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Volumetric Analysis of Concentration and Composition of Airborne Fungal Spores in a Working Environment with Meteorological Study

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Occurrence in concentration and composition of airborne fungal isolates with their seasonality in indoor and outdoor environments of a working environment of Vaniyambadi city of Tamilnadu was carried out by Burkard's volumetric sampler on agar plates from January to December 2012. During the study period, the fungal spores considerably varied from indoors to outdoors as well as from season to season both in concentration and composition. It was found that indoors had more numbers of fungal spores in comparison to outdoors in the environments. Incidence of fungal species was predominated with more number of propagules during mid winter months like December, January and February in comparison to other months. Aspergilli were predominant in working area followed by penicilli and cladosporia. Fruit deteriorating fungi like *Alternaria, Curvularia, Geotrichum* and *Trichothecium* were recorded in the current study. *Aspergillus* spp was also found with the highest frequency and had the maximum contributed members i.e., *Aspergillus flavipes, A. flavus, A. fumigatus, A. niger, A. terreus* and *A. ustus.* Of the 28 isolated fungal taxa, indoor air distributed with 25 species and outdoor air with 19 species. Seasonal occurrence recorded that the winter was the maximum spore load in the working environments followed by rainy and summer seasons. Statistical significance of fungal spores between indoors and outdoors was found non significance difference in their means at peH0.05 level with the acceptance of null hypothesis. Meteorological parameters were found to control the fungal spores in the environment confirmed by Pearson's correlation analysis.

Key words: Volumetric analysis, working environment, Null hypothesis, Seasonal occurrence.

Fungal growth in indoor air of working environments has long been regarded as detrimental, basically because of its tendency to reduce perceived air quality. Working environments like vegetable market, where the spoiled plant materials and dumped debris are often act as reservoirs of saprophytic fungi, those who generally grow over them. The activity of these fungi is to carry out pathogenicity to the vegetables and fruits, their degradation and deterioration because of their requirements for prime sources of carbon, nitrogen and other nutrients[1]. A wide spectrum of fungi has been reported as a result of indoor aerobiological research performed throughout the world in places such as dwelling houses, poultry sheds, and dairies [2,3]. Fungi usually enter the working envirnments from outdoors but their concentration may well be higher in indoor air when there are indoor sources. Increasingly, fungi in indoor air are being proposed as a cause of adverse health effects [4,5]. The health risks connected with exposure to fungi involve not only immunosuppressed patients but also perfectly healthy persons among whom hyper- reactivity to

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the fungal allergen may develop; such hyper-reactivity may cause respiratory disorders and may exacerbate asthma[6,7]. There are reports of the possible onset of respiratory disorders after hypersensitization to various genera of fungi. In particular, various strains of *Aspergillus* and *Penicillium* seem to be chieûy involved in the genesis of asthma and allergic alveolitis (pulmonitis because of hypersensitivity) [8]. Many epidemiological studies in several countries have consistently detected an association between respiratory symptoms and reported home dampness and mould growth, but causality in these studies has not been established [9,10]. Besides allergic and respiratory problems, some indoor moulds, when ingested or inhaled, could produce mycotoxins, including aûatoxins and microbial volatile organic compounds which may lead to several health complaints, for example headache, dizziness, and inability to concentrate, consistent with mycotoxicosis [11,12]. Indoor fungal growth may therefore lead to multiple health effects if neglected. In this study an attempt was made to evaluate the qualitative and quantitative fungal burden of indoor air of a working environment (Veg. market) of Vaniyambadi city of Tamilnadu, India which may help in predicting possible risks of indoor fungi to employees and stored products.

MATERIALS AND METHODS

The present study was carried out in indoor and outdoor environments of one of the working environment (vegetable market) of Vaniyambadi city, Tamilnadu, India for constant one year from the month of January to December 2012.

Study sites

Vaniyambadi is one of the municipalities of the district Vellore, Tamilnadu, India. Vaniyambadi is located at 12.68°N 78.62°E degree of longitude and latitude respectively. It has an average elevation of 363 meters (1190 feet) and lies on the banks of the Palar river.

Air Samplings

Aeromycological samplings were taken for continuous one year from January 2012 to December 2012, at monthly intervals, between 10 to 11 A.M. from indoors of the working environment, 30 feet away from the market, running the Burkard's personal volumetric sampler on agar plates at 5ft height from the floor in indoors and at 10ft height from the ground at outdoors to avoid dust. Sabouraud Dextrose Agar (SDA) was used for the sampler containing with streptomycin/ penicillin ($50mg^{-l}$) which was carried to the study sites with sterilized container and kept in the sampler in order to run the sampler for five minutes to receive the the air borne fungal spores on the media plate. Altogether 24 Petriplates were employed in the indoors and outdoors of the working area. After operation of sampler, each set of plates were brought separately to the Department of Biomedical Engineering, Sathyabama University, Chennai-600119, India with utmost care and incubated in culture room at 25 ± 3^0c upside down for 15 days with constant observation after 3-4 days of incubation. During the sampling time, meteorological parameters like, temperature and relative humidity were recorded together from the two sites. 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 colony forming units (CFUs) that could not be identified directly from plates were sub cultured in PDA/CDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa[13,14]. The total number of fungal CFUs counted from the working environment was converted to CFUs m^{-3} of air. Annual and monthly percentage occurrence of individual fungus was determined with the seasonal variation. Different statistical tests, like Pearson's correlation coefficient analysis, t test and z test were analyzed to find out the significance of two means, variance of fungal distribution in between indoors and outdoors of the working environment.

RESULTS

In the present aeromycospora study, a total of 17,760 fungal CFUs m^{-3} of air were recorded from both indoors and outdoors of the working environment, of which, indoors recorded with 11,840 fungal CFUs m^{-3} and it was followed 5,920 fungal CFUs m^{-3} from outdoors. Relative abundance of fungal species, their CFUs

Table 1. Relative incidence of airborne fungal spores recorded from indoors of the working Environment during 2012

S. No.	Name of fungi	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual Occurrence
1	*Absidia* sp.	0	4.2	0	2.6	0	3.5	0	3.7	0	4.0	4.1	4.3	2.3
2	*Aspergillus flavipes*	0	0	5.6	5.3	0	4.1	15.3	0	0	4.0	1.4	0	2.3
3	*A. flavus*	23.0	0	0	2.6	0	3.5	0	11.1	2.3	0	0	2.2	1.7
4	*A. niger*	29.4	42.0	26.4	18.4	12.9	7.1	0	14.8	34.8	48.9	35.6	38.6	29.7
5	*A. ustus*	0	0	0	2.6	0	10.0	0	0	2.3	0	1.4	1.0	1.2
6	*Alternaria alternata*	0	0	9.4	0	12.9	7.1	11.5	0	0	6.1	8.2	7.6	5.0
7	*Alternaria* sp.	0	6.4	0	0	0	7.1	15.3	3.7	0	2.0	1.4	1.0	2.2
8	Brown Sterile mycelia	0	0	0	5.3	0	0	0	0	0	0	0	0	0.33
9	*Candida sp.*	1.2	0	0	10.5	0	10.7	0	7.4	2.3	0	1.4	2.2	2.3
10	*Cladosporium herbarum*	10.5	0	11.3	0	6.4	3.5	0	14.8	6.9	0	6.2	8.7	6.4
11	*C. resinae*	0	6.4	0	0	0	7.1	19.2	0	0	2.0	0	1.0	2.02
12	*C. sphaerospermum*	3.5	0	0	5.3	0	0	0	0	2.3	0	0	0	1.01
13	*Fusarium oxysporum*	7.0	0	0	0	12.9	3.5	11.5	7.4	2.3	0	2.7	4.3	3.9
14	*Geotrichum candidum*	1.2	4.2	3.7	0	9.6	0	0	0	0	0	1.4	1.0	1.7
15	*Monilia sitophilla*	9.4	0	9.4	0	9.6	7.1	0	11.1	2.3	0	1.4	0	3.9
16	*Mortierella* sp.	1.2	0	0	0	3.2	0	0	0	0	0	0	1.0	0.50
17	*Mucor racemosus*	0	4.2	0	5.3	0	3.5	7.7	0	4.6	2.0	2.7	2.2	2.3
18	*Penicillium citrinum*	14.1	21.3	26.4	23.6	19.3	14.2	0	22.2	13.9	14.2	16.4	13.0	16.5
19	*Penicillium frequentans*	10.5	0	0	15.7	9.6	0	15.3	0	11.6	12.2	8.2	7.6	7.8
20	*Trichoderma* sp.	3.5	4.8	3.7	0	0	0	0	0	6.9	2.0	2.7	1.0	2.3
21	*Trichothecium roseum*	4.7	6.4	1.9	2.6	0	0	0	3.7	4.6	0	2.7	2.2	2.7
22	White sterile mycelia	1.2	0	1.9	0	3.2	3.5	3.8	0	2.3	2.0	1.4	1	1.5

Table 2. Relative incidence of airborne fungal spores recorded from outdoors of the working environment during 2012

S. No.	Name of fungi	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual Occurrence
1	*Aspergillus flavipes*	0	10.0	0	0	4.2	0	0	6.6	0	0	2.6	2.3	2.0
2	*A. flavus*	0	0	22.2	0	0	4.7	0	0	10.5	0	0	6.9	3.3
3	*A. fumigatus*	10.0	0	0	15.7	0	4.7	8.3	0	5.3	0	0	0	3.3
4	*A. niger*	20.0	30.0	0	0	8.3	9.5	8.3	20.0	0	33.3	15.7	18.6	15.2
5	*A. terreus*	5.0	0	16.6	0	4.2	0	8.3	0	10.5	3.7	7.8	9.3	5.7
6	*Alternaria alternata*	2.5	0	0	0	12.5	9.5	8.3	0	10.5	3.7	7.8	9.3	15.2
7	*Alternaria* sp.	0	10.0	0	15.7	0	0	16.6	0	0	11.1	10.5	0	4.7
8	*Cladosporium cladosporioides*	20.0	20.0	11.1	15.7	0	14.3	0	13.3	21.0	22.2	21.0	16.2	15.8
9	*Cladosporium herbarum*	10.0	20.0	0	10.5	16.6	9.5	0	33.3	0	7.4	13.1	13.9	11.5
10	*Curvularia lunata*	7.5	0	11.1	0	8.3	14.3	8.3	0	21.0	0	5.3	6.9	6.7
12	*Fusarium oxysporum*	0	0	0	5.3	0	4.0	0	0	5.3	0	0	2.3	1.3
13	*Monilia sitophilla*	10.0	0	16.6	0	25.0	9.5	0	0	0	3.7	0	0	5.4
14	*Mucor racemosus*	2.5	0	0	5.3	0	4.7	0	13.3	0	0	0	0	1.6
15	*Penicillium citrinum*	5.0	0	5.5	26.3	8.3	4.7	25.0	13.3	5.3	7.4	15.7	16.2	10.8
16	*Penicillium frequetans*	0	10.0	0	0	4.2	0	8.3	0	1	3.7	1	2.3	2.0
17	*Rhizopus stolonifer*	2.5	0	5.5	1	1	4.7	1	0	0	3.7	2.6	2.3	2.0
18	*Saccharomyces cerevisiae*	2.5	0	5.5	0	0	4.7	0	0	0	3.7	2.6	2.3	2.0
19	White sterile mycelia	2.5	0	11.1	0	4.2	4.7	8.3	0	5.3	0	0	2.3	2.7

contribution and monthly occurrence in each environment of the working area were found different from each other. Relative and monthly incidence of fungal spores in the indoors and outdoors of the working environment is given in Table 1 and 2 respectively, which showed the total abundance of fungi isolated during the study period. In fungal composition, a total of 28 fungal species were isolated comprising of 19 genera from both outdoors and indoors. *Aspergillus* was found with the highest frequency and had six members i.e., *Aspergillus flavipes, A. flavus, A. fumigatus, A. niger, A. terreus, A. ustus* but quantitatively, *Penicillium* was isolated highest in its contribution

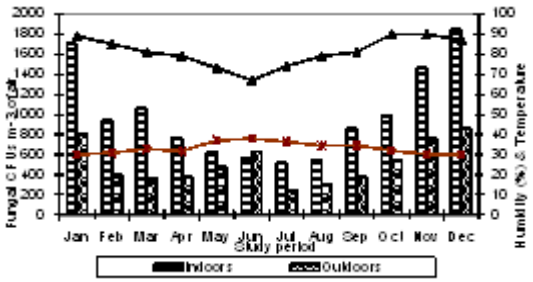


Fig. 1. Monthly incidence of fungal CFUs m-3 of air and meteorological parameters in the working environment

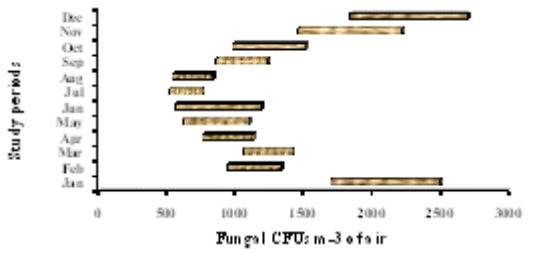


Fig. 2. Disrtibution of fungal spores in the working environment during the study period

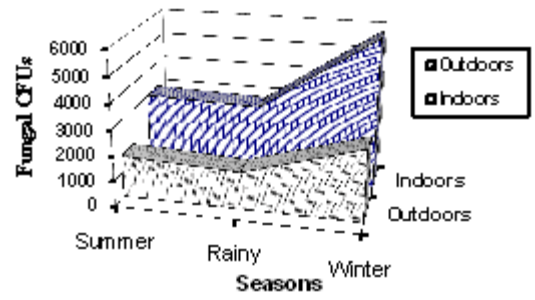
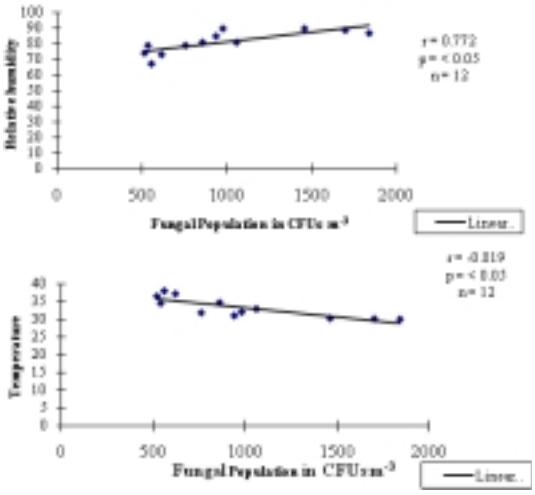


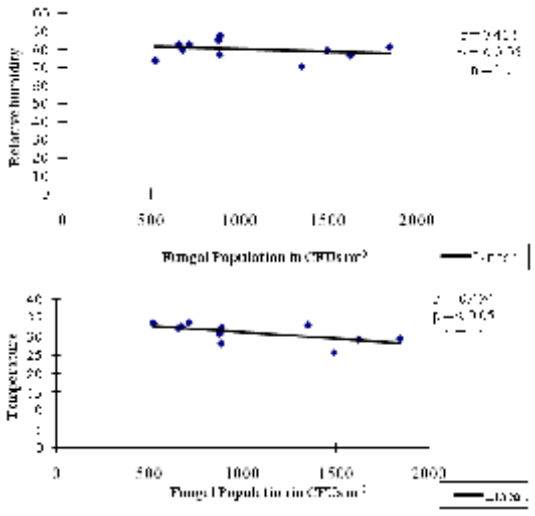
Fig. 3. Seasonal variation of fungal spores in working environment

to total CFUs m^{-3} of air followed by *Aspergillus*. Out of the total isolated fungal taxa, Indoors contributed 22 species under 15 genera and outdoors contributed with 19 species pertaining to 12 genera. *Alternaria alternata, Aspergillus niger, Curvularia, Penicillium citrinum, Cladosporium* spp., *Trichothecium* and



'n ' number of observations, 'r' correlation co-efficient and 'p' Probability level.

Fig. 4. Pearson's coefficient of correlation of fungal population of CFUs m^{-3} of air recorded in Indoors with Temperature (°c) and Relative humidity (%)



'n ' number of observations, 'r' correlation co-efficient and 'p' Probability level.

Fig. 5. Pearson's coefficient of correlation of fungal population of CFUs m^{-3} of air recorded in Outdoors with Temperature, (°c) and Relative humidity (%)

Trichoderma were found as the predominant fungal spores, in the working environment. Based on the annual occurrence, *Aspergillus niger* was found to be the highest in indoors and outdoors. It was found that *Penicillium citrinum* also recorded with the second highest in both indoors and outdoors followed by *Cladosporium*, *Curvularia* and *Alternaria.* Some phytopathogenic fungi were also appeared in both the area during our study. Monthly incidence of fungal spores with recorded temperature and humidity in indoors and outdoors showed a clear view of the distribution of fungi with their relation (Fig 1). The distribution of fungal CFUs recorded from the working environment is given Fig 2, which showed that the months of July and August harbored the least fungal spores than other months. The winter months like December and January recorded with the maximum spores in indoors as well as outdoors. Seasonal occurrence of fungal spores in both the sites is given in Fig 3, which showed the winter season harbored the maximum fungal spores in the working environments followed by summer and rainy both in outdoors and indoors. Moreover, winter months contributed the maximum spores due to the abundant occurrence of *Cladosporium,* penicilli and aspergilli in the month of December and January. T-test and z-test results between two samples mean of the indoors and outdoors fungal spora were non-significance at pe”0.05 level and null hypothesis were accepted. F-test for two sample variance between indoors and outdoors was also found not significance at pe”0.05 level. Pearson's coefficient of correlation of fungal CFUs m^{-3} of air recorded in Indoors and outdoors with the meteorological parameters like temperature (^{o}c) and relative humidity (%) showed that positive correlation between the relative humidity and negative relation between temperatures (Fig 4 and 5).

DISCUSSION

The utility of Burkard's volumetric sampler on agar plates in the current study is considered as the right choice for quantitative study to find out the total fungal spores present per cubic meter of air since it is the widely used technique for the sake of volumetric sampling by earlier workers [15,16]. Indoors was found to harbor more fungal spores in comparison to outdoors of the working environment was agreed with the previous work by Kakde and Kakde [17]. Aspergilli and penicilli contributed the maximum both in indoors and outdoors, but their concentration was more in indoors compared to outdoors, which was confirmed with the work made by Usha et al[18]. Based on the species distribution, species belongs to *Aspergillus* comprised of 6 species in the working environment was in agreement with the findings of many others[17,18,19,20]. A number of studies on aerospora of indoors and outdoors, particularly of occupational sites are found worldwide[15,21,22,23]. Indoors spore load had a greater resemblance with outdoors qualitatively but the concentration greatly depended on atmospheric parameters and the local vegetation with accordance to their occurrences. The present findings found the dense spores in indoors than outdoors contribute to the prevailing atmospheric conditions and socio economic conditions leading to the degree of cleanness of the market area[17]. During early nineties, Cosentino and Palmas[24] described in their work that *Cladosporium*, *Alternaria, Penicillium* and *Stemphyllum* were prevailing in the greenhouse; *Penicillium, Cladosporium*, yeasts, *Trichoderma* and *Rhizopus* occurred more frequently in the carpentries; *Penicillium, Candida, Mucor* and *Geotrichum* were the most common genera identified in the dairies, which is more or less similar to our results. Nayak and Behera [25] described that in tropical environments the fungi like, aspergilli and penicilli are mostly the dominant ones and are known for allergenicity[8]. They are well known for the cause of allergic alveolities[23]. The meteorological parameters had direct effect on air borne fungal spores in the outdoors & indoors of the market environments confirmed with our present study[8]. Statistical test of non-significance was found between the sample means and two means variance of the distribution of fungal spores, indoors and outdoors of the working environments at pe”0.05 level with the acceptance of null hypothesis. Our present study reports that due to the high incidence of fungi and their allergenic nature, they may incite potential health hazards to the workers in the market environments, not only to the customers but also to the market dwellers. Pearson's coefficient of correlation of fungal CFUs

m^{-3} of air recorded in Indoors and outdoors with the meteorological parameters like temperature (^oc) and relative humidity (%) (Fig 4 and 5) confirmed that the prevalence of the fungal spores in the working environments are always dependant mostly on prevailing relative humidity and temperature on the area agreed with the previous workers[8,21].

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