

RESEARCH ARTICLE

Diversity of air-borne mycoflora from indoor environment of library

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ABSTRACT

Aeromycoflora from Library is known to be significant in respect to allergies and also involve in deterioration of cellulosic and non-cellulosic library materials. Airborne fungal flora of the Central library of R.T.M. Nagpur University Campus, Nagpur have been isolated at regular intervals for a month employing gravity plate exposure technique. Altogether 1595 colonies categorized under 23 genera and 39 species were isolated. Deuteromycota exhibited greater count of isolates with higher incidence followed by Ascomycota. Member of Basidiomycota did not persist. *Cladosporium* dominated the air of library exhibiting significant incidence. *Aspergillus* was recorded with greater count of species whereas *Botrytis*, *Chaetomium*, *Cladosporium*, *Cunninghamella*, *Drechslera*, *Nigrospora*, *Phoma*, *Phytophthora*, *Pyricularia*, *Pythium*, *Rhizopus*, *Torula*, *Trichoderma* and *Trichothecium* were encountered on agar jelly with individual species.

Keywords: Aeromycoflora, indoor, library, allergy, saprophytes, biodeterioration; frequency.

INTRODUCTION

Libraries are the true backbone of research academy and well-organized collection of information resources in the form of books, periodicals, newspapers, films, recorded music that made accessible to defined community for reference or borrowing (Kayarkar and Bhajbhuj, 2014). The books and documents in libraries are valuable heritage as they carry all kinds of knowledge through the barrier of time and have a capacity to pass them to the academicians in future. Documents and books are considered as precious legacies that remind people of their culture, religions and ethnic tradition (Kalbende *et al.*, 2012). They deserve to be maintained and conserved in their original conditions in libraries in inbuilt indoor atmosphere.

Libraries are store houses of mostly cellulosic and rarely non-cellulosic materials (Verma *et al.*, 2013). The books are made from paper which is a

polymer of cellulose. A binding gum used in books may be organic or synthetic. The library material and other articles of cellulosic and non-cellulosic substrates contributing to pollute indoor environment, may be unhygienic affecting the health of library visitors and library staff (Prester, 2011; Lanjewar and Sharma, 2014).

The high moisture content and moderate temperature in indoor environment of library are conducive for the growth of microbes and accelerate the deterioration process that affect the physical and chemical properties of library collection (Dalal *et al.*, 2011). The fungal propagules surviving as scavengers and bring about the biodeterioration of cellulosic and non-cellulosic materials (Thakre and Bhajbhujje, 1989; Kalbende *et al.*, 2012). Human endoparasites may able to provoke any infection, childhood asthma, allergies and mycotoxicity (Aimanianda *et al.*, 2010). The increased awareness among human population has made the study of fungal airspora essential and hence the aeromycology has acquired a prominent position in various fields of environmental sciences.

Several investigations have been carried out on indoor environmental microfungal organisms in many different parts of the globe due to their relationship with plants, animals and human. Fungal organisms in indoor environment caused biodeterioration of books and other library material (Dalal *et al.*, 2011). They are responsible for causing diseases in humans like athlete's foot and skin infection like dermatophytosis or ring worm infection or tinea (EFSA, 2011). Since diverse fungal species constitute the major components of aeromycoflora, are major cause of respiratory ailment of human beings as well as important agents of degradation of cellulosic and non-cellulosic material in indoor closed environment, there is a great need for understanding, aerobiological studies from indoor environment for central library of Mahatma Jyotiba Phule Educational Campus, RTM Nagpur University, Nagpur (M.S.) India. Presently, prevalence of aeromycoflora has so far not been reported earlier from this place, it seemed to be worthwhile to undertake a more comprehensive and systematic study of the diversity of aeromycoflora from indoor environment for central library during winter season.

MATERIAL AND METHODS

1. Selection of Sampling Site:

The central library (presently called P.V. Narasimha Rao Library) of the Mahatma Jyotiba Phule Educational Campus, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (M.S.) India has been selected as sampling site as it is one of the oldest library established in 1966.

2. Culture medium:

The jelly medium of Potato Dextrose Agar (PDA) consisting of 200 gram potato; 20 gram dextrose and 20 gram agar powder in one liter of water was prepared and a slightly cooled sterile liquid medium was transferred aseptically to each sterile Petri plates under laminar air flow. It was allowed to jellify at room temperature. The junctures of the petri dishes having semi-solid medium were sealed by parafilm.

3. Spore Sampling and identification:

The Petri-plates containing sterilized PDA jelly were exposed in triplicates for 10 minutes then they were sealed with parafilm; brought to the laboratory and incubated at room temperature for appearance of fungal colonies. The colonies appearing were picked up and cultured. They were identified to species level on the basis of their characteristics, reverse colour of colonies and spore morphology. Finally, aeromycoflora was authenticated by authority. The colonies were counted and recorded in terms of percent frequency (Kayarkar and Bhajbhujje (2014).

RESULT AND DISCUSSION

Airborne fungal propagules are a major cause of respiratory ailments of human causing allergies, asthma, and pathogenic infections of the respiratory tract (Adams *et al.*, 2013). They are also well known agents of plant disease and means for dissemination of many saprotrophic fungi. Some of the propagules are toxic and causing serious health hazards in human being, as well as their higher concentration in the air creates environmental pollution (Ilanovici *et al.*, 2011).

The prevalence of heavy load of indoor aeromycoflora in library during winter season is receiving the great attention with the framework of potential hazards to library visitors including students and the faculties.

Great concern has been expressed about potential health hazards to library staff remaining engaged in library and visitors with special focus on allergenic or toxigenic aeromycoflora and their association with air quality. The present survey aims to record diversity of airborne mycoflora from indoor environment of P.V. Narasimha Rao library by gravity plate exposure technique for a period of a month (February, 2017) as majority findings revealed prevalence of higher concentration of aeromycoflora during winter season (Luka *et al.*, 2014; Bhajbhujje, 2015).

In the present study, gravity plate exposure technique which has been proved to be more appropriate over others was employed for detection of indoor aeromycoflora to record fungal diversity. This has been agreed with the findings of Kayarkar and Bhajbhujje (2014); Verma *et al.*, (2013); Lanjewar and Sharma (2014) and Katre (2016) who reported the greatest count of fungal isolates as well as higher fungal colony count of indoor airborne mycoflora by gravity plate exposure test. This technique was preferred for isolation of airborne fungal flora due to certain advantages (Kayarkar and Bhajbhujje, 2014; Dongre and Bhajbhujje, 2015).

During the period of aeromycological survey, altogether 1595 fungal colonies classified under 23 genera and 39 species have been encountered on agar jelly in petri-plates (Table 1 & 2). Deuteromycota dominated with 76.1% airspora exhibiting highest concentrations followed by Ascomycota with 16.9% air spora. Sterile mycelia contributed 4.6% while Oomycota had 1.9% airspora. Zygomycota had least count of 0.6%. Fungal spores from Basidiomycota did not appear on nutrient jelly (Fig 1). It is in agreement with the results of Adams *et al* (2013) who obtained the higher count of fungal isolates in indoor environment of library for Deuteromycota. The count of isolates and their concentration in the indoor environment varied with climatic changes. Of the total isolates, Deuteromycota exhibited highest count of isolates followed by Ascomycota and Zygomycota. Least count of isolates was associated with Oomycota and Sterile mycelia (Fig. 2). Members of Deuteromycota produce enormous resistant thick walled conidia asexually and remain dormant in unfavorable indoor environment for longer duration and able to germinate on the onset of optimum temperature and high relative humidity (Adams *et al.*, 2013; Jyoti and Malik 2013; Katre, 2016).

Ascomycota contributed second highest incidence of indoor air spora (Fig. 1). It may be possible in response to prevalence of significant concentration of viable resistant fungal propagules including spores in indoor environment for Ascomycota and also favorable indoor microclimate in library for their proliferation (Luka *et al.*, 2014). Members of Ascomycota are well known saprophytes involved in the biodegradation of organic substrate of cellulosic nature (Verma *et al.*, 2013). Under moist environment, the library material including books, journals, newspapers, documents etc. form the ideal organic substrate for the development of saprophytic fungal organisms (Adams *et al.*, 2013). *Aspergillus* contributed 14.5% while *Penicillium* contributed 2.1% airspora. *Aspergilli* and *Penicilli* are reported abundantly on the nutrient rich substrates, involved in degradation (Jyoti and Malik, 2013; Thakur and Jite, 2015). Due to biodegradation, books may be damaged, deteriorated or discolored in pictures and prints (Kayarkar and Bhajbhujje, 2014).

The genus *Cladosporium* was recorded dominant saprophyte against other genera recorded in this group (Fig 3). Naresh *et al.* (2013) have reported greater concentration of conidia of *Cladosporium* in the moist atmosphere. It is reported to be major constituent of fungal bio-aerosol (Lanjewar and Sharma, 2014). *Cladosporium* is most correlated with meteorological parameters, may be attributed to appearance of dry conidia in chains, which can easily carried through air reasonably dispersion of spores was more influenced by meteorological parameters. Even moderate concentrations of *Cladosporium* spores in the indoor air are known to cause allergic diseases in humans (Gonclaves *et al.*, 2010). The dry conidia in chains are easily carried through air to a long distance away from existing area hence dispersion of spores was more influenced by meteorological parameters. Ascomycetous genus, *Aspergillus* exhibited greater count of species followed by *Alternaria* (5), *Fusarium* (3), *Helminthosporium* (2) and *Penicillium* (2). Remaining genera were recorded with individual species (Fig. 4). Kayarkar and Bhajbhujje (2014) reported higher count of species of *Aspergillus* from indoor environment of library. *Rhizopus* of Zygomycota; *Aspergilli* and *Penicilli* of Ascomycota as well as *Alternaria*, *Cladosporium*, *Curvularia* and *Fusarium* of Deuteromycota contributed as major components; represented a group of taxa of favorable environment that can exploit virtually any organic

Table 1: Distribution of indoor aeromycoflora of campus central library, Nagpur.

S.No.	Fungal organism	Total colonies	Percent Contribution	
			Species	Genus
A.	Oomycota	30	1.88	1.88
1.	<i>Phytophthora infestans</i> de Bery	12	0.75	0.75
2.	<i>Pythium aphanidermatum</i> (Els.) Fitz	18	1.13	1.13
B.	Zygomycota	9	0.6	0.6
3.	<i>Cunninghamella elegans</i> Kuwab & Hoshino	1	0.06	0.06
4.	<i>Rhizopus stolonifer</i> (Eh.Ex.Rr.)Lind	7	0.44	0.44
5.	<i>Syncephalastrum racemosum</i> Cohn ex J.Schrot	1	0.06	0.06
C.	Ascomycota	269	16.86	16.86
6.	<i>Aspergillus amstelodami</i> Thom & Church	23	1.44	14.48
7.	<i>Aspergillus flavus</i> Link.	20	1.25	
8.	<i>Aspergillus japonicus</i> Link	12	0.75	
9.	<i>Aspergillus ochraceus</i> Wilh	1	0.06	
10.	<i>Aspergillus niger</i> Van Tieghen	152	9.53	
11.	<i>Aspergillus sulphureus</i> (Fres.)T&C	16	1.00	
12.	<i>Aspergillus terreus</i> Thom.	3	0.2	
13.	<i>Aspergillus versicolor</i> Tiraboschi	4	0.25	
14.	<i>Chaetomium glabosum</i> Kunze & Schm	4	0.25	
15.	<i>Penicillium glaucum</i> Link	14	0.88	
16.	<i>Penicillium oxalicum</i> Thom.	19	1.19	
17.	<i>Phoma</i> spp.	1	0.06	0.06
D.	Basidiomycota	-	-	-
E.	Deuteromycota	1214	76.12	76.12
18.	<i>Alternaria alternata</i> Keissler	41	2.57	3.26
19.	<i>Alternaria porri</i> (Ellis) Cif	1	0.06	
20.	<i>Alternaria solani</i> (E & M.) J & G.	2	0.13	
21.	<i>Alternaria tenuissima</i> Wiltshire	7	0.44	
22.	<i>Alternaria triticina</i> Prasada & Prabhu	1	0.06	
23.	<i>Botrytis cinerea</i> Persoon	4	0.25	
24.	<i>Cladosporium cladosporoides</i> (F) de Vries	1083	67.9	67.9
25.	<i>Curvularia lunata</i> (Wakker) Boedijn	19	1.19	1.25
26.	<i>Curvularia ovoidea</i> (Hiroe & N.Vatan) MuntCvetk	1	0.06	
27.	<i>Drechslera rostrata</i> (Drechsler) Richardson &Fraser	4	0.25	0.25
28.	<i>Fusarium dimerum</i> Penzig	3	0.20	0.70
29.	<i>Fusarium moniliformae</i> Sheldom	1	0.06	
30.	<i>Fusarium semitactum</i> Berk & Ravenel	7	0.44	
31.	<i>Helminthosporium tetramera</i> McKinney	10	0.63	0.76
32.	<i>Helminthosporium solani</i> Durieu & Mont	2	0.13	
33.	<i>Nigrospora oryzae</i> (Berk & Broome) Petch	9	0.6	0.6
34.	<i>Pyricularia</i> spp.	1	0.06	0.06
35.	<i>Torula</i> spp.	5	0.31	0.31
36.	<i>Trichoderma</i> spp.	6	0.38	0.38
37.	<i>Trichothecium roseum</i> Link	7	0.44	0.44
F.	Other types	73	4.58	4.58
38.	Sterile white mycelium	59	3.7	3.7
39.	Sterile black mycelium	14	0.88	0.88
	<i>Sum of total colonies</i>	1595	100.04	100.04

Table 2 : Division wise count of genera & species and their percent contribution

S.No.	Division	Count of Genera	Count of Species	Total colonies	Percent contribution
1	Oomycota	2	2	30	1.9
2	Zygomycota	3	3	9	0.6
3	Ascomycota	4	12	269	16.9
4	Deuteromycota	12	20	1214	76.1
5.	Sterile mycelia	2	2	73	4.6
	Total	23	39	1595	100.1

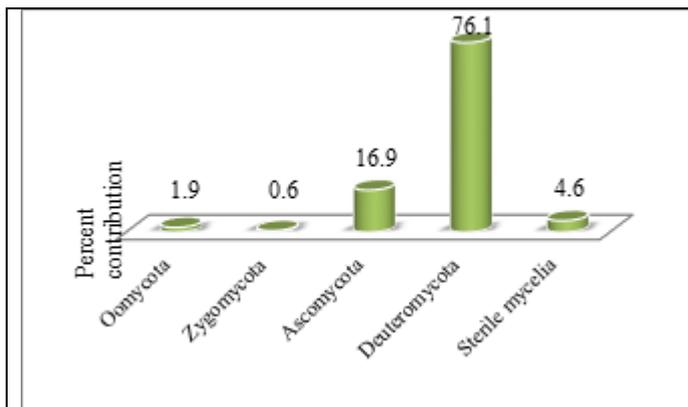


Fig. 1 : Division wise contribution of indoor aeromycoflora for library under study.

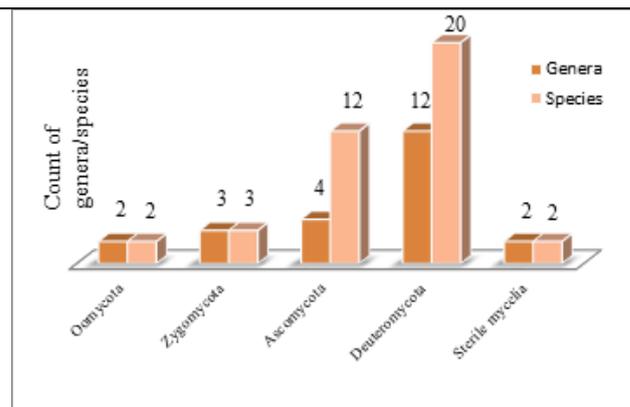


Fig.2 : Count of genera and species in indoor aeromycoflora for library under study.

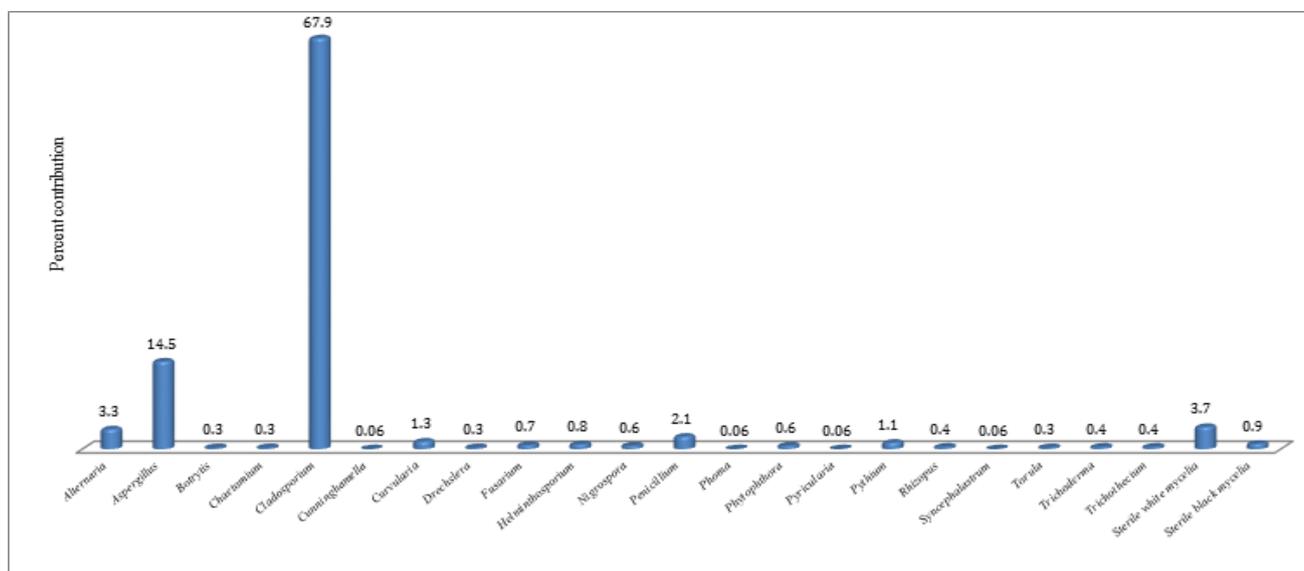


Fig. 3: Percent contribution of the genera recorded in the aeromycoflora of library under study.

substrate provided oxygen, temperature and relative humidity and accumulate toxic secondary metabolites (EFSA, 2011; Adams *et al.*, 2013; Thakur and Jite, 2015).

Various investigations concerns to fungal proliferation and sporulation suggest that optimum temperature;

high relative humidity, nutritive substrate creates favorable microclimates for a profuse growth, proliferation and sporulation of indoor airspora resulting to higher population of fungal isolates (Prester, 2011; Bhajbhujje, 2015). Fluctuation in any of these parameters mostly temperature resulted inhibition of fungal growth and increase dormancy.

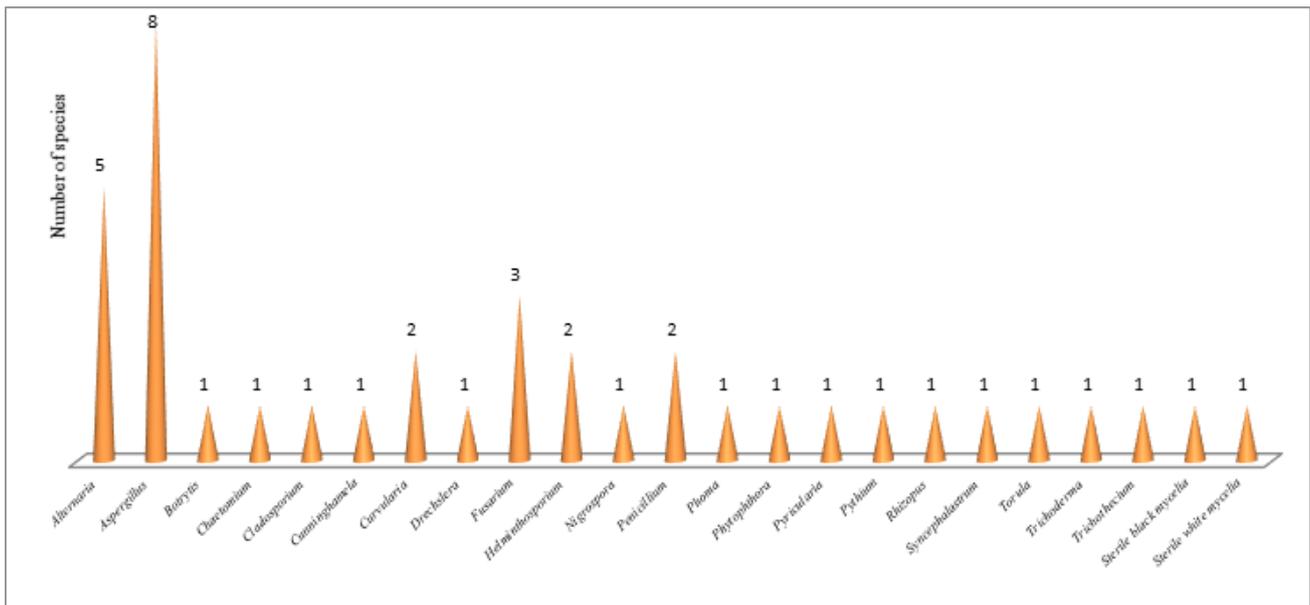


Fig. 4 : Genera & species wise distribution of aeromycoflora of Library

Members of Deuteromycota are involved in biodegradation of cellulosic and non-cellulosic material and liberate enormous conidia in the indoor environment. These conidia have implication to asthmatic and allergy patients. A sector of visitors and faculties of library inhaling conidia develops hay fever, woodworker's lung or apple store hypersensitivity; susceptible individuals can become sensitized to the protein on the spore surface and develops allergies (Wikipedia, 2017).

Ascomycota conidia have implication to asthmatic and allergy patients. During mycelial and reproductive growth, *Alternaria solani* secretes mycotoxins such as *Altersolarol-A*, *Alternaric acid*, *Dibenzopyron*, *Tetranic acid*, *Altartoxin-I and II*, *Alternariol*, *Alternariol monomethyl ether*, *Tentoxin*, *Tenuazonic acid*, *Altartoxins*, *Stemphylltoxin III* (Holensein and Stoessi, 2008) whereas *Alternariol monomethylether*, *Tenuazoic acid* and *Altartoxins* were secreted by *Alternaria alternata*, can affect respiratory system, skin, and nails in humans (Skjoth, 2012). Altartoxin induced micro-mutation in diverse group of animal (EFSA, 2011).

The inhalation of conidia of *Curvularia* and its deep skin inoculation causes health risk to persons with weak immune system and also has health risks related to major barrier breaks such as corneal perforation, major surgery peritoneal or venous catheter presence and injection drug use (MBL, 2012).

Fusarium is reported to degrade carpet, mattresses, damp walls, polyester, polyurethane foam, humidifier pans and produce a diverse range of mycotoxins including Trichothecenes (T-2 toxin, HT-2 toxin, deoxy-nivalenol and nivalenol), Zearalenon and Fumonisin many of which have significant impacts on human health (MBL, 2012). The fumonisins have been linked to esophageal cancer, liver cancer and neural tube defects. Deoxy-nivalenol is known immune modulatory and produces emesis and growth retardation in animals whereas Zearalenone is naturally occurring endocrine disturbing chemical. It induces gastro-intestinal effects and precocious pubertal changes. Apart from these, many other secondary metabolites (moniliformin, beauvericin and fusaproliferin) are known to be secreted by different *Fusaria* and their effects on human health, either alone or in combination with other mycotoxins, was largely unexpected (MBL, 2012). *F. moniliformae* was reported to cause keratitis, wound and infections of the eyes and fingernails, invasive mycosis in immune-compromised people. Its inhalation and deep skin inoculation health risks to persons with weak immune system *Fusarium* is also associated with allergy and vomiting. Trichothecenes causes a serious feed refusal and vomiting in animals contaminated feed (EFSA, 2011).

Aspergillus niger was reported second dominant. It has potential to produce *ochratoxin-A* and degrade polysaccharide (Anderson *et al.*, 2012). *A. flavus*

secretes aflatoxin B₁, B₂, G₁ and G₂ and other toxic compounds including *strigmatocystin*, *cyclopiazonic acid*, *kojic acid*, *β-nitropropionic acid*, *aspartoxin*, *aflatrem*, *gliotoxin* and *aspergillilic acid*. The other species are known to carry mycotoxins in their spores or produced volatile metabolites that are reported responsible for causing toxigenic effect to flora and fauna (Wikipedia, 2017).

Penicillium secretes penicillic acid, causing systemic penicilliosis in AIDS patients in Southern Asia and proved to be nephrotoxic in pigs and broilers may cause tremors, coagulopathy and enteritis. Moreover, 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 (EFSA, 2011).

Members of Mucorales of Zygomycota, contributed 0.6% colonies of the total, caused a common disorder mucormycosis accounting for 70-80% human population (Marisa *et al.*, 2011). *Rhizopus stolonifer* has been linked with a common disorder, Zygomycosis, allergies, and mold sensitivity. Its spore's inhalation caused septic arthritis, renal infections, gastritis and severe pulmonary infection, and difficulty in breathing (Smith, 2013). Its metabolic products induced significant inhibition of human serum vitamin C and Fe, Cu concentrations to a greater extent and the inhibition was proportional with an increase in concentrations of the metabolic products (Al-Jubury *et al.*, 2012).

CONCLUSION

Indoor aeromycoflora is reported to be significant in respect of allergic diseases and responsible for deterioration of library material. Present investigation revealed that members of Deuteromycota exhibited higher incidence followed by Ascomycota. *Cladosporium cladosporoides* and species of *Aspergillus* dominated the air of library. Impact of airborne fungal spores including their release, dissemination, deposition and effect is of great significant to identify the health hazards and physiological disorders in human beings. Exposure to indoor airborne inhalant mould allergens develops respiratory symptoms, airways disorders and allergies. Thus clean indoor

environment is of prime importance for maintenance of good health.

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