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ORIGINAL ARTICLE

# Aeromycoflora Analysis in Outdoor Environment in Various Localities at Agra

**Neeraj Sharma and P.K. Mathur**
Department of Botany, B.S.A. College, Mathura
Email: neerajsharma16166@gmail.com

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## ABSTRACT

*Aerobiology concerns mainly with the study of passively air borne microorganisms and their spores in both indoor (intramural) and outdoor (extramural) environments. The outdoor air is never completely free from the incidence of microbial propagules, which are collectively termed as "air spora." In India, studies on airspora in relation to some phytopathological problems were initiated by Mehta. Fungal spores are among the most commonly encountered bio-particles in air. It is known beyond any doubt that the airborne fungal spores play an important role in the etiology of respiratory allergic disorders. 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.*

***Key words**: Aeromycoflora, Allergy, Outdoor environment, Agra region*

## INTRODUCTION

Gregory (1952) coined the term "Air spora". His classic book entitled "*Microbiology of the atmosphere*" is a useful source of information on pollen flora, fungal spores including his extensive microscopic examination of plant parts has been described. 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 (Mallaiah, 1999). 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 (Bajaj, 1988; Durham, 1998; Verma and George, 1997).

Airborne fungal spores have been widely recognized as major allergens capable of causing asthma, allergic rhinitis and other allergic diseases (Baruah, 1961). Studies on prevalence of allergenic fungal spores and their allergenicity have been made at several places in India. Several workers have recorded the presence of a wide variety of spores inside several buildings and working environments of Manipur (Singh and Singh, 1988; Devi and Singh, 1997 and Devi, 1998) and these are known to cause asthma and allergic rhinitis. Composition of airborne fungal spores is characteristics of each biogeographical zone depending upon the type of vegetation (Sahney and Chaurasia, 2009).The number and type of fungal spores depend upon on the time, day, wheatear and season (Verma and Chile, 1994). Large numbers of studies have been undertaken in different parts of India and other countries which have established the prevalence of airborne fungi (Mishra, *et al.*, 2008). In recent past increased attention is being directed to indoor environments as a source of biopollution. The occupational environments (ie. bakery, granary, hospitals etc.), which deal with the organic source and the byproducts provide a conducive atmosphere for the growth and proliferation of fungal allergens (Singh, 1998; Singh, *et al.*, 1998 and Rafiyauddin, *et al.*, 1997).

## MATERIALS AND METHODS

Present study was undertaken to find out the outdoor aeromycospora of different sites of different socio-economic groups at Agra city for two years (January, 2009 to December, 2010). 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 are 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

The present investigation was carried out in outdoor environments at selected site during a period of two years (January- December, 2009 and January-December, 2010). The survey of outdoor environment near the selected indoor sites mentioned above during the years (2009 & 2010) was undertaken for providing the comparative view of fungal spores. The survey made at the outdoor environment of four colonies (I. Kaushalpur, II. Government Quarters, Judge's Compound, III. ADA'S colony, Sanjay Place, and IV. Surya Nagar revealed the presence of 101 types of fungal spores and these are listed below in Table-1.

**Table 1:** List of fungal types trapped by volumetric samplers from different outdoor sites of Agra

| S.No. | Class | Fungal types |
|---|---|---|
| 1. | **Myxomycetes:** | *Physarum* Pers. |
| 2. | **Phycomycetes:** | *Cunnighamella* Matr., *Phytophthora* de Bary. & *Sclerospora* Sacc. |
| **3.** | **Ascomycetes :** | *Apiorhynocostoma* Petrak., *Asterina* Lev., *Bombardia* Fr., *Calospora* Nitschke, *Chaetomium* Kunj ex Fr., *Claviceps* Tul., *Cucurbitaria* Gray. Ex. Grev., *Didymosphaeria* Fuck., *Hypoxylon* Bull. Ex. Fr., *Hysterium* Tode ex. F., *Lacanidion* Endl., *Leptosphaeria* Ces & de not., *Lophiostoma* Ces. De not., *Massarina* Sacc., *Melanospora* Corda., *Nectria* Fr., *Nodulosphaeria* Rabh., *Ottthis* Nke., *Parodiella* Berl., *Passereniella* Berl. *Pleospora* Rabh. *Pringsheimia* Schultz. *Rosellina* Ces. & de Not. *Sodaria* Ces & de Not.*Sporomia* de Not. *Sydowia* Agharkari., *Tiechspora* Fuckel. *Trematosphaeria* Fuck. *Valsaria* Ces & de Not. & *Xylaria* Hill ex Grev. |
| **4.** | **Basidiomycetes:** | Basidiospores, *Ganoderma* Kartz., Rust spores & Smut spores. |
| **5.** | **Deuteromycetes:** | *Alternaria* Nees., *Arthrinium* Kunz. Ex Fr., *Aspergilli*, *Beltrania* Penzig., *Beltraniella* Subram., *Botrytis* Pers., *Cephaliophora* Thaxt., *Ceratophorum* Thaxt., *Cercospora* Fr.,*Cladosporium* Link., *Clasterosporium* Schw., *Cordana* Presuss., *Corynespora* Guessow., *Curvularia* Boed., *Dactylium* Nees., *Deightoniella* Hughes., *Dendryphiopsis* Hughes, *Dictyosporium Corda*., *Diplodia* Fr., *Drechslera* Ito., *Epicoccum* Link., *Exosporium* Link., *Fusariella* Sacc., *Fusarium* Link., *Haplosporella* Speg., *Harknessia* Cook., *Helminthosporium* Klotzsch., *Hirudinaria* Ces., *Humicola* Traaen., *Memnoniella* Hohn., *Microsporum* Gruby., *Monilia* Pers., *Nigrospora* Zimm., *Oidium* Sacc. Link., *Papularia* Fr., *Periconia* Tode ex Schw., *Pestalotia* de Not., *Phaeotrichoconis* Subram., *Pithomyces* Ber., *Prathigade* Subram., *Pseudotorula* Subram., *Pyricularia* Sacc., *Ramularia* Sacc., *Scopulariopsis* Bain., *Sirodesmium* Sacc., *Spicaria* Auct., *Spondylocladiella* Linder., *Sporidesmium* Link., *Stemphylium* Wallr., *Stigmina* Sacc., *Tetracocosporium* Szabo., *Tetraploa* Berk. & Br., *Torula* Pers. Link., *Trichoconis* Clements., *Trichothecium* Corda., *Ulocladium* Presuss., & *Venturia* Pers. |
| **Other types:** Pollen grains, hyphal fragments, algal parts, insect parts and insect scales. | | |

It is evident from Table-1 that one member of Myxomycetes; four from Phycomycetes; thirty from Ascomycetes; four from Basidiomycetes and sixty two members from Deuteromycetes were observed from various outdoor sites of Agra city. Thus, the maximum number of spores of Deuteromycetes followed by Ascomycetes was present in the air of different outdoor sites of Agra city.
The percentage contribution of above mentioned types of fungal spore with their taxonomic lasses from outdoor environment of various sites is shown in Table- 2.

**Table 2:** Percent contribution of various fungal spores and their taxonomic classes

| S.No. | Class | Total no. of fungal types from each class | Contribution (%) |
|---|---|---|---|
| **1** | Myxomycetes | 1 | 0.98 |
| **2** | Phycomycetes | 4 | 3.92 |
| **3** | Ascomycetes | 30 | 29.41 |
| **4** | Basidiomycetes | 4 | 3.92 |
| **5** | Deuteromycetes | 62 | 61.76 |

It is clear from Table-2 that Deuteromycetes contributed to 61.76% fungal airspora, Ascomycetes being the next with 29.41% contribution followed by Phycomycetes and Basidiomycetes contributing 3.92% each and only 0.98% contributed by Myxomycetes. Fungal genera trapped by exposed Petri-plate method during the study period. A total of 48 fungal species belonging to 32 genera were recorded from different sampling sites. The class Phycomycetes contributed 14.58%, Ascomycetes 2.08%, Basidiomycetes 2.081% and Dueteromycetes contributed 81.25%.

Aerobiological studies on various parts of Agra city have been undertaken in recent years (Singh and Chauhan, 2000; Kulshreshtha and Chauhan, 2000; 2001; 2002; 2003; Kulshreshtha, 2002; Chauhan and Kulshrestha, 2006; Chauhan and Goyal, 2006; Chauhan et al., 2004; 2008 and Verma and Chauhan, 2006). Kulshrestha and Chauhan (2001) have studied the aeromycoflora of the intramural environment of the S.N. Medical College, District Hospital and G.G. Nursing Home in Agra. The survey conducted during January- December, 2000 showed the presence of major spore types of *Aspergillus, Cladosporium, Penicillium, Alternaria, Curvularia* and *Fusarium.* Variation in different months was also observed and attributed to changes in the climatic conditions

Kulshrestha and Chauhan (2002) have studied the aeromycoflora of vegetable and fruit markets at Agra between January and December, 2000 using a Rotorod air sampler. A total of 38 fungal forms were identified. The dominant fungal types were *Aspergillus*, *Cladosporium*, *Alternaria*, Smut spores and Basidiospores. Maximum fungal spores were recorded during the months of February and March with temperature ranging between 8.1- 31.0$^{o}$C and RH between 83-93%. Kulshrestha and Chauhan (2003) have made an assessment of outdoor aeromycoflora of various historical monuments of Agra city for a period of one year from January- December, 2001 using Rotorod volumetric sampler. Maximum concentration of fungal spores was encountered from Taj Mahal followed by Dayal Bagh Temple, Sikandra, and Red Fort. *Alternaria* was found to be dominant at each site followed by *Aspergilli*, *Cladosporium*, *Curvularia* and Sumt spores.

Chauhan, *et al.,* (2004) have recorded the airborne fungi from six cites of Agra city with special reference to their allergenic significance. A total of 56 fungal types were recorded from a total catch of 28,290 spores/m$^{3}$ of air. The skin prick tests for the clinical investigation were made. A total of 17 fungal antigens were tested. Out of them, *Rhizopus nigricans* showed maximum allergenicity while *Candida albicans* showed minimum allerginicity. Patients of bronchial asthma with rhinitis were highest followed by bronchial asthma and allergic rhinitis. Maximum number of patients had the symptoms between the age group of 31-40 years and male were more sensitive than females.

Chauhan and Kulshrestha (2004) have also made a comparative study on the incidence of airborne fungal spores at two gardens of Agra city, namely, Motilal Nehru Park and Paliwal Park during January to December, 2001 using Rotorod volumetric sampler. Maximum concentration of fungal spores was encountered from Paliwal Park situated in the heart of the city. *Alternaria* was found to be most dominant at both the sites followed by smut spores, *Aspergillus*, *Cladosporium* and *Curvularia*. The maximum number of fungal spores was trapped in the months of February, March, October, and November with moderate temperature (22.3$^{0}$C) and relative humidity above 70%.

Airborne fungi from six sampling sites of Agra city were studied with special reference to their allergenic significance (Kulshrestha and Chauhan, 2000; Chauhan and Kulshrestha, 2006; Chauhan, *et al.*, 2004; 2008). A total of 56 fungal types were recorded from total catch of 28, 290 spores/m3 of air. The population of taxonomic groups of fungi was Myxomycetes, Phycomycetes, Basidiomycetes, Ascomycetes and Deuteromycetes. Among them *Alternaria* was most dominant fungal type at each site. A skin prick test for the clinical investigation was made. A total of 17 fungal

antigens were tested. Out of them *Rhizopus nigricans* showed maximum allergenicity (20.95%), while, *Candida albicans* showed minimum allergencity (2.06%). Patients of bronchial asthma with rhinitis (62.30%) were highest followed by bronchial asthma (25.61%) and allergic rhinitis (12.07%).

Singh and Chauhan (2000) and Chauhan and Goyal (2006) have recorded the pollen calendar of Agra with special reference to their allergenic significance. Pollen grains of 35 species belonging to 23 angiosperm families have been identified out of a total catch of 24,220/$m^3$ of air annually. High occurrence of pollen grains in air belonged to Asteraceae (522/$m^3$) and *Parthenium hysterophorous* contributed the maximum (17.91%) of the total airspora. Higher counts of pollen were found in ecozones surrounded by agricultural fields, parks and gardens. He skin prick tests for the clinical investigation were made. Patients of bronchial asthama with rhinitis (62.30%) were highest followed by bronchial asthma (25.61%) and allergic rhinitis (12. 07%). Maximum number of patients had symptoms between the age group of 31-40 years and male were more sensitive than females. Maximum sensitivity was caused by *Amaranthus spinosus*, followed by *P. artenium, P. hysterophorus, Chenopodium album, Cynodon dactylon* and *Cassia occidentalis.*

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