



Research article

Study on airborne fungal diversity in Kokrajhar Science College Campus, Assam, India

Junuwara Almina^{1*}, Miniswring Basumatary², Suraj Kumar Nath³
and Mohesh Gogoi^{1,2}

¹Department of Botany, Science College, Kokrajhar, BTC, Assam, India

²Institutional Biotech Hub, Science College, Kokrajhar, BTC, Assam, India

³Department of Botany, Bodoland University, Kokrajhar, BTC, Assam, India

*Corresponding Author: mohesh.gogoi@rediffmail.com

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Abstract: The present study deals with an investigation on the distribution of airborne fungi within Science College campus, Kokrajhar that has been conducted during 2018. The airborne fungal colonies were isolated through the settle plate method and studied. Total 117 airborne fungal colonies were isolated, of which 20 species belonging to 6 Classes and 9 genera could be identified in the study. Among them, *Curvularia* species were found the most prevalent in the college campus followed by *Pythium*, *Penicillium*, *Rhizopus*, *Botrytis* and *Geotrichum* in the month of May. In August, *Aspergillus* species was found to be highest followed by *Curvularia*, *Penicillium*, *Rhizopus* and *Paecilomyces* and *Trichoderma* respectively. Conspicuously *Curvularia* and *Aspergillus* were more abundant in both the indoor and outdoor environment of the college campus in May and August respectively.

Keywords: Airborne - Fungi - Aeroallergen - Diversity.

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INTRODUCTION

Assessing fungal microflora diversity in a place through proper investigation is prerequisite to understand the availability of both harmful and beneficial species in relation to human health. Moreover, it is also important to know the distribution of fungal species in the air, its association with human health problems and the quality of air. The fungal spores are known as environmental bio-particles causing allergic manifestation in human beings. Some certain airborne fungi are pathogenic that causes serious diseases in both plants and animals. Plants and animals including human have varied sensitivity to the airborne fungi, and the sensitivity causes different levels of allergic reaction when come in contact with fungal spore. The distribution of airborne fungi is very unpredictable although some studies reported the regional distribution patterns of microorganisms in soil and water (Green *et al.* 2004). Fungal spores are ubiquitous in the earth's atmosphere (Bowers *et al.* 2009). Meanwhile, environmental factors like humidity and wind speed affect the distribution and release of spores (Nielsen 2003). Indoor environments are potential sources of fungal spores and harmful organic compounds such as mycotoxins which can be harmful to human health (Cabral 2010).

Although air is the primary medium for the dispersal of microorganisms, the study on the distribution of airborne fungi has not been done to that extent. Several studies have shown that a wide range of micro-fungi are present in air and water supplies etc. (Pakshir *et al.* 2007, Hedayati *et al.* 2011). The recent studies done by Bowers *et al.* (2013), Cao *et al.* (2014) etc. has indicated that specific fungal spores are linked with the occurrence of public health problems. Several studies were being carried out to investigate the diversity of fungi in places *viz.* hospitals, educational settings, industrial sites etc. of various parts of Assam, India. However, in our region, such studies have not been done yet and no such evidence was found. In the present study, the college campus was selected as it is a hub for more than 1500 students. Moreover, it is situated in low land surrounded by the moist place that makes a favorable ground for a number of microorganisms. The identification of fungal microflora can help us to find the problems associated with allergy, respiratory diseases,

fungal infections in a particular environment like school & colleges, hospitals, industries and home. In the present study, an attempt has been made to investigate the distribution of airborne fungi and to identify the prevalent fungal species within the campus and to understand the cause of various health issues related to fungi. Such a study is of great concern to evaluate the potential health hazards of allergenic and toxigenic fungi and to make a database of distribution of airborne fungi in a particular area.

MATERIALS AND METHODS

Study area

The Kokrajhar Science College is located in the western part of Kokrajhar town approximately 500 meters away (Fig. 1). The campus covers an area of 4.5 Acres and it is situated in the eastern part of the Gaurang River in between 26°24' N latitude and 90°15' E longitude, at an average elevation of 30–45 meters above mean sea level. The campus is bounded by human settlement urban villages in the East and South, Dotma-Kokrajhar road in the West and Paddy field with human settlement in the North. The area is characterized by almost plain topography with sparse vegetation. The average temperature ranges from minimum 15°C to maximum 35°C throughout the year. The average humidity remains almost the same with variation from 62% in winter to 87% in the post-monsoon period. The average annual rainfall of the district is 3102.4 mm with 110 annual average rainy days. The maximum rainfall occurs during the period from April to August.

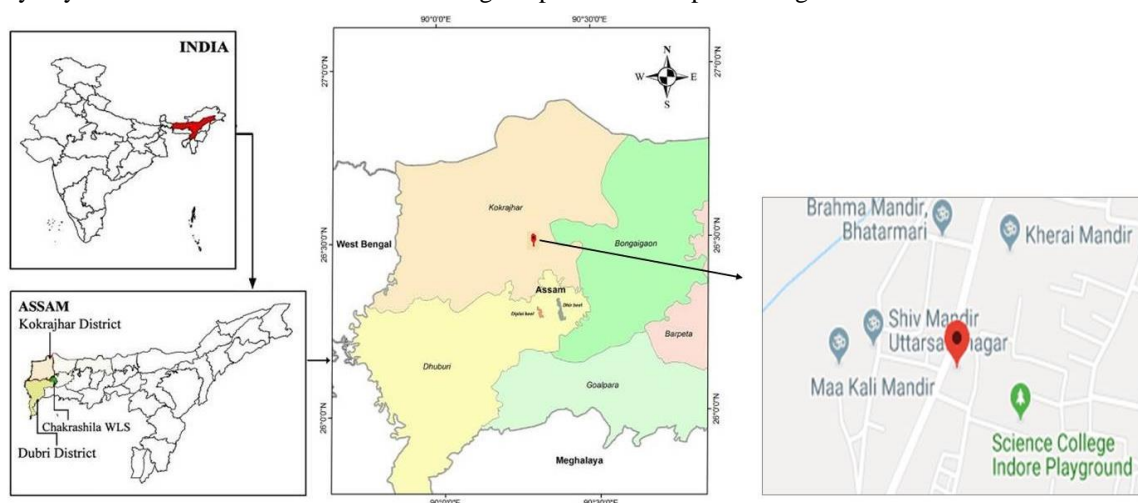


Figure 1. Map showing the location of the study area.

Methodology

The isolation of the airborne microflora was done through the settle plate method using PDA media. First the Potato dextrose agar (PDA) media was prepared and aseptically poured into petridishes and allowed to solidify; these were then exposed in air at 1.5 meter height for 15 minutes in 5 different locations Site-1 (Backside of college campus), Site-2 (Botanical Garden), Site-3 (Auditorium), Site-4 (Classroom), Site-5 (Central Library) of the college campus. After exposure for 15 minutes, the petridishes were collected and incubated at $28^{\circ}\pm 2^{\circ}$ C for 3–5 days. Thereafter, colonies grown were counted in each petridish and their mean was considered. Subcultures were also done for some species for better observation of the individual characteristics. The fungal colonies were identified with the help of authentic literature and by comparing the morphological (color, texture, shape, diameter appearance of colony) and microscopic characteristics (reproductive structures, conidia structure along with conidiophores size, shape and mycelium structure). Microphotography was done by using LABOMED Lx 400 microscope at IBT hub laboratory, Science College, Kokrajhar. The lacto phenol and aniline blue wet mount preparation was used for staining and observing under microscope.

RESULTS

A total of 117 colonies belonging to 9 different fungal genera were isolated (Table 1). The highest number of airborne fungal colonies were found in Site-3 (Auditorium=40) followed by Site-2 (Botanical Garden=32), Site-5 (Central Library=18), Site-1 (Backside of college campus=15), and Site-4 (Classroom=12) respectively. From 5 sources of the sample, the *Culvularia* was found to be most prevalent accounting 41.37% of the total spore in May and 28.56% in August (Table 2 & 3). The airborne fungus of the genus *Aspergillus* was most encountered with a relative prevalence in terms of isolation equal to 45.71%, followed by *Curvularia* (28.56%), *Penicillium* (14.29%), *Rhizopus* (5.71%), *Trichoderma* (2.86%) and *Peicilomyces* (2.86%) respectively in the month of August (Table 3). Total fungal concentration in indoor environment was (10 cfu/plate/15min) in May and (13

cfu/plate/15min) in August. In the case of the outdoor environment, the colony forming unit was 11 cfu/plate/15min in the month of May and 12 cfu/plate/15min in the month of August (Table 1). Both temperature and humidity were high during the study period. The study indicates that the outdoor and indoor air of the college campus harbor variety of pathogenic and allergenic airborne fungal spores that may serve as the main source of contamination in indoor environments of the campus viz. auditorium, library, offices, classrooms, etc. The *Curvularia* species was observed to be the most common fungi isolated from both indoor and outdoor air of different sites of the college campus (Table 2 & 3). The indoor air of Site-3 had the highest fungal population and Site-4 having the least population (Table 1). *Curvularia* (41.37%) was the most prevalent isolated fungus followed by *Pythium* (31.04%), *Penicillium* (10.34%) and *Rhizopus* (10.34%) that reflects the findings of Fernstrom & Goldblatt (2013) who reported these species to be the permanent outdoor air flora (Table 2). *Botrytis cinerea* is known as the fungal air species in an outdoor environment that account for 3.45% of the total fungal species in the month of May (Table 2). Common fungal species in air include *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium notatum*, *P. chrysogenum* (Li & Kendrick 1995). The airborne fungi *Curvularia*, *Geotrichum*, *Aspergillus* and *Penicillium* were revealed to be associated with various common health problems. The prevalence of such aeroallergens fungi may due to the influence of favorable ecological conditions inside the college campus. Besides their allergic nature, some are non-allergic and beneficial and they can be used for various purpose like producing antibiotics, enzymes production, biodegradation process and even as biological controlled agents (Shen *et al.* 2001).

Table 1. Number of colonies grown in the cultured medium exposed in the air of different sites of the college campus in two different months.

Sites	Number of colonies (cfu/plate/15min)		
	May	August	Total
Site-1	08	07	15
Site-2	17	15	32
Site-3	21	19	40
Site-4	02	10	12
Site-5	07	11	18
Total	55	62	117

Table 2. Occurrence of airborne fungi in different sites of the college campus in the month of May.

S.N. Identified species	Number of identified colonies					Total spore load (%)
	Site 1	Site 2	Site 3	Site 4	Site 5	
1 <i>Curvularia lunata</i> (Wakker) Boedijn	2	3	4	0	0	09 (31.03%)
2 <i>Carvularia</i> sp.	0	1	2	0	0	03 (10.34%)
3 <i>Pythium aphanidermatum</i> (Edson) Fitzp.	1	1	2	0	0	04 (13.79%)
4 <i>Pythium insidiosum</i> De cock	0	1	1	0	0	02 (06.90%)
5 <i>Pythium diclinum</i> Takunaga	1	0	0	0	1	02 (06.90%)
6 <i>Pythium</i> sp.	0	0	1	0	0	01 (03.45%)
7 <i>Penicillium chrysogenum</i> Thom	0	2	0	1	0	03 (10.34%)
8 <i>Rhizopus oryzae</i> Went & Prins. Geerl.	1	0	0	0	2	03 (10.34%)
9 <i>Botrytis cinerea</i> Michelle S. Oliveira	0	0	1	0	0	01 (03.45%)
10 <i>Geotrichum candidum</i> Link	0	0	1	0	0	01 (03.45%)
Total	05	08	12	01	03	29 (100%)

Table 3. Occurrence of airborne fungi in different sites of the college campus in the month of August.

S.N. Identified species	Number of identified colonies					Total spore load (%)
	Site 1	Site 2	Site 3	Site 4	Site 5	
1 <i>Aspergillus niger</i> Van Tieghem	0	2	2	0	2	06 (17.14%)
2 <i>Aspergillus flavus</i> Link	1	2	1	1	0	05 (14.29%)
3 <i>Aspergillus</i> sp.	0	1	0	0	1	03 (08.57%)
4 <i>Aspergillus fumigatus</i> Fresen.	0	0	2	0	0	02 (05.71%)
5 <i>Curvularia lunata</i> (Wakker) Boedijn	3	2	0	2	1	08 (22.85%)
6 <i>Curvularia oryzae</i> Bugnic.	1	1	0	1	0	02 (05.71%)
7 <i>Penicillium notatum</i> Thom	1	0	3	0	1	05 (14.29%)
8 <i>Rhizopus oryzae</i> Went & HC Prinsen Geerlings	0	0	1	1	0	02 (05.71%)
9 <i>Trichoderma viridi</i> Pers.	0	0	1	0	0	01 (02.86%)
10 <i>Peicilomyces lilacenus</i> (Thom) Samson	0	0	1	0	0	01 (02.86%)
Total	06	08	11	05	05	35 (100%)

DISCUSSION

The study on airborne fungi has attained maximum importance due to their ubiquitous nature of existence and the increase of allergies and infection caused by them (Larsen & Gravesen 1991). Globally, more than 180 genera of fungi are associated with allergies and serious human infections (Horner *et al.* 2004). It has also been reported that micro-fungi cause allergies, spoilage of foods and many other adverse effects and some may even produce mycotoxins that can adversely affect human and animal health (Burge & Rogers 2000). So, indoor and outdoor airborne fungal spore monitoring in the college campus may be very useful in future from the allergological point of view. Isolation and identification of airborne fungi are of great importance to find the causes associated with the minor health problem in a particular working environment. This can be achieved by isolation and identification of disease causal agents on appropriate culture media.

The microfungi can live in extreme conditions in almost all places and regions. The airborne fungi such as *Aspergillus*, *Penicillium* etc. are causative agents of fungal allergies (de Ana *et al.* 2006). Besides causing allergies, the *Aspergillus* species are also responsible for mycotoxin production (Nielsen 2003). *Aspergillus* and *Penicillium* spores are the most widespread aeroallergens in the world (Asan *et al.* 2003). *Aspergillus fumigatus*, *A. niger*, *Penicillium notatum* and *P. chrysogenum* identified in our study are also observed to be widespread in the campus that implies the findings of Asan *et al.* (2003). Fungal concentrations, in the indoor environment of the campus, were high in the library and auditorium this may be due to the presence of paper, house dust etc. As per the cases of health issues reported by the college community, it can be inferred that the airborne fungi *Aspergillus*, *Curvularia* and *Penicillium* are responsible for health issues like skin irritation, eye infection, allergy, asthma etc. Moreover, *Trichoderma viride* species also found in the campus accounting 2.86% of the total spore load in August which is beneficial to be used as biocontrol agent having the capacity to inhibit mycelial growth of other fungi plant pathogen such as *Sclerotium rolfsii* (Basumatary *et al.* 2015). This species can be tested as a biological control agent against some fungal diseases. Furthermore, some isolated species were also found to be utilized in beneficial ways such as *Curvularia lunata* is used as antimicrobial agents (Avinash *et al.* 2015), *Penicillium* species are used to produce different kinds of antibiotics and pectinase enzyme (Banu *et al.* 2010) and *Rhizopus* species are used for the production of Lactic acid from renewable organic materials (Zhang *et al.* 2007) and *Pythium* is used as biological control agent for their rapid germination of sporangia (Whipps & Lumsden 2008).

The wide distribution of airborne fungi may be due to multiple factors which account for dispersal of fungal spores into the air and water at the same time. The fungi available in the air mainly originate from soil, plants and water. Fungal spores in aquatic environments may be transferred to the air by wave action. Their distribution is related to wind, humidity, temperature, rainfall, altitude, vegetation, and some specific reservoirs of contamination (Asan *et al.* 2003). Relative humidity was consistently less than 70% in May that increased to 80% in August that makes the environment more prevalent of fungal spores load in August. Both *Curvularia* and *Phytium* were more abundant in the outdoor environment this implies that these fungi enter houses from outside. Spore percentage of *Aspergillus* was highest in August whereas the species not encountered in the month of May. A positive correlation was observed for the concentration of airborne fungi with humidity in both indoor and outdoor environment.

CONCLUSION

The fungal species *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium notatum*, *P. chrysogenum*, *Carvularia* sp. identified in the present study are aeroallergens and may cause infections. Some of them are non-pathogenic and have the potential to be utilized as biotic resource for other purposes such as antibiotic, enzyme production and biodegradation of waste materials. The study generated a database about the distribution and composition of airborne microflora within the college campus that will be beneficial for future use since no such studies was being conducted earlier. A detailed study is required to evaluate the total microbial diversity of the campus as microbes are ubiquitous in nature. And their availability also varies as per the season, so continuous season wise study is recommended for complete evaluations of fungus species present in the college campus.

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