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STUDIES ON SEASONAL VARIATION OF INDOOR AIRBORNE FUNGAL SPORES IN RABBIT HOUSE

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ABSTRACT

The indoor airborne fungal spore survey has been conducted for one year to assess the seasonal variation of the fungal flora in a rabbit house situated at Hessaraghatta village, near Bangalore city. The investigation was carried out by using an Andersen two stage viable sampler, at monthly intervals over a period of 12 months from January 2011 to December 2011. A total of 1.16 x 10^4 CFU/m^3 belonging to fifteen different genera, excluding some unidentified ones were recorded. The differences in distribution among these fungi for seasonal and meteorological factors were correlated and the mean significant difference was expressed statistically at 0.05% and 0.01% level of significance.

KEYWORDS: **Indoor air, Andersen sampler, Meteorological factors, Seasonal variations and Health hazards.**

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INTRODUCTION

Aerobiological studies are widely used to determine the fungal spectrum of the air. Airborne microfungi are one of the important indoor air biocontaminants and are the most numerous and diverse particles found both in indoor and outdoor environments. The main source of airborne fungi in indoor air is usually from outdoor environment[1] and also from some indoor environmental factors such as dampness and high humidity levels that encourage fungal growth[2]. The concentrations and types of airborne microfungi in the atmosphere are affected by many biological and environmental factors[3]. They vary greatly, by nature, with time, season, geographical, climatic and other physical factors[4]. Moreover, meteorological factors, affect the numbers and types of airborne microorganisms[5]. It is well documented that, more than 80 genera of fungi are associated with symptoms of respiratory tract allergies[6] and over 100 species are involved with serious human and animal infections[7]. The air in intensive livestock buildings usually contains high concentrations of airborne microorganisms[8]. Especially fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, etc., which grow routinely on livestock buildings[9] i.e. in the indoor environments, releases highly infectious agents such as fungal spores and mycotoxins[10]. They can cause or trigger lung-related diseases in animals and in humans[11]. Recent reports have shown that most experimental animals do not reach optimal quality and workers are subjected to high risk exposure[12]. Farm workers are exposed to large concentrations of airborne fungi when working with animal material[13]. Hence, there is an immediate need for the specific identification of microorganisms under different weather conditions as well as to carry out studies on exposure-response relationships. Presently, an increasing interest on the airborne biological particles as indicators of the quality of the environment in general and more specifically, of the atmosphere has emerged. Hence, a study was carried out to determine the variations in the indoor environment of rabbit house for the fungal distribution concurrent with seasonal changes and meteorological factors.

MATERIALS AND METHODS

(i) Sampling site and time

Hessaraghatta, a village situated 10km away from Bangalore city, has several rabbit houses among which, two rabbit houses were selected for the present study. Indoor air samples were collected at monthly intervals over a period of 12 months from January 2011 to December 2011.

(ii) Collection of samples[14]

The Andersen two-stage viable sampler was placed in the center of the rabbit house, 1.5 m above the ground level. Malt Extract Agar (MEA) was used as the sampling medium. The sampling time was limited to 5 minutes with an air flow rate of 28.3 L/min.

(iii) Collection of meteorological data

The meteorological data such as temperature, relative humidity, wind speed and rainfall were collected from the Department of Statistics, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore.

(iv) Treatment of samples

The indoor-air sampled MEA plates were incubated for 5 to 7 days at room temperature between 25°C to 30°C; identification of fungal colonies were based on morphological and microscopic observations, followed by further identification and confirmation at Agharkar Research Institute, Pune. The results obtained at each stage of the sampler were converted to Colony Forming Units per cubic meter (CFU/m^3) of air sampled and the total concentration was obtained by adding the CFU/m^3 from each stage of the sampler.

(v) Statistical analysis

The statistical analysis was performed using SPSS-16, 2007 version software. One way ANOVA and Pearson correlation was used for

determining the coefficients between CFU/m^3 and meteorological data (temperature, rainfall, wind speed and relative humidity), and the significant differences were expressed at 0.05% and 0.01% level of significance.

RESULTS

The indoor-air sampling for fungi in rabbit house for the year 2011, resulted in a total of 11642.66 CFU/m^3 (Table 1). Among the various organisms isolated, CFU's for all genera were determined and only fifteen genera could be identified through microscopic examinations. According to the CFU's, *Cladosporium* sp. proved to be predominant throughout the year with a maximum CFU's of 6698.89 while *Scopulariopsis* sp. was the least dominant with a CFU's of 28.32.

Table 1

Seasonal variations in fungal CFU per cubic meter of air sampled during summer, rainy and winter seasons, from January 2011 to December 2011.

Genera	Season			Total
	Summer	Rainy	Winter	
	March-June	July-October	November-February	
Acremonium sp.	70.8	-	70.82	141.62
Alternaria sp.	-	84.97	99.15	184.12
Arthrinium sp.	184.13	-	-	184.13
Aspergillus sp.	439.07	269.11	382.43	1090.61
Curvularia sp.	56.65	70.8	-	127.45
Cladosporium sp.	2733.68	1473.07	2492.9	6699.65
Fusarium sp.	127.46	70.82	141.63	339.91
Mucor sp.	-	155.79	141.62	297.41
Nigrospora sp.	-	113.3	226.62	339.92
Pencillium sp.	354.09	566.55	552.4	1473.04
Phoma sp.	-	56.65	-	56.65
Pithomyces sp.	184.12	28.32	99.15	311.59
Rhizopus sp.	-	99.14	-	99.14
Scopulariopsis sp.	28.32	-	-	28.32
Trichoderma sp.	56.64	-	212.46	269.1
Total	4234.96	2988.52	4419.18	11642.66

From Table 1 and Table 2, it can be observed that during summer*, Alternaria* sp.*, Mucor* sp.*, Nigrospora* sp.*, Phoma* sp. and *Rhizopus* sp. were not isolated, likewise during the rainy season, *Acremonium* sp.*, Arthrinium* sp.*, Scopulariopsis* sp. and *Trichoderma* sp. were not isolated, while during winter, *Arthrinium* sp.*, Curvularia* sp.*, Phoma* sp.*, Rhizopus* sp. and *Scopulariopsis* sp. were not isolated.

Table 2
Descriptive statistics for ANOVA showing the mean distribution of CFU's for the various fungi during the different seasons.

Genera	Season	Mean ± SD	P	F
Acremonium sp.	Summer	17.70±3.54	0.872	.451
	Winter	17.70±1.35		
	Rainy	.00±.00		
Alternaria sp.	Summer	24.78±4.95	0.675	.533
	Winter	.00±.00		
	Rainy	21.24±2.71		
Arthrinium sp.	Summer	.00±.00	7.567	.012*
	Winter	46.03±3.34		
	Rainy	.00±.00		
Fusarium sp.	Summer	35.40±4.24	0.039	.962
	Winter	31.86±2.92		
	Rainy	28.32±3.46		
Cladosporium sp.	Summer	623.22±43.96	1.691	.238
	Winter	683.42±6.87		
	Rainy	368.26±2.58		
Aspergillus sp.	Summer	95.60±3.34	1.268	.327
	Winter	109.76±5.47		
	Rainy	67.27±1.78		
Curvularia sp.	Summer	.00±.00	1.235	.336
	Winter	14.16±2.83		
	Rainy	17.70±.70		
Penicillium sp.	Summer	138.10±13.35	0.349	.715
	Winter	88.52±10.99		
	Rainy	141.63±2.00		
Nigrospora sp.	Summer	56.65±6.54	1.800	.220
	Winter	.00±.00		
	Rainy	28.32±3.27		
Mucor sp.	Summer	35.40±1.82	5.285	.030*
	Winter	.00±.00		
	Rainy	38.94±2.68		
Pithomyces sp.	Summer	24.78±4.95	0.722	.512
	Winter	46.03±6.05		
	Rainy	7.08±1.41		
Rhizopus sp.	Summer	.00±.00	1.485	.277
	Winter	.00±.00		
	Rainy	24.78±4.06		
Phoma sp.	Summer	.00±.00	1.000	.405
	Winter	.00±.00		
	Rainy	14.16±2.83		
Scopulariopsis sp.	Summer	.00±.00	1.000	.405
	Winter	7.08±1.41		
	Rainy	.00±.00		
Trichoderma sp.	Summer	53.11±6.16	2.235	.163
	Winter	14.16±1.63		
	Rainy	.00±.00		

P: P value, F: Frequency, SD: Standard deviation, Highly significant at 0.01%

DISCUSSION

The dominant indoor organisms such as *Cladosporium* sp., *Aspergillus* sp. and *Penicillium* sp. showed highest distribution throughout the year[15], whereas, *Scopulariopsis* sp., *Phoma* sp. and *Rhizopus* sp. species showed lesser distribution, only during winter and rainy season, as these organisms require wet and higher humidity conditions[16] for their growth. Thus, a distinct seasonal variation was observed in the airborne fungal flora of the selected rabbit house in Hessaraghatta village of Bangalore city. The varied distribution of all the fifteen fungal genera for monthly intervals and yearly indoor CFU numbers significantly justifies the involvement of seasonal variations and meteorological factors. The release of fungal spores from the indoor environment was found to be driven by the energy from external sources and is significantly affected by environmental factors[17]. The weather conditions probably have the greatest influence on the number and type of fungal spores. Correlation

of climatic data with the incidence of aerospora show that parameters such as temperature, rainfall, relative humidity and wind speed played a significant role. The distribution and aerosolization of all the species was found to be maximum during summer when compared to the winter and rainy seasons. The CFU's of all fifteen fungi increased during hot and humid conditions and was influenced by temperature, relative humidity and wind speed. Bhat and Rajasab[18] also reported the distribution of a large number of organisms or spores during the summer season. The major cause for this spore release may be due to the air currents prevailing in the indoor environment at higher temperatures during summer season, causing spore detachment and dispersion[19].

Table 3

The statistical correlation for distribution of fungal species for their CFU's with meteorological factors.

Genera		Temperature	Relative humidity	Wind speed (km/h)	Rainfall (mm)
Acremonium sp.	r	.700*	-.304	.140	.020
	p	.011	.337	.664	.952
Alternaria sp.	r	-.703*	.062	-.225	-.391
	p	.011	.848	.483	.208
Arthrinium sp.	r	.304	.015	.601*	.563
	p	.337	.964	.039	.057
Fusarium sp.	r	.658*	.006	-.109	.488
	p	.020	.986	.737	.108
Cladosporium sp.	r	.535	-.379	.256	.176
	p	.073	.224	.422	.585
Aspergillus sp.	r	.013	.001	.473	.009
	p	.968	.997	.120	.979
Curvularia sp.	r	-.215	.515	-.023	.683*
	p	.502	.087	.943	.014
Penicillium sp.	r	.184	.210	-.249	.196
	p	.567	.512	.436	.542
Nigrospora sp.	r	.436	-.230	-.406	-.196
	p	.157	.472	.190	.541
Mucor sp.	r	.015	-.011	-.512	-.402
	p	.964	.973	.089	.195
Pithomyces sp.	r	.341	.305	.208	.667*
	p	.278	.335	.516	.018
Rhizopus sp.	r	-.025	.251	.051	-.052
	p	.939	.431	.874	.872
Phoma sp.	r	-.319	.159	-.125	-.124
	p	.312	.621	.699	.701
Scopulariopsis sp.	r	.431	-.749**	-.085	.252
	p	.162	.005	.792	.430
Trichoderma sp.	r	.645*	-.461	-.125	-.159
	p	.023	.131	.698	.621
Temperature	r	1	-.408	.141	.345
	p	.	.188	.662	.273
Relative humidity	r	-.408	1	.268	.191
	p	.188	.	.400	.551
Wind speed km/h	r	.141	.268	1	.095
	p	.662	.400	.	.769
Rainfall mm	r	.345	.191	.095	1
	p	.273	.551	.769	.

**** Correlation (0.05) and ** Correlation (0.01) is significant level 2-tailed. r is Correlation Co-efficient and p is P value.***

Another principal physical factor affecting the dispersion of spores in indoor environment is the wind speed[20]. The release of spores from different fungal species is mainly a function of air velocity so that the increase in velocity causes an increase in the spore release rate but in the present study, the distribution of organisms was found to be lesser during the increased wind speed. The air velocity required for the spore release is an independent factor and is solely dependent on each fungal type[21]. The presence of fungal spores according to statistical correlation was lesser during high humidity and rainfall period. The release of spores gradually decreases under wet conditions and show variation in distribution[22]. Several authors reported a negative correlation between rainfall and spore concentration i.e., the rainfall washes all the spores in the outer atmosphere and simultaneously also decreases the spore concentration in the indoor environment and this release of spores from the wet wall differs for different fungi under identical conditions[23]. The fifteen isolated organisms have been associated with some of the health related disorders. All the organisms are allergic in nature; either they cause type-I allergic response such as hay fever and asthma (*Trichoderma* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Nigrospora* sp., *Arthrinium* sp. and *Acremonium* sp.) or type-III hypersensitive reactions such as bronchoblastomycosis and allergic fungal sinusitis (*Scopulariopsis* sp., *Trichoderma* sp., *Cladosporium* sp., *Alternaria* sp., *Acremonium* sp. and *Curvularia* sp.) [24, 25, 26]. They also cause several diseases or disorders such as mycotoxicosis (*Trichoderma* sp., *Pneumonitis* sp. and *Penicillium* sp.), zygomycosis (*Rhizopus* sp. and *Mucor* sp.), diabetes, ketoacidosis (*Rhizopus* sp.), facial eczema (*Pithomyces* sp.), onycamycosis (*Scopulariopsis* sp., *Curvularia* sp. and *Fusarium* sp.), pneumonia (*Curvularia* sp. and *Penicillium* sp.), cerebral abscess (*Curvularia* sp.), mycetoma and mycotic eye infections (*Fusarium* sp. and *Penicillium* sp.), bronchopulmonary *aspergillosis* (*Pneumonitis* sp.), external ear infections, respiratory, urinary tract infections, penicillosis, endophalmitis, otomycosis, endocarditis and peritonitis (*Penicillium* sp.) phaeohyphomycosis (*Phoma* sp.), eye and nails infections (*Acremonium* sp.) and bakes asthma (*Alternaria* sp.) [27, 28, 29]. They also produce several toxins such as trichothecene (*Trichoderma* sp., *Fusarium* sp. and *Acremonium* sp.), cyclic peptidies, gliotoxin, isocyanides, T-2 toxin, trichodesmin (*Trichoderma* sp.), achratoxin-A (*Penicillium* sp.), sporidesmin (*Pithomyces* sp.), zearalenone and vomitoxin (*Fusarium* sp.), aflatoxins (*Aspergillus* sp.) and tenanzoic acid (*Alternaria* sp.) [30, 31, 32]. Thus, safety measures such as fumigation, maintenance of clean environment, avoiding the dumping of wastes, to keep the microbial load to a minimum has to be employed as has been observed and studied at the Neyveli Lignite Corporation Limited[33].

CONCLUSION

The distribution of the fungal organisms isolated in the present study could have a potential and significant effect on the health of the rabbits and the working laborers. The results of the present study could be incorporated while taking suitable measures to prevent health hazards of animals and workers, living or working in such infectious environments.

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