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Diurnal periodicity of aero allergenic fungal spores in bed rooms of houses of the different areas in Pondicherry

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Diurnal periodicity of aero-allergenic fungal spores in the bed rooms of houses of the different areas of Pondicherry city was carried out by implementing Petri plate sedimentation method during 2008. Composition and concentration of fungal spores considerably varied from time to time in the day-night periods as well as from bed room to bed room. Occurrence of fungal species was predominated with more number of propagules during mid night and early morning periods in comparison to other time's air samples. *Aspergillus* was recorded with the highest frequency and had eight species i.e., *A. fumigatus, A. awamori, A. niger, A. flavus, A. flavipes, A. ochraceous, A. glaucus* and *A. wentii. Penicillium* was isolated in qualitatively and quantitatively next to *Aspergillus*. Out of the 28 isolated fungal taxa, *Aspergillus fumigatus, A. awamori, A. niger, Rhizopus stolonifer* and *Alternaria alternata* were the predominant aeroallergens, which caused diifferent types of respiratory lung diseases in atopic human beings. *Penicillium* spp were the second highest recorded fungus in bed room environments are well known for the cause of allergic alveolities. *A. flavus,* a mycotoxin producing fungus was abundantly recorded. *Alternaria alternata,* which is accounted as a human allergen for sporosis inducer and an agent for hay fever and other pathologies, was also intermittently recorded. Besides the above prominent human allergens, a number of other fungi were recorded; many of them were prominent plant pathogens and saprophytic field and storage fungi.

Key words: Diurnal periodicity, Aeroallergens, bed room, sedimentation method

INTRODUCTION

The incidence of allergic respiratory diseases is increasing alarmingly and the airborne fungal spores are mostly responsible for causing a significant proportion of such diseases in most of the countries (Holgate, 1999). The trend of allergic diseases in India has gone up more than 25% of the total human population (Sharma *et al.*, 2007). People who are immune-compromised would be at risk since they are easily infected even by non-pathogenic organisms (Simon *et al.*, 2008). The quality of indoor air could be an issue for public health, since the health of people residing in these indoors (house environments) is affected (Michael *et al*, 2007). If respiratory allergic diseases are to be treated correctly, knowledge of airborne fungi both qualitatively and quantitatively in indoors should be given more preference. The indoor environments of the houses are not anthropogenically polluted but it lacks a hyginic condition of living due to the lack of scientific information on aeromycology in the country like India. The objectives of the present study are to determine the diurnal incidence of aero-allergenic fungal spores in bed rooms of houses of the different areas in Pondicherry region.

MATERIALS AND METHODS

The present study was carried out in bed rooms of three houses of different areas of Thattanchavady in Pondicherry city during July to September 2008.

Study sites

The study sites were in Thattanchavady locality near to the JIPMER, Pondicherry, which lies within 11° 46" and 12° 30" N latitudes and 79° 36" and 79° 53" E longitudes. It is not an industrially developed place but a unique medical (JIPMER) unit of Govt, of India and other governments offices are there that cater to the needs of the Puducherry state. The present study was conducted in bed rooms of three distinct houses located in Veema Nagar, Ananda Nagar and V.V.P. Nagar at 1 to 1.5 km apart from each other in the above area.

Air Samplings

Air samplings were made diurnally from 1 AM to 12 mid night at one hr interval for one day continuously exposing Petri plates at 150 cm height from the floor in the kitchen. Three replicates of Petri plates (0 = 9 cm) containing Potato Dextrose Agar (PDA) medium with Streptomycin/ Penicillin (50 mg^{-1}) were carried to the bed rooms inside a sterilized container and exposed to the air for five minutes to receive the sedimented air borne fungal spores on the media plates. Altogether 72 Petri plates were exposed in the bed room of each house. Each bed room was air sampled at 15 day intervals and continued for total three months to complete the samplings. The exposure time was standardized to get 10-30 number of fungal colonies/colony forming units (CFUs) per plate. Each set of plates exposed in different bed rooms were brought separately to the Microbiology Laboratory, Department of Plant Science, KMCPGS (Autonomous), Pondicherry with utmost care and incubated in culture room at 25±3°C upside down for 15 days with constant observation after 3-4 days of incubation. Fungal colonies developed in plates were counted for individual species and to get the total number CFUs. Microscopic slides stained with lacto phenol cotton blue were prepared from each CFUs and observed microscopically to identify them up to species level. The laboratory experience and taxonomic literature were employed to identify the fungal taxa (Gilman, 1957; Ellis, 1971; Barnett and Hunter, 1972; Ellis, 1976; Onions *et al.*, 1986).

RESULTS

During the study period, a total number of 216 Petri plates covering seventy two air samplings, three replicates in each samplings, 24 times in each bed room were exposed and each plate provided on an average of 15-18 colony forming units (CFUs). In qualitative study, altogether 28 fungal species under 18 genera were isolated from the bed rooms of all houses. Relative incidence of isolated fungi in the bed rooms of different area houses in Pondicherry city are given in Table 1. Among the recorded taxa, members of Deuteromycotina were most prominent in their occurrences followed by the members of Zygomycotina. *Aspergillus* spp, *Penicillium* spp, *Fusarium oxysporum* White sterile mycelia, *Curvularia* sp., *Candida* sp., *Absidia spinosa* and *Rhizopus stolonifer* were recorded frequently from the bed rooms.

Diurnal occurrence of air borne fungal CFUs recorded in the bed rooms of three area houses are given in Fig. 1. Among all the 24 air samplings of the diurnal time period, the highest peaks were found at 1 AM, 3 AM, 5 AM and 2 PM. It showed that the occurrences of airborne fungal spores were more during these peak periods in the bed rooms. The line graph showed a unique pattern of circadian periodicity of fungal distribution in the bed rooms of the three area houses. The trend of diurnal incidence of fungal spores was found same in all the bed rooms studied.

Among all the fungal species, *Aspergillus* spp.was dominant ones followed by *Penicillium, Fusarium oxysporum* and White sterile mycelia. Eight species of *Aspergillus* i.e., *A. niger, A. flavus,. A. awamori, A. flavipes, A. ochraceous, A. fumigatus, A. glaucus,* and *A. wentii* were given in Fig. 2 with their occurrence. Four species of *Penicillium* were also isolated i.e., *Penicillium* sp., *P. oxalicum*, *P. fellutanum* and *P. italicum,* (Fig. 2). *Aspergillus fumigatus* was isolated with highest percentage (10.13%) followed by *Aspergillus niger* (9.86%) and White sterile mycelia (10.74%) in the bed room environments of Veema Nagar house (Table 1). In Ananda Nagar, *Aspergillus awamori* was the maximum (9.29%) contributer followed by *Aspergillus fumigatus* (7.48%) and *A.flavus* (7.25%). *Aspergillus fumigatus* was the highest (9.87%) recorded fungus followed by *Penicillium italicum* (7.84%) and *A. awamori* (7.33%) in their occurrence in the bed rooms of V.V.P. Nagar (Fig. 3). *Trichothecium roseum*, contributed 1.86%, 3.17% and 3.29% from the total fungal CFUs recorded from

Table 1: Relative incidence of isolated fungi in the bed rooms of different area houses in Pondicherry region

Fungi	Relative incidence (%)		
	Veema Nagar	Ananda Nagar	V.V.P.Nagar
Aspergillus fumigutus	10.13	7.48	9.87
A. niger	9.86	5.21	5.56
White sterile mycelia	7.46	4.76	4.55
A. flavus	7.20	7.25	3.54
A. awamori	6.40	9.29	6.32
Penicillium oxalicum	5.60	5.66	4.30
Fusarium oxysporum	5.60	6.35	4.81
A. flavipes	5.33	3.17	5.31
Penicillium fellutanum	5.06	4.31	2.78
Pencillium sp.	4.80	4.54	3.54
Curvuluria lunata	4.26	3.85	4.81
Gray sterile mycelia	4.00	2.95	4.05
Neurospora sp.	3.46	2.27	4.30
Aerobasidium pullulans	3.20	0.45	–
Rhizopus stolonifer	2.93	4.31	3.29
A. wentii	2.66	3.40	1.77
Cladosporium hurbarum	2.13	2.72	1.77
Alternaria alternata	2.13	2.72	3.04
Trichothecium roseum	1.86	3.17	3.29
Candida sp.	1.60	1.81	3.29
Absidia spinosa	1.33	1.59	1.27
A. glaucus	1.06	3.63	3.79
Mucor sp.	0.80	0.68	0.76
Cladosporium cladosporioides	0.53	–	0.25
Helminthosporium sp.	0.26	1.36	2.03
A. ochraceous	0.26	1.81	2.78
Penicillium italicum	–	5.22	7.84
Gliocladium sp.	–	–	0.50
Drechslera sp	–	–	0.50

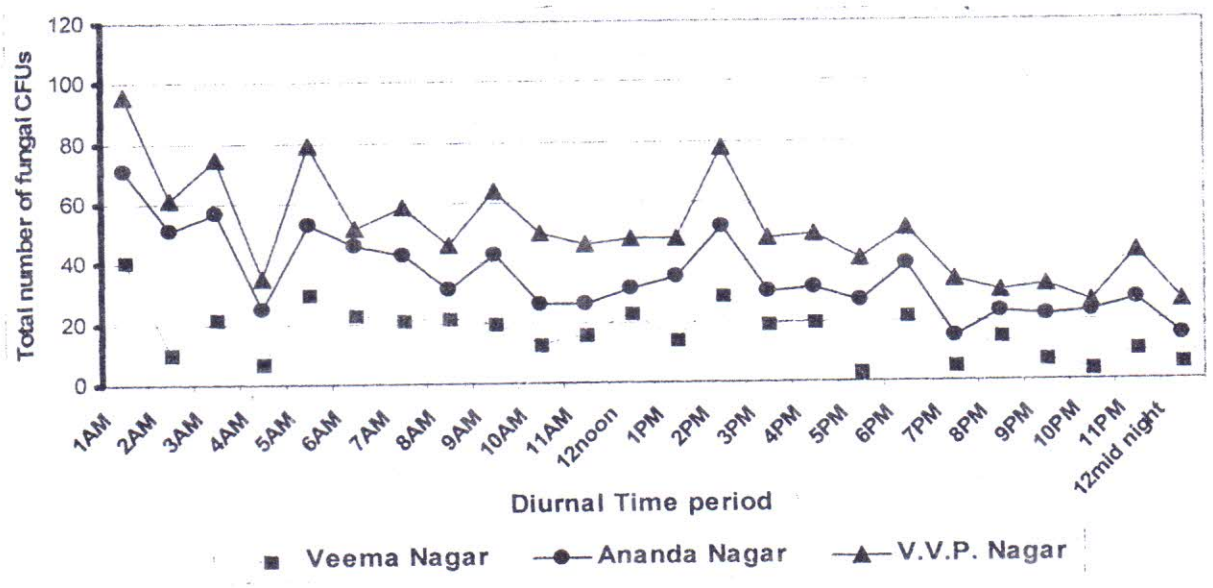

Fig 1. Diurnal occurrence of airborne fungai CFUs recorded in bed rooms of different area houses of Pondicherry Union territory

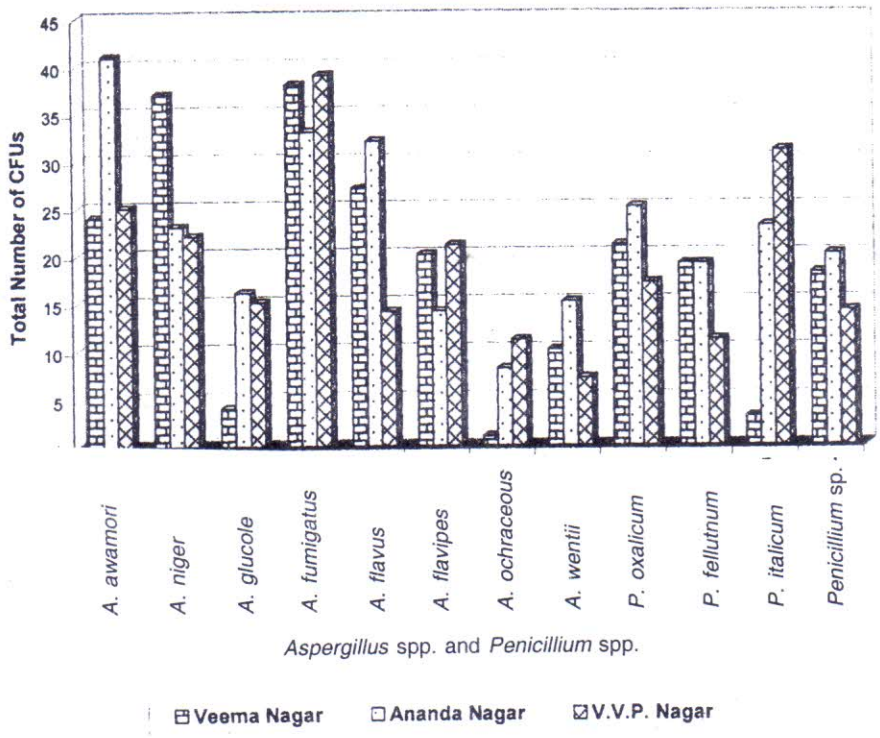

Fig 2. Distribution of *Aspergillus* spp. and *Penicillium* spp. in bed rooms of different area houses

bed rooms of Veema Nagar, Ananda Nagar and V.V.P. Nagar respectively. *Absidia spinosa*, *Helminthosporium* sp., *Neurospora* sp., *Drechslera* sp., *Alternaria alternata*, *Rhizopus stolonifer* and *Mucor sp.* were also recorded intermittently from the bed room environments. *Cladosporium herbarum* and *C. cladosporioides* were also recorded from bed room air of three houses studied.

Among the isolated fungal taxa, *Aspergillus fumigatus, A. niger, A. awamori, Penicillium* spp., *Rhizopus stolonifer* and *Alternaria alternata* were predominant aeroallergens (Fig. 3) that caused different types of respiratory/lung diseases in atopic human beings. *Aspergillus fumigatus* causes bronchopulmonary aspergillosis disease. *Aspergillus flavus*, a mycotoxin producing fungus was abundantly recorded from the bed room environments.

Number of fungal CFUs recorded in bed rooms of three area houses showed the bed room environments of Ananda Nagar harbored maximum number of fungal spores followed by V.V.P. Nagar and Veema Nagar. The distribution of various fungal species and their abundance in different bed rooms are given in Fig. 2. In comparative analysis, the occurrence of *Aspergillus* spp. was recorded with highest percentage in bed rooms of all three houses i.e., (43%) in Veema Nagar, (41%) in Ananda Nagar but it was 39% in V.V.P. Nagar (Fig. 2). *Aspergillus*, *Penicillium*, *Fusarium*, White sterile mycelia, *Candida*, *Absidia*, *Mucor* and *Curvularia* were in descending order according to their diurnal occurrence. *Neurospora* sp. and *Aerobasidium pullulans* were also recorded from all the three houses.

DISCUSSION

Study of aeromycospora employs a number of sampling methods of which gravity settling of spores on culture medium is one widely used method by workers (Hedayati *et al.*, 2005; Nanda *et al.*, 2000; Abdel Hafez, 1989) both in indoor and outdoor environments but its use in indoor environment is more appropriate as the deposition of spores is less affected by wind turbulence (Infante *et al.*, 1992). The settled viable spores germinate, develop to mycelia and sporulate on the broad spectrum medium. It facilitates the microscopic study of the colonies and enables the identification of the species. It is highly suitable for qualitative study but the result can not be set forth quantitatively as it is not possible to express them as a unit of air volume.

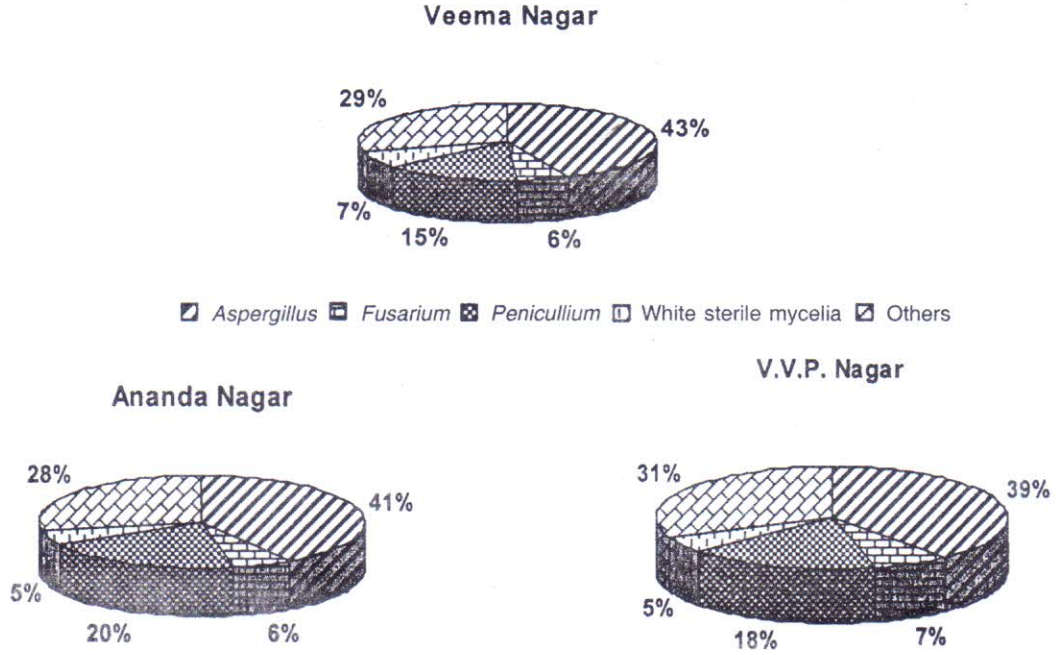

Fig 3. Distribution of major fungal spores isolated from bed rooms of different area houses.

Out of the isolated fungal species, most of them belong to the members of Deuteromycotina followed by the members of Zygomycotina, which occurr at high levels at the bed room environments as reported by other workers (Infante *et al.*, 1992; Li, 1995). A number of aspergilli comprises high incidence of *A. fumigatus, A. awamori, A. niger, A. flavus, A. flavipes, A. ochraceous, A. glaucus* and *A. wentii* have been reported in the present study similar to findings of many others (Jurado *et al.*, 1990; Dharmage *et al.*, 2002; Hedayati *et al.*, 2005). Banerjee and his co workers (1987) have isolated *Cladosporium, Aspergillus, Penicillium, Alternaria, Mucor, Rhizopus, Aureobasidium* and *Fusarium* from the house environments in Durham, North Corolina (U.S.A.), which is similar to our findings. Higher number of mould fungi are associated with high shade and high levels of organic debris near the home and poor landscaping (Banerjee *et al.*, 1987). Lumpkins *et al.*, (1973) have described the same group of deuteromycetaceous fungi from both indoors and outdoors and he has also reported that there is a common reservoir pool for both indoor and outdoor microflora from where they represent in the ambient air.

Diurnal incidence of air borne fungal spores has showed that the mid night and early morning time periods are predominated with more number of propagules in comparison to other timings. Since the sedimentation rate is highest and the air turbulence is zero during these diurnal periods, the contributions of the maximum fungal CFUs are recorded in the Petri plates during these times' air samples (Bhatti *et al.*, 1986; Nayak *et al.*, 1998; Hyvarinen *et al.*, 2001).

Our study has showed that *Aspergillus* and *Penicillium* are the most common fungi recovered inside the bed rooms, these finding are in agreement with the finding of several other researchers (Dharmage *et al.*, 2002; Unlu *et al.*, 2003). It is well known that spores of *Aspergillus, Cladosporium,* and *Penicillium* generated in damp building can cause bouts of asthma and/or rhinitis among atopic occupants; as well as having a role in such individual cases of allergic disease (Hyvarinen *et al.*, 2001; Hedayati *et al.*, 2005). Fungi are now seen as having a wider role in respiratory ill health (Fung *et al.*, 1998). During our study, *Cladosporium* is also recorded in bed rooms but their occurrence is very less in copmarision to *Aspergillus* and *Penicillium*. *Cladosporium* spp. are winter loving fungi generally occur in more numbers during the months between October to March all over the world in indoors and outdoors (Li and Hsu, 1997; Nayak *et al.*, 1998; Hedayati *et al.*, 2005).

The occurrence of *A. flavus,* the causal agent of "aflatoxicosis" in birds, needs a detailed investigation in view of its role in Farmer's lung disease besides, type I hypersensitivity disorders (Singh *et al.* 1990). Singh *et al.*, (1990) have reported *Aspergillus fumigatus* as an opportunistic pathogen in immune suppressed patients which causes respiratory infections leading to bronchopulmonary aspergillopsis. It is also noted that *Penicillium* spp. predominated in air samplings next to *Aspergillus* spp. in the bed room environment. In tropical environments these fungi are among the dominant ones (Nayak and Behera,1996) and are well known for allergenicity and are causal agents of allergic alveolities (Cordasco *et al.*, 1995). *Helminthosporium* sp. is a plant pathogenic fungus which is also recorded from bed room air. *Rhizopus, Alternaria, Curvularia* and *Drechslera* are saprophytic fungi and weak pathogens of vegetables and crop plants, were reported in the present study. Although *Alternaria alternata* was not abundantly recorded, it is accounted as a human allergen for sporosis inducer, an agent for hay fever and other pathologies (Nayak *et al.*, 1998; Cordasco *et al.*, 1995). Besides these prominent human allergens, a number of other fungi are recorded; many of them are prominent plant pathogens and saprophytic field and storage fungi.

Recently, attention has been focused on the fungi in relation to human disease, especially, in sick building syndrome (Li, 1995; Hyvarinen *et al.*, 2001). However, the presence of fungi in building does not necessarily imply a cause and effect relationship with illness, but should alert physicians and healthcare professionals to do more vigorous environmental testing.

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