RESEARCH ARTICLE

Comparative studies on indoor Aeromycoflora from the laboratories

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ABSTRACT

Laboratory is the basic need of scientific research provided with several equipments, materials including cellulosic and non-cellulosic substrates. These substrates are degraded by diverse group of fungal microbes in a set of climate, indirectly polluting the indoor environment. In the present study aeromycoflora from various laboratories was reported for a month at an interval of a week. A population of 3368 fungal colonies falls under 19 genera and 28 species have been confined by culture plate exposer method. Ascomycota contributed with more than half of the total colonies recorded while Oomycota had least colonies. Zygomycota and Deuteromycota contributed moderate count of colonies. No member of Basidiomycota did persist. *Aspergillus* had higher colony count as well as greater species number. The sub-dominant air spora included *Cladosporium cladosporoides, Mucor pusillus* and *Rhizopus stolonifer*. The genus *Fusarium* had 3 species; *Penicillium, Curvularia*, *Alternaria* recorded with 2 species and others with single species. Prevalence of diverse group of fungal organisms on cellulosic material in laboratories depends on changing indoor environment. The climate of Lab IV was comparatively more ideal for fungal sporulation during survey

Key words: Aeromycoflora, Indoor, microbes , fungal colonies, bio-pollutant, Laboratories.

INTRODUCTION

Laboratories are the backbone of science research academy that provides controlled conditions to perform scientific research in innovating new creatures. They are store houses of mostly cellulosic and rarely non-cellulosic materials (Verma *et al*., 2013). The high moisture content, various gases, many chemicals, solid bi-products, numerous particulates and mostly cellulosic material available in the laboratories contribute to pollute indoor environment that may be hygienic affecting the health of researchers around the globe (Lanjewar and Sharma, 2014). The spores of fungal origin surviving in atmosphere are important components of bioaerosol as well as considered to act as

indicator of the level of atmospheric bio-pollution (Ananna *et al*., 2013).

Airborne fungal spores are ubiquitous in nature and can survive in both wet and dry environment through scavenging nutrients from the atmosphere (Verma *et al*., 2013). Concentration of airborne fungal spores has been linked to wind, relative humidity, temperature and rainfall (Jadhav and Lal, 2011). The ambient temperature and relative humidity play an important role in increasing fungal population in the indoor environment (Kayarkar and Bhajbhuje, 2014).The biological phenomenon of fungal organism concern to their ease of dispersion makes fungi one of the chief agents of contamination of variety of substrates including cellulose materials in the laboratories (Bhajbhuje, 2013). The saprobic fungal organisms undergo rapid proliferation in response to high relative humidity and are implicated to rapid degradation of cellulosic materials (Kayarkar and Bhajbhuje, 2014).

Majority of fungal airborne micro-propagules are pathogenic to human beings causing allergic problems including asthma due to differential deposition in the respiratory system. More than 80% micro fungal genera have been associated with respiratory disorders (Ghosh *et al*., 2011). About 20% of the human population is easily sensitized by normal fungal spore concentrations (up to 106 spores/m³) and all fungal spores in the indoor environment are regarded as potentially allergenic (Pathak, 2012). The appearance of respiratory allergy by fungal organisms is accounted at 20-30% among atopic individuals and up to 6% in general human population (EFSA, 2011). Indoor Aeromycoflora is responsible for causing several serious diseases to animals and other organisms because of their chemical and genetical properties. Fungal organisms in indoor environment caused spoilage of stored grains and food stuff (Nafis and Sharma, 2012), fabrics, leather and other similar articles (Ramamurthy *et al*., 2011) and bio-deterioration of books and other material (Thakre and Bhajbhuje, 1989).

The investigation on common airborne fungi and their distribution in a particular region can be helpful in identifying association between fungal sensitization and clinical diagnosis and clinical prevention of the seasonal allergic diseases (Chelak and Sharma, 2012). Since diverse group of species of aeromycoflora are considered major cause of respiratory ailment of human beings, causing allergies, asthma and pathogenic infections of the respiratory tract, plant diseases and as well as important agents of degradation of cellulosic and non-cellulosic material in indoor closed environment, thus there is a great need for understanding, aerobiological studies from indoor environment for laboratories of Post Graduate Department of Botany, Mahatma Jyotiba Phule Educational Campus, RTM Nagpur University, Nagpur (M.S.) India.

MATERIALS AND METHODS

Aeromycoflora from four different laboratories was isolated employing culture-plate exposure method (Bhajbhuje, 2013) at weekly intervals from 16thJanuary to 15th February 2014. Petri-plates containing sterilized Potato Dextrose Agar (PDA) medium were exposed for 10 minutes in triplicates inside different corners of four laboratories. The exposed petri plates were incubated at 25 ± 1^{0}C for 4-5 days. The colonies appeared on agar plates were recorded as percentage for individual species (Lanjewar & Sharma, 2014). The identification of species were made on the basis of micro- & macro morphology; reverse and surface colony coloration, grown on Czapek's medium and finally authenticated by authority.

RESULTS AND DISCUSSION

The present survey aims to record prevalence of diverse group of microfungal flora in indoor environment from various corners of four laboratories of sampling sites. During survey, aeromycoflora from laboratories such as Mycology & Plant Pathology (Lab.-I); Plant Physiology (Lab.-II); Plant Taxonomy & Anatomy (Lab.-III); and Plant Biotechnology Laboratory (Lab.-IV) has been isolated and recorded. The culture plate exposure technique has proved to be more appropriate over others to record fungal diversity, has been used in the present study for isolation of aeromycoflora. This is in agreement with the findings of Bhajbhuje (2013); Lanjewar and Sharma (2014). The air borne fungi from indoor environment include a very large and heterogeneous group of organisms having an enormous diversity. During aeromycological survey, a population of total 3368 fungal colonies classified under 19 genera and 28 species have been recorded. The count of isolates and

their concentration in the indoor environment varied with climatic changes. Of the total isolates, Deuteromycota dominated with 9 genera and 13 species exhibiting highest count of isolates followed by Ascomycota, representing 4 genera and 9 species. Least count of two genera with single species was confined with Oomycota, Zygomycota and Sterile mycelia (Table 1).

Table 1: : Report on fungal air spora from indoor environment of Laboratories

Sr. No.	Fungal organism	Number of fungal colonies				Total colonies	% Contribution	
		[2]Lab.-I	Lab.-II	Lab.-III	Lab.-IV		Species	Genera
A	Oomycota	5 (0.14)[1]	2 (0.05)	2 (0.05)	3 (0.08)	12 (0.35)	0.36	0.36
1	*Phytophthora infestans* de Bery	3	1	1	1	6	0.18	0.18
2	*Pythium aphanidermatum* (Els.) Fitz	2	1	1	2	6	0.18	0.18
B.	Zygomycota	131 (3.9)	220 (6.5)	122 (3.6)	121 (3.6)	594 (17.6)	17.6	17.6
3	*Mucor pusillus* Lindt	65	109	60	58	292	8.7	8.7
4	*Rhizopus stolonifer*(Eh.Ex. Rr.)Lind	66	111	62	63	302	8.9	8.9
C.	Ascomycota	422 (12.5)	420 (12.5)	324 (9.6)	552 (16.3)	1716 (50.9)	50.9	50.9
5	*Aspergillus flavu s*Link.	242	304	214	330	1090	32.36	49.46
6	*Aspergillus fumigatus* Fres.	58	26	26	84	194	5.76	
7	*Aspergillus niger* Van Tieghen	99	61	56	106	322	9.56	
8	*Aspergillus sulphureus*(Fres.)T&C	3	8	6	15	32	0.95	
9	*Aspergillus terreus*Thom.	8	7	8	5	28	0.83	
10	*Chaetomium glabosum*Kunze&Schm	1	1	1	3	6	0.18	0.18
11	*Penicillium citrinum*(C & S) Pitt.	5	5	7	4	21	0.62	1.12
12	*Penicillium oxalicum*Thom.	4	5	5	3	17	0.50	
13	*Phoma glomerata*(Corda)Wr.&Hocha	2	1	1	2	6	0.18	0.18
D.	Basidiomycota	-	-	-	-	-		
E.	Deuteromycota	231 (6.8)	184 (5.5)	195 (5.7)	370 (10.9)	980 (29.1)	29.1	29.1
14	*Alternaria alternata* Keissler	7	5	5	8	25	0.7	2.0
15	*Alternaria solani*(E.&M.) J & G.	8	13	9	15	45	1.3	
16	*Botryodiplodia sp*	2	2	2	5	11	0.3	0.3
17	*Cladosporium cladosporoides*(F) de Vries.	123	123	126	292	664	19.7	19.7
18	*Curvularia lunata*(Wakker) Boedijn.	3	2	7	4	16	0.5	1.3
19	*Curvularia tetramera*(McKinney) Boedijn.	5	5	10	8	28	0.8	
20	*Fusarium moniliformae*Sheldom	31	10	10	10	61	1.8	4.9
21	*Fusarium oxysporum*Schlecht.	21	9	11	10	51	1.5	
22	*Fusarium solani*(M.) App &Wollenw)	25	10	11	10	56	1.6	
23	*Helminthosporium tetramera*McKinney	2	1	1	2	6	0.18	0.18
24	*Nigrospora sp.*	1	2	1	1	5	0.14	0.14
25	*Pyricularia sp*	1	1	1	2	5	0.14	0.14
26	*Trichothecium roseum* Link	2	1	1	3	7	0.20	0.20
F.	Other types	18 (0.5)	13 (0.4)	15 (0.4)	20 (0.6)	66 (2.0)	2.0	2.0
27	*Sterile black mycelia*	10	7	9	10	36	1.1	1.1
28	*Sterile white mycelia*	8	6	6	10	30	0.9	0.9
	Sum of total colonies	807	837	658	1066	3368		
	Per cent contribution	24.0	24.8	19.5	31.6			

1. Values in parenthesis indicate per cent contribution of indoor fungal flora over total colonies recorded
2. Lab.-I : Mycology and Plant Pathology Laboratory; Lab.-II : Plant Physiology Laboratory;
Lab.-III: Plant Taxonomy and Anatomy Laboratory; Lab.-IV: Plant Biotechnology Laboratory.

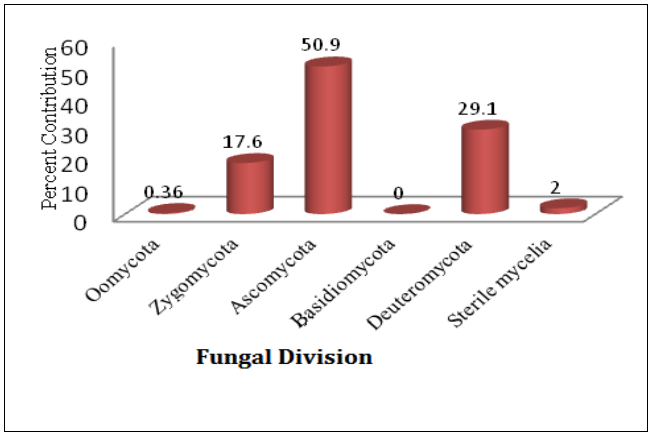

Fig. 1: Division wise percent contribution

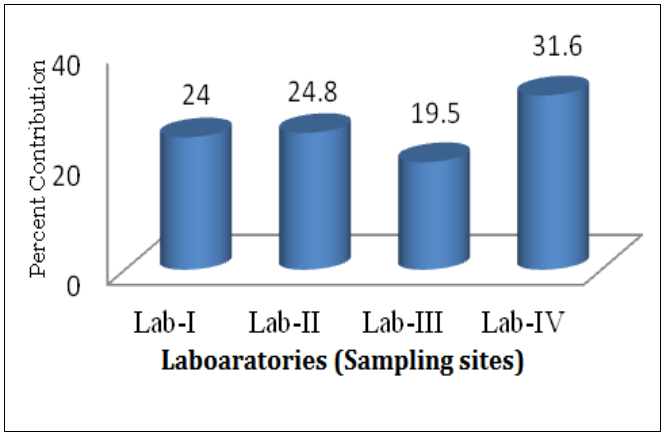

Fig. 2: Laboratory wise percent contribution

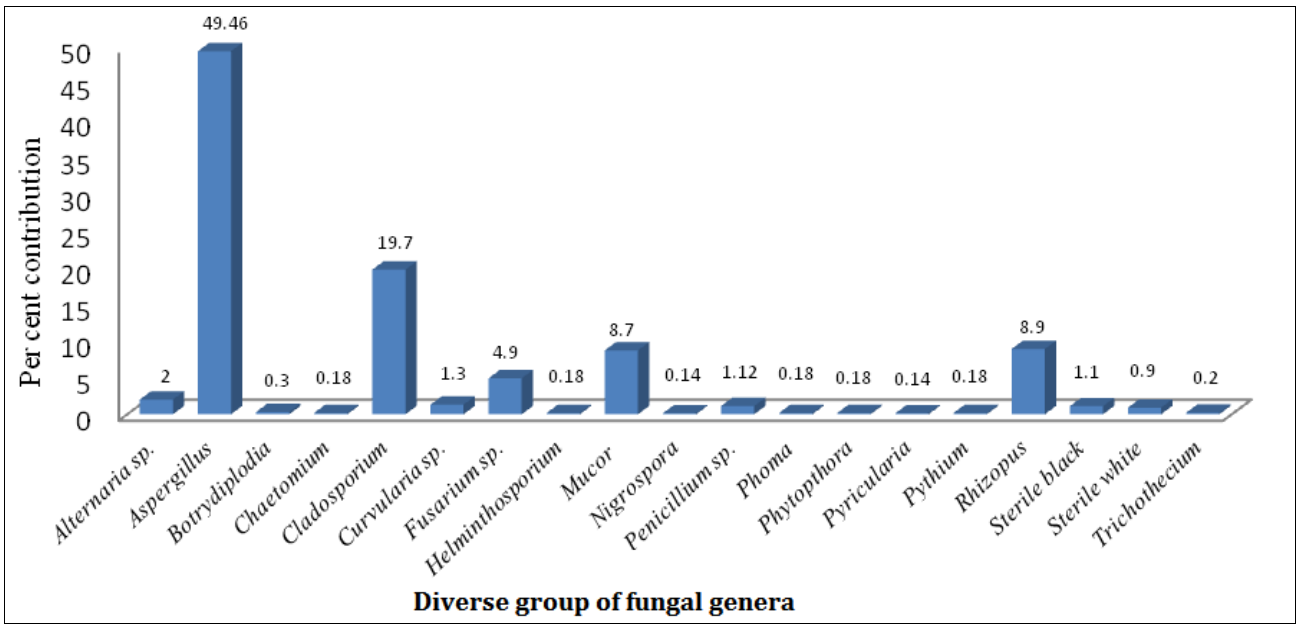

Fig. 3: Genera wise percent contribution

Deuteromycota contributed highest count of isolates followed by Ascomycota over Oomycota, Zygomycota and Sterile mycelia (Table1). The dominant Deuteromycota genera included *Alternaria, Cladosporium, Curvularia, Fusarium, Helminthosporium and Trichothecium*. These results confirmed with the earlier findings of Adams *et al*. (2013); Luka *et al*. (2014). The higher count of fungal isolates in indoor environment for Deuteromycota were reported from indoor environment of residential area; *Chawri bazaar* metro-railway station (Ghosh *et al*., 2011; Nafis and Sharma, 2012); rice mill (Lanjewar and Sharma, 2014). The occurrence of comparatively higher count of fungal isolates of Deuteromycota may be attributed to prevalence of diverse viable fungal spores with high indoor humidity of laboratories. Members of Deuteromycota produce enormous resistant thick walled conidia asexually; remain dormant in unfavourable indoor environment for longer duration and able to germination on the onset of favourable condition of optimum temperature and high relative humidity (Adams *et al*., 2013).The micro-fungal propagules were found scattered inside the laboratories understudy. Out of total count of 3368 colonies encountered, Ascomycota dominated with 50.9% exhibiting highest concentration followed by Deuteromycota with 29.1% of the total fungal air spora. The concentration of air spora was recorded decreasing for Zygomycota contributing 17.6% while sterile mycelia represented 2% of the total colony count. Oomycota had least count. Fungal spores from Basidiomycota did not appear in indoor environment of area understudy (Fig.1).

Comparative studies on indoor aeromycological survey revealed that all the laboratories understudy were heavily contaminated by airborne fungal flora. Out of the total indoor aeromycoflora encountered, Lab.-IV contributed 31.6% exhibiting highest concentration of fungal air spora. Lab.-III had least fungal air spora (19.5%). Lab.-I and Lab.-II dominated with nearly same concentrations of 24% and 24.8% of the total aeromycoflora respectively (Fig.2). The observation in the work depicted the fact that a comparatively rich fungal air spora existed in the Lab.-IV with higher concentration of fungal viability. The fungal organisms require more than 65% humidity for their growth provided nutrient rich substrates. The activities inside the laboratories such as regular functioning of air conditioners as well as use of autoclave for media sterilization are responsible for creation of ideal indoor environment like the optimum temperature and relative humidity for airborne fungal spore germination and their proliferation for Lab.-IV. Healthy fungal spores with optimum temperature and high relative humidity favour the indoor fungal growth (Kayarkar & Bhajbhuje, 2014).

Members of Ascomycota were recorded predominant inside all the laboratories understudy followed by Deuteromycota and Zygomycota. Members of Oomycota and Sterile mycelia exibited low concentration. The members of Basidiomycota did not persist in indoor environment. Lab.-IV contributed 16.3% fungal airspora belonging to Ascomycota exhibiting highest concentration while the least count was recorded in Lab III. Remaining two laboratories contributed nearly equal concentrations of indoor aeromycoflora (Table1).

Members of Deuteromycota were recorded sub-dominant in all the laboratories. Lab.-IV also had higher concentrations of Deueromycotou*s* fungal air spora followed by Lab.-I. The members of Zygomycota, were recorded more dominant in Lab.-II, contributing highest, 6.5% fungal air spora.Lab-I Lab-III and Lab-IV had nearly same concentration. Few colonies were encountered for members of Oomycota and Sterile mycelia from area understudy (Table 1). Out of the total colonies encountered on agar plates, *Aspergillus* represented 49.5% of the total colony count followed by *Cladosporium* (19.7%), Rhizopus (8.9%), *Mucor* (8.7%) and*Fusarium* (4.9%). Among these, *Aspergillus flavus , A. niger and A. fumigatus* were observed most dominant followed by *Cladosporium* and two members of Mucorales viz., *Rhizopus stolonifer* and *Mucor pusillus.*The genera*, Alternaria, Curvularia,* and *Penicillium* were recorded most significant or equally dominant. Other members, *Alternaria solani*, *Fusarium moniliformae, F. oxysporum, F. solani* and *Sterile black mycelia* contributed 1.1–1.8% of the total fungal air spora (Table 1).

Major components included most frequently encountered genus *Aspergillus* while minor components included less frequent and sporadic types. Other stable components recorded were *Botryodiplodia*, *Chaetomium,* and *Sterile mycelia*. The genera, *Phytophthora, Pythium, Phoma, Nigrospora, Helminthosporium, Pyricularia* and *Trichothecium* were rare in samples; found prevalent only 2-4 times during sampling (Fig.3). This is in agreement with the findings of Lanjewar & Sharma (2014) who reported frequent appearance of these fungi in indoor environment of rice mill. Diverse group of fungal species of saprophytic nature inside laboratories grew profusely on organic substrates such as cellulosic and non-cellulosic materials with different shades against other group of microbes, producing allergens, enzymatic proteins, secondary metabolites and other toxins that caused many respiratory disorders (MBL, 2012).

Aspergillus was dominant in indoor environment of laboratories contributing 49.7% (Fig.3). Several investigators including Nafis and Sharma (2012) and Luka *et al*., (2014) have reported higher concentration of *Aspergillus niger, A. fumigatus, A. flavus* and *A.nidulans.* Kayarkar and Bhajbhuje (2014) confirmed the airborne nature of *Aspergillus terreus.* Most prominent laboratories are provided with humidifiers and air-conditioning systems depending upon research work to be done. These activities help to make the environment extremely supportive for fungal proliferation on nutrient rich substrate like cellulosic materials. The precious legacies of cellulosic substrate in the form of books, documents, papers, wooden almirahs, cabinet, leather articles including shoes and non-cellulosic materials including microscopes, lenses, films etc. available in laboratories form ideal nutrient rich substrates for growth and development of microfungi as bio-deteriogens. The cellulosic products are rich source of sugar while lipids, glycides of leather provide protein rich substrate for many fungal species. Microbial deterioration of cellulose fibers is a very well established fact inside laboratories. *Aspergilli* and *Penicilli* are abundantly reported on these nutrient

rich substrates, involved in degradation (Thakre and Bhajbhuje, 1989, Lanjewar and Sharma, 2014). This substrate may act as carbon and nitrogen source for microfungal organisms. The relative humidity, temperature and rainfall play a key role in the proliferation of indoor fungal air spora.

The liberation of spores follow dispersion mechanism; both are interrelated and related to wind velocity, weather and other existing environmental conditions. The spores liberation of *Aspergilli* and *Penicilli* were favored by high air humidity and while those of *Alternaria,Cladosporium* and *Helminthosporium,* were liberated mechanically by the action of wind (Ianovici, 2008). Spore dispersal of Ascomycota is therefore favored by high relative humidity and low temperature, while, slightly increasing temperature with low humidity supports spore dispersal of Deuteromycota. The occurrence of such conditions at different times in different geographical regions may help to explain differences in the observed periodicities (Chelak and Sharma, 2012). Deuteromycetous fungus, *Cladosporium cladosporoides* predominated, and it is the most important genus in terms of defining variations in total count. This genus is reported to be a major constituent of fungal bio-aerosol(Lanjewar & Sharma, 2014). *Cladosporium* was most correlated with meteorological parameters, may be attributed to dry conidia in chains easily carried through air hence dispersion of spores was more influenced by meteorological parameters than *Alternaria* spores (Ianovici, 2008).

In Zygomycota, a count of total 594 fungal colonies were recorded representing two genera belongs to Mucorales, contributed 17.6% of the total colony count, caused a common disorder mucormycosis in human population (Wikipedia 2014). *Mucor pusillus* contributed 8.7% air spora, has been linked with zygomycosis, allergies, and mold sensitivity. Its spore's inhalation caused mucocutaneous & rhinocerebral infections, septic arthritis, renal infections, gastritis and severe pulmonary infection, and difficulty in breathing (Wikipedia 2014). Detailed investigations on epidemiology and clinical presentations of these unusual infections may improve early diagnosis and treatment.

The report of present study revealed, the microfungal organisms from indoor environment reported under Ascomycota, appeared to be dominated by 51 per cent (Fig 1). Among these most prevalent fungi, *Aspergilli* and *Penicilli* were the most abundant and widely distributed microfungal organisms on the globe (Verma *et al*., 2013). Members of the genus *Aspergillus* are known obligate saprophyte and survive in the indoor and outdoor environment provided favourable climatic condition and nutritive substrate (Adams *et al*., 2013). *Aspergillus flavus, A. niger,* and *A. fumigatus* had highest count of occurrence. *A. niger* has potential to produce *ochratoxin-A* and degrade polysaccharide (Wikipedia 2014), *A. flavus* secretes aflatoxin B_1, B_2, G_1& G_2 and other toxic compounds including *strigmatocystin, cyclopiazonic acid, kojic acid, β-nitropropionic acid, aspertoxin, aflatrem, gliotoxin*and*aspergillic acid* (Wikipedia, 2014). Inhalation of diverse group of fungal spores carrying mycotoxins such as aflatoxin, secalonic acid, zearalenone and trichothecenes may affect the immunological response of the lung tissues or cause other hazards to human health. *Aspergillus fumigatus* has been reported finding the ergot alkaloids, an array of potent chemicals, was used as primary raw material for synthesis of hallucinogen, and LSD (Wikipedia 2014).

Deuteromycota contributed with 29.1% of the total colony count (Fig 2). During mycelial and reproductive growth, *Alternaria solani* secretedmycotoxins such as *Altersolarol-A*, *alternaric* acid, *dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl ether, tentoxin, tenuazonic acid, altertoxins, stemphyltoxin III* (Wikipedia 2014) whereas *Alternariol monmethylether, tenuazoic acid* and *altertoxins* were secreted by *Alternaria alternata*, can affect respiratory system, skin, and nails in humans (Skjoth, 2012) and also induced reduction in seed germination and seedling emergence with chromosomal abnormalities in plants (Bhajbhuje, 2013). Alterotoxin induced micro-mutation in diverse group of animals (EFSA, 2011). The genera *Curvularia* represented 1.3% of total fungal air spora in indoor environment.Its conidial inhalation from environment have been reported to cause infection to lungs, heart, nose, skin and eye which results to sneezing, coughing, general weakness, swelling around the eyes, and partial loss of vision. *Fusarium*, a most prevalent toxin-producing deuteromycetous fungal organism contributed with 4.9% of total colony count in indoor environment of the area understudy (Table1), reported to degrade carpet, mattresses, damp walls, polyester, polyurethane foam, humifier pans and

produce a diverse range of mycotoxins includes tri-chothecenes (T-2 toxin, HT-2 toxin, deoxy-nivalenol & nivalenol), zearalenon and fumonisins many of which have significant impacts on human health (MBL, 2012).

CONCLUSION

Environmental microfungal population is seemed to act as an indicator of the level of environmental bio-pollution. Microbial components to indoor air are receiving the greater attention with the framework of potential health hazards to diverse group of biotic elicitors including human beings. Exposure to fungal air spora has been linked to a range of detrimental health effects like allergies and respiratory disorders in both infants and adults. Moreover, most of the fungal microorganisms are well known saprophytes involved in the biodegradation of organic substrate of cellulosic nature.Hence the clean indoor environment is the basic need for good health.

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