Study of Airborne Fungi in the Homes of Asthmatic Patient's

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Abstract:

Fungi are eukaryotic organisms belonging to a kingdom that is distinct from plants and animals. All fungi depend on an external source of organic material for growth; these organic materials are digested by fungal enzymes and subsequently absorbed. Fungi are introduced into the indoor environment through natural open windows, doors, small ventilators and also mechanical ventilation systems. Indoor ambient concentrations of fungal spores are influenced by several factors including temperature, humidity, rainfall, and water intrusion into building structures, and also the extent of movement of outdoor air into a building.

Homes of asthmatic patient's of East and South parts of Nagpur city were surveyed during the study period. Study was conducted to reveal the prevalence of airborne fungi in asthma patient's home during winter season (November, 2013 to February, 2014). Altogether 12 species were isolated and reported from the studied environments. Species of *Cladosporium* were found to be dominant followed by species of *Aspergillus, Curvularia, Trichoderma, Rhizopus, Fusarium, Alternaria, Mucor and Nonsporulating fungi.* Six species of *Aspergillus* were isolated from the studied environments i.e. *Aspergillus flavus, A. niger, A. terrus, A. tameri, A. fumigatus* and *A. spp.* mostly found in the ranges of relative humidity 47-60% and the temperature ranges from 27-33°C.

In the last decades, much interest has also grown for the fungi present in indoor environments, since exposure to airborne biological agents in both the occupational and residential environments could be associated with a wide range of adverse health effects with major public health impact, including infectious diseases, acute toxic effects, allergies and cancer. The present study was aim to find out the prevalence and concentration of fungal spores in residential homes of asthmatic patients located in two different sites of Nagpur city.

Keyword – Asthmatic patient's, homes, Relative humidity, Temperature, *Aspergillus*.

Introduction:

In the last decades, much interest has also grown for the fungi present in indoor environments, since exposure to airborne biological agents in both the occupational and residential environments could be associated with a wide range of adverse health effects with major public health impact, including infectious diseases, acute toxic effects, allergies and cancer Hugues, et al., (1995)[4]; Dales, et al., (1997)[5]

In most situations exposure occurs to complex mixtures of toxins and allergens (and chemicals) and a wide range of potential health effects have to be considered. Three major groups of diseases associated with bioaerosol exposure can be distinguished: 'infectious diseases', 'respiratory diseases' and 'cancer'. Infectious and respiratory diseases are most common; however, valid incidence or prevalence data for most diseases caused by biological agents are lacking Lacey, (1991)[3].

Respiratory symptoms and lung function impairment are probably the most widely studied among bioaerosol-associated health effects. They can range from

acute-mild conditions that (at least initially) hardly affect daily life, to severe chronic respiratory diseases that require specialist care. Generally, occupationally related respiratory symptoms result from airway inflammation caused by specific exposures to toxins, pro-inflammatory agents or allergens. In addition to asthma and Chronic obstructive pulmonary disease (COPD)), organic dust exposed workers may develop hypersensitivity pneumonitis (HP) and organic dust toxic syndrome (ODTS) Morey, (2001)[10].

Moisture and dampness in the home environment supports the mold growth on biodegradable materials Dales et al., (1997) [5]. However, limited information is available for both determining the source and nature of mold contamination in indoor environment. Therefore, a scientifically sound evaluation of fungal concentration is required for overall investigation of mold in indoor environment of patients home. The type and concentration of fungi in residential buildings differ and depending upon the building maintenance, material use in the building, furniture and carpeting, ventilation system, extend of indoor plants, indoor temperatures, relative humidity and other abiogenic and biogenic factors including environmental parameters Ren et al., (2001)[9]

The present study is unique because it describes fungi found in patient's home whom is important to take care and avoid fungal exposure levels, prevention measures and also help doctor's to diagnose and treat the allergic individuals.

Material and Methods:

Air samples were collected from the main drawing halls and bedrooms of asthmatic patient's homes of East and South parts of Nagpur city. Preliminary survey was conducted to choose the homes where asthmatic individuals/ patients were suffering. Air samples were taken from 10 residential buildings to sample the indoor and outdoor (control) air during winter season at fortnight intervals i.e. November, 2013 to February, 2014. Colony forming units (CFUs/m3) of drawing rooms and bed rooms were counted and calculated together.

CFU counts and identification:

Qualitative results were expressed in terms of genus, species predominance (taxa), whereas quantitative estimation was recorded as CFUs/m^3. Isolated colonies were analyzed, identified and counted their CFUs/m^3. Identification of fungi is based on available texts and reference materials (Ellis, Raper and Thom; and Nagamani)[18-20]. Fungi that failed to sporulate after 10 to 15 days were recorded as nonsporulated and yeasts were counted separately i.e. white, orange and yellow.

Air sampler system-Mark-II (Hi-Media Lab. Pvt. Ltd., India) was used to collect airborne fungal propagules by using two different agar medium strips (i.e. PS-640 and PS-290). The sampling period was 5 minutes with an airflow rate of 280 lt/min., drives the impeller at constant speed of 4100 RPM. After sampled the air, media strip were removed and covered with their original plastic cover and later incubated at normal room temperature in laboratory for 7–15 days, after which the resulting colonies were counted.

Fungi were identified up to genus and species level by colony structure and microscopic examination of the spore structure. Temperature and relative humidity were recorded by digital thermo-hygrometer at the time of sampling. The fungi detected per unit volume of air calculated as under:

$$CFU/m^3 = \frac{\text{Colonies on agar strip x 25}}{\text{Sampling time in minutes (5)}}$$

Result and Discussion:

The number of CFU/m^3 counted on PS-640 medium strips which were exposed in indoor air of living rooms and bed rooms was higher than on PS-290 medium strips which were exposed in outdoor air (control) i.e. 5331 and 3813 CFU/m^3, respectively (Table. 1). However, there was no significant difference between the numbers of CFUs/m^3 from the main drawing halls and bedrooms.

Varieties of fungi were found among the sampled air of 10 homes of two different localities i.e. south and east part of Nagpur city. In all 16 different fungal species belonging to 10 genera were recorded and identified (Table. 1). *Aspergillus* species are the most frequently isolated fungi which were counted altogether 18.76% in indoor air while in outdoor (control) air was counted 19.34%. *Curvularia tetramera* was the second most dominant fungi which is counted 17.47% and (9.18% control), followed by *Cladosporium* spp. 17.12% and (14.26% control), *Trichoderma* spp. 10.43% and (2.13% control), *Rhizopus* spp. 8.44% and (4.43% control), *Alternaria* spp. 7.03% and (5.25% control), Mucor spp. 6.45% and (3.11% control), Fusarium spp. 5.63% and (3.77% control), Torula spp. 1.99% and (1.15% control), Penicillium spp. 1.41% and (1.64% control), Yeasts 2.81% and (24.59% control), and nonsporulated fungi 2.46% and (11.15% control), to the total count (Table 1).

Anderson (1985)[21] reported that *Penicillium* occurred almost constantly during the year while *Cladosporium* and *Alternaria* showed significant seasonal variations. In dust, humidity, more particularly, affects not only the fungal growth and the sporulation, but also determine prevalence of species. Humidity in homes may thus reduce or annihilate the seasonal variation of some mesophilic species. More moulds can be observed under this conditions mesophilic species may develop in mattresses all year round. Santra and Chanda, (1989)[2] investigated the indoor airspora of residential quarters at Calcutta. Spores of *Aspergilli* were predominant followed by *Helminthosporium, Alternaria, Curvularia, Penicillium, Rhizopus, Cladosporium* and *Candida*. They recorded that the seasonal periodicity of *Aspergillus* was maximum during the post monsoon period. In present investigation *Aspergillus* species also recorded maximum CFUs count (18.76%) in winter season.

Beguin (1995)[4] isolated molds ranging from less than 1000 CFU/g to 7,00,000,00 CFU/g of dust. *Alternaria alternata* reported quite frequently. Seasonal fluctuations noted for fungi may suggest that weather conditions even inside the

homes may have an impact. Hugues studied the mould biodiversity in homes II, analysis of mattress dust and reported species of *Alternaria, Penicillium, Aspergillus, Cladosporium, Mucor, Trichoderma* and *Pithomyces* in high concentration. *Aspergillus fumigatus* reported in rare and detected in small quantities. The same species were isolated and identified presently in indoor and outdoor air of homes except the *Pithomyces*.

Author had reported 14 species of *Aspergillus* in indoor air and only 6 species in outdoor air of residential quarters in rainy season, in the previous study Giri and Saoji, (1998)[6]. Presently *Aspergillus* recorded only 6 species in indoor and outdoor environment of homes that might be due to no rains, less humidity and high temperature. The most dominant species of *Aspergillus* reported in the present study was *A. niger, A. flavus* and *A. fumigatus*. The most frequent occurring species causing allergic fungal sinusitis (AFS), sinus aspergilloma and also reported potent hepatocarcinogenic are *Aspergillus fumigatus* and *Aspergillus flavus* (Ronald et al., 2003 and Hedayati et al., 2007) [12-13].

Fungi are well-known sources of allergens that play a role in the development of HP. Many fungal species have also been described as producers of type I allergens (IgE binding allergens), and IgE sensitization to common outdoor and indoor fungal genera like *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium* spp. is strongly associated with allergic respiratory disease, especially asthma Alan P. Knutsen *et al.*, (2012) [16].

Sahay et al., (2008)[14] reported the moulds in indoor environments of buildings and homes by Bio-Scan400 TM. They recorded 58 different fungal taxa from the surface samples of US buildings, *Aspergillus* and *Penicillium* counted dominant fungal taxa followed by *Cladosporium spp., Curvularia spp., Nigrospora, Alternaria, Chaetomium* and *Stachybotrys*. In present study *Nigrospora, Chaetomium* and *Stachybotrys* were not recorded, but the species of *Aspergillus, Cladosporium, Curvularia* and *Alternaria* were recorded dominantly.

Nayak and Nanda, (2010)[15] studied the fungal spores in bed rooms of homes in Pondicherry city, they reported eight species of *Aspergillus* out of which *A. niger, A. flavus, A. fumigatus* and *A. glaucus* were predominant with highest frequency. Present study also reported six species of *Aspergillus* out of which *A.niger, A.flavus* and *A. fumigatus* were recorded in highest concentration. *A. flavus* causes a broad spectrum of disease in humans, ranging from hypersensitivity reactions to invasive infections associated with angioinvasion. *Aspergillus fumigatus, A. flavus* are is the second leading cause of invasive and non-invasive Aspergillosis. Several species of *Aspergillus* are allergenic, including *A.niger, A. flavus, A. oryzae* and *A. fumigatus* Beaumont et al., (1984)[1]. It is too alarmic as these are isolated and counted dominantly in present study.

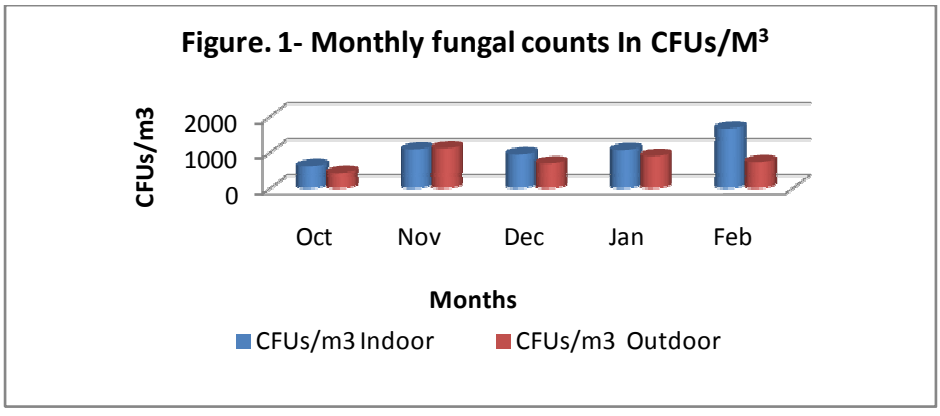
Many occupational studies have shown positive associations between endotoxins exposure and health effects including reversible (asthma) and chronic airway obstruction, respiratory symptoms (symptoms of asthma, bronchitis and bysinosis) and increased airway responsiveness, keratitis, endophthalmitis, cutaneous infection, wound infection, endocarditis, pericarditis, central nervous

system infection, rhinosinusitis, allergic fungal sinusitis, sinus aspergilloma, osteorticular infection (trauma), and urinary tract infection (urinary tract Aspergillosis) mainly caused by *Aspergillus flavus* (Hedayati et al., 2007)[13].

Results of several studies in which subjects were exposed to airborne fungi which have β(1→3)-glucans, suggest that these agents play a role in bioaerosol-induced inflammatory responses and resulting respiratory symptoms (Ronald et al., 2003)[12].

Table. 1- Isolated fungal taxa/species and their total CFU's/m3 and Percentage contribution in Indoor and outdoor environments of home

Sr.No.	Fungal taxa/species	Media strip PS-640		Media strip PS-290	
		Total CFU's (Indoor)	%	Total CFU's (Control)	%
1	Aspergillus niger	331	6.21	350	9.18
2	Aspergillus flavus	319	5.98	225	5.90
3	Aspergillus fumigates	181	3.40	69	1.80
4	Aspergillus terrus	106	1.99	19	0.49
5	Aspergillus tamerri	19	0.35	50	1.31
7	Aspergillus zonatus	44	0.82	25	0.66
8	Cladosporium spp.	913	17.12	544	14.26
9	Curvularia tetramera	931	17.47	350	9.18
10	Alternaria spp.	375	7.03	200	5.25
11	Mucor spp.	344	6.45	119	3.11
12	Rhizopus spp.	450	8.44	169	4.43
13	Fusarium spp.	300	5.63	144	3.77
14	Trichoderma spp.	556	10.43	81	2.13
15	Penicillium spp.	75	1.41	63	1.64
16	Torula spp.	106	1.99	44	1.15
17	Yeasts white	69	1.29	406	10.65
18	Yeasts orange	38	0.70	288	7.54
19	Yeast yellow	44	0.82	244	6.39
20	Non-sporulated	131	2.46	425	11.15
Total CFU's/m3		**5331**	**100**	**3813**	**100**



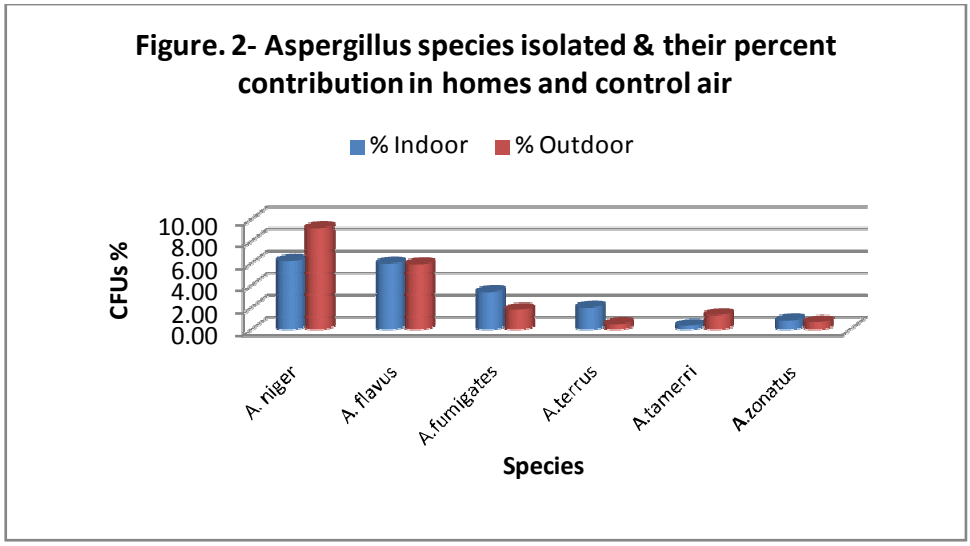


Figure. 2- Aspergillus species isolated & their percent contribution in homes and control air

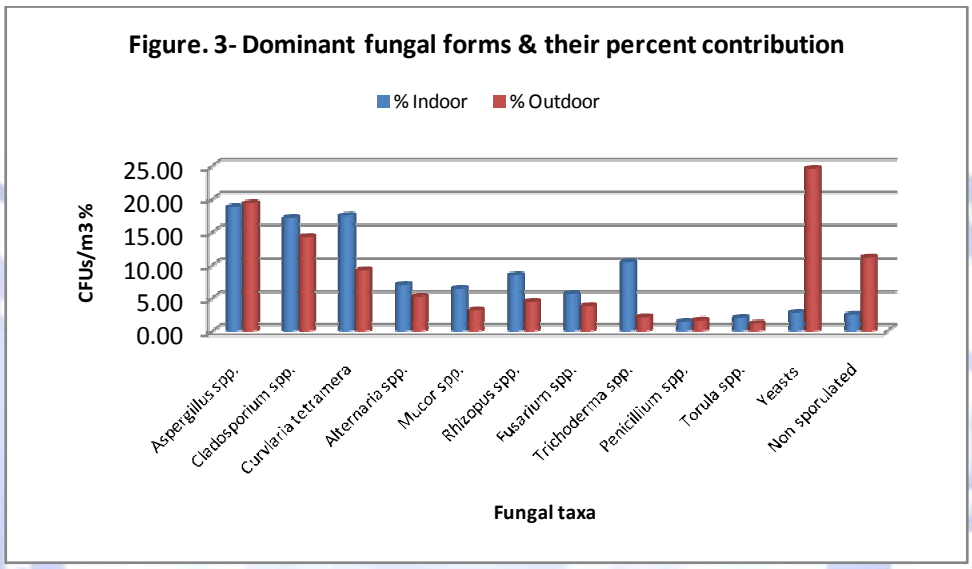


Figure. 3- Dominant fungal forms & their percent contribution

Conclusion:

General household and building activities have been reported to influence the changes in fungal concentrations. Such activities include cleaning, dusting, vacuuming, vegetable peeling, and presence of indoor plants and pets. Although significantly higher numbers of total culturable fungi were measured for samples collected in indoor air on the PS-640 medium strips than the PS-290 medium strips which are used in outdoor air (control), some specific genera, such as *Aspergillus, Cladosporium, Curvularia, Alternaria, Trichoderma, Mucor, Rhizopus and Penicillium* had a significantly higher number of CFUs/m^3. However, very less difference for *Aspergillus flavus, A. zonatus*, and *Penicillium* spp. between these two media was observed. These results indicated that PS-640 had best performance on the counts of *Aspergillus, Cladosporium, Curvularia, Trichoderma* and *Alternaria*. A. flavus is the second most important Aspergillus species causing human infections.

Cladosporium, Alternaria, Curvularia, Aspergillus, Penicillium, Rhizopus, Mucor and Trichoderma are grow mainly outdoors and indoors Hedayati et al., (2007)[13]. Outbreaks of Aspergillosis involving the skin, oral mucosa or

subcutaneous tissues are more often associated with *Aspergillus flavus* than other species. This is quite distinct from what is observed for outbreaks caused by *Aspergillus fumigatus*, i.e. life threatening pulmonary or sinuses diseases in severely immunocompromised patients (Myoken et al., 2003)[11].

Damp spots, water leakage or report of water damage, observation of mold or mildew growth, etc. from actual investigation at the time of sampling the air in homes have often been used as surrogate measures for the number of fungi in several published epidemiologic studies Dales,(1997), Giri and saoji, (1998) Darmage, et.al. (1999); Dales et al., (1999)[5–8]. Present results suggest that an actual measurement of fungal concentration in a home is more reliable than questionnaires for assessing residential exposure in epidemiologic studies.

In this small sample, we further confirm that fungal concentrations show substantial variations or trends among seasons, as is consistent with our previous study Giri, (2013)[17]. Summer and Rainy seasons were more likely to have higher concentrations of fungal propagules in indoor air than spring/winter. In addition, the seasons are associated with indoor temperature and relative humidity. Summer and autumn tend to have higher temperature and be more humid than spring/winter, which favor fungal growth. However, the question of how many samples in a year or in which season samples should be taken to best represent house hold fungal concentration is not clear.

The presence of fungal propagules in indoor air cannot be reliably predicted by home characteristics. Actual measurements are required for fungal exposure assessment, and the use of only one medium to collect samples in one location in a home might be adequate to represent residential levels of fungi in indoor air.

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