium*, *Curvularia*, *Alternaria*, *Fusarium*, *Trichoderma*, *Pestalotia*, *Rhizopus* and *Colletotrichum*. In the dry winter (December - February), *Alternaria*, *Cladosporium*, *Curvularia* and *Rhizopus* showed its peak. Hot humid summer (April) is the most favourable season for the occurrence of *Colletotrichum*. Similarity has been found in fungal biodiversity in the indoor and outdoor air. However, higher number of colony was recorded from indoor (57.23%) than that of outdoor air.

Introduction

The atmosphere of earth contains air borne viruses, bacteria, protozoa, pollen grains, different propagules and vegetative cells of algae, fungi, lichens, bryophytes and pteridophytes. Among these, fungal spores play a significant role in childhood asthma, allergies, mycotoxicity, biodeterioration and infections of man and animals (Burge 1985, Amanianda *et al.* 2010). Airborne fungi are considered to act as indicator of the level of atmospheric bio-pollution (Kakde *et al.* 2001). About 20% of the human population is easily sensitized by normal fungal spore concentrations (up to 10^6 spores/m³) and all fungal spores should be regarded as potentially allergenic. Numerous plant diseases such as rusts, smuts, mildews, leaf spots, etc. are caused by air borne fungi (Kendrick 2000).

Occurrence of fungal spores in the air varied season to season remarkably because of variation of weather conditions. Warm and dry weather favours the development, sporulation and dispersal of conidia of *Cladosporium*, *Epicoccum* and *Alternaria* and the greatest daily concentration of conidia of these genera usually occurs at noon and after noon. It is also varied with vegetation types under and around the study area. The greatest concentrations of *Alternaria* spores were noted at the harvesting time (Chakraborty *et al.* 2003, Stepalska and Jerzy 2005, Kasprzyk 2008).

Surveys on these aspects have been made in different countries of the world following impaction or sedimentation method (Li and Kendrick 1995, Khan *et al.* 1999, Ianovici and Tudorica 2009, Sharma 2011). The sedimentation method is still quite popular in India and some other countries. The method is cheap and simple and is also recommended by Polish Standards (Fleischer *et al.* 2006, Sekulska *et al.* 2007, Sharma 2011).

In Bangladesh, study on aerobiology and air borne bio-particle has been done (Khan and Alio 1984 and Pasha and Hossain 2011). But no investigation on air borne fungi has been carried out particularly in the Dhaka metropolitan where 14.5 million people breathe. The present investigation has therefore, been undertaken to study the monthly distribution pattern of mycoflora in the air of the Dhaka University campus.

¹Author for correspondence: Department of Botany, Jagannath University, Dhaka, Bangladesh <ksh1968@gmail.com>

Materials and Methods

Ten different locations of the Dhaka University Campus (23°42'0" N and 90°22'30" E) were selected for the sampling of air borne fungi. Among these locations five were inside the buildings of Arts Faculty, University Medical Centre, Science Library, Shahidullah Hall, Mycology and Plant Pathology Laboratory of the Botany Department. Remaining five locations were at the open areas of Mol Chottor, Hakim Chottor, Teachers Students Centre, Mukarram Bhaban and Botanical Garden. In this investigation, gravity plate sampling method (Sharma 2011) was followed for isolation of air borne fungi at the selected locations.

Sampling was done monthly at noon from February 2011 to January 2012 excluding the months of October and December. Three culture media *viz.* Czapek's Dox Agar (CDA), Potato Dextrose Agar (PDA) and Emmons version of Sabouraud Agar (SA) media were used (Emmons *et al.* 1977). During the selected day of each month, about 20 ml sterile culture media were poured onto each pair of Petri plates (9 cm in dia.). To check bacterial contamination, a drop of 50% lactic acid was added with each plate under laminar air flow. Each pair of Petri plates were sealed with paraffin strip. At each selected location, nine Petri plates containing sterile culture media (3 plates for each medium) were exposed horizontally for ten minutes on a 1.5 m high tripod stand. After that, the exposed Petri plates were sealed with paraffin strip and taken into the laboratory and incubated at $25 \pm 2^{\circ}\text{C}$ for five days. The fungal colonies developed on the culture media were examined and identified with the help of standard mycological books and manuals.

Per cent abundance and frequency of the fungal colonies was calculated by adopting the formula of Pathak 2012. Temperature and relative humidity of the selected months at the sampling sites were determined by a digital Hygro-thermometer machine (Mextech-1). Monthly precipitation of the Dhaka city was collected from the official website of Bangladesh Meteorological Department.

Results and Discussion

Only viable fungal spores or mycelial fragments of saprophytes and facultative parasites those settled on culture media were formed colonies. A total of 12068 colonies were recorded from the 900 culture plates exposed for ten minutes from the ten sampling points. Nine hundred culture plates make 5.73 m^2 sampling area, therefore, on an average, ca. 2106 colony forming units (CFU) per square meter were settled during ten minutes exposure. Out of this, 219 (1.82%) colonies were sterile mycelia (Table 1). Sharma (2011) reported that *Mycelia sterilia* were 9.19% of the total fungi settled on PDA media at the selected tea garden during the summer. Some fungi need specific culture medium and/or physical stimulation for spore formation and some others are rigorously non-spore forming. Moreover, fungal sporulation highly depends on weather condition. Consequently, fewer non-spore forming fungi were also found in the present study.

Table 1 shows that the spore forming colonies were distributed into ten genera *viz.* *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Penicillium*, *Pestalotia*, *Rhizopus* and *Trichoderma* under the class Zygomycetes and Deuteromycetes. In accordance with Sharma (2011), the member of Ascomycetes and Basidiomycetes were totally absent and anamorphic fungal genera were recorded as a dominant fungal group.

All the fungi recorded in Table 1 were grown on the three culture media used in the present investigation, none was absent on any culture media. Their abundance on the different culture media, however, varied. The highest number of colony was found on CDA (4253) followed by PDA (4172) and SA (3642). Per cent abundance of the fungi on these three media reveals that *Aspergillus*, *Cladosporium*, *Alternaria*, *Curvularia* and *Pestalotia* were preferred to grow on

CDA, whereas, *Penicillium*, *Trichoderma* and *Rhizopus* were on PDA and *Fusarium* and *Colletotrichum* were on SA.

Among the identified fungi *Aspergillus* was one of the most dominating genera at all the locations and months in the air of the campus (Table 1). Its collective per cent abundance was 42.60 on the three nutrient media followed by *Penicillium* (27.61), *Cladosporium* (8.49), *Curvularia* (4.76), *Alternaria* (4.65), *Fusarium* (3.25), *Trichoderma* (2.56), *Pestalotia* (2.04) and *Rhizopus* (1.36). The abundance of *Colletotrichum* was the lowest (0.87). This result is in agreement with similar variations that have been reported from India (Sharma 2011). Following gravity plate method, highest per cent abundance of *Aspergillus* (41.35) in the air of Darjeeling tea garden followed by *Mucor* (10.34), *Penicillium* (9.19), *Rhizopus* (8.04), *Trichoderma* (5.74), *Curvularia* (3.44), *Nigrospora* (2.29), and *Cladosporium* (1.14) was observed. The variation in aeromycoflora and its abundance with the present study might be due to the difference of weather condition and vegetation between the study areas.

Table 1. Air borne fungi on three culture media during February, 2011 to January, 2012 at the Dhaka University campus.

Fungal genera	Collective number of fungal colonies on three culture media (*)			Total colony	Frequency (%)
	CDA*	PDA	SA		
<i>Alternaria</i>	247 (2.05) **	186 (1.54)	128 (1.06)	561 (4.65)	28.89
<i>Aspergillus</i>	1853(15.36)	1693 (14.04)	1594 (13.21)	5141 (42.60)	100.00
<i>Cladosporium</i>	394 (3.27)	352 (2.92)	277 (2.30)	1023 (8.49)	46.67
<i>Colletotrichum</i>	35 (0.29)	32 (0.26)	39 (0.32)	105 (0.87)	7.78
<i>Curvularia</i>	227 (1.88)	185 (1.53)	164 (1.36)	576 (4.76)	31.11
<i>Fusarium</i>	47 (0.39)	140 (1.16)	205 (1.70)	392 (3.25)	22.22
<i>Penicillium</i>	1116 (9.25)	1237 (10.26)	977 (8.10)	3331 (27.61)	100.00
<i>Pestalotia</i>	99 (0.82)	56 (0.46)	92 (0.76)	247 (2.04)	14.44
<i>Rhizopus</i>	51 (0.42)	60 (0.50)	53 (0.44)	164 (1.36)	12.22
<i>Trichoderma</i>	106 (0.88)	123 (1.02)	80 (0.66)	309 (2.56)	18.89
Sterile mycelia	78 (0.65)	108 (0.90)	33 (0.27)	219 (1.82)	14.44
Total colonies	4253(35.25)	4172 (34.58)	3642 (30.17)	12068 (100)***	

*CDA = Czapek's Dox Agar, PDA = Potato Dextrose Agar and SA = Sabouraud Agar. **Per cent abundance of fungal colonies within parenthesis. ***Total culture plates used = 900 (5.73 m²), number of CFU settled = 2,106/m².

Pathak (2012) from Madhya Pradesh, India found abundance of *Aspergillus* and *Penicillium* which were 32 and 9%, respectively by using particle sampler. The abundances of these two fungi were also quite higher in the present investigation. Sedimentation method does not permit exact quantitative determination. Some earlier observations reported that results of sedimentation method are usually higher than numbers obtained with the use of air samplers (Fleischer *et al.* 2006). However, data collected by sedimentation method allow the drawing of correct conclusions on types of fungi present in the air and can give a rough approximation of fungal concentration.

Table 1 also showed that *Aspergillus* and *Penicillium* were found in the highest per cent frequency (100) followed by *Cladosporium* (46.67), *Curvularia* (31.11), *Alternaria* (28.89), *Fusarium* (22.22), *Trichoderma* (18.89), *Pestalotia* (14.44), *Rhizopus* (12.22) and *Colletotrichum* (7.78). Pathak (2012) recorded *Aspergillus* in the highest per cent frequency (95.85) followed by *Penicillium* (84.80), *Alternaria* (83.30), *Cladosporium* (54.15), *Curvularia* (41.70), *Rhizopus*

(41.66), *Fusarium* (39.15), *Mucor* (33.35), *Epicoccum* (33.3), *Phoma* (29.15), *Nigrospora* (21.35) and *Trichoderma* (16.65). The variation in aeromycoflora and its frequencies with the present study might be due to the variation of meteorological parameters of the study areas and methods used in the investigations.

Tables 2 and 3 show variations in sedimentation of fungi on the three culture media among seasons and locations. Number of colony of five fungal genera *viz.* *Aspergillus*, *Fusarium*, *Penicillium*, *Pestalotia* and *Trichoderma* showed its peak and near to peak during warm rainy monsoon (June - September). Among them *Aspergillus*, *Penicillium* and *Pestalotia* found at its peak in indoor location. Whereas, two others found at its peak in outdoor location. *Alternaria*, *Cladosporium*, *Curvularia* and *Rhizopus* showed its peak and near to peak during dry winter (December - February) and *Colletotrichum* found its peak in hot humid summer (April).

Table 2. Monthly total fungal colonies of air borne fungi on three culture media and climatic factors during February, 2011 to January, 2012 at 10 different sampling sites of the Dhaka University campus.

Fungal genera	Collective number of air borne fungi in ten different months									
	Feb	Mar	April	May	June	July	Aug	Sep	Dec	Jan
<i>Alternaria</i>	104	62	58	42	18	33	25	40	95	84
<i>Aspergillus</i>	123	193	335	509	624	714	843	1072	379	349
<i>Cladosporium</i>	204	80	73	61	61	67	53	50	198	162
<i>Colletotrichum</i>	7	18	33	13	10	7	6	0	5	6
<i>Curvularia</i>	89	65	33	47	27	19	37	24	136	99
<i>Fusarium</i>	25	28	30	35	46	57	73	62	23	13
<i>Penicillium</i>	313	254	219	209	250	339	411	546	347	443
<i>Pestalotia</i>	33	25	10	0	0	0	109	12	11	47
<i>Rhizopus</i>	25	11	0	11	7	5	7	15	47	36
<i>Trichoderma</i>	19	21	0	15	22	57	89	63	11	12
Sterile mycelia	33	22	18	12	0	0	79	11	14	30
Total colonies	975	786	809	954	1065	1298	1732	1895	1266	1288
Relative humidity (%)	54.0	47.8	72.8	72.0	81.1	61.6	63.3	72.2	52.4	57.1
Temp.(°C)	24.3	27.5	29.2	31.1	31.7	27.1	26.9	31.5	21.3	20.2
Precipitation (mm)	0.0	6.8	8.6	6.3	15.5	12.7	15.1	18.4	0.0	7.0

Cladosporium is the fungal genera most correlated with meteorological parameters. This may be attributed to the size and nature of conidia. *Cladosporium* produces dry conidia in chains easily carried through air. Therefore, dispersion of *Cladosporium* spores is more influenced by meteorological parameters than *Alternaria* spores (Awad 2005). In accordance with the present study, Levetin (1995) reported that members of dry-air spores (*Cladosporium*, *Alternaria* and *Curvularia*) were found in greatest abundance in the atmosphere characterised by low humidity, generally during warmer afternoon hours.

Table 3 shows that fungi which were dominant in the indoor air were also recorded in significant concentration in the outdoor air and *vice versa*. The table also shows that similarity has been existed in fungal biodiversity in the indoor and outdoor air. This is in agreement with the report from India (Kotwal *et al.* 2010). In comparison to 5 outdoor sampling sites, higher contribution (57.23%) were found in 5 indoor sampling sites. In accordance with the present investigation Sekulska *et al.* (2007) also reported that amount of fungi was higher in the indoor than that of outdoor air.

Table 3. Number of indoor and outdoor air borne fungi on the three culture media at ten different sampling sites of the Dhaka University campus during February, 2011 to January, 2012.

Fungal genera	Collective number of air borne fungi at ten different sampling sites of DU campus													
	Indoor							Outdoor						
	AF*	UMC	SL	SH	MPPL	Total	MCH	HCH	TSC	MBH	BG	Total		
<i>Alternaria</i>	42	42	36	56	81	257	15	31	49	71	138	304		
<i>Aspergillus</i>	537	624	589	843	677	3270	383	420	377	332	359	1871		
<i>Cladosporium</i>	148	67	94	90	151	550	25	12	89	112	235	473		
<i>Colletotrichum</i>	7	6	0	1	24	38	11	17	15	8	16	67		
<i>Curvularia</i>	50	33	62	44	86	275	21	24	68	65	123	301		
<i>Fusarium</i>	18	22	35	18	63	156	32	18	10	33	143	236		
<i>Penicillium</i>	360	364	419	313	541	1997	225	224	309	275	301	1334		
<i>Pestalotia</i>	24	13	44	24	53	158	7	11	15	0	56	89		
<i>Rhizopus</i>	11	7	20	12	21	71	7	3	17	18	48	93		
<i>Trichoderma</i>	9	1	1	3	32	46	18	44	39	33	129	263		
Sterile mycelia	29	17	20	15	8	89	4	14	14	47	51	130		
Total colony	1235	1196	1320	1419	1737	6907**	748	818	1002	994	1599	5161		

*AF = Arts Faculty, UMC = University Medical Centre, SL = Science Library, SH = Shahidullah Hall, MPPL = Mycology and Plant Pathology Laboratory, MCH = Mol Chottor, HCH = Hakim Chottor, TSC = Teachers Students Centre, MBH = Mukarram Bhaban and BG = Botanical Garden. ** 57.23% of the total colony.

Among the fungi, found in the present investigation, *Atlermaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus* were reported as pathogenic to plants and/or human and strongly allergenic to human. *Colletotrichum* and *Pestalotia* were reported as only plant pathogenic (Burge 1985, Kendrick 2000, Kotwal *et al.* 2010). The present study contributes to our knowledge of airborne spores in the Dhaka city. Regular monitoring of airborne fungi can be helpful in the prevention of fungal allergic diseases in the city.

References

- Aimanianda V, Bayrz J, Bozza S, Kniemeyer O and Perruccio K 2010. Clever cloak prevents immune recognition of air borne fungal spores. 4th advances against Aspergillosis, Asp. Newsl. **460**: 1117-1123.
- Awad A 2005. Vegetation: A source of air fungal bio-contaminant. Aerobiol. **21**: 53-61.
- Burge HA 1985. Fungus allergens. Clin. Rev. Allergy **3**: 319-329.
- Chakraborty PS, Gupta-Bhattacharya and Chanda S 2003. Aeromycoflora of an agricultural farm in West Bengal, India. Grana. **42**: 248-254.
- Emmons CW, Binford CH, Utz JP and Kwon-Chung KJ 1977. Medical Mycology, 3rd ed., Lea & Febiger, Philadelphia, PA. pp. 535.
- Fleischer RM, Bober-Gheek B, Bortkiewicz O and Rusiecka-Ziolkowska J 2006. Microbiological control of airborne contamination in hospitals. Indoor and Built Environ. **15**(1): 53-56.
- Ianovici N and Tudorica D 2009. Aeromycoflora in outdoor environment of Timisoara City (Romania). Not. Sci. Biol. **1**(1): 21-28.
- Kakde UB, Kakde HU and Saoji AA 2001. Seasonal variation of fungal propagules in a fruit market environment, Nagpur (India). Aerobiologia **17**: 177-182.
- Kasprzyk I 2008. Aeromycology- main research fields of interest during the last 25 years. Ann. Agric. Environ. Med. **15**: 1-7.
- Kendrick B 2000. The fifth kingdom. 3rd ed. Focus Publishing, R. Pullins Co. Newburyport MA 01950, USA. pp. XI+373.
- Khan MR and Alio S 1984. An aerobiological study of the Dhaka City and its sub-urban areas. Bangladesh. J. Bot. **13**(2): 214-219.
- Khan ZU, Khan MAY, Chady R and Sharma PN 1999. *Aspergillus* and other moulds in the air of Kuwait. Mycopathol. **146**: 25-32.
- Kotwal SG, Gosavi SV and Deore KD 2010. Aeromycoflora of outdoor and indoor air of residential area in Nashik. Asian J. Exp. Biol. Sci. **SPL**: 24-30.
- Levetin E 1995. Fungi. p. 87-120, *In*: Ianovici N and D Tudorica 2009. Aeromycoflora in outdoor environment of Timisoara City (Romania). Not. Sci. Biol. **1**(1): 21-28.
- Li De-Wei and Kendrick B 1995. A year-round comparison of fungal spores in indoor and outdoor air. Mycologia. **87**(2): 190-195.
- Pasha MK and Hossain MS 2011. Airborne bio-particulate objects at Chittagong University campus. Bang. J. Bot. **40**(2): 189-191.
- Pathak K 2012. An extramural aeromycological investigation of dental college hospital associated environment. Int. J. Env. Sci. **2**(4): 1952-1961
- Sekulska M, Stryjakowska, Piotraszewska-Pajak A, Szyszka A, Nowicki M and Filipiak M 2007. Microbiological quality of indoor air in university room. Polosh J. Env. Stud. **16**(4): 623-632.
- Sharma K 2011. Comparative study of aeromycoflora in relation to soil mycoflora of Darjeeling tea garden, India. Recent Research in Science and Technology **3**(5): 84-86.
- Stepalska D and Jerzy W 2005. Variation in fungal spore concentrations of selected taxa associated to weather conditions in Cracow, Poland in 1997. Aerobiol. **21**: 43-52.

(Manuscript received on 24 June, 2013; revised on 27 November, 2013)