

Dynamics of Aspergilli in the Aeromycospora of Medical College of Gorakhpur City and Evaluation of Their Allergenic Properties

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Abstract— Study of aeromycoflora of a particular locality is very significant, especially when the people in general and patients in particular are exposed to this fungal flora. It is helpful in locating the aeroallergens. Study of airborne fungi forms the basis of research in aeromycology with respect to medicine. Of all these airborne fungi, *Aspergillus* spp. have been reported to be the most prevalent ones. Therefore, in the present investigation, the periodicity of spores of *Aspergillus* spp. in the air of General Ward/OPD/Campus of B.R.D. Medical College, Gorakhpur has been investigated and their allergenic properties have been evaluated. Their monthly, seasonal and temporal variations have been studied and recorded from March, 2013 to February, 2014. The maximum number of *Aspergillus* spp. was recorded in the months of March, May, October and November (9 species in each) and the minimum number in February (3 species only). The maximum number of isolates of aspergilli was recorded in April (32 isolates). The summer season had the highest number of *Aspergillus* species (12) and isolates (78) than winter and rainy seasons. A comparison of aeromycological data of *Aspergillus* spp. with that of the clinical data of medical college revealed that patients suffering from the allergic disease -Aspergillosis (Bronchopulmonary Aspergillosis and Aspergilloma etc.) are frequent visitors of Gorakhpur medical college.

Index Terms— Aeromycoflora, Aeromycology, *Aspergillus*, Allergens.

I. INTRODUCTION

Fungal spores occur very numerous in the air and, on account of their dimensions (several micrometers), are classified as bioaerosols¹. They are always observed in natural air and their concentration changes depending on environmental conditions. *Aeromycology* investigates their occurrence in the air of the indoor-outdoor environment. The methods of sampling can be divided into the gravimetric method when the spores fall onto a catching surface by force of gravity, and the volumetric method consisting of analysis of spores contained in a given air unit. The content of fungal spores in air is characterized by a specific seasonal and diurnal cycle. Among other things, these cycles depend on climate and weather conditions, on the accessibility of fresh substrates for the development of the fungus, circadian cycle of light and darkness, and other environmental hardly definable factors. Many fungi undesirably affect human health, cause immunotoxic diseases, and are a frequent cause of allergic diseases. Knowledge of concentrations of airborne

fungal spores is especially important for agricultural and occupational medicine. Aeromycology has its application in agrobiolgy, particularly with respect to pathogenic fungi, and in the conservation of the artistic heritage. Study of aeromycology of an area is significant for several applications, such as forecasting of plant diseases as well as studies about allergy, litter decomposition and allied aspects of microbiology². Determination of patterns of distribution of aeromycospora over urban areas has been chiefly concerned with the allergic disorders in man³.

Aspergillus Pier Antonio Micheli species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate. These species are common contaminants of starchy foods and grow in or on many plants and trees. In addition to growth on carbon sources, many species of *Aspergillus* demonstrate oligotrophy where they are capable of growing in nutrient-depleted environments, or environments in which there is a complete lack of key nutrients. Species of *Aspergillus* are common saprobes, responsible for natural degradation of a variety of organic matter and deterioration in storage of a number of commodities including grains, vegetables, fruits, paper, textiles and leather etc⁴⁻⁹. In India, damage to cultural properties by fungal biodeterioration is enormous. Paper manuscripts and paintings are damaged by fungi, including *Aspergillus* and other fungal genera¹⁰.

Species of *Aspergillus* are important medically and commercially also. More than 60 *Aspergillus* species are medically relevant pathogens¹¹. Occasionally, some species of this genus are opportunistic pathogens in the respiratory tracts of birds and animals, including man and cause serious diseases¹². **Aspergillosis** is the group of diseases caused by *Aspergillus* spp. In humans, the major forms of disease are allergic broncho-pulmonary aspergillosis, acute invasive aspergillosis, disseminated invasive aspergillosis and aspergilloma, a "fungus ball" that can form within cavities such as the lungs. Therefore, the present investigation has been done to study the dynamics of aspergilli in the aeromycospora of B.R.D. Medical College of Gorakhpur City with reference to the monthly, seasonal and temporal distribution of their spores and evaluation of their allergenic properties.

II. MATERIALS AND METHODS

A. Site Selected for Study:

The General Ward/OPD/Campus of B.R.D. Medical College, Gorakhpur was selected for air sampling. The air almost always contains spores, but their number and types

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depend on the time of day, weather, season and geographical location¹³. Gorakhpur is situated in the Tarai belt of North-Eastern corner of Uttar Pradesh. This district lies between Lat. 26°13'N and 27°29'N and Long. 83°05'E and 83°56'E. It has humid, sub-tropical climate. Based on temperature and rainfall, the year can be divided into three seasons – summer (March to June), rainy (July to October) and winter (November to February). The summer has high temperature (maximum upto 45°C), decreased humidity and hot winds, whereas the winter is characterized by low temperature (minimum upto 4°C). In rainy season, there is a heavy and frequent rainfall (a maximum precipitation of about 349 mm.). The temperature is moderately high with high relative humidity and it drops slowly at the end of the season.

A. Isolation of *Aspergillus* spp. from *Aeromycoflora*:

The spores of *Aspergillus* were trapped by “Gravity Plate

Method” of Frankland and Hart. The culture medium used was Martin’s Streptomycin – Rose Bengal Agar. Five Petri plates of 80 mm. diameter containing this culture medium were exposed for five minutes in the area of investigation, three times on each day of sampling (Morning - 8.00 hr., Noon - 12.00 hr. and Evening - 18.00 hr.). The Petri plates were placed on a stand, at a height of one meter above the ground level. This sampling was done for one year (March, 2013 to February, 2014) at 15 days intervals. The exposed Petri plates were incubated for seven days at 25±2°C. The fungi appeared in the mixed culture were isolated by single spore culture and identified¹⁴. Total number of colonies per plate and number of colonies of individual species were recorded separately. Quantitative studies were made by calculating the average colony count per Petri plate during one month.

III. OBSERVATIONS

Table – 1
Monthly Variations in Number of *Aspergillus* Micheli species and Isolates (March, 2013 to Feb., 2014)

| Sr. No. | <i>Aspergillus</i> species | Months/No. of Isolates | | | | | | | | | | | |
|---------|--|------------------------|------|-----|------|------|------|------|------|------|------|------|------|
| | | Mar. | Apr. | May | June | Jul. | Aug. | Sep. | Oct. | Nov. | Dec. | Jan. | Feb. |
| 1 | <i>A. aculeatus</i> Iizukais | - | - | 1 | - | 2 | - | 1 | 1 | - | - | - | - |
| 2 | <i>A. amstelodami</i> (Mangin) Thom & Church | - | - | - | - | - | - | - | - | 1 | 1 | - | - |
| 3 | <i>A. carneus</i> (van Tiegham) Blochwitz | - | 4 | - | - | - | - | - | - | - | - | - | - |
| 4 | <i>A. chevalieri</i> (Mangin) Thom & Church | 1 | 1 | 1 | - | - | - | - | - | - | - | - | - |
| 5 | <i>A. flavipes</i> (Bain. & Sart.) Thom & Church | 2 | - | - | 1 | - | - | - | 1 | - | - | - | - |
| 6 | <i>A. flavus</i> Link | 1 | 3 | 1 | 1 | - | - | 2 | 1 | 1 | 1 | - | 1 |
| 7 | <i>A. fumigatus</i> Fresenius | 1 | - | 1 | 2 | - | - | - | 1 | 1 | - | - | - |
| 8 | <i>A. nidulans</i> (Eidam) Wint. | 3 | 5 | 4 | 1 | 2 | - | - | 1 | 1 | 1 | 1 | - |
| 9 | <i>A. niger</i> van Tieghem | 5 | 5 | 3 | 3 | 2 | 2 | 3 | - | 2 | 1 | 2 | 1 |
| 10 | <i>A. niveus</i> Blochwitz | - | - | - | - | - | - | - | - | - | 1 | - | - |
| 11 | <i>A. ochraceus</i> Wilhelm | 1 | - | 1 | 2 | - | - | - | 1 | 1 | - | - | - |
| 12 | <i>A. regulosus</i> Thom & Raper | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 13 | <i>A. sydowii</i> (Bain. & Sart.) Thom & Church | 1 | 10 | 6 | - | 4 | 2 | 2 | 4 | 4 | 6 | 3 | 1 |
| 14 | <i>A. tamarii</i> Kita | - | - | - | - | - | - | - | 3 | - | - | - | - |
| 15 | <i>A. terreus</i> Thom | - | 4 | 2 | - | - | - | 2 | - | 2 | - | 2 | - |
| 16 | <i>A. versicolor</i> | - | - | - | - | - | 1 | - | 1 | 1 | 1 | 2 | - |

| | | | | | | | | | | | | | |
|-----------------------|---------------------------|----|----|----|----|----|----|----|----|----|----|----|----|
| | (Vuillemin) Tiraboschi | | | | | | | | | | | | |
| Total Species | 16 | 09 | 07 | 09 | 06 | 04 | 03 | 05 | 09 | 09 | 07 | 05 | 03 |
| Total Isolates | 156 | 16 | 32 | 20 | 10 | 10 | 05 | 10 | 14 | 14 | 12 | 10 | 03 |

Table – 2

Seasonal and Temporal Variations in Number of *Aspergillus Micheli* species and Isolates
(March, 2013 to Feb., 2014)

| Seasonal Variations | | | Temporal Variations | | |
|---|---|--|------------------------------------|-------------------------------------|-------------------------------------|
| Summer (March, 2013 to June, 2013) | Rainy (July, 2013 to Oct., 2013) | Winter (Nov., 2013 to Feb., 2014) | Morning (8.00 hr.) | Noon (12.00 hr.) | Evening (18.00 hr.) |
| 78 Isolates | 39 Isolates | 39 Isolates | Maximum colony counts in April (8) | Maximum colony counts in April (14) | Maximum colony counts in April (11) |
| 12 Species | 11 Species | 10 Species | Minimum colony counts in Feb. (0) | Minimum colony counts in Feb. (0) | Minimum colony counts in Feb. (0) |

IV. RESULTS AND DISCUSSION

Sixteen species and 156 isolates of *Aspergillus* were trapped from the air of B.R.D. Medical College Campus of Gorakhpur during one year of investigation (March, 2013 to February, 2014).

Table – 1 and Table – 2 reveal that –

- The maximum number of species were encountered in the months of March, May, October and November (9 species in each) and the minimum in February (3 species).
- Seasonal variation was observed in quality and quantity of aspergilli. Summer season appeared as the richest, with maximum number of species (12) and isolates (78). In rainy season, 11 species and 39 isolates whereas in winter, 10 species and 39 isolates were reported.
- *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. ochraceous*, *A. sydowii* and *A. terreus* were present in all the three seasons. Among all these species, *A. sydowii* was the dominant one with 43 isolates in one year. The least dominant species were *A. fumigatus* and *A. ochraceous* with only 6 species.
- The species isolated in at least two seasons of the year were *A. aculeatus* and *A. flavipes* (in summer and rainy seasons) and *A. versicolor* (in rainy and winter seasons).
- The species restricted to only one season or only one month of the year were *A. amstelodami* and *A. niveus* (in winter), *A. carneus*, *A. chevaleri* and *A. regulosus* (in summer) and *A. tamarisii* (in rainy season).

- If calculated quantitatively, April month was the richest in the number of isolates of aspergilli (32 isolates) and February was the poorest (3 isolates only).

This pattern of seasonal variation in the occurrence of *Aspergillus* spp. over the crop fields has also been reported by other workers¹⁵⁻¹⁶.

- Colony counts during the three sampling hours (8.00, 12.00 and 18.00 hrs.) also showed variation. The maximum colony counts were observed in the morning (8 colonies), noon (14 colonies) and evening (12 colonies) in April month and the minimum in the morning, noon and evening of February month (0 colony). However, no precise pattern in the distribution of isolates was recorded during different sampling hours.

V. CONCLUSION

It can be concluded from the present investigations that there is a wide range of variation in the periodicity of mycoflora of *Aspergillus* spp. in different seasons during the year. Although the highest number of species and isolates of *Aspergillus* in the aeromycospora of B.R.D. Medical College, Gorakhpur are observed in summer (12 species and 78 isolates), their number is not drastically reduced in other seasons (rainy and winter). This fungus genus has been reported to be the most dominant one in different earlier studies too¹⁷⁻²³. Also, the seasonal distribution of aspergilli in the aeromycospora of B.R.D. Medical College is different from that of urban areas and agricultural fields. It can also be inferred that the incidence of airborne spores of *Aspergillus* spp. of clinical significance shows greater variation in response to the environmental conditions. Their side effects on the patients and attendants visiting medical college should be examined in detail, especially of those with less body immunity.

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REFERENCES

- [1]. I. Kasprzyk, "Aeromycology – main research fields of interest during the last 25 years." *Ann Agric Environ Med*, 15(1), 2008, pp. 1-7.
- [2]. P.H. Gregory. "The microbiology of the atmosphere." 2nd ed. Leonard Hill Aylesbury Bucks. 1973.
- [3]. M.K. Agarwal and D.N. Shivpuri. "Fungal spores and their role in respiratory allergy." *Advances in pollen spore research*, 1, 1974, pp. 78-128.
- [4]. M.S. Abd-Alla, K.M. Atalia and M.A.M. El-Sawi. "Effect of some plant waste extracts on growth and aflatoxin production of *Aspergillus flavus*." *Annals Agric. Sci.*, 46, 2001, pp. 579-592.
- [5]. O.P. Agrawal. "An Overview of Problems of Biodiversity of Cultural Property in Asia." In: *Biodeterioration of Cultural Property 3*. Aranyanak, C. and Singhasiri, C. Eds. Proceedings of the 3rd International Conference, Bangkok. 1995, pp. 14-34.
- [6]. C. Aranyanak. "Microscopical Study of Fungal Growth on Paper and Textile." In: *Biodeterioration of Cultural Property 3*. Aranyanak, C. and Singhasiri, C. Eds. Proceedings of the 3rd International Conference, Bangkok. 1995, pp. 82-102.
- [7]. Irene Arroyo. "The role of fungi in the deterioration of movable and immovable cultural heritage." *E-Conservation Magazine*, Spain. 2007, pp. 40-50.
- [8]. Sunita Bansod and Mahendra Rai. "Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*." *World Journal of Medical Sciences*, 3(2), 2008, pp. 81-88.
- [9]. M. Barkat and A. Bouguerra. "Study of antifungal activity of essential oils extracted from seeds of *Foeniculum vulgure* Mill. for its use as food conservative." *African Journal of Food Science*, 6(9), 2012, pp. 239-244.
- [10]. Mamta Srivastava, M.K. Arya and Neeraj Srivastava. "Cellulolytic fungi causing biodeterioration of Webster's dictionary in Gorakhpur." *International Journal of Biological Technology*, 2 (Special Issue), 2011, pp. 216-220.
- [11]. E.S. Beneke and A.L. Rogers. "Medical Mycology Aannual." Burges Pub. Co., U.S.A, 1970.
- [12]. C. Thom and M. Church. "The Aspergilli." The Williams & Wilkins Company, Baltimore, 1926.
- [13]. P.H. Gregory. "The microbiology of the atmosphere." 1st Ed. Wiley Interscience, New York, 1961.
- [14]. K.B. Raper and D.I. Fennel. "The Genus *Aspergillus*." The Williams and Wilkins Co., Baltimore, 1965.
- [15]. T. Sreeramalu and A. Ramalingam. "A two year study of the air spora of paddy fields near Visakhapatnam." *Indian J Agr Sci*, 36, 1966, pp. 111-132.
- [16]. Kamal and N.P. Singh. "An investigation on myco-organic content of air over sugarcane field at Gorakhpur (U.P.). I. Aspergilli." *Proc Nat Acad Sci, India*, 44(B), 1974, pp. 134-138.
- [17]. E. Rati and A. Ramalingam. "Air borne Aspergilli at Mysore." *Asp Allergy and Applied Immunol*, 9, 1976, pp. 139-149.
- [18]. B.S.V. Rajan S.S. Nigam and P.K. Shukla. "A study of the atmospheric fungal flora at Kanpur." *Proc Ind Acad Sci*, 35(B), 1952, pp. 33-37.
- [19]. B.P.R. Vittal and P. Ponnusamy. "A preliminary study of the atmospheric fungal flora of Madras." *Kavaka*, 1, 1979, pp. 79-82.
- [20]. E.D. Hamilton. "Studies on the air spora." *Acta. Allergol.* 13, 1939, pp. 143-175.
- [21]. S.L. Kalra and D.G. Dumbrey. "Aerobiology of army medical campus, Poona (Part I) : pollen, spores and mites." *Armed Forces Medical Journal (India)*, 13, 1957, pp. 3-16.
- [22]. J. Rubulis. "Air borne fungal spores in Stockholm and Eskilstuna, Central Sweden." *Nordic Aerobiology*, 1984, pp. 85-93.
- [23]. G.P. Agarwal. "An outlook of certain opportunistic fungi in India emerging as potential human pathogens." *J. Indian Bot. Soc.*, 71, 1992, pp. 1-10.