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Airborne Viable and Settled Dust-Bound Micro-Fungi in Residential Homes

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Abstract: ***Recent reviews of the effects of home dampness and fungi have shown several positive associations between fungal exposure and increased risk of adverse respiratory symptoms in children's and adults. Present study was conducted to study the airborne viable and settled dust bound micro-fungi in residential homes of two different localities namely Ayodhya Nagar and Raghuji Nagar area of Nagpur city. Air and dust sampling were carried out simultaneously at monthly intervals from July, 2017 to December, 2017 (6 months) by using Hi-Media Air sampler (Hi-Media-LA002); and Eureka Forbes Mini vacuum cleaner (Eureka Forbes Co. ltd. India) were used for dust collection from various sources.***

Total 23 fungal species were isolated and counted their CFUs/m3 from the indoor air of residential homes. Nine species of Aspergillus were recorded and it was dominant throughout the study period followed by the species of the genera Curvularia (2 spp.), Cladosporium (2 spp.), Alternaria (2 spp.), Trichoderma, Mucor, Rhizopus and Yeasts. While 19 species were isolated from the settled dust samples collected from the various sources, these are Ceiling fan/table fan dust isolated 4 species of Aspergillus, out of which Aspergillus niger and Aspergillus flavus are the dominant.

Sofa dust isolated 6 species of Aspergillus of which Aspergillus niger is the dominant followed by Aspergillus flavus, Aspergillus fumigatus, A. ocharceous, A. terrus and A. zonatus. Carpet dust also isolated Aspergillus species dominantly followed by Cladosporium, Penicillium, Curvularia, Rhizopus, Alternaria, Trichoderma and Yeasts. Seven species were reported from the bed dust collected from two different localities. Bed dust isolated 5 species of which 3 were Aspergillus niger, A. flavus and A. fumigatus and one each of Penicillium chrysogenum and Curvularia tetramera. Indoor airborne mould exposure causes neurologic dysfunction and cognitive deficits including memory loss, irritability, anxiety, depression, numbness, tingling and tremor.

Keywords: Airborne, Viable, Settled dust bound Micro-fungi, Residential homes, and Aspergillus.

I. INTRODUCTION

The inhalation of fungal spores and also house dust of dwelling homes cause acute symptoms in allergenic individuals. The risk of respiratory symptoms, such as cough and wheeze or asthma as well as respiratory infections and general symptoms like headache and tiredness, is higher for occupants in residential buildings Peat et. al., [12], Borneheg et al., [2]. Many fungal genera were observed in homes and it is well described correctly as the “weeds of home” which are also responsible for dust allergy.

Allergic reactions to fungi (single or clusters of conidia, hyphae elements, spores, crystals) in air include rhinitis, asthma and extrinsic allergic alveolitis or hypersensitivity pneumonitis Hedayati et.al., [8]. Although it has not always been possible to find a high degree of correlation between the concentration of fungal spores and the incidence of asthmatic symptoms, the role of spores has been clearly identified in specific atopic individuals. Traditionally, allergists have assumed that mold-induced asthma was entirely due to an allergic reaction. It is clear, however, that some species such as *Aspergillus fumigatus* have particular properties that can result in more severe symptoms caused by direct lung infection allergic bronchopulmonary aspergillosis (ABPA) Dales [3] and Hedayati et al. [8].

Dwelling homes are one of the most important indoor environments. It may serve as a reservoir and source of allergens. The fungal spores in dwelling houses may come from many sources within the building. They may come from fungi growing in condensation on walls, paint works, and on foods or spores may come from outside and accumulate in house dust and grow, if the humidity is high enough.

They may then become dispersed by human activity such as sweeping, bed making, and building repairing work etc. Verhoeff & Burge [15]. Indoor airborne mould exposure causes neurologic dysfunction and cognitive deficits including memory loss, irritability, anxiety, depression, numbness, tingling and tremor etc. Luke Curtis et al., [11]. The purpose of this study was to assess quantitatively and qualitatively the occurrence of airborne viable and settled dust bound micro-fungi in indoor environment of residential homes which are mainly responsible for various human health hazards.

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II. MATERIAL AND METHODS

A. Selection of Sampling Site

To find out the dust borne and airborne fungi prevalent in the indoor environment of residential homes were carried out. Ayodhya Nagar and Raghuji Nagar were selected for the sampling. The air sampling was conducted in bed room, drawing room and kitchen and simultaneously dust sampling also takes place from various sources like fan dust, bed dust, sofa dust and carpet dust in order to get complete analysis of fungi present in indoor environment of residential homes.

B. Sampling and Isolation Methods

Air was sampled by using a Hi-Media Air Sampler with Rose Bengal Agar strip (RBS-640). The sampling duration was 4 minutes and the samples were taken at fortnight intervals over a period of 6 months from July, 2017 to December, 2017. (Figure. 1).

C. Air Sampling

Indoor and outdoor (control air) air samples were collected from each home using a Hi-Air sampler (Hi-Media, Mark-II Ltd. India). RBS-640 medium strips were used (Hi-Media Lab.). Sampler was run in homes for 4 minutes and exposed strips were brought back to the laboratory and incubate it up to 7 to 15 days for the development of colonies (Figure. 2).

D. CFUs Count and Identification

Exposed media strips were incubated in an inverted position at 27 ± 2^0C; after four to five days of incubation, the colony forming units (CFUs) were visually counted and the total fungal count was expressed as colony forming units per cubic meter of air (CFUs/m^3). The fungi detected per unit volume of air calculated as under:

$$\text{CFU/m}^3 = \frac{\text{Colonies on agar strip x 25}}{\text{Sampling time in minutes (4)}}$$

Isolated genera/species were identified by macroscopic and microscopic analysis with the help of standard published literature [4, 5, 11, 13].

E. Dust Collection and Isolation of fungal Colonies (CFUs/g):

Dust was collected from various sources was vacuumed for 2-5 minutes, using a Eureka Forbes Mini vacuum cleaner (Eureka Forbes Co. ltd. India). After vacuumed dust was collected in air tight plastic bags and brought back in the laboratory and kept in refrigerator.

Temperature and relative humidity measurements in each homes, was made possible with the help of hygrometer. Dust was sieved through a 2 mm sieve; collected dust is suspended for 20-30 minutes in 250 ml Erlenmeyer flask with 90 ml sterile water to make a suspension. Further serial dilutions 10-2 to 10-6 are made while the suspension is in motion, by withdrawing 1ml or 10 ml into additional dilution blanks having 9 ml or 90 ml sterile water in test tubes or flasks respectively Waksman [16]. After solidification of the medium, the petri dishes are incubated in an inverted position for 3-7 days at room temperature till the colonies appear. To get uniform results two replicate plates are prepared for each sample.

F. Fungal Analysis

The number of fungal colonies appearing on dilution plates are counted, averaged and multiplied by the dilution factor to determine the number of colony forming units (cfu)/g (or ml) of the sample.

$$\text{No. of colony forming Units /g of dust/soil (cfu g-1)} = \frac{\text{No of colonies (av. of replicates)}}{\text{Dry weight of the dust}} \text{ X Dilution factor}$$

To obtain distinct colonies, 1 ml of the final dilution is distributed over the surface of a solidified agar medium, which has been poured into dishes 2-3 days before, so that the agar surface is dry when the suspension is added. The calculated concentrations of dust borne fungi were colony forming units (CFUs)/g of dust, and those of airborne fungi were CFUs/m^3 of air were counted separately.

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III. RESULTS AND DISCUSSION:

Dust sampling is often a surrogate measure for respiratory exposure to fungi. This practice assumes that fungi in dust are representative of past and continuing airborne exposure. It is likely that cumulative or average fungal exposure is more relevant to health outcomes than a single day's exposure. However, I saw very close associations are found between the airborne and dust bound micro-fungi in the studied indoor environments.

A. Species Composition of Air Sampling

Altogether 23 fungal species were isolated and counted their CFUs/m^3 from the indoor air of dwelling homes in two different localities. Eight species of *Aspergillus* were recorded and it is dominant throughout the study period followed by the species of the genera *Curvularia* (2 spp.), *Cladosporium* (2 spp.), *Alternaria* (2 spp.), *Trichoderma*, *Mucor*, *Rhizopus* and *Verticillium* (Table 1) & (Figure 4).

B. Species Composition of Dust Sampling

Total 19 species were isolated from the dust samples collected from the various sources. Ceiling fan dust isolated 4 species of *Aspergillus*, out of which *Aspergillus niger* and *Aspergillus flavus* are the dominant. Sofa dust isolated 6 species of Aspergillus of which *Aspergillus niger* is the dominant followed by *Aspergillus flavus*, *Aspergillus fumigatus*, *A. ocharceous*, *A. terrus* and *A. zonatus*. Carpet dust also isolated Aspergillus species dominantly followed by *Cladosporium*, *Penicillium*, *Curvularia*, *Rhizopus*, *Alternaria*, *Trichoderma* and *yeasts*. Seven species were reported from the bed dust collected from two different localities. Bed dust isolated 5 species of which 3 were *Aspergillus niger*, *A. flaus* and *A. fumigatus* and one each of *Penicillium chrysogenum* and *Curvularia tetramera* (Table 2.), (Figure. 3).

Carpeted floors contained higher dust borne fungal concentrations than smooth floors. Carpeting may provide a micro environment that maintains the cultivability of fungi, or may even encourage fungal growth under some circumstances. There are differences between studies in the relations between home characteristics and indoor-dust fungal concentrations. These differences might arise from the varied methods of sampling and analysis, but also from cultural differences in home furnishings and housing stock, and atmospheric conditions. It is essential to obtain reliable and precise identification of the fungi found in homes. For example, the identification of *Aspergillus* species in the air of the home was described by Miller et al., [10]. The development of an allergy requires repeated exposure to the allergens; such exposure obviously occurs more frequently in contaminated homes or buildings. Hence, persons living and working in contaminated areas are more likely to be affected. Furthermore, the severity of effect may be dependent on the length of exposure. There is no apparent sex bias in known allergic manifestations. Factors influencing the severity of fungal related disease include the concentration, size and shape of the invading organism. The production of toxins and volatiles by these organisms is also an important factor Ronald et al., [14].

Beguin [1] studied the mould diversity in homes (Analysis of mattress dust) at Belgium and recorded the frequent species of *Aureobasidium pullulans*, *Alternaria alternata*, *Penicillium chrysogenum*, *Aspergillus restrictus* and *Aspergillus penicilloides* which are frequently isolated and revealed important concentrations in dust samples. In present investigation also shows the presence of *Alternaria alternata*, *Penicillium chrysogenum* and *Aspergillus* species. In earlier study Giri [7] reported 37 species of which the dominants are *Aspergillus*, *Penicillium*, *Cladosporium*, *Curvularia*, *Mucor*, *Rhizopus*, *Alternaria*, *Trichoderma*, *Fusarium* and Non-sporulating fungi. Author also studied the airborne fungi in the homes of asthmatic patient's in 2013 to 2014 in east and south parts of Nagpur city and recorded 12 fungal species Giri & Matey [6]. The *Cladosporium* were found to be dominant followed by species of *Aspergillus*, *Curvularia*, *Trichoderma*, *Rhizopus*, *Fusarium*, *Alternaria*, *Mucor* & Non-sporulating fungi. While in present investigation total 23 fungal species were isolated and recorded in studied environment. Here the species of Aspergillus (8 spp.) were found to predominant followed by the species of the genera *Curvularia* (2 spp.), *Cladosporium* (2 spp.), *Alternaria* (2 spp.), *Trichoderma*, *Mucor*, *Rhizopus* and *Verticillium spp.* This result clearly indicates that the variation found in fungal species might be due to indoor conditions of homes and other environmental parameters like temperature and relative humidity.

El-Gali Z et. al., [18] reported the airborne and dust-borne fungi in the atmospheric air of ElBeida city, Libya. The dominant fungal genera are *Penicillium* spp. (54.4%), *Cladosporium* spp. (37.5%), *Rhizopus* spp. (29.8%) *Alternaria spp.* (28.7%), *Fusarium solani* (25.4%) and *Trichothecium spp.*. The other fungal species were also isolated these are *Rhizopus nigricans*, *Alternaria alternata*, *Trichothecium roseum* and *Curvulara spp.* In present investigation also isolated the genera/species of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus terrus*, *Aspergillus tamerri*, *Aspergillus ochraceous*, *Aspergillus carboniferous*, *Aspergillus spp.*, *Cladosporium herbarum*, *Cladosporium sp.*, *Curvularia tetramera*, *Curvularia geniculata*, *Curvularia sp. Alternaria spp. Mucor spp. Rhizopus spp.*, *Fusarium spp.*, *Trichoderma spp.*, *Nigrospora spp.*, *Penicillium chrysogenum*,

Penicillium spp., *Torula spp.*, *Yeast spp.*, and Non-sporulating fungi. The species of *Aspergillus terrus*, *Aspergillus* spp., *Curvularia geniculata* and *Nigrospora spp.*, was not isolated from the settled dust, but they are isolated from the dust collected from various sources of residential homes.

Zhiguo Fang et al., [19] studied and reported that the profile distribution and characteristics of culturable airborne fungi in residential homes in Bejing. They isolated 24 genera and 65 species of fungi in homes of which species of *Penicillium*, *Cladosporium*, *Aspergillus* and *Alternaria* are the most common fungi in indoor air and range that CFUs/m^3 from 62 to 3498 colonies. In present study also isolated the fungal colonies more in indoor air of residential homes and that ranges from the colonies CFUs/m^3 are 37.5 to 256. The lowest colonies CFU of *Aspergillus tamerri* and the highest colonies CFU is of *Cladosporium herbarum* which is 256.25 CFUs/m^3.

Table 1. Total fungal genera/species isolated from the air of homes (CFUs/m3) and dust-bound fungi (CFU/g-l) and their percent contribution

Fungal genera/species		Number of Colonies from Air	CFU/m3	%	Number of Colonies from Dust	CFU/g-l	%
1	Aspergillus niger	56	350	8.30	79	3.95	11.45
2	Aspergillus flavus	39	243.75	5.78	65	3.25	9.42
3	Aspergillus fumigatus	22	137.5	3.26	36	1.8	5.22
4	Aspergillus terrus	09	56.25	1.33	00	00	0.00
5	Aspergillus tamerri	06	37.5	0.89	14	0.7	2.03
6	Aspergillus ochraceous	18	112.5	2.67	07	0.35	1.01
7	Aspergillus carboniferus	11	68.75	1.63	19	0.95	2.75
8	Aspergillus spp.	23	143.75	3.41	00	00	0.00
9	Cladosporium herbarum	41	256.25	6.07	22	1.1	3.19
10	Cladosporium sp.	27	168.75	4.00	18	0.9	2.61
11	Curvularia tetramera	48	300	7.11	29	1.45	4.20
12	Curvularia geniculata	25	156.25	3.70	00	00	0.00
13	Curvularia sp.	40	250	5.93	36	1.8	5.22
14	Alternaria soloni	19	118.75	2.81	12	0.6	1.74
15	Mucor spp.	15	93.75	2.22	24	1.2	3.48
16	Rhizopus spp.	34	212.5	5.04	28	1.4	4.06
17	Fusarium spp.	12	75	1.78	19	0.95	2.75
18	Trichoderma spp.	39	243.75	5.78	71	3.55	10.29
19	Nigrospora spp.	11	68.75	1.63	00	00	0.00
20	Penicillium chrysogenum	23	143.75	3.41	10	0.5	1.45
21	Penicillium spp.	46	287.5	6.81	27	1.35	3.91
22	Torula spp.	09	56.25	1.33	19	0.95	2.75
23	Yeast spp.	38	237.5	5.63	69	3.45	10.00
24	Non-sporulating fungi	49	306.25	7.26	87	4.35	12.61
Total		675	4218.75	100	691	34.55	100

Figure 1. Air sampling was carried out in residential homes by using Hi-Media Air Sampler and Room temperature maximum and minimum and relative humidity was recorded by Hygrometer.

Air sampling in kitchen

Air sampling in Drawing room

Air sampling in kitchen

Figure 2. Fungal colonies grown on RBS-640 media strips exposed in residential homes.

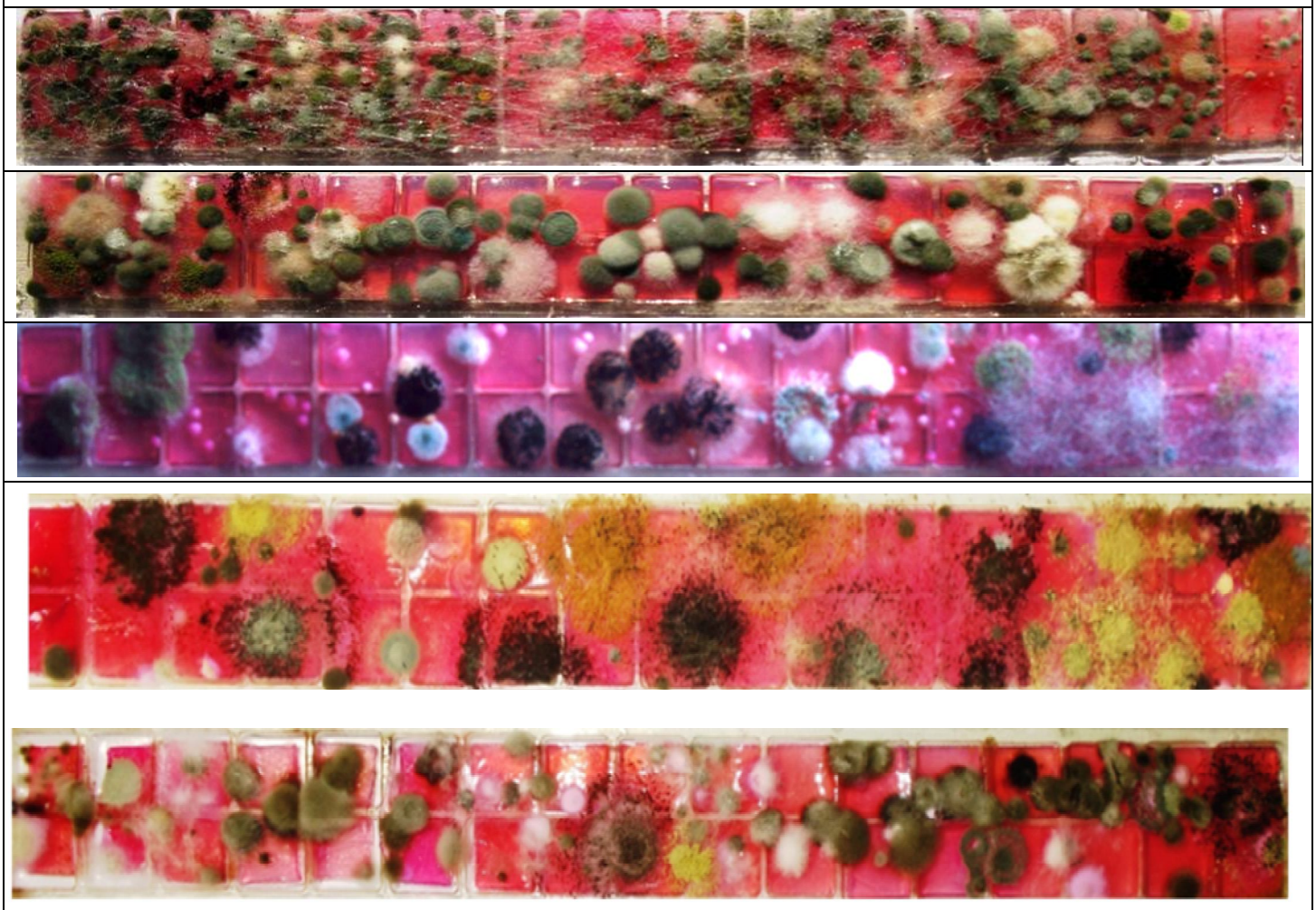
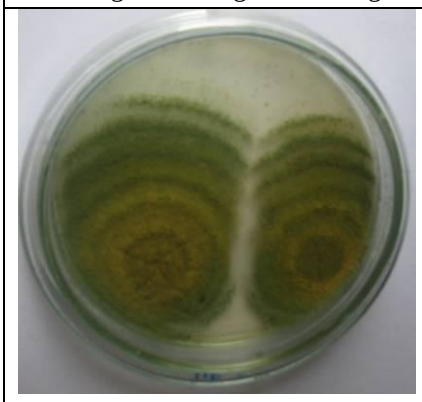


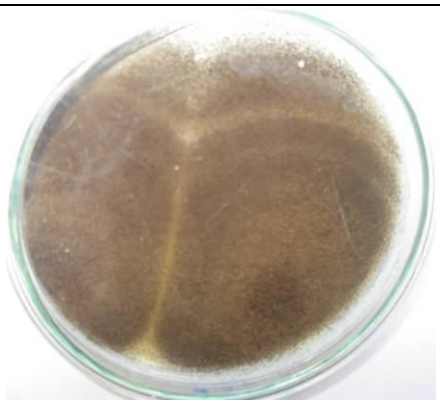
Table. 2. Fungi isolated from the dust collected from various sources

Sources	Fan dust	Carpet dust	Bed dust	Sofa dust
Fungi isolated	Aspergillus niger, A. flavus, Penicillium, Curvularia, Rhizopus, Alternaria, peniciliium spp., Cladosporium spp., Trichoderma	Aspergillus niger, A. flavus, A. fumigates, A. ochraceous, A. terrus and A. zonatus, Penicillium spp., Cladosporium, Curvularia, Alternaria, rhizopus, Mucor, Trichoderma and yeasts.	Aspergillus niger, A. flavus, A. fumigates, Penicillum chrysogenum, Trichoderma, Nigrospora, Curvularia tetramera, Mucor and yeasts.	Aspergillus spp., Cladosporium, Penicillium, Curvularia, Rhizopus, Alternaria, Trichoderma and yeasts.

Figure 3. Fungal colonies grown on culture petri-plates (Czepaks' Dox Agar Medium) which are isolated from the dust.

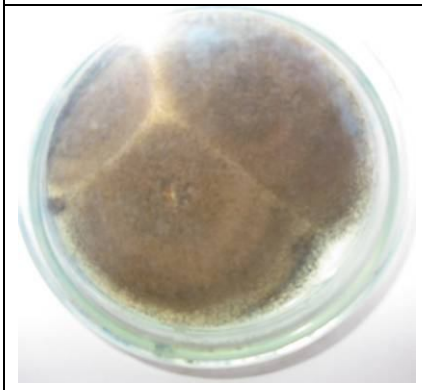


Aspergillus zonatus (15 days old)

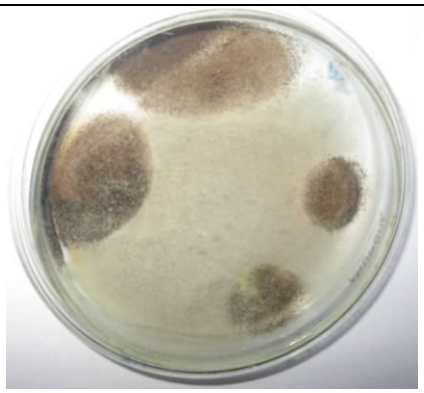


Aspergillus niger (10 days old)

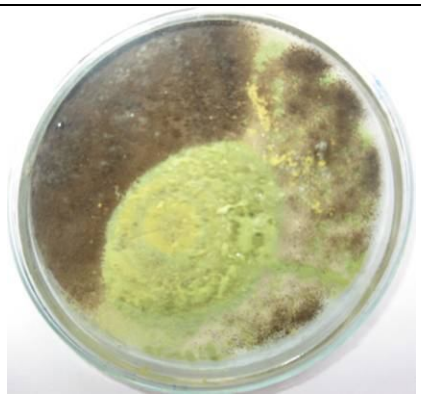
Aspergillus niger and Alternaria spp. (15 days old)



Aspergillus niger (10 days old)

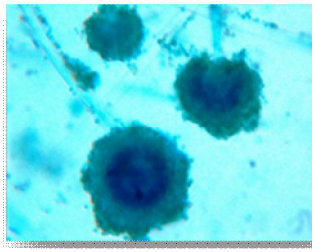
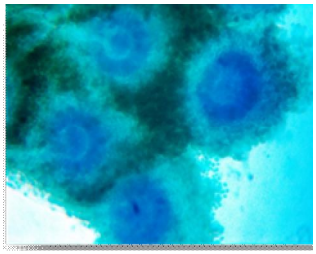
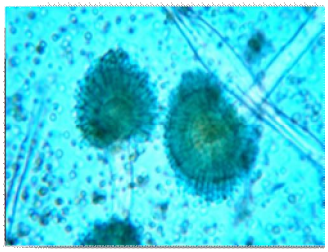
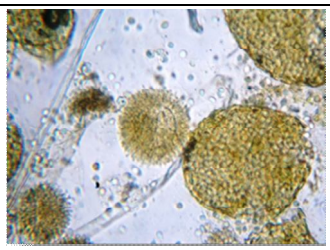


Aspergillus niger and Mucor spp. (10 days old)



Aspergillus niger and Aspergillus flavus (15 days old)

Figure 4. Micro-photographs of isolated fungal genera/species from the indoor air of residential homes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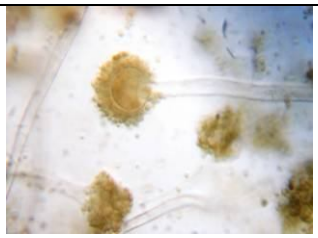
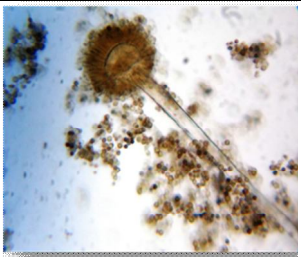
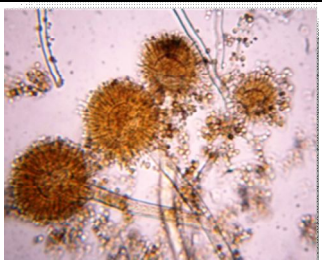
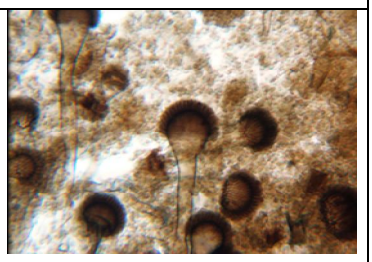
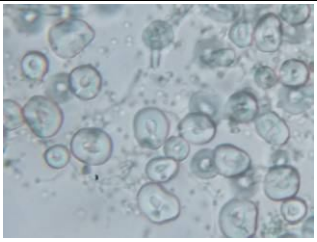
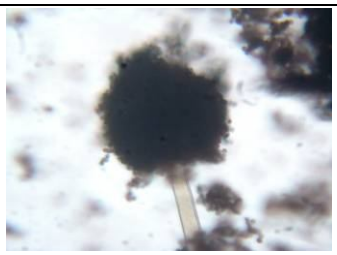
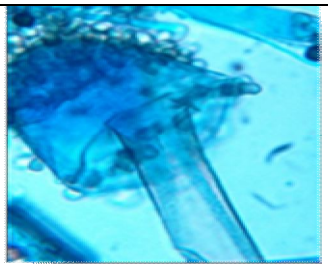
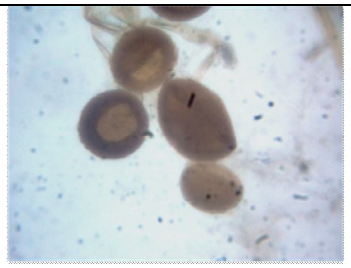
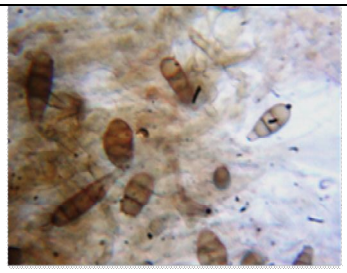
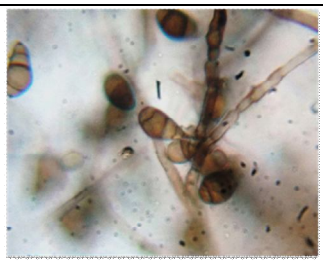
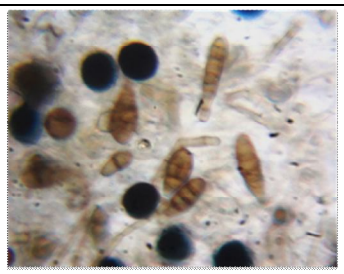
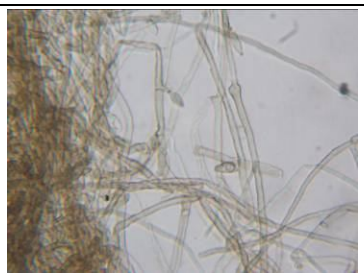
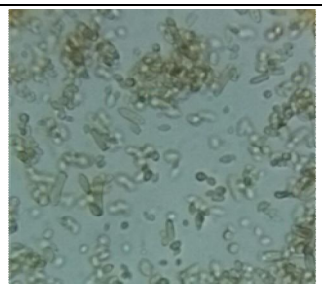
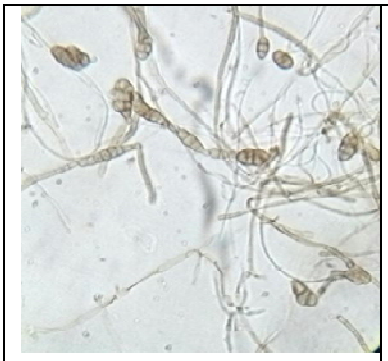
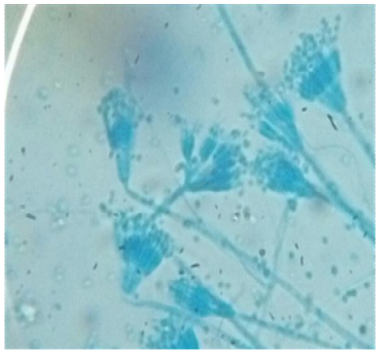
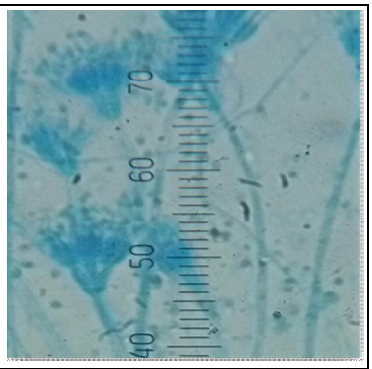
			
Aspergillus flavus	Aspergillus flavus	Aspergillus versicolour	Aspergillus fumigatus
			
Aspergillus hulle cells	Aspergillus spp.	Aspergillus niger	Aspergillus zonatus
			
Mucor spp	Rhizopus spp.	Curvularia tetramera	Curvularia geniculata
			
Nigrospora & Alternaria alternata	Cladosporium herbarum	Cladosporium spp.	Cladosporium spp.

			
Alternaria soloni	Alternaria spp.	Penicillium chrysogenum	Penicillium chrysogenum

IV. CONCLUSION

Indoor airborne and dust bound micro fungal exposure frequently causes adverse human health effects with injury to dysfunction of multiple organs and systems including respiratory, nervous, immune, haematological and skin. Indoor mold is also a common cause of life-threatening systemic infections in immune-compromised patients [12]. With all the caveats discussed above, several epidemiological studies have shown associations between culturable fungi and increased risk of adverse respiratory symptoms Hedayat et al., [8]; Lacey [9]. Measures of culturable airborne fungi, dust borne fungi, and home characteristics, likely provide different and complementary information regarding the presence of fungi that may be inhalable on a short-term or chronic basis Wickmen et al., [17]. It is essential to understand the ecological and methodological reasons for the differences which is found in the genera/species and also the fungal levels obtained from airborne and dustbound fungi. It gives good interpretation of epidemiological studies of the health effects of airborne and dust bound fungi in residential homes. There is considerable evidence in the medical literature validating the many different health effects reported in airborne mould exposed patients. Viable and non-viable airborne spore counts can vary considerably over several periods of time may be necessary to characterise airborne fungal spore levels accurately. However, airborne fungal measurements fail to take into consideration non-airborne fungal contamination such as fungal contamination in dust or surfaces which is often visible to the naked eye and mycotoxins in air, dust and on surfaces. Therefore in present investigation, both the sampling methods used to analyse the airborne fungal presence and the dust bound fungi in indoor environment of residential homes. In order to get a more complete assessment, therefore it is recommended that airborne fungi and the fungi present in settled dust on the various sources must be tested or collect the samples simultaneously for the analysis of indoor moulds.

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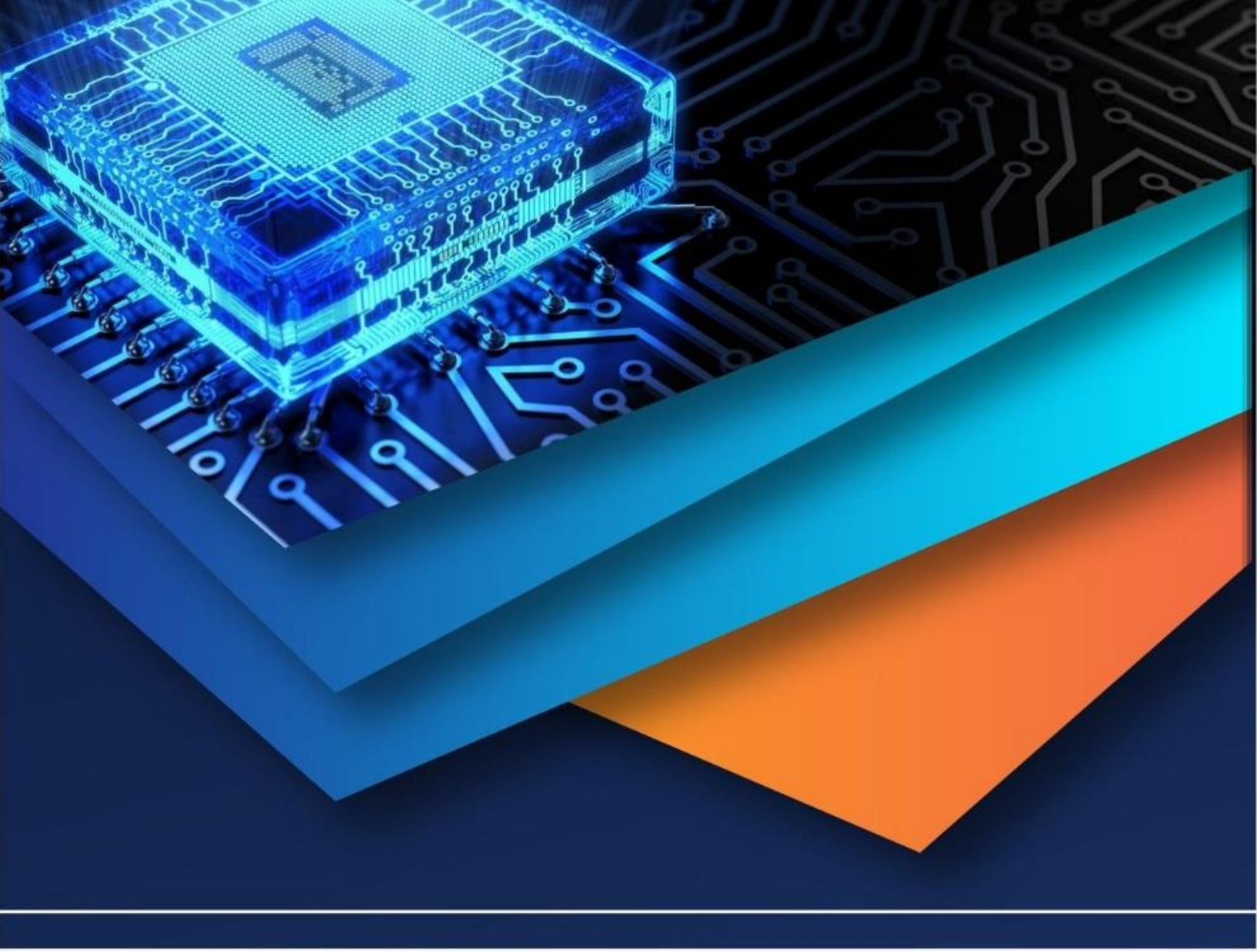

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