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Study of Fungal Diversity in Air of Some Buildings near a Forest Area

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ABSTRACT
Air contains large number of microorganisms including bacteria and fungi and their estimation is important as an index of cleanliness for any particular environment. The microbial quality of indoor air of different buildings near a forest area located in Nanda Ki Chawki, Dehradun was conducted. Samples were collected in PDA Petri plate. Total 30 samples were taken. Sampling was conducted in the month of July 2019, total 10 fungal species were observed. Environmental condition plays a crucial role for the growth of the fungal spores. The outcome of this study shows that the quality of air in these selected buildings is highly contaminated with fungi like *Mucor* sp, *Aspergillus flavus*and*A.niger*and moderately contaminated by *Botrytis* sp, *Collentrotricum gloesporieda*, *Colletrotricum* sp., *Fusarium solani*, *Fusarium* sp.,*Penicillium* sp. and *Trichoderma* sp.

Key words: Fungal spores, *Aspergillus* sp, Fungal Diversity, *Mucor* sp, *Penicillium* sp, *Fusarium* sp, *Collentrotricum* sp, *Tricoderma* sp.

I. INTRODUCTION

Air quality is a measure of the suitability of air for breathing by people, plants and animals. Air quality means the condition of air around us which is mainly affected by factors like gases, moisture, minute particles, pollen grains, microbial contamination and other pollutants. Among these, microbiological quality is one important factor considered as source of infection and cause several communicable diseases in human beings. Many times spores of bacteria and fungi may cause allergy. Microbiological quality of air in buildings of school, college, offices, bus stand, railway stations, hospitals and other public places is more important if near to traffic of highways, farm fields, forests, landfills of wastes, etc. In these places dust particles, pollen grains and aerosols carry microbes in air.

Hospital is a place where a patient is admitted for treatment, however, poor air quality may increase chances of cross-contamination and patients may get secondary infection and disease like severe fungal allergies, asthma, lungs problem, etc. The patients whose immune system is weak particularly children, aged peoples and those who are suffering from cancer, hepatitis, AIDS, heart or respiratory conditions such as asthma, etc. are more vulnerable for secondary infection caused by air contaminants. Thus air quality directly affects our health. Some health problems which is caused by poor air quality are given below:

- Skin diseases like athlete's foot , jock itch , ringworm , yeast infection etc.
- More frequent or more severe asthma attacks.
- Worsened symptoms of emphysema and bronchitis.
- Irritated eyes, nose, throat and lungs.
- Coughing, wheezing and bronchial restriction.

As there are many cases seen due to the poor air quality in India:-

Sudharsanam et.al,(2008) reported *Aspergillus flavus, Aspergillus niger, Candida non-albicans* in intensive care unit hospital in south Chennai, India. Similarly, Srishti choudhary, (2019) reported *Candida auris(C.auris*) in blood culture of 62 year old patient who die within two weak in ICU of New Delhi hospital. As the cases describe above shown that still the condition of air near hospitals is not good, it may cause many serious diseases.

The climate of Dehradun is temperate due to its location at the foot of the Himalayas. The climate of Dehradun is the same as of a north Indian city i.e. , cool winters , warm summers , rainy monsoons and a balmy spring . The climate of Dehradun also depends upon the altitude, the more high we go,and the morecold we will feel.Dehradun receives the rainfall in between June and September. Though in December and January

it receives winter rainfall, but the maximum rainfall is recorded in between July and August. Dehradun gets an average rainfall of 2073.3mm annually. Such specific climate and relatively higher forest corner favour fungal diversity in monsoon and post monsoon season in Dehradun city. Therefore, It is assumed that fungal spores in air remain higher in those area near forest, river alpine etc.

In present study, air quality for fungal spore contamination was studied in corridor of some buildings nearby a forest area, Nanda-ki-Chawki, Dehradun Uttarakhand.

II. MATERIALS AND METHOD

In the present experimental work all the chemicals and culture media were used of High Media, Mumbai India. All the glassware used of borosilicate quality. Major instruments used were Laminar Air Flow, Hot Air Oven, Autoclave, Water bath, Microscope etc. The samples were collected from differentbuildings near forest area, Nanda ki Chawki Prem Nagar, Dehradun.

A. Place of study:

This was a laboratory based observational study, carried out in the Department of Microbiology Taq Gene Training & Research Institute (TGTRI), Dehradun, Uttarakhand as a part of summer training research cum dissertation from July 4, 2019 to August 16, 2019.

B. Sampling of air at various sites:

Sample collection method was based on the adhesive property the media used, that traps the airborne particles on to their surface when culture plates containing media are exposed face upwards to the atmosphere to collect particles settling by gravity.

Samples were collected from different wards of a hospital, a laboratory of a research center and a student hostel building. The culture plates of Potato dextrose agar (PDA) medium were exposed at different places for 10 min in sampling area randomly. All plates were transfer in a BOD incubator at 22°C for 3 to 7 days in invert position.

After sufficient incubation all plates were observed for growth of fungal colonies. Number of fungal colonies was counted and reported.pure cultures of each fungal colony were prepared on PDA plates.

C. Isolation of fungal isolates:

Fungal colonies were observed for various culture characters from day third to seven or sometimes more. Each fungal isolate was observed under microscope. Small quantity of mycelia structure was mounted on a microscope slide in distilled water and Lacto Phenol Cotton Blue stain separately.

Various morphological structures viz. mycelia and modification of mycelia, spores or conidia, sporangiophore, other structures were observed carefully. Measurements of mycelia thickness, length and breadth of fungal spores, colour and shape of spores, etc. were observed separately. All fungal isolates were identified on basis of morphological and culture characteristics. Identification of fungi is carried out on material mounted in a slide which carry one drop of distilled water for the characterization of their primary structure. Then cover slip is put on the slide and then observed under the microscope. For the secondary structure of fungi we use lacto phenol cotton blue in slide.

III. RESULTS AND DISCUSSION:

Samples collected from Hospital Ward, corridor, Hostel, Lab and Canteen show higher fungal contamination. As in table-1 we observe the colony forming unit of different fungal species isolated from samples.

Concerning quantitative results we found that Hospital Corridor was highly contaminated with fungal species as it has high cfu value than others, this may happen due to poor sanitation or ventilation condition of ward. This high fungal contamination in Hospital corridor can cause many serious problems and also it is a major problem for immunosensitive patients.

In table-1 we also observe that in Girls Hostel-I the contamination of fungus is also high which is from $0.1x10^2$ cfu/unit to $2.8x10^1$cfu/unit, that create also a major problem as it cause many skin infections and nosocomial infection which is dangerous for girls.

As the samples were conducted in rainy season which is favorable season for the growth of fungus. Due to humidity or moisture in the environment of Hospital corridor or Girls Hostel-I the colony forming unit is high. On the other hand we observe that in Hospital ward the fungal contamination relatively very low as compared to other locations which is about $0.1x10^1$to $0.6x10^1$ cfu/unit. This could happen because there is proper ventilation and sanitation.

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Table- 1: The mean numbers of colonies growing from plates exposed at each site during sampling are indicated

S. No	Site	Samples	Average number of fungal colonies (cfu/unit)
1	Hospital Ward	Sample 1 Sample 2 Sample 3 Sample 4 Sample 5	0.3×10^1 0.3×10^1 0.1×10^1 0.6×10^1 0.4×10^1
2	Hospital corridor	Sample 6 Sample 7 Sample 8 Sample 9 Sample 10	2.8×10^1 2.8×10^1 1.6×10^1 0.8×10^1 2.3×10^1
3	Girls Hostel-I	Sample 11 Sample 12 Sample 13 Sample 14 Sample 15	0.1×10^2 1.7×10^1 2.8×10^1 1.8×10^1 0.7×10^1
4	Girls Hostel-II	Sample 16 Sample 17 Sample 18 Sample 19 Sample 20	1.6×10^1 0.1×10^1 0.7×10^1 0.2×10^1 0.6×10^1
5	Research Laboratory	Sample 21 Sample 22 Sample 23 Sample 24 Sample 25	0.1×10^1 0.1×10^1 0.3×10^1 9.5×10^1 0.9×10^1
6	Canteen of Hospital building	Sample 26 Sample 27 Sample 28 Sample 29 Sample 30	0.2×10^1 0.8×10^1 0.6×10^1 5.4×10^1 0.1×10^1

The isolated fungi were *Aspergillus flavus, Aspergillus niger, Mucor* sp., .*Penicillium* sp., *Fusarium solani, Fusarium* sp., *Tricoderma* sp., *Colletrotricum gloesporieode,*. This shows that the quality of air is still not so good. As it seen in Table.1 that in Hospital Corridor there is higher fungal contamination as compared to other places and research lab has least fungal contamination. It is also seen that in Girls Hostel I the amount of fungus is high which not a good is. In Canteen there are average amount of fungus is found but it is quite dangerous. Some fungi occur in more than one place. As we seen that *Mucor sp*. grow at all 6 locations. It is also recorded that the amount of *Aspergillus flavus* is high in Hospital Corridor, which is not good for immunosensitive patients. Some non-infectious fungus is also reported like *Penicillium* sp. which is very helpful for drug production and also used as an antibiotic.

MICROSCOPY:

Fungus is characterized by observing their microscopic characters. As in microscope we observe the shape of spores, size of spores, colour of spores, of all fungi as given in Table 3. By observe the microscopic character we can find that which type of fungus is that.

As we observe in table-3 the *Aspergillus flavus* in microscope show green colour spore which are circular in shape and their size varied from 0.71-2.84µm. The presence of *Aspergillus flavus* in air can cause Aspergillosis among patients with weak immunity, or tend to cause serious intestinal problems as well as liver cancer. In our sample we also identify Aspergillus niger whose

spores is dark brown in colour and size of spores varied from 0.47-2.84µm.

Next fungus which is isolate I our sample is *Penicillium* which in microscope show flask-shaped phialides and spores which is blue in colour are produced in dry chains from the tips of the phialids. The size of spore varied from 0.35-4.97µm. *Mucor* sp is also majorly isolated from our air samples. The spore of Mucor is black in colour and their size varied from 1.06-4.26µm.

In this research we also identify *Fusarium solani* whose spores are white in microscopy and there shape is crescent whose size is varied from 1.42-4.26µm in microscope.

Table 3.

S. No.	Fungi	Microscopy					
		Colour of spore/ conidia	**Shape of spore/ conidia**	**Size of spore/conidia**	**Opacity of spore/conidia**	**Thickness of mycelia**	**Septum in mycelia**
1	*Aspergillus flavus*	Green	Circular	0.71-2.84µm	Translucent	1.42-4.26µm	Present
2	*Aspergillus niger*	Dark brown	Circular	0.47-2.84µm	Opaque	1.42-5.68µm	Absent
3	*Penicillium* sp.	Blue	Circular	0.35-4.97µm	Opaque	1.42-2.84µm	Present
4	*Mucor* sp.	Black	Circular	1.06-4.26µm	Transparent	4.26-9.94µm	Absent
5	*Trichoderma* sp.	Green	Circular	0.71-1.13µm	Transparent	1.42-2.84µm	Present
6	*Botrytis* sp.	Green	Circular	0.85-2.84µm	Transparent	1.42-4.26µm	Present
7	*Colletrotricum gloesporioide*	White	Rod	7.1-15.62µm	Transparent	4.26-5.68µm	Absent
8	*Fusarium solani*	White	Crescent	5.68- 17.04µm	Translucent	1.42-4.26µm	Present
9	*Fusarium* sp.	Grey	Crescent	0.56-1.13µm	Transparent	1.42-4.26µm	Present
10	*Colletrotricum* sp.	White	Rod	4.26-14.2µm	Transparent	1.42-7.1µm	present

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Table 4.

S. No.	Fungal isolate	Frequency per site
1.	*Aspergillus flavus*	15
2.	*Aspergillus niger*	10
3.	*Penicillium* sp.	9
4.	*Mucor* sp.	21
5.	*Tricchoderma* sp.	1
6.	*Botrytis* sp.	1
7.	*Colletrotricum gloesporiode*	1
8.	*Fusarium solani*	3
9.	*Fusarium* sp.	1
10.	*Colletrotricum*sp.	3

According to the above table, we observe that *Mucor* sp. has high frequency then others fungal sp., and it almost isolate from each sampling location. Some fungal isolates are found least in number like *Tricoderma* sp., *Botrytis* sp, *Colletrotricum gloesporiode*, *Fusarium* sp. On the other hand *Aspergillus flavus* and *Aspergillus niger* are also found in high amount in air. *Penicillium* sp, also found in more than one place of sampling.

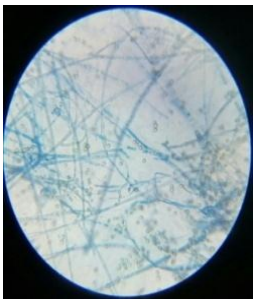

Fig-1: Colony of *Aspergillus flavus* Microscopic view of *Aspergillus flavus*

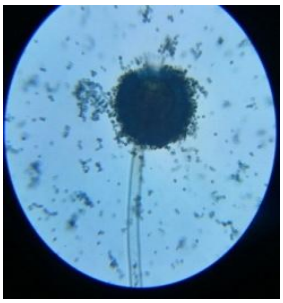

Fig-2: Colony of *Aspergillus niger* Microscopic view of *Aspergillus niger*

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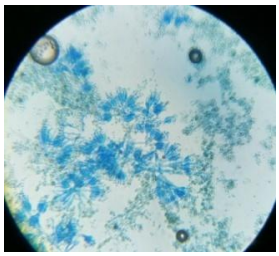


Fig-3: Colony of *Penicillium* sp**.** Microscopic view of *Penicillium* sp

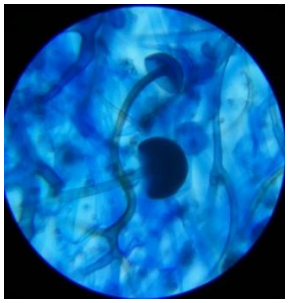
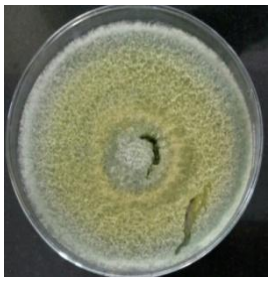


Fig-4: Colony of *Mucor* sp .Microscopic view of *Mucor* sp



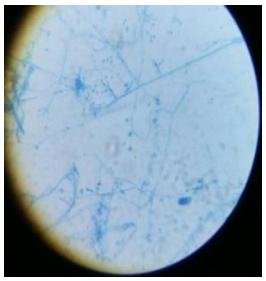


Fig-5: Colony of *Trichoderma sp.* Microscopy view of *Trichoderma sp*

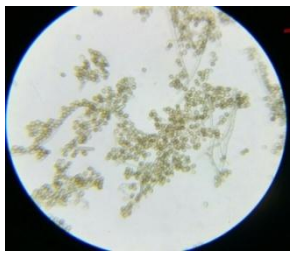


Fig-6: Colony of *Botrytis sp* Microscopic view of *Botrytis sp*.

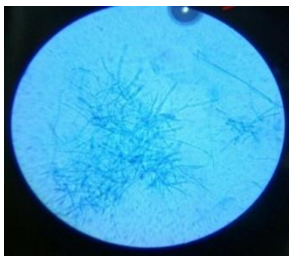
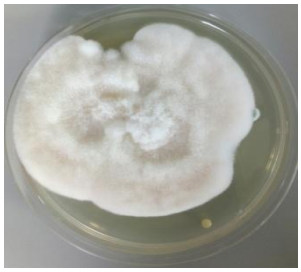


Fig-7: Colony of *Colletrotricum gloesporioide* Microscopic view of *Colletrotricum gloesporioide*



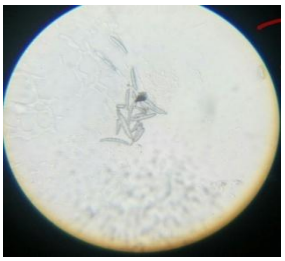
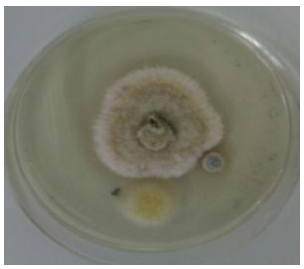


Fig-8: Colony of *Fusarium solani* Microscopic view of *Fusarium solani*



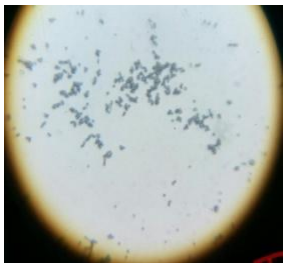


Fig-9: Colony of *Fusarium* sp Microscopy of *Fusarium* sp

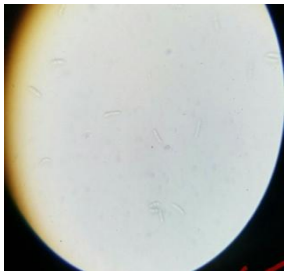


Fig-10: Colony of *Colletrotricum* sp**.** Microscopy view of *Colletrotricum* sp.

IV. CONCLUSION:

Based on the results of the present study the most common fungus isolate from air was *Mucor* sp. and *Aspergillus* in Hospital Ward and Corridor. *Aspergillus* is the most frequent cause for nosocomial infections immunecompromised patients. By observing this result we conclude that the air of Hospital Ward and Corridor is relatively contaminated with fungal spores which may cause various diseases so we require efficient ventilation

system in this Hospital and also we require switching high efficiency particulate air filters, avoid opening windows, for natural ventilation, regular disinfection, other chemical compounds can be used for disinfecting against fungal and their spores.

We can also used air-conditioning unit with a high-efficiency particulate air (HEPA) filter attachment which will trap outdoor mold spores. Regular cleaning of the ward &corridor of Hospital, Hostels must require. Utensils in the canteen must be properly rinsed before used. We must avoid the source of fungus.

Although the presence of mold contamination in the healthcare environment may increase fungal infections. For the prevention of these fungal infections regular surveillance & cleaning is necessary.

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