International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.6, pp 306-312, 2015

Volumetric samplings of airborne fungal spores in different college libraries: A Preliminary study

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Abstract: The fungal spores are unanimous in their distribution i.e., present in the indoors and outdoors of the environment. They are dispersed in a very high concentration and hence remain suspended in the air for an extended period of time. An aeromycological survey of the indoor sites of the libraries (before and after disturbance of book selves) in four different colleges in Pondicherry city was carried out by employing Burkard's volumetric sampler on agar plates. Air samplings were made for isolating the prevalent fungi from the study sites in between 9AM to 1PM. During the study period, altogether, 21fungal species under 14 genera were isolated, among which *Cladosporium herbarum* was recorded as the dominant followed by *Aspergillus* sp. and *Penicillium* sp. Occurrence of *Aspergillus niger* was found very amazing since they were available in all the college libraries, but it was more in concentration in Bharathidasan College. All together, six species of Aspergillus were isolated i.e., *Aspergillus awamori, A. japonicus, A. flavus, A. fumigatus, A. niger and A. tamarii*. *Penicillium* spp were also isolated from both the sites of the library environments i.e., *Penicillium citrinum, P. chrysogenum and P. oxalicum.* Allergenic fungi like, *Aspergillus fumigatus, A. niger, Penicillium* sp., *Rhizopus stolonifer* were recorded from the two sites i.e., before and after disturbance of books in the the library environments. The prevalence of spores was found more in the after disturbance of books in comparison to the before disturbance of books in the libraries. The diversity of fungal species were analyzed in the indoors of libraries mainly after disturbance of books contributed the maximum than the before disturbance of books but when it comes to the concentration of fungal load (spores) it also showed that after disturbance was dominated over the before disturbance of books in the libraries.
Key words: Library environments, Aeromycospora study, Burkard's volumetric air sampler.

Introduction

Air is the most valuable content of our life as well as of the environment. Different microbial particulates are present in the aerial atmosphere. The availability of fungi in the aerial atmosphere concern for both allergists and biologists with an interest in health and environmental biopollution problems. The air we breathe contains spores of many different fungi. Air is a hostile environment for fungi because of high irradiance and low water availability. Spores of most fungi do not survive significant periods in air because of the absence of food and energy and those who survive have quite specific mechanisms to prevent damage from desiccation and irradiation. Based on different studies it was found that people who are healthy, atopic (sensitive), already suffer from allergies, asthma or compromised immune systems and occupy damp or moldy buildings are at an increased risk of health problems such as inflammatory and toxic responses to mold spores, metabolites and other components [1,2,3,4]. The most common health problems like, allergic symptoms, respiratory

infections and exacerbation of asthma and rarely hypersensitivity pneumonitis, allergic alveolitis, chronic rhino sinusitis and allergic fungal sinusitis are due to the allergenic airborne mould fungi[4]. A person's reaction to molds depends on their sensitivity and other health conditions, the amount of mold present, length of exposure and the type of mold or mold products. Fungi in the indoor air of library environments cause biodeterioration of books and other materials present in the library, as these books provide a congenial atmosphere for the growth and sporulation of fungi and handling of moldy books and papers causes inhalation of spores which may creates respiratory and cardiac problems with allergic reactions among library workers and visitors in the library[5]. All these substrates sometimes may lead to other problems like dermatitis to the library visitors. Therefore the present study is an attempt to carry out the volumetric aeromycological survey of four College libraries of Puducherry city to record the allergenic fungi in the indoors.

Materials and Methods

Study sites

The present aeromycological study was carried out in four government college libraries situated in Pondicherry city. The four Colleges are namely Kanchi Mamunivur Center for Post Graduate Studies (KMCPGS), Lawspet, Tagore Arts College (TAC), Lawspet, Bharathidasan Government College for Women (BGCW), Muthialpet and Pondicherry University Community College (PUCC), Lawspet, Pondicherry, India during the period from January to March 2015. Pondicherry is situated 160 km away from Chennai in the Tamilnadu territory and on the coastal belt of Bay of Bengal. Pondicherry is located between 11 degree 46' and 12 degree 30' of north latitude and between 79 degree 36' and 79 and 52' of east longitude. The layout of Puducherry is located within Tamil Nadu presents a peculiar picture of territorial jurisdiction. The city has little above seven lakh population and has a number of places of interest for the people of Pondicherry. The Library seems to be the favorite place of interest for all students including the academic fraternity. The Libraries that were selected for my study are situated in Lawspet and Muthialpet area of the city and all these places are surrounded on all sides by schools, colleges, hospitals, residential quarters, hotels and bus stand etc.

Sterilization, media preparation, precaution and identification

Glassware and media used throughout the study period were steam sterilized at 15 lb pressure per square inch in an autoclave for 15 minutes. Before sterilization, the glassware were properly cleaned in liquid detergent, washed thoroughly and dried in oven. Single glass distilled water was used, throughout the study period. The Sabouraud's Dextrose agar medium used during the study period was supplemented with streptomycin (50 mg^{-1}) to check the bacterial growth. To maintain the aseptic condition, sterilized inoculation chamber or 'Laminar air flow' was used for plating and culturing. Knives and needles were perfectly sterilized over the spirit lamp flame before use. Agar agar melted in 500 ml of boiling distilled water was added with peptone, dextrose, yeast extract and rose Bengal dye and the volume was made up to 1000 ml. by adding rest amount of distilled water. It was dispensed into ten 250ml flasks each having 100 cm^3 of agar solution. The flasks were plugged with non-absorbent cotton and wrapped with the aluminum foil. Flasks were autoclaved at 15 lb pressure for 15 minutes. Media flasks were kept outside for 3-4 days to see infection if any. During plating and inoculation all precautions were taken to maintain an aseptic condition.

Air Samplings

The Burkard's Volumetric Air Sampler on agar plates was used in the present study. The air sampling was taken by running the Burkard's sampler for five minute at the indoor sites, before disturbing the books and after disturbing the books of the selves in the Library environments of the colleges. The Volumetric Air Sampler is designed for short-term sampling in domestic or industrial environments particularly where no power supplies are available. The Burkard's Volumetric Air Sampler is a perfect air quality monitor used in culture rooms, indoor environments and domestic environments for collecting fungi and other bio-particulates directly onto the SDA mediated petriplates supplemented with streptomycin ($50mg^{-1}$). It was designed to record the total number of bioaerosols per cubic meter of air of the sampling sites.

Air samples for culturing fungi were collected by the Petriplates supplemented with SDA medium (Sabouraud Dextrose Agar) in the operating samplers. Air samplings were made at intervals for isolating the prevalent fungi from the study sites in between 9AM to 12noon. The sampler was run at the height (1.5-2m)

above the ground just to the breathing level based on the substrates available in the Libraries. After operation, the Petriplates were brought to the laboratory in the Pre-sterilized polythene bags and incubated at 25±3 ºC for 3-7 days. After three days of incubation, the fungal colonies were counted for individual species and the total number CFUs were calculated. Microscopic slides stained with lacto phenol cotton blue were prepared from each CFUs and observed microscopically under the light microscope to identify directly them up to species level. The colony forming units (CFUs) that could not be identified directly from plates were sub cultured in CDA/PDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal species [6,7,8,9,10]. Cultured fungi on agar plates of different library sites were identified up to their species level. The percentage contribution of spores was found out taking the average of the two readings of each experimental site.

Calculation of Percentage contribution of an individual fungus:

$$\% \text{ occurrence of fungus} = \frac{\text{Total CFUs recorded by individual fungus}}{\text{Total CFUs recorded by total fungi}} \times 100$$

Results

Based on fungal composition, altogether 21 fungal species under 14 genera were isolated from all the indoor sites of the four different libraries. Occurrence of airborne fungal spores before and after disturbance of books in the college library environments is given in Table 1 & 2.

In total, 8100 fungal CFUs m^{-3} of air were isolated from all libraries, moreover, indoor of the Library had some difference in their spore profiles. Occurrence of spores after disturbance of books was found the maximum with 59 % and the before disturbance of books had only 41% of the fungal spores. The fungal spores were recorded with the maximum in after disturbance of books due to the abundance of *Cladosporium, Aspergilli* and *Penicillium* were found to be the highest in all the four different libraries of the indoors.

In analyzing the diversity of fungal species, in the indoor area mainly after disturbance of books contributed the maximum number (22 species under 14 genera) and before disturbance of books it contributed 20 species of 10 genera but when it comes to the concentration of fungal load (spores) after disturbance of books it was dominated over the before disturbance of books in all the four libraries. Percentage occurrence of each fungus recorded in indoor sites on the libraries, before and after disturbance of books selves are given in Table 1 and 2 respectively.

Among the recorded fungal members, Hyphomycetous fungi were most prominent in their occurrence followed by the members of Zygomycetes in both before and after disturbance of the books in the libraries studied. Among all, *Cladosporium herbarum* was found to be the dominant one before and also after disturbance of books of all the five different libraries followed by *Aspergillus niger and Penicillium chrysogenum* (Table 1 and 2). Besides these, *Aspergillus flavus, A. fumigatus, Penicillium citrinum and P. oxalicum* were recorded frequently from the Library environments.

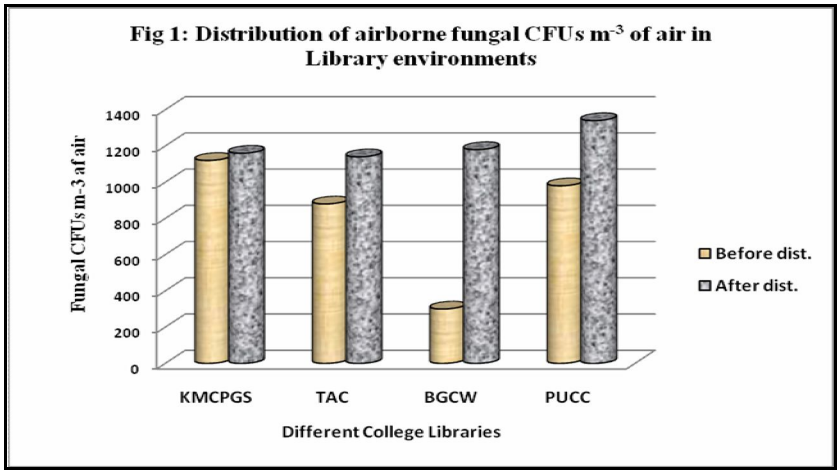
Table 1: Percentage occurrence of air borne fungal spores m^{-3} of air in the library environments of four colleges before disturbance of books on the selves.

Sl. No.	Name of the fungi	Different College Libraries			
		KMCPGS	TAC	BGCW	PUCC
01.	*Absidia* sp.	1.78	-	-	-
02.	*Aspergillus awamori*	5.35	-	-	1.96
03.	*A. flavus*	3.57	-	40.0	-
04.	*A. niger*	5.35	2.27	40.0	3.92
05.	*A. tamarii*	-	4.54	20.0	-
06.	Brown sterile mycelia	1.78	-	-	-
07.	*Cladosporium herbarum*	48.21	31.81	-	73
08	*Curvularia resinae*	12.5	-	-	
09.	*Curvularia* sp.	-	4.54	-	-
10.	*Fusarium oxysporum*	5.35	15.90	-	-
11.	*Fusarium* sp.	-	-	-	-
12.	Grey sterile mycelia	1.78	-	-	-
13.	*Monascus rubur*	3.57	-	-	1.96
14.	*Mucor* sp.	-	2.27	-	1.96
15.	*Penicillium chrysogenum*	-	4.54	-	-
16.	*P. citrinum*	8.92	15.90	-	-
17.	*P. oxalicum*	-	2.27	-	-
18.	*Rhizopus stolonifer*	-	2.27	-	-
19.	*Syncephalastrum racemosum*	-	2.27	-	-
20.	White sterile mycelia	5.35	6.81	-	-

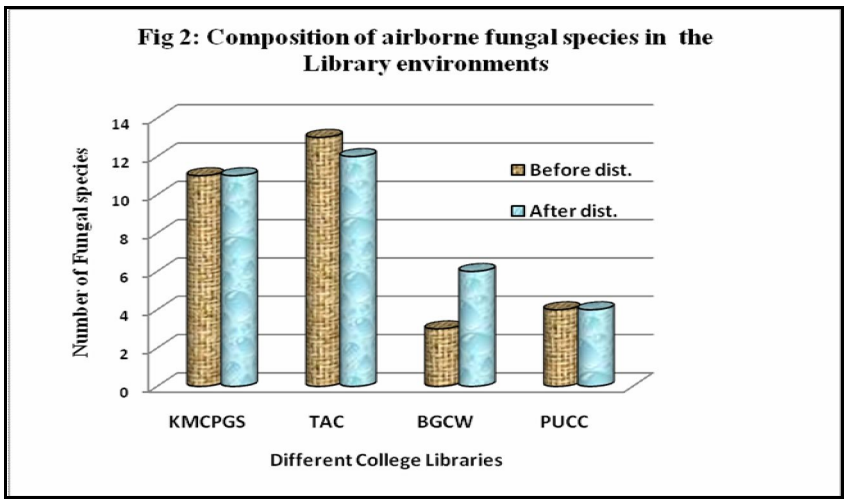
Table 2: Percentage occurrence of air borne fungal spores m^{-3} of air in the library environments of four colleges after disturbance of books on the selves.

Sl. No.	Name of the fungi	Different College Libraries			
		KMCPGS	TAC	BGCW	PUCC
01.	*Absidia* sp.	1.72	-	-	-
02.	*Aspergillus awamori*	1.72	5.26	-	-
03.	*A. flavus*	-	-	3.50	-
04.	*A. fumigatus*	5.17	-	22.5	-
05.	*A. japonicus*	5.17	-	-	-
06.	*A. niger*	5.17	7.01	70.01	2.5
07.	*A. tamarri*	-	5.26	14.06	-
08.	*Cladosporium herbarum*	36.2	42.01	-	74.05
09.	*Curvularia resinae*	1.72	-	-	-
10.	*Curvularia* sp.	-	1.75	-	-
11.	*Fusarium oxysporum*	10.34	8.77	-	-
12.	*Fusarium* sp.	-	-	-	1.25
13.	Grey sterile mycelia	-	-	3.50	-
14.	*Monascus rubur*	-	-	3.44	-
15.	*Mucor* sp.	5.17	3.50	3.44	-
16.	*Penicillium chrysogenum*	-	5.26	-	-
17.	*P. citrinum*	17.24	10.52	-	-
18.	*P. oxalicum*	-	1.75	-	-
19.	*Rhizopus stolonifer*	1.72	-	-	-
20	*Syncephalastrum racemosum*	3.44	3.50	-	-
21.	White sterile mycelia	6.89	5.26	-	-

Occurrence of airborne fungal CFUs m^{-3} of air in library environments of four Colleges are give in Fig 1, which showed that the distribution pattern among library environments. Pondicherry community college harbored maximum CFUs due to the availability of more *Cladosporium herbarum* spores in the samplings followed by KMCPGS and BGCW colleges.



Occurrence of *Aspergillus niger* was found very amazing since they were available in all the college libraries, but it was more concentration in Bharathidasan college. All together, six species of aspergilli were isolated i.e., *Aspergillus awamori, A. japonicus, A. flavus, A. fumigatus, A. niger and A. tamarii*. Five species were isolated from before disturbance of books and six species were recorded from after disturbance of books from the four libraries. Three species of *Penicillium* were also isolated in both before and after disturbance of books in all the library environments i.e., *Penicillium citrinum, P. chrysogenum and P. oxalicum. Cladosporium herbarum* was isolated in more numbers from Community college both in before and after in comparison to other college libraries (Fig 1). Fig 2 explained the total number of fungal species recorded from all library environments, of which Tagore Arts College was dominated and it was followed by KMCPGS and least number were recorded from BGCW and PUCC colleges.



Discussion

Aeromycological study employs a number of sampling methods of which, gravity settling of spores on culture medium is the one widely used by workers [11,12,13,14] 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 [15]. It is highly suitable for qualitative study but the result cannot be set forth qualitatively as it is not possible to express them as a unit of air volume. The present study used the volumetric Burkard's sampler on agar plates, which was found to be more effective than settling plate technique in Library environments, since it gave the quantitative data of airborne fungi in the per cubic meter of air, which was not possible by the former method.

Like our present results, Ferdowsi et al[16] isolated and found the genera of *Aspergillus, Curvularia, Penicillium, Rhizopus* and *Alternaria* from the library environments. Occurrence of aeromycoflora was recorded only in the indoors without and after disturbance of books in different Library environments. It was found that the fungal spores were the maximum in the after disturbance of books followed by the before disturbance of books in the libraries. But the differences in fungal spore load in the indoors site i.e., before and after disturbance of books of the Libraries were dependant on the abundance of *Cladosporium*, *Aspergillus niger* followed by *Penicillium chrysogenum*. The lesser Library activities were recorded before disturbance of books than the after disturbance. The abundance of fungi more in the after disturbance of books may be due to the dispersion of fungal mass from the substrates available in the papers present in the books of the Library environment. The library atmosphere was particularly dense in spores; this is certainly due to the fact that the extremely cramped locality was a site of intense activity [5]. The variations in the number of CFUs in different points of the Libraries do not appear to be significant.

A number of *Aspergillus* sp. (6 species) such as *A. flavus, A. fumigatus, A. japonicas, A. niger, A. tamarii and A. Awamori* were reported to be of high incidence in the present study similar to the findings of Singh et al[17]. According to Pasanen et al [18], it was found that despite of the relatively low level of humidity in the atmosphere; library environments harbor more fungal spores. It is the water from condensation present on the surfaces, which favour the growth of aspergilli. Among all, *Cladosporium herbarum* was found to be the dominant one before and also after disturbance of books in the libraries followed by *Aspergillus niger and Penicillium chrysogenum*. Besides these, *Aspergillus flavus, A. fumigatus, Penicillium citrinum and P. oxalicum* were recorded frequently from the Libraries.

Out of the isolated fungal species, *Aspergillus* and *Penicillium* sp were more recorded prominent moulds contaminating all rested manuscripts and periodicals account for two third of contaminations in library[19]. Fungal growth and sporulation on paper materials adversely affect and cause symptoms such as staining, discolorations, loss of luster, disfigurement, powdering of paper material reduction is strength of the paper and odor due to mildewing [5]. These observations are in conformity with those reported by Szezepanowska and Lovett [20]. Many of fungi reported in this survey were potential health hazards to library users [21]. *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., and *Aspergillus* sp. were the most common fungal species found from library [22]. The occurrence of total fungi are *Aspergillus* sp., *Curvularia lunata, Penicillium* and *Rhizopus* were found from the library environment, but *Aspergillus niger* were most dominated aerospora and exhibited the highest concentration in the section of library[23]. *Aspergillus flavus*, a well-known fungus for production of mycotoxin (alfatoxin), cause asthma in workers of food processing plants[2]. Health effects are associated with exposure to fungal spores and considered to be genus-specific. In an enclosed environment, fungal spores can become airborne in large concentrations and posses potential health hazards of occupational allergies or other lung disorders to atopic and sensitized individuals[1]. The large concentration of spores of *Aspergillus flavus* and *Aspergillus niger* from May to September and *Cladosporium* and *Penicillium citrinum* from November to February correlated with the higher prevalence of symptoms in workers during these period[2]. Among the isolated fungal taxa, *Aspergillus fumigatus, A. niger, Penicillium* sp*., Rhizopus stolonifer* were predominant aeroallergens that can cause different type of respiratory/lung diseases in atopic human beings. *Aspergillus fumigatus* causes broncho-pulmonary aspergillopsis diseases. *Aspergillus flavus*, a mycotoxin producing fungus was abundantly recorded from the two sites i.e., before and after disturbance of books in the Library environments.

Conculsion

Presence of microfungi in the indoor environment plays a very important role for both allergists and biologists who have an interest in health and environment biopollution problems. So the present indoor aeromycological study of the library environments would help the atopic human beings those who are suffering from allergenic disorders in these environments to diagnosis their dysfunctions. Moreover it would support the mycologists to categorize the allergenic fungi in order to segregate them from others in the indoor environments and would help them to learn how to prevent them from these environments.

Acknowledgement

The author is grateful to UGC, New Delhi for financial support in the form of Major Research Project to do the research in our centre.

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