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RESEARCH PAPER

Aeromycological Investigation of B.R.D. Medical College, Gorakhpur, with special reference to spores of *Aspergillus* species and their Pathogenic Properties

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ABSTRACT

Aeromycology deals with the studies of airborne fungal spores. The human society and especially immunocompromised patients are exposed to the fungal spores present in the air and therefore, aeromycological investigation of a particular locality is very important. It helps locate the aeroallergens. A thorough investigation of airborne fungi forms the basis of research in aeromycology with respect to medicine. Data from previous studies reveals that various species of the fungus genus Aspergillus are most prevalent among all airborne fungi. This forms the basis of present investigation. The Campus, General Ward and Outdoor Patient Department of Baba Raghav Das Medical College, Gorakhpur were selected to record the periodicity of spores of Aspergillus species and to study and evaluate their allergenic properties. The period of investigation was from March, 2008 to February, 2009 and parameters taken were monthly, seasonal and temporal variations of spores of different species of Aspergillus. The months of March, May, October and November were recorded to have maximum number of Aspergillus species (10 species in each month) and minimum number in February (04 species only). The number of isolates of Aspergilli was maximum in April month (33 isolates) and minimum in February month (only 04 isolates). In seasonal variations, highest number of Aspergillus species (13 species) and isolates (81 isolates) was in summer season (March, 2008 to June, 2008), as compared to winter (09 species and 40 isolates) and rainy season (10 species and 43 isolates). The clinical data of B.R.D. Medical College, Gorakhpur have been compared with that of aeromycological data of Aspergillus species collected, which reveals that the patients of various allergic diseases like Bronchopulmonary Aspergillosis, Aspergilloma, Asthma, Alveolitis etc. frequently visit Medical College, Gorakhpur.
Keywords: Aeromycology, Fungal Air Spores, Aspergillus species, Aeroallergens.

INTRODUCTION

Air is a natural environment for spores of many genera and species of fungi. Despite their small size and a significant dispersion, they have a great impact on human health and different areas of our activities, such as agricultural production *etc.* "Bioaerosol" is a term used for fungal spores which are several micrometres in dimension and occur very numerously in the air (Kasprzyk, 2008). They are always observed in natural air and changes in their concentration depend on environmental conditions. Occurrence of these fungal air spores in the indoor and outdoor environment is studied under "Aeromycology". Gravity Plate Method of sampling of these fungal spores is used when these spores fall onto a catching surface (e.g., a culture medium) by earth's gravitational force, and Volumetric Method includes analysis of these fungal spores present in a given air unit. These air spores are characterised by their seasonal and diurnal cycles, which depend on conditions of climate and weather, on the availability of suitable substrates for proper growth of these fungal spores, circadian cycle of light and darkness, and some other environmental factors. These fungal spores adversely affect human health by causing severe diseases like immunotoxic and allergic diseases (Srivastava *et al*., 1990; Kasprzyk, 2008). Also, a detailed study of these airborne fungal spores is very significant for agriculture, agrobiology including pathogenic fungi, occupational medicines and in the conservation of our cultural heritage (Srivastava & Srivastava, 2007). Aeromycology of an area has its significance for several reasons, such as forecasting of plant diseases as well as studies about allergy, litter decomposition and allied aspects of microbiology (Gregory 1973). Airborne cellulolytic fungi play important role in decomposition of glazed and unglazed papers (Srivastava, 2007). The patterns of distribution of aeromycospora over urban areas is directly concerned with the allergic disorders in man (Agarwal & Shivpuri, 1974).

Pier Antonio Micheli described the fungus genus *Aspergillus,* various species of which are highly aerobic and found in almost all oxygen-rich environments. They grow on or inside various plants and contaminate starchy foods too. These *Aspergillus* species can survive on carbon sources as well as on the nutrient deficient substratum as oligotrophs. Various species cause natural degradation of a variety of organic matter by growing as saprophytes. They cause biodeterioration of various commodities in storage including grains, vegetables, fruits, papers, textiles and leathers. (Agarwal, 1995; Aranyanak, 1995; Arroyo, 2007; Bansod & Rai 2008).

Aspergillus species are very significant in medical science as over 60 species are reported as pathogens (Beneke & Rogers 1970). They cause respiratory tract diseases in humans, birds and animals (Thom & Church 1926). The term "Aspergillosis" is used for a group of diseases caused by various species of *Aspergillus*, which include "Broncho-pulmonary Aspergillosis, Acute Invasive Aspergillosis, Disseminated Invasive Aspergillosis and Aspergilloma (a "fungus ball" which is formed within the cavities lungs)."

Keeping in view of the above-mentioned importance of the fungus genus *Aspergillus*, the present research work has been performed to study diversity of spores of *Aspergillus* species in the air of Baba Raghav Das Medical College, Gorakhpur. Various parameters have been taken in this investigation including monthly, seasonal and temporal variations of spores of *Aspergillus* species and evaluation of their possible allergenic properties.

MATERIALS AND METHODS

(i) Site Selected for Investigation

The campus, General Ward and Outdoor Patient Department (OPD) of B.R.D. Medical College, Gorakhpur was selected for air sampling from March, 2008 to February, 2009. The air almost always contains fungal spores, but their number and types depend on the time of day, weather, season and geographical location (Gregory 1961). District Gorakhpur is located in the Tarai region of North-Eastern Uttar Pradesh and 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. The full year can be classified into three seasons on the basis of temperature and rainfall, named "Summer" (March to June), "Rainy" (July to October) and "Winter" (November to February). The summer season has a high temperature of up to 45^0C with decreased humidity and hot air winds. The winter is characterized by low temperature of up to 4^0C. In the rainy season, there is a heavy and frequent rainfall, with a maximum precipitation of about 349 mm. It has a moderately high temperature with high relative humidity. It drops gradually till the end of rainy season.

(ii) Isolation of Spores of Aspergillus species from Aeromycoflora

"**Gravity Plate Method**" (Frankland & Hart 1887) was used to isolate spores of *Aspergillus* from air. Martin's Streptomycin – Rose Bengal Agar culture medium was used. Five Petri plates of 80 mm. diameter containing this culture medium were exposed in the area of investigation for five minutes, three times on each day of sampling (Morning - 8.00 hr., Noon - 12.00 hr. and Evening - 18.00 hr.). These five 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, 2008 to February, 2009) at 15 days intervals. The exposed Petri plates were incubated for seven days at 25±2°C for fungal growth. The mixed cultures obtained were purified by single spore culture technique and identified (Raper & Fennel 1965). Total number of colonies per Petri plate and number of colonies of individual species were recorded separately. Quantitative studies were done by calculating the average colony count per Petri plate during one month.

RESULTS AND DISCUSSION

In the present investigation of one year (March, 2008 to February, 2009), a total of 16 species and 164 isolates of the fungus genus *Aspergillus* have been collected and reported from the air of B.R.D. Medical College, Gorakhpur.

Table – 1, Bar Chart -I and Table – 2, Line Graph – A and B reveal that

I) The months of March, May, October and November have maximum number of *Aspergillus* species (10 species in each month). The month of February show minimum number (only 04 species).

II) **Seasonal Variation** in the quality and quantity of various species of *Aspergillus* has been observed. The maximum number of *Aspergillus* species (13 species) and isolates (81 isolates) are recorded in summer season (March, 2008 to June, 2008). It gradually reduces in rainy season (July, 2008 to October, 2008) – 10 species and 43 isolates, and further in winter season (November, 2008 to February, 2009) – 09 species and 40 isolates.

III) Seven species of *Aspergillus* viz. *A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochraceous, A. sydowii* and *A. terreus* are found to be present in all the three seasons*, i.e.,* summer, rainy and winter. *Aspergillus sydowii* is recorded as the most dominant species with 43 isolates in one year. *Aspergillus regulosus* is the least dominant one with only 01 isolate in one year.

Table 1. Monthly Variations (March, 2008 to Feb., 2009) in Number of Species and Isolates of *Aspergillus* Micheli.

Sr. No.	*Aspergillus* species	Months/No. of Isolates											
		Mar.	Apr.	May	June	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
1	*A. aculeatus* Iizukais	-	-	1	-	2	-	1	1	-	-	-	-
2	*A. amstelodami* angin) Thom & Church	-	-	-	-	-	-	-	-	1	1	-	-
3	*A. carneus* (van Tiegham) Blochwitz	1	4	-	-	-	-	-	-	-	-	-	-
4	*A. chevalieri* (Mangin) Thom& Church	1	1	1	-	-	-	-	-	-	-	-	-
5	*A. flavipes* (Bain. & Sart.) Thom & Church	2	-	1	1	-	-	-	1	-	-	-	-
6	*A. flavus* Link	1	3	1	1	-	-	2	1	1	1	-	1
7	*A. fumigatus* Fresenius	1	1	1	2	-	-	-	1	1	-	-	-
8	*A. nidulans* (Eidam) Wint.	3	5	4	1	2	1	-	1	1	1	1	1
9	*A. niger* van Tieghem	5	5	3	3	2	2	3	1	2	1	2	1
10	*niveus* Blochwitz	-	-	-	-	-	-	-	-	1	1	-	-
11	*ochraceous* Wilhelm	1	-	1	2	1	1	-	1	1	-	-	-
12	*regulosus* Thom & Raper	1	-	-	-	-	-	-	-	-	-	-	-
13	*sydowii* (Bain. & Sart.) Thom &Church	1	10	6	-	4	2	2	4	4	6	3	1
14	*A. tamarii* Kita	-	-	-	-	-	-	-	3	-	-	-	-
15	*A. terreus* Thom	-	4	2	-	-	1	2	-	1	-	2	-
16	*A. versicolor* (Vuillemin) Tiraboschi	-	-	-	-	-	-	-	1	1	1	2	-
Total Species	**16**	10	08	10	06	05	05	05	10	10	07	05	04
Total Isolates	**164**	17	33	21	10	11	07	10	15	14	12	10	04

BAR CHART 1.

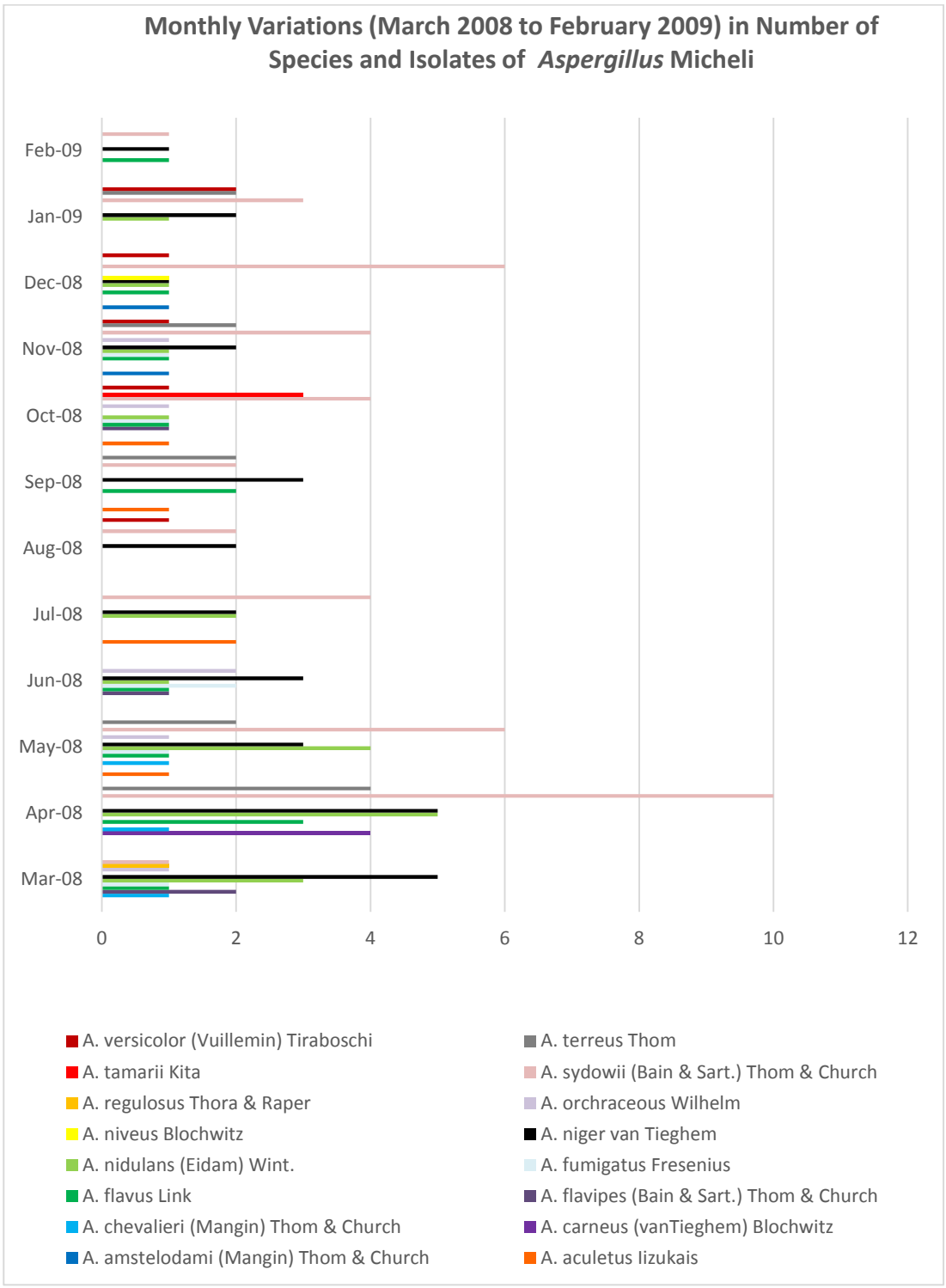
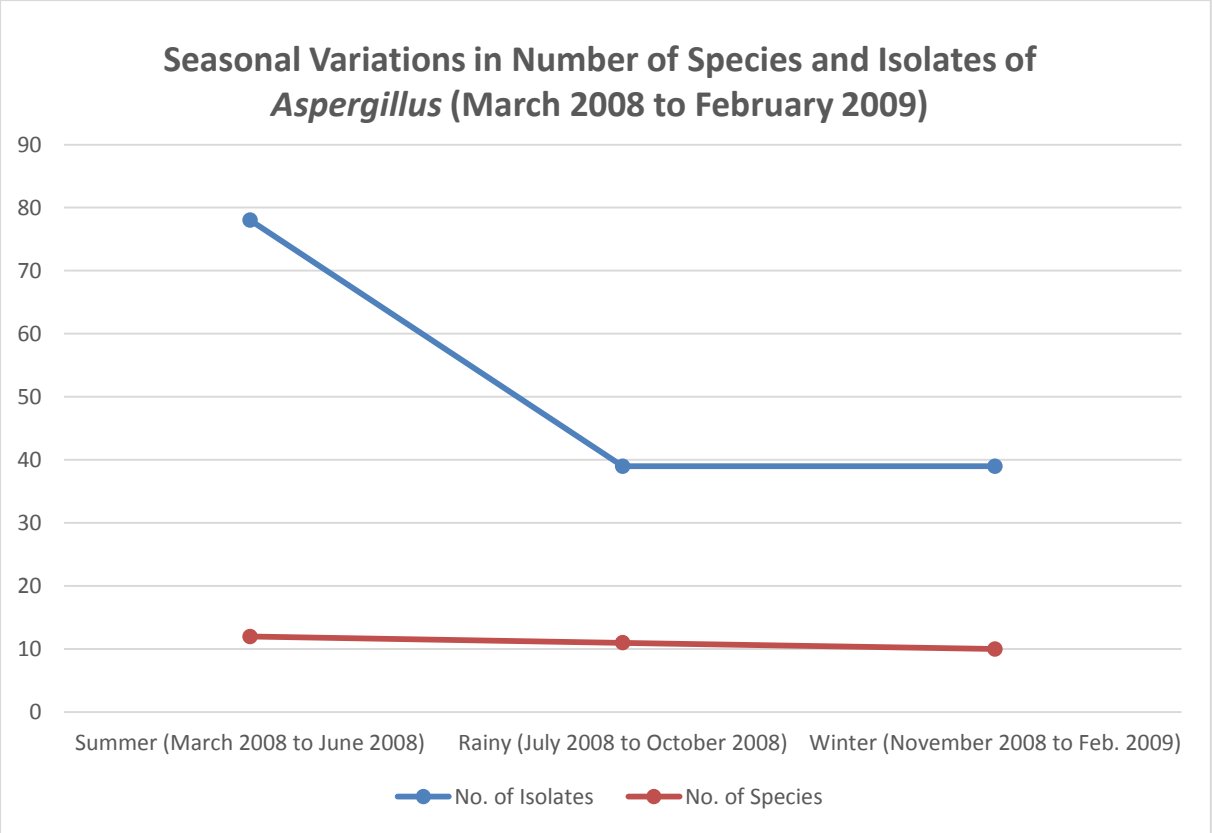

Monthly Variations (March 2008 to February 2009) in Number of Species and Isolates of Aspergillus Micheli
Feb-09
Jan-09
Dec-08
Nov-08
Oct-08
Sep-08
Aug-08
Jul-08
Jun-08
May-08
Apr-08
Mar-08
0
2
4
6
8
10
12
A. versicolor (Vuillemin) Tiraboschi
A. terreus Thom
A. tamarii Kita
A. sydowii (Bain & Sart.) Thom & Church
A. regulosus Thora & Raper
A. orchraceous Wilhelm
A. niveus Blochwitz
A. niger van Tieghem
A. nidulans (Eidam) Wint.
A. fumigatus Fresenius
A. flavus Link
A. flavipes (Bain & Sart.) Thom & Church
A. chevalieri (Mangin) Thom & Church
A. carneus (vanTieghem) Blochwitz
A. amstelodami (Mangin) Thom & Church
A. aculetus Iizukais

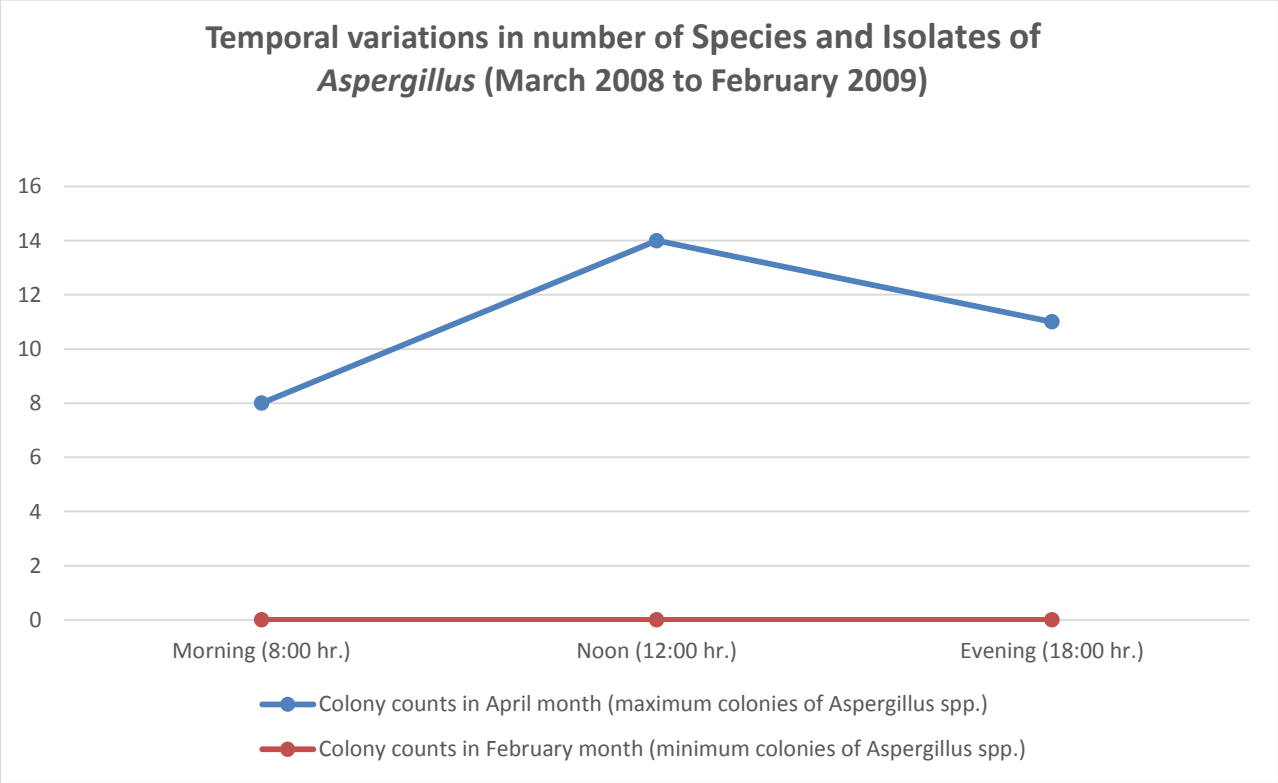
Table 2. Seasonal and Temporal Variations in Number of Species and Isolates of *Aspergillus* Micheli (March, 2008 to Feb., 2009).

Seasonal Variations			Temporal Variations		
Summer (March, 2008 to June, 2008)	**Rainy (July, 2008 to Oct., 2008)**	**Winter (Nov., 2008 to Feb., 2009)**	**Morning (8.00 hr.)**	**Noon (12.00 hr.)**	**Evening (18.00 hr.)**
81 Isolates	43 Isolates	40 Isolates	Maximum colony counts in April (09)	Maximum colony counts in April (15)	Maximum colony counts in April (12)
13 Species	10 Species	09 Species	Minimum colony counts in Feb. (01)	Minimum colony counts in Feb. (01)	Minimum colony counts in Feb. (01)

LINE GRAPH – A



LINE GRAPH - B



IV) *Aspergillus aculeatus* and *A. flavepes* are isolated in summer and rainy seasons, whereas *A. versicolor* in rainy and winter season.

V) Two species *viz*., *A. amstelodami* and *A. niveus* are restricted only to winter season; three species *viz*., *A. carneus, A. chevaleri* and *A. regulosus* to summer season and one species *viz*., *A*. *tamarii* to rainy season.

VI) Quantitative observation reveals that maximum number of isolates of *Aspergillus* species is in the month of April (33 isolates) and minimum number in February month (only 04 isolates).

Some other workers like Sreeramalu & Ramalingam (1966) and Kamal & Singh (1974) have also reported the same pattern of seasonal variations in the air spores of *Aspergillus* species over the crop fields.

VII) **Temporal Variations** have also been reported in *Aspergillus* species. The parameter used for this variation is colony count during three sampling times/hours *viz*., morning (8.00 hour), noon (12.00 hour) and evening (18.00 hour). The month of April shows maximum colony counts in all the three hours (09 colonies in morning, 15 colonies in noon and 12 colonies in evening). The month of February shows minimum colony counts in all the three hours (01 colony only in all the three hours of morning, noon and evening). However, this investigation does not show a precise pattern of distribution of *Aspergillus* species isolates during different sampling hours.

CONCLUSION

The present investigation clearly shows that the aeromycoflora of *Aspergillus* species has a wide range of variation, seasonal as well as temporal, in their periodicity in different seasons during one year. Summer months (March to June, 2008) have the maximum number of species (13 species) and isolates (81 isolates) of *Aspergillus* species present in the air of B.R.D. Medical College, Gorakhpur. However, this number is not recorded to be drastically reduced in other two rainy and winter seasons. Earlier studies have also reported this fungus genus *Aspergillus* to be the most dominant one (Rati & Ramalingam, 1976; Rajan *et al*., 1952; Vittal & Ponnusamy, 1979; Hamilton, 1939; Kalra & Dumbrey, 1957; Rubulis, 1984; Agarwal, 1992). However, seasonal distribution pattern of air spores of *Aspergillus* species of B.R.D. Medical College, Gorakhpur is different from distribution pattern reported from urban areas and agricultural fields. Also, a great variation has been observed in the incidence of airborne spores of clinically significant *Aspergillus* species in response to environmental conditions. The side effects of these Aspergillus species on immunocompromised patients and attendants visiting medical college must be examined.

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