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Research Article

DIURNAL VARIATION OF AEROMYCOFLORA AT ASSAM DOWN TOWN UNIVERSITY CAMPUS- A PRELIMINARY INVESTIGATION

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ABSTRACT
Aeromycological study was carried out at three different time i.e 7AM, 1PM and 7PM in Assam down town University, Panikhaiti, Guwahati (Latitude: 26.2016 N and Longitude: 91.8614 E) by settle plate method for two occasions at monthly interval. A total of 123 fungal colonies were recorded, belonging to 8 genera and 19 species. Diurnal fluctuation of aeromycoflora were observed and a total of 55, 33 and 36 colonies were recorded at 7AM, 1PM and 7PM respectively in two occasions. *Cladosporium spp*, represented highest amount occurrence (29.26%) followed by *Aspergillus niger* accounting for 11.38% of total aeromycoflora. There are six species of *Penicillium* which occurred in the first occasion and present occurrence varied from 2.43-3.25%. Sterile brown mycelium occurred 9.75% of total aeromycoflora. Other species of fungi i.e. *Curvularia, Geotrichum (yeast), Penicillium*, *Mucor*, *Bortrytis*, *Aspergillus flavus*, *Aspergillus versicolor,* Red yeast, sterile colonies varied in the range of 2-5% of total aeromycoflora. Significance and environmental impact of aeromycoflora recorded in the study was discussed in the light of available literature.

Keywords: *Aeromycoflora, Aeroallergens, Environmental monitoring, Air pollution*

INTRODUCTION
Air is composed of complex mixture of biotic and abiotic components aerosolized in gaseous medium. Pollen grain, fern spores, moss, fungal spores, and hyphal fragments, algal spores and filaments, bacteria, actinomyceties, plant particles, lichens, animal hairs etc. observed as predominant biotic components and contribute 10-20% of total particulate matter (Chandramauli *et al*., 2005). Air borne microbes originate from earth crust, crop debris, soil, rivers, volcanic activities, animals, plants including anthropogenic activities. The air of indoor environment may contain as high as 10^9 cfu/M^3 viable filamentous fungi and yeast (Pieckova and Jesenska,1999),80 cfu/M^3 (Shelton *et al.,* 2002), while over 16 cfu /M^3 of viable bacteria (Chandramauli *et al.* 2005). In general indoor environment acquire microflora from outdoor environments and maximum microbes enter the open houses than air conditions houses and which may be a source of harmful mycotoxin, ß-Glucans, and volatile organic compounds (Dong and Yao, 2010). (Larsen and Gravesen, 1991) studied air borne out door fungi in Copenhagen. Globally, more than 180 fungi are associated with allergies and serious human infections (Horner *et al*., 1995; Moelling and Broecker, 2020).
It has also been reported that micro fungi cause allergies, spoilage of foods and many other effects and some more even produce mycotoxin that can adversely affect human and animal health (Burge & Rogers, 2000). Common health effect caused by exposure to particulate air pollution (PM2.5 and PM10)includes allergies, bronchitis, COPD, coughing, eye irritation, cardiovascular disease, rhinitis, cancer and increased risk of contracting SARS virus (Moelling and Broecker ,2020).
Never the less microbial exposure of human have been shown to be beneficial as far as human micro biome is concerned which provide immune regulations protecting against nonspecific infections and injudicious use of sanitizer pose a risk to reduction of population(Graham *et al.*, 2021).

Fungi occupy a significant place in earth's biotic environment and are found in diverse habitats in rocks, air, water and soil environment and they contribute substantially towards decomposition of forest litter besides agricultural, environmental and ecosystem benefits (Hyde *et al.,* 2019). However, spores of pathogenic fungi like *Fusarium solani, Pyricularia oryzae, Puccinia graminis, Phytophthora infestans and Exobasidium vexans* etc. are in airborne cause destructive plant disease in different crop plants (Klinkowski, 1970). Horizontal and vertical distribution of airborne fungal spore is largely governed by seasonal change and local climatic condition (Gregory 1961).There is ample opportunity of forecasting of plant disease by monitoring air borne spore load over a crop field before onset of disease and success has been achieved in many situation (Mehta, 1952; Tilak, 2004).

Research on Aerobiology in India: (Cunningham, 1873) studied air spora in Calcutta and marked the beginning of aerobiological study in India. Subsequently studies on aerobiology resumed in Jaipur, Delhi, Kolkata (Bose Institute), Bangalore, Vishakhapatnam, Aurangabad, Gauhati after a long gap and vast amount of scientific information were generated (Sreeramulu, 1967; Sinha, 1976; Tilak 2004;Vittal, 2005). Establishment of Indian Aerobiological Society (IAS) in 1980 was a mile stone in aerobiological Research and Late Prof Sunirmal Chanda was the First President. Subsequently an All India Coordinated project on Aerobiology was sponsored by CSIR in 1980 and 41 centers participated in 20 States all over India. In this project qualitative analysis of fungal and pollen grain were carried out (Nair and Joshi 1980-83). Another All India coordinated project entitled "Aeroallergens and human health"1994-98 was sponsored by Ministry of Environment and Forest Government of India and successfully accomplished (Anonymous 2000). A comprehensive review of current scientific progress of Indian aerobiology was published by Vittal,2005 and a glimpse of progress of clinical aerobiology in India was well documented (Singh 2017).

Climate of Assam is warm and humid in summer and cool and dry in winter. Mean annual rainfall is 2340 mm and 92% of total rainfall recorded during wet monsoon period. Mean minimum and maximum temperature range from 23°-31° C and 10°-25°C respectively. Soil is predominantly alluvial and acidic (pH 4.2-5.8). Geoclimatic condition of Assam is conductive for growth and survival of various fungus species. There is therefore a need to assess quantitative as well as qualitative composition of airborne fungi in local environment in Assam, under changing climate conditions.

Present work aims to determine the quantitative and qualitative composition of fungus species in air of Assam down town University campus by conventional petri plate's exposure method and discuss the role of fungi in plants, man and environment in the light of available scientific information.

MATERIALS AND METHODS

Site of Experiment: The work covers almost 2 months from 29th January to 12th April, 2021. The average temperature throughout these period was from minimum 13˚C to maximum 27˚C.The average humidity remains almost the same with variation from 73% to 63% from January to March.

Mycological Technique: The isolation of airborne mycoflora was accomplished by settle plate method as per Turner, 1966. Potato Dextrose Agar (PDA) media contained in 9 cm petriplate in triplicate was used for the study. Plates were exposed for 10 minutes by placing over a wooden support at a height of 1.5 meter from ground level at 5 meter apart.

Incubation: The petri plates were collected after exposure and placed inside sterile polythene bag and incubated at 28º±2º C for 4-5 days in the laboratory.

Observation: Aeromycoflora appearing on triplicate petri plates were examined under light microscope (Trinocular Olympus research Microscope) under 100x, 400x and 1000x after staining with aniline blue over a microslide. Microscopic characteristic like conidial ontogeny, atypical reproductive structure, conidiophore, philaides, their size, shape were recorded. A few microphotograph of aeromycoflora were shown in Plate 1 (a-1). The fungi were identified with the help of available literature (e.g Burnett and Hunter, 1972; Subramanium, 1970). Qualitative and quantitative composition and percent occurrence of individual species of aeromycoflora were recorded as per following formula and shown in the table 1.

Percent distribution of species = Total colonies of a given species / Total no of species x100

RESULTS AND DISCUSSION

Table 1: Diurnal fluctuation of different aeromycoflora at different point of time

Expo-sure date	Fungal species	7AM	1PM	7PM	Total	Frequency %
29.01. 21	*1.Bortrytis*	0	1	0	1	0.81 %
	2. Cladosporium spp. I	25	8	3	36	43.90 %
	3. Cladosporium spp. II	1	0	0	1	0.81%
	4. Cladosporium spp. III	5	0	0	5	4.06%
	5. Cladosporium IV	0	2	0	2	1.62%
	6. Curvularia	0	0	1	1	0.81%
	7. Geotrichum (yeast)	1	0	0	1	0.81%
	8. Green Penicillium	0	2	0	2	0.81%
	9. Mucor	0	0	1	1	0.81%
	10. Penicillium spp. I	2	1	0	3	2.43%
	11. Penicillium spp. II	1	2	0	3	2.43%
	12. Penicillium spp. III	1	1	2	4	3.25%
	13. Penicillium spp. IV	0	0	1	1	0.81%
	14. Penicilliumspp. V	0	0	1	1	0.81%
	15. Red Penicillium	0	1	0	1	0.81%
	16. Red yeast	1	0	0	1	0.81%
	17. Sterile brown colony	0	2	2	4	3.25%
	18. Sterile green colony	0	0	1	1	0.81%
	18. Sterile white colony	3	2	2	7	5.69%
	20. Sterile yellow colony	0	0	1	1	0.81%
04.03. 21	*21..Aspergillus niger*	5	5	3	14	11.38%
	22.Apergillus flavus	1	0	0	1	0.81%
	23.Aspergillus versicolor	0	0	1	1	0.81%
	24.*Cladosporium sp*	0	1	10	11	8.94%
	25.*Penicillium sp*	1	0	2	3	2.43%
	26.Pink yeast	0	0	1	1	0.81%
	27.Sterile white	3	5	4	12	9.75%
	28.Sterile Brown	3	0	0	3	2.43%
	Total	55	33	36	123	

It was evident from the table 1 that a total of 123 fungal colonies were recorded belonging to 8 genera and 19 species in two occasions. Diurnal fluctuation of aeromycoflora were observed and there were a total of 55,33 and 36 colonies recorded at 7AM, 1PM, and 7PM respectively in two occasions. *Cladosporium spp*. represented highest percent occurrence (29.26%) followed by *Aspergillus niger* accounting for 11.38% of total aeromycoflora in the second occasion. (Debnath *et al.*, 2015) observed a total

aeromycoflora over tea field of 652 belonging to 17 genera and Cladosporium *spp.* represented highest frequency of 78 percent a trend observed in our present study.
(Bhat and Vinya, 2015) working on aeromycoflora of Mahe by settle plate method recorded a total of 15 fungal species where Cladosporium contributed 50 percent of total mycoflora which is in close conformity with our observation. Similar observation was made by (Mahobia *et al.*, 2015) who studied aeromycoflora of Nawapara, Rajim District, Chattisgarh by settle plate method and observed percent contribution *Cladosporium spp*. to be the maximum (22.24%).
(Kunjam and Jadhav, 2015) working on aeromycoflora at Panabaras observed maximum occurrence of *Aspergillus niger* (16.87%)which is in close conformity of our observation of highest population of *Aspergillus niger* (11.38%)in second occasion.
There were six species of *Penicillium* which occurred in the 1st occasion and percent occurrence varied from 0.81-2.43%.Sterile brown mycelium occurred 9.75% of total aeromycoflora. Other species of fungi *i.e Botrytis, Curvularia , Geotrichum (yeast) , Penicillium , Mucor, Penicillium spp, Aspergillus flavus, Aspergillus versicolor,* Red yeast, sterile colonies varied in the range of 2-5% of total aeromycoflora. Accumulation of water droplets were observed on the surface of growing colonies of *Cladosporium, Aspergillus niger* and *Aspergillus flavus, Red Penicillium* and these droplets are known to contain secondary metabolites of fungi including mycotoxin. Environmental, agricultural and medicinal importance of aeromycoflora and their potential impact on society have been well documented (Hyde *et al.*, 2019). (Horner *et al*., 1995) reported allergenic potential of aeromycoflora *Mucor, Cladosporium, Botrytis, Saccharomyces, Red yeast, Aspergillus* and *Penicillium.* Conidia of *Aspergillus and Penicillium* are microscopic, globose, spherical and vary from ±2.5-5µ in diameter. These spores or conidia enter the respiratory air way through inhalation and reach the interior of lung tissues, colonies and cause infection in immune compromised individuals (Horner et al. 1995).

Table 2: Types of inhibitions shown by *Aspergillus flavus*

Plates AM/PM (Interacting Fungi)	Types of Inhibition	Pictures
Plate C-7PM (04.03.2021)	Type B: Growth superficial over the contending organism. Here, the *Aspergillus niger* inhibits *Cladosporium* spp. along with some bacterial inhibition.	
Plate C-1PM (04.03.2021)	Type D: growth around the contending organism. Here, *Aspergillus niger* inhibits the growth of sterile white mycelia.	

Figure 1: Plate B- 1PM (Date- 29.01.2021) Penicillium colonies shows water droplets. These droplets are known to contain secondary metabolites of fungi including mycotoxin.

Interference Competition

Some colonies of aeromycoflora appearing on exposed plates exhibit distinct inhibitory interference with adjoining colonies which varied depending on fungal species. Such inhibition of adjoining fungal colonies may be due to production of secondary metabolites involving, toxin, antibiotic or antifungal compounds. This phenomenon was observed by Potter asearly as 1924.He recognized five types of inhibitions i.e. A. Mutually intermingling, B. Over growing, C. Slight inhibition, D. Growth around and E. Inhibition at distance. We have observed type C and type D inhibitions as shown in table 2.

CONCLUSION

The fungal species *Aspergillus niger, Aspergillus flavus, Aspergillus versicolor, Penicillium species, Curvularia species* identified in the present study are aeroallergens and may cause infections. Some of them are nonpathogenic and have some potential to be utilize as biotic resource for other purposes such as antibiotics, enzyme production and biodegradation of waste materials. Mucor species has been recorded as one of the aeromycoflora in our study which has a late been implicated as causative agent of mucormycosis associated with immunocompromised patients associated with Covid-19. The study generates an interesting trend of rice biodiversity of aeromycoflora in Assam.

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