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FUNGAL AIR SPORA SURVEY OVER COW SHED IN BANGALORE, INDIA

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ABSTRACT

A fungal air spora survey of indoor and outdoor air samples of cow shed at Hessaraghatta village of Bangalore city, India was carried out by Andersen two stage sampler using an malt extract agar media were collected fortnightly from January 2011 to December 2011. Indoor air harbored maximum fungal spores (7808.36 CFU/m^3) in comparison to outdoor air (7610.68 CFU/m^3). Altogether, total 29 species belonging to 13 genera with unidentified fungi were isolated from indoor environment of the cow shed, when compared to 26 species belonging to 12 genera with unidentified fungi were isolated from outdoor environment of the cow shed. Summer season was found to harbored maximum fungal spores in comparison to winter and rainy. The present study helped in preparation of fungal calendars for the region, which may useful for clinicians to identify the cause of fungal spore related problem affecting human life.

Key Words : Cow shed, Fungal calendar, Seasonal variations, Meteorological, Andersen

INTRODUCTION

Fungi are the causal agents for different diseases in animals, plants and human beings. The basic sources of microbes are soil, water, animals and humans and they are originate in many different forms and affect visibility, climate, human health and the quality of life[1]. Airborne microbial quantity and quality vary with time of day, year and location[2]. Various factors, such as the type of collection medium, the identification process, the period of the day in which the collection of fungi takes place, the sampling frequency and duration, influence the airborne fungi monitoring[3]. Fungi are common in indoor and outdoor environment. Nearly 10% of people worldwide have fungal allergy[4]. Numerous studies have shown that exposure to fungi may be associated with acute toxic effects, allergies and asthma[5].

AIMS AND OBJECTIVES

To determine the concentration and better characterize the inhalation exposure to these fungi in terms of monthly, seasonal distribution with relation to meteorological factors of indoor and outdoor fungal spores of cow shed of Bangalore, India.

MATERIAL AND METHODS

The site selected for fungal sampling was Hessaraghatta village, situated from Northwest of Bangalore, India. The indoor and outdoor air samples were collected fortnightly from January 2011 to December 2011 using Andersen two stage air sampler[6]. Sampler was placed in the center of the cow shed and 1.5 meters above the ground level. Standard 90 mm petridish with malt extract agar was used as sampling media are used as collecting surfaces on each stage. Air flow was 28.3 L/min during the sampling and the sampling time is limited to 5 minutes. The number of fungal colonies on each plate is counted and expressed as number of colony forming unit per cubic meter (CFU/m^3) of air, as per the following conversion formula. The air sampled plates were incubated for 5 to 7 days at room temperature between 25^{0}C to 30^{0}C and colony morphological charac-teristics were observed microscopically by using manuals and references slides[7,8]. Statistical analysis was performed using Pearson Correlation was used for determining the coefficients between indoor and outdoor fungal spores of the cow

shed, seasonal variation and meteorological data. The meteorological data were collected from the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, India **(Table 1)**.

Table 1 : Meteorological data recorded at Hesaraghatta village, Bangalore

Monthly average				
Year 2011	**Temperature ^{0}C**	**Relative humidity %**	**Wind speed km/h**	**Rainfall mm**
January	27.8	89.9	4.8	Nil
February	29.5	87.0	4.8	16.6
March	32.9	85.0	5.1	Nil
April	32.6	89.3	4.5	57.4
May	32.1	90.0	5.0	126.0
June	29.1	94.0	9.0	30.0
July	27.8	94.0	7.6	95.8
August	27.3	94.0	6.4	253.2
September	28.0	94.0	5.8	59.7
October	28.6	93.0	2.9	122.6
November	26.6	89.0	4.8	38.0
December	26.9	91.0	4.5	5.2

RESULTS AND DISCUSSION

The present study was conducted to analyze the monthly incidence of airborne fungi, seasonal variation and influence of meteorological parameters in indoor and outdoor fungi of cow shed at Hessaraghatta village, Bangalore, India. During the study period, a total number of 15419.04 CFU/m^3 was isolated from both indoor and outdoor environment of the cow shed, of which indoor environment of the cow shed contributed to 7808.36 CFU/m^3 and the outdoor environment of the cow shed contributed 7610.68 CFU/m^3. Similar airborne and closely related fungal assemblages have been recorded many researchers[9,10]. With respect to the qualitative analysis, altogether 29 species belonging to 13 genera with unidentified fungi were isolated from indoor environment of the cow shed, when compared to 26 species belonging to 12 genera with unidentified fungi were isolated from outdoor environment of the cow shed. Among the total number of isolated fungal species from indoor environment of the cow shed *Aspergillus* (27.36%) was represented by 6 species viz., *A. flavus, A. niger, A. oryzae, A. ochraceus, A. fumigatus* and *A. terreus* followed by 3 species of cladosporium (18.2%) viz., *C. cladosporioides, C. herbarium* and *C. acremonium*, 3 species of fusarium (13.9%) viz., *F. oxysporum, F. moniliforme* and *F. solani*, 3 species of penicillium (13.14%) viz., *P. versicolor, P. citrinum* and *P. nigricans*, 1 species of curvularia (4.07%) viz., *C. lunata* along with *Acremonium* sp., (2.07%), *Altern-aria alternata* (5.51%), *Ascomycetes* sp., (0.85%), *Mucor* sp., (2.66%), *Neurospora* sp., (0.72%), *Rhizopus* sp., (4.06%), *Scopulariopsis* sp., (0.4%) and *Trichoderma* sp., (3.2%) as shown in **Table 2**.

Table 2 : Fungal spores recorded (CFU/m^3) in the indoor air of the cow shed during January-December, 2011

S/N	Genera and species	Total	%
1	*Acremonium* sp.	162.38	2.07
2	*Alternaria alternata*	430.66	5.51
3	*Ascomycetes* sp.	67.07	0.85
4	*Aspergillus* sp.	501.26	6.41
5	*A. flavus*	462.43	5.92
6	*A. niger*	476.55	6.1
7	*A. oryzae*	328.29	4.2
8	*A. ochraceus*	49.42	0.63
9	*A. fumigatus*	98.84	1.26
10	*A. terreus*	222.39	2.84
11	*Curvularia* sp.	218.86	2.8
12	*C. lunata*	169.44	2.16
13	*Cladosporium* sp.	635.4	8.13
14	*C. cladosporioides*	395.36	5.06
15	*C. herbarum*	257.69	3.3
16	*C. acremonium*	134.14	1.71
17	*Fusarium* sp.	338.88	4.33
18	*F. oxysporum*	208.27	2.66
19	*F. moniliforme*	271.81	3.48
20	*F. solani*	268.28	3.43
21	*Mucor* sp.	208.27	2.66
22	*Neurospora* sp.	56.48	0.72
23	*Rhizopus* sp.	317.7	4.06
24	*Penicillium* sp.	335.35	4.29
25	*P. versicolor*	257.69	3.3
26	*P. citrinum*	250.63	3.2
27	*P. nigricans*	183.56	2.35
28	*Scopulariopsis* sp.	31.77	0.4
29	*Trichoderma* sp.	250.63	3.2
30	Unidentified	218.86	2.8
Total		7808.36	100

When these findings were compared with that of outdoor environment of the cow shed it was observed that all the fungal genera and species remain the same, with variation in their percentage occurrence. Few of the fungal species like *A. ochraceus, Neurospora* sp. and *P. nigricans* were not found in the outdoor environment of the cow shed are presented in **Table 3**. In the naturally continuous mixing of indoor and outdoor air, the concentration of fungi can be two to five times higher than the outdoor level[11]. However, the spore concentration has been observed to be much lower in outdoor (control) environment. Because many people spend as much as 90% of their time indoors, the health risk of indoor air pollutants is of critical public health concern. Studies have shown association

between reported indoor dampness and health outcomes, including respiratory symptoms, headache and upper respiratory airway infections[12].

Table 3 : Fungal spores recorded (CFU/m^3) in the outdoor (control) air of the cow shed during January-December, 2011

S/N	Genera and species	Total	%
1	*Acremonium* sp.	310.64	4.08
2	*Alternaria alternata*	826.02	10.85
3	*Ascomycetes* sp.	321.23	4.22
4	*Aspergillus* sp.	815.43	10.71
5	*A. flavus*	201.21	2.64
6	*A. niger*	109.43	1.43
7	*A. oryzae*	218.86	2.87
8	*A. fumigatus*	271.81	3.57
9	*A. terreus*	74.13	0.97
10	*Curvularia* sp.	180.03	2.36
11	*C. lunata*	130.61	1.71
12	*Cladosporium* sp.	554.21	7.28
13	*C. cladosporioides*	232.98	3.06
14	*C. herbarum*	169.44	2.22
15	*C. acremonium*	254.16	3.33
16	*Fusarium* sp.	275.34	3.61
17	*F. oxysporum*	130.61	1.71
18	*F. moniliforme*	275.34	3.61
19	*F. solani*	278.87	3.66
20	*Mucor* sp.	342.41	4.49
21	*Rhizopus* sp.	236.51	3.1
22	*Penicillium* sp.	324.76	4.26
23	*P. versicolor*	158.85	2.08
24	*P. citrinum*	289.46	3.8
25	*Scopulariopsis* sp.	67.07	0.88
26	*Trichoderma* sp.	331.82	4.35
27	Unidentified	229.45	3.01
Total		7610.68	100

Based on comparative analysis, dominant fungal species in indoor environment of the cow shed were *Cladosporium* (8.13%), *Aspergillus* (6.41%) and *Aspergillus niger* (6.1%) but *Scopulariopsis* (0.4%) and *Aspergillus ochraceus* (0.63%) were least recorded **(Table 2)**. Whereas in outdoor environment of the cow shed the dominant fungal species recorded were *Alternaria alternata* (10.85%) and *Aspergillus* (10.71%) while *Scopulariopsis* (0.88%) and *Aspergillus terreus* (0.97%) were least recorded **(Table 3)**. Monthly variation of total fungal spores in the indoor environment of the cow shed showed maximum spore distribution in May (780.13 CFU/m^3) followed by February (773.07 CFU/m^3) and January (755.42 CFU/m^3) compared to other months of the year

(Table 2), whereas the monthly incidence of fungal spores of outdoor (control) environment of the cow shed showed highest distribution during March (790.72 CFU/m^3) followed by June (783.66 CFU/m^3) and January (716.59 CFU/m^3) when compared to other months of the year **(Table 3)**. Most cow shed investigations have reported *Alternaria alternata*, *Aspergillus* and *Cladosporium* as the predominant fungal species. The dominance of *Aspergillus, Penicillium* and *Cladosporium* in all monitored locations was in agreement[13].

Seasonal occurrence of fungal spores in both indoor and outdoor air of the cow shed **(Fig. 1)**, revealed almost similar percentages of fungal spores in the respective seasons studied. During summer season, maximum spores were recorded 2742.81 CFU/m^3 followed by winter 2643.97 CFU/m^3 and rainy 2421.58 CFU/m^3 in indoor. In outdoor summer season 2756.93 CFU/m^3 contributed maximum, followed by winter 2548.66 CFU/m^3 and rainy 2305.09 CFU/m^3 season. Over all, maximum spore formation due to the abundance of *Aspergillus flavus* (218.86 CFU/m^3) was observed during summer season, followed by winter season with maximum occurrence of *Aspergillus* spp., (218.86 CFU/m^3) and rainy season *Cladosporium* spp., (141.20 CFU/m^3) were recorded. The possible reason could be due to the vigorous growth of plant in summer simultaneously allowing the growth of airborne fungi along with increased temperature and therefore favoring fungal growth[14]. The winter season in India belongs to the months between (November and February), summer (March to June) and rainy (July to October). The CFU/m^3 growth for cowshed was lowest during the rainy season when the temperature around 23^{0}C. The reason could be due to the washing of fungal spores from the atmosphere due to precipitation. Immediately after rainy seasons the CFU/m^3 increases since they was a increase in temperature and abundance dead plant material favoring the spore relation conditions for fungi, ventilation was also inadequate there. Such observations have been recorded by investigation carried out by Aydogdu & Asan[15].

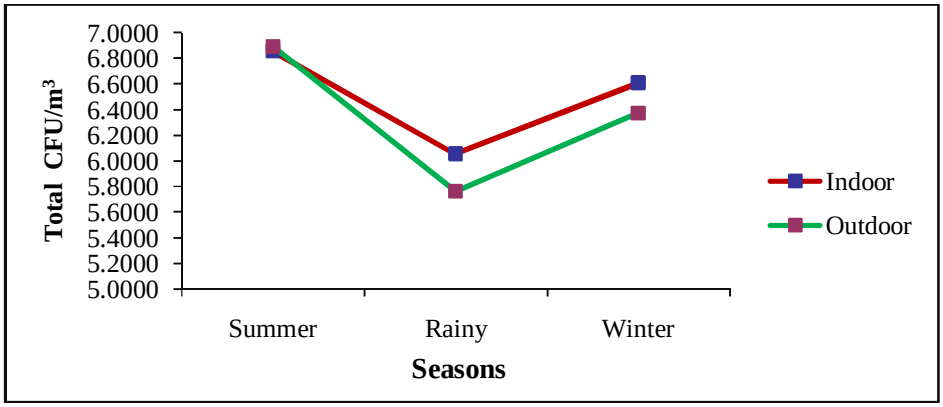

Fig. 1 : Seasonal variations of fungal spores recorded in indoor and outdoor air of the cow shed during January-December, 2011

Distribution of CFU's varied with meteorological factors **(Table 1)** at higher temperature more than 32^{0}C, the total CFU's distribution was slightly more during March 709.53 CFU/m^3 reached maximum in their CFU's, when the temperature was less than 26^{0}C, the CFU's reduced between November 476.55 CFU/m^3. The high relative humidity from June to September (94 %) reduced the distribution of organisms (2449.82 CFU/ m^3). The CFU's got gradually increased to 2915.78 CFU/m^3 with less relative humidity (85 %) between the months of January and April. In the wind speed showed less impact on the distribution of CFU's with less variation in their CFU's. Almost during the months from May to October with wind speed 9 km/h or higher, the distribution of organisms seems to be less (3777.10 CFU/m^3) and at less wind speed between the months of November to April (4.5 km/h) the organism number was more with 4031.26 CFU/m^3. During the maximum rainfall in the month of August (253.2 mm) distribution of organism was

628.34 CFU/m^3 but during January 755.42 CFU/m^3 and March 709.53 CFU/m^3 though no rainfall, the total organism distribution became higher during these periods. In cow shed differences were observed with respect to the CFU's and spore concentrations of individual fungal types with average monthly concentrations but no significant difference was observed when the total overall yearly concentrations were considered. The possible reason might be the insignificant changes of environmental factors as well as the fungal growth substrates at the cow shed during the one year sampling. It is an established fact that temperature and relative humidity are two important factors for fungal spore generation, release and dispersal particularly in indoor environments[16]. Higher temperature and relative humidity in the months of rainy season and associated higher concentration of culturable fungal spores in the cow shed are supporting the observations of earlier researchers. Outdoor airborne fungi sometimes influence the levels of airborne concentration in indoors[17].

The correlation of coefficient between meteorological data with cow shed fungal CFU's collected during the sampling time showed significance at different concentration levels **(Table 4)**. Some people are extremely allergic to the presence of particles in their environment. Those are the ones at risk of being affected by these fungi. As a result, adequate care must be taken to prevent the inhalation of fungal spores as prolonged exposure to unusually large numbers of spores, often as a result of occupational circumstances, can result in the development of allergic alveolitis[18-25].

Table 4 : Comparison between indoor and outdoor air of the cow shed

S/N	Variables	Indoor	Outdoor
1.	Concentration	6.51±0.94	6.34±0.95
2.	Temperature	23.27±1.99	23.27±1.99
3.	Relative humidity	67.32±7.28	67.32±7.28
4.	Wind speed	5.43±1.60	5.43±1.60
5.	Rain fall	67.04±73.71	67.04±73.71

CONCLUSION

This study was carried out in the animal rearing houses it clearly revealed the concentration of different fungal species in the environment. Fungal spores are ubiquitous and quite dominant in the indoors as against the outdoor environments. These spores enter the enclosed areas through the normal air intake and sometimes through the ventilation air stream. The data of fungal spore helped us to prepare the fungal spore calendar on this region. The seasonal periodicity information of the major allergenic spores will be valuable for susceptible patients to take respiratory protection measures during the peak time. The fungal spore calendar and prediction model will be helpful to forecast the allergenic fungal spore load in the air of Hessaraghatta village, Bangalore, India, Respiratory allergic problems and hospital admission with relevant diseases of that zone are related to the presence of airborne allergenic fungal spores.

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