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ORIGINAL ARTICLE

Indoor Aeromycoflora Analysis of Government Quarters at Agra

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ABSTRACT

The inception of aerobiology was marked by a preliminary concept of the study of seasonal atmosphere diffusion of pathogenic fungal spores several decades back. Other aeroallergens including bacteria and virus associated with respiratory diseases over short distances, mainly indoor are also transferred to long distances. In India, studies on airspora in relation to some phytopathological problems were initiated by Mehta in 1952. This was followed by the work of several others. The spores of phytopathogenic fungi contribute a small but significant portion of airborne fungal spore populations. Among the phytopathogenic fungi whose spores disperse mainly through air are rust, powdery mildews, leaf spotting fungi e.g. Cercospora, Alternaria, Helminthosporium, Drechslera, Pyricularia and others. These fungi sporulate abundantly to their successful aerial dispersal. With the increasing problems of pollution aerobiological studies have gained new impetus. The large segment of the air borne spore flora is composed of fungal spores and therefore, studies on aeromycology have become important.

***Key words**: Aerobiology, Microflora, Fungi, Allergy*

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INTRODUCTION

The inception of aerobiology was marked by a preliminary concept of the study of seasonal atmosphere diffusion of pathogenic fungal spores several decades back. Other aeroallergens including bacteria and virus associated with respiratory diseases over short distances, mainly indoor are also transferred to long distances. Blackely (1873) discovered pollen and spores in the air at Manchester, England. The term aerobiology was first coined by Meier (1930). Aerobiology (from Greek ἀήρ, aēr, "air"; βίος, bios, "life"; and -λογία, -logia) is a branch of biology that studies organic particles, such as bacteria, fungal spores, very small insects, pollen grains and viruses, which are passively transported by the air (Singh and Mathur, 2012). Aerobiologists have traditionally been involved in the measurement and reporting of airborne pollen and fungal spores as a service to allergy sufferers (Larsson, 1993). Aerobiology is a scientific discipline that deals with the transport of organisms and biologically significant materials through the atmosphere (Isard and Gage, 2001). Aerobiology also encompasses the generation, uptake, translocation, dispersion, viability, deposition and infection/ infestation of seeds, viruses, fungi, bacteria and other agents, including insects such as aphids, and mosquitoes, which act as virus vectors. Aerobiology is a field which focuses on the study of particles which are small enough to be passively carried in the air. These particles are often biological in origin. An important medical application of aerobiology is the study of the transmission of airborne diseases. It is known that many bacteria and viruses can be spread through the air, possibly within droplets. A global rise in the prevalence of asthma and other allergenic

ailments all over the world has been reported since the last few decades and experts are puzzled over the cause of this phenomenon. Almost 20% of the child population in US and other industrialized countries are afflicted from the disease (Anonymous, 1998). Fungal spores, pollen grains, dust mites and insect parts are known to cause various allergenic disorders in human beings since time immemorial (Kramer, *et al*., 1960; Bhargava, *et al*., 1966; Anisworth, *et al*., 1973; D'Silva and Freitas, 1981). The presence of these bio-pollutants in the indoor environment cause several health problems not only in rural areas but in towns as well as in big cities. Outdoor allergens usually include pollen grains and fungal spores, while indoor allergens are usually mixture of outdoor environment only, which comprise house dust mite, cockroaches, cat, dog and mice, beside fungal spores and pollen grains (Sharma, *et al*., 2005). Aspergillus spp. and Penicillium spp. are less common in outdoors and are considered as major indoor fungi (Lacey, 1981; Burge, *et al*., 1982; Beaumont, *et al*., 1985; Licorish, *et al*., 1985). Changes in the lifestyle and increase in indoor allergen exposure, due to higher temperature existing indoors and humidity have been suggested to be the potential cause of fungal allergy.

The present study was carried out with the aim to study indoor aeromycospora of houses people of Agra of different socio-economic groups. The information thus collected will be used to correlate with the respiratory disorders in people living in different localities and different climatic conditions.

MATERIALS AND METHODS

Present study was undertaken to find out the indoor aeromycospora of houses from selected site of at Agra city for two years (January, 2009 to December, 2010). Study was carried out on five houses from each site selected in Agra city. In the present research work Lower Middle Class area of Government quarters, Judge's compound has been selected. Samples were collected with the help of-

(A) ROTOROD SAMPLER:

Perkins (1957) developed a battery operated rotorod sampler, sampling at a constant rotation speed. It is a battery operated small motor with constant high speed is used to whirl thin brass rod about its axis. The collecting arms of this sampler are made up of brass having 0.159 cm cross sectional area. It is square in shape and slightly bent inwards. The vertical arms are 6 cm long and 4 cm apart from the axis. The motor operating with 6.9 volts battery gives rotation speed of approximately 2300 r. p. m. In the present study, an adhesive transparent cellophane tape was cut into strips of approximately 4x6 cm which were applied on the sampling surface of the rods. The edges of the tape strips were trimmed to the width of rod with the help of sharp razor blade. The cello tape on the arms was coated with melted glycerine jelly. After exposure tape strips were carefully removed and cut into four equal parts (1.5 cm each) and placed on the glass slide and mounted in glycerine jelly for microscopic studies.

Collection Efficiency:

This sampler has 85% collection efficiency. The sampler operates on the impaction principle small air borne particles is deposited on the strips due to the process of impaction when the sampler is run. The rotorod sampler is useful for short period sampling upto 2 h. The conversion factor for the sampler is 5. This sampler provides volumetric data (number of spores/m^3) which enables to analyze microbial populations.

Sampling Rate:

It is the volume of the air swept over by the collecting surface per minute. The volume of air can be calculated on the basis of the dimensions.

= 2 (arms) x 0.159 cm x 6 cm x 8 x 2300 x 10^3

= 48.0 x 10^3 x 2300 liter/minute.

= 100 liters/minute approximately.

Sampling Method:
Airspora of different sites as mentioned above were studied by Rotorod sampler for two years (2009 and 2010). The Rotorod sampler was run for half an hour once a month at constant height of 1 meter above the ground level at each site between 10-11 am. The exposed tape strips were mounted on the slides by glycerine jelly.

Scanning:
It was carried out as described by Tilak and Srinivasulu (1967). The conversion factor for the sampler is 5. If total number of spores from catches is 7, the total number of spores/m^3 of the air will be 5x7=35. The number is the total number of spores/m^3 of air.

(B) TILAK AIR SAMPLER:
This sampler is run by electric/battery power supply of AC-220 V and provides a continuous sampling of air. The electric clock fitted in the instrument and is synchronized with the drum. Air is sucked through the orifice of the projecting tube at the rate of 5 liters per minute and it impinges and sticked on the slowly rotating drum. The drum completes one circle in eight days, thus giving the trace of catches for 8 days.

Collection Efficiency:
The sampler has 75% collection efficiency. The main advantage of this sampler is, to provide volumetric data (number of spores/m^3) which enables to analyze microbial population both qualitatively and quantitatively.

Sampling Methods:
The air sampler was installed at a constant height of one meter above ground level for air monitoring inside the rooms. The slides were prepared after the method of Tilak and Sriniasulu (1967) and mounting was done with the help of glycerin jelly. Cello tape was divided into 16 equal parts and each cello tape segment was mounted on a labeled clean glass slide with glycerin jelly as mountant. Glycerin jelly has the best optical property for visual examination.

Scanning:
The mounted slides were examined after a few days. The calculated conversion factor for this sampler is 14. If the total number of spores/m in the air are 6 than 14x6=84 will be the total number of spores/m^3 of air at particular site at that time.
All the slides were examined directly under the microscope and total number of each spore type was counted.

$$\% \text{ of occurrence} = \frac{\text{No. of individual fungal spores}}{\text{Total fungal spores trapped}} \times 100$$

The number of fungal spores was counted and the percentage value was determined.

RESULTS AND DISCUSSION

GOVERNMENT QUARTERS, JUDGE'S COMPOUND:
This colony consists of blocks of four houses (two at the ground floor and two at top floor). There is some gap between the blocks. The houses are small with 2-3 rooms and separate kitchen and toilet facilities. The construction is very poor and the plaster at most places is broken and the walls show the presence of moisture. Between blocks the area is full of garbage and stray animals (cows, pigs and dogs) are free to move around). Sanitation is also poor. 4-5 or sometimes more members (both children and adults) live in the same house. In winters all the members sleep in small rooms (3-4 members/room). In summers some of the male members sleep in open or inside the room using air coolers making the rooms much humid.

Year 2009: The spores of various fungal types trapped during January, 2009 to December, 2009 their number and percentage collected from the indoor environment from site II (Government Quarters, Judge's compound) is shown in Table- 1.
Spores of *Alternaria* spp. followed by Aspergilli, rust spores, *Cladosporium* spp., *Curvularia* spp. and *Epicoccum* spp. were present in all the seasons. Spores of *Lophiostoma*, *Pleospora*, basidiospores, *Cercospora* and *Nigrospora* were recorded only during few months of the year.
Year 2010: The spores of various fungal types trapped during January, 2010 to December, 2010, their number and percentage collected from indoor environment of site II (Government Quarters, Judge's compound) is shown in Table- 2.

Table 1: Fugal spore trapped during January, 2009 to December, 2009 from indoor environment from Site Government Quarters, Judge's compound

Fungal types	No. of spores	Contribution (%)	Seasonal Frequency		
			Rain	Winter	Summer
Phycomycetes					
Cunnighamella sp.	05	0.57	+	---	---
Rhizopus arhizus	30	3.11	++	+	---
Rhizopus microsporus	15	1.41	+	---	+
Deuteromycetes					
Alternaria alternata	119	12.22	++	+++	++
Aspergillus flavus	101	10.0	+++	+	+++
Aspergillus niger	85	8.71	+++	++	+++
Aspergillus terreus	28	2.78	++	+	+
Aspergillus ustus	10	1.29	+	---	+
Aureobasidium pollulans	11	1.11	---	++	---
Candida sp.	23	2.33	+	---	++
Cladosporium herharum	65	6.35	+++	+++	+
Curvularia lunata	78	7.75	++	+++	+
Colletotrichum sp.	14	1.48	+	+	---
Epicoccum purpurascens	122	12.13	++	+++	+
Fusarium oxysporium	28	2.78	+	+	++
Geotrichum sp.	15	1.51	---	+	---
Helminthosporium sp.	43	4.33	+++	--	+
Nigrospora sp.	20	2.01	+	++	---
Penicillium citrinum	50	5.21	++	++	+
Penicillium purpurogenum	12	1.26	+	+	---
Trichoderma sp.	45	4.44	++	---	++
Trichothecium roseum	26	2.43	+	++	---
Ulocladium sp.	02	0.19	--	+	---
Unidentified colonies	13	1.37	------	-------	-----
Total	**960**	**94.11**			

---Absent; + present; ++ moderately present; +++ abundantly present.

It is evident from the data in Table- 2 that more or less similar type of fungal spores are seen in the year 2010 as recorded in the year 2009, except that a few are absent and the number of spores of a fungus and its concentration is also different. The spores of *Cunnighamella* sp., *Rhizopus arhizus, Rhizopus microspores, Alternaria alternate, Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus ustus, Aureobasidium pollulans, Candida sp., Cladosporium herharum, Curvularia lunata, Colletotrichum* sp, *Epicoccum purpurascens, Fusarium oxysporium, Geotrichum* sp., *Helminthosporium* sp., *Nigrospora* sp., *Penicillium citrinum, Penicillium purpurogenum, Trichoderma* sp., *Trichothecium roseum, Ulocladium* sp. and unidentified colonies were trapped by both the samplers and exposed Petri-plates. Among these *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cladosporium herharum, Curvularia lunata, Epicoccum purpurascens, Penicillium citrinum, Trichoderma* spp. are the most dominating once. However, *Ulocladium* spp. was absent and *Colletotrichum* spp. was represented by only one spore.

Maximum contribution was of *Alternaria* (20.11%) followed by Aspergilli (14.02%), rust spores (12.1%), *Cladosporium* (9.54%), *Curvularia* (6.2%) and *Epicoccum* (4.23%). Spores of *Lophiostoma* (0.7%), *Pleospora* (1.2%), basidiospores (1.3%), *Cercospora* (0.7%) and *Nigrospora* (2%) were recorded only during few months of the year. The spores of *Chaetomium, Pleospora, Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Cladosporium herharum, Cladosporium herharum, Curvularia lunata, Epicoccum purpurascens, Epicoccum purpurascens, Fusarium oxysporium* and *Penicillium citrinum* were recorded in all seasons.

From the indoor environment of site (Government quarters, Judges compound) more or less similar types of fungal spores were seen in both the years (2009 & 2010) as recorded from site I, except that a few were absent and the number of spores of a fungus and their concentration was low. The spores of *Cunnighamella* sp., *Rhizopus arhizus, Rhizopus microspores, Alternaria alternate, Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus ustus, Aureobasidium pollulans, Candida sp., Cladosporium herharum, Curvularia lunata, Colletotrichum* sp, *Epicoccum purpurascens, Fusarium oxysporium, Geotrichum* sp., *Helminthosporium* sp., *Nigrospora* sp., *Penicillium citrinum, Penicillium purpurogenum, Trichoderma* sp., *Trichothecium roseum, Ulocladium* sp. and unidentified colonies were trapped by both the samplers and exposed Petri-plates. Among these *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cladosporium herharum, Curvularia lunata, Epicoccum purpurascens, Penicillium citrinum, Trichoderma* spp. are the most dominating once.

Table 2: Fungal spore trapped during January, 2010 to December, 2010 from indoor environment from Site Government Quarters, Judge's compound

Fungal types	No. of spores	Contribution (%)	Seasonal Frequency		
			Rain	Winter	Summer
Phycomycetes					
Cunnighamella spp.	04	0.59	+	---	---
Rhizopus arhizus	30	3.18	++	+	---
Rhizopus microsporus	13	1.39	+	---	+
Deuteromycetes					
Alternaria alternata	101	12.32	++	+++	++
Aspergillus flavus	97	9.94	+++	+	+++
Aspergillus niger	84	8.94	+++	++	+++
Aspergillus terreus	23	2.98	++	+	+
Aspergillus ustus	10	1.39	+	---	+
Aureobasidium pollulans	14	1.19	---	++	---
Candida sp.	23	2.38	+	---	++
Cladosporium herharum	46	6.85	+++	+++	+
Curvularia lunata	65	7.95	++	+++	+
Colletotrichum spp.	01	1.49	+	+	---
Epicoccum purpurascens	85	12.42	++	+++	+
Fusarium oxysporium	27	2.98	+	+	++
Geotrichum sp.	15	1.59	---	+	---
Helminthosporium sp.	41	4.37	+++	--	+
Nigrospora spp.	22	2.38	+	++	---
Penicillium citrinum	47	5.46	++	++	+
Penicillium purpurogenum	11	1.29	+	+	---
Trichoderma spp.	15	1.77	++	---	++
Trichothecium roseum	12	2.48	+	++	---
Ulocladium spp.	00	00	--	---	---
Unidentified colonies	11	1.39	–	–	–
Total	**995**	**96.81**			

---Absent; + present; ++ moderately present; +++ abundantly present.

Singh (2007) has reviewed the studies made on airborne mould biodiversity of certain indoor work environments in North-Eastern (NE) India during the last five decades.

Indoor workplace environments were either industrial or non-industrial. Industrial indoor workplace included Cinema halls, saw mills, rice mills, paper mills and baker, while the non-industrial workplace included hospital wards, poultry farm, library, pig farm, hostel kitchens, Food Corporation of India grain godown and potato storage chambers. Rooms occupied by asthmatic patients, Medical wards. They found *Aspergillus* sp. Contributing 33% of the total population whereas *Penicillium* sp. Contributing 15.2% of the total population. These studies revealed rich biodiversity of moulds in the indoor workplace environments of N.E. Further, fluctuations in fungal airspora were observed as influenced by meteorological parameters in addition to available indoor substrates. Most of the indoor workplace environments present higher population of *Penicillium* and *Aspergillus* indicating possible sources of indoor contamination. All the studies reviewed by Singh (2007) revealed rich biodiversity of moulds in the indoor workplace environments of North Eastern India as well as in other parts of the country. Further, a fluctuation in fungal airspora was observed as influenced by meteorological parameters (outdoor and indoor) in addition to available indoor substrates.

Govind, *et al*. (2002) have made an interesting comparative study of culturable fungi in asthmatic patient's bedroom and inside hospital ward. It is often observed that several patients fail to improve from asthmatic attack while treated at home. The condition of the patient improves if transferred to hospital and gives better response to treatment. This was found to be associated with higher fungal spore load in the patient's bedroom as compared to that of hospital ward.

Sharma and Dutta (2002) have observed the fungal airspora of some indoor environment of Medical Wards, Paper Mill, Poultry, Bakery, F.C.I. Godown of greater Silchar and adjacent areas from January 200 to June 2000, using Petri dish exposure method. A total of 57 fungal spores were recorded during the study period. Some of the dominant fungal forms encountered were *Aspergillus flavus, A. fumigatus, Penicillium brevicampactum, Humicola* sp*., Geotrichum* sp*.,* and *Curvularia* sp*.* These fungi are known to be causative agents for several kinds of skin and respiratory problems of human beings. Species of *Aspergillus* are known to cause Aspergillosis.

Bhuvaneswari and Vittal (2005) have made a survey on airborne fungi in the residences of asthmatics in Chennai city, using 2-stage Anderson viable sampler. Petri-plates with Sabouraud's Dextrose Agar were exposed in the air sampler. A total of 100 asthmatic patient's residences were studied for the presence of airborne fungi. Altogether 95 species classified into 37 genera were isolated. Among the environments studied, an average of 322.78 CFU/m^3 of air and bedroom recorded 298.98 CFU/m^3 of air. Among the fungi isolated *Aspergillus niger* was dominant in all the sites. The second and third dominant fungi varied with the environment.

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