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Mold Allergens in Indoor Play School Environment

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Abstract

Moulds are found in nature for the breakdown of leaves, wood, and other plant and animal materials. The spores of these moulds can be easily drifted by wind and travel distant places. Presence of these moulds in air can cause allergies to susceptible individuals. These moulds can directly enter into a building or their spores being carried by air in homes, offices and play schools. They are usually found to be growing on wood, dry wall, wall paper, fabric, ceiling tiles and carpeting. Air borne fungi can cause asthma and allergic rhinitis. The present study aimed to isolate and identify airborne fungi in different play schools of Visakhapatnam area. Gravity Petri-plate method is used to isolate filamentous fungus. The study was carried out for ten months viz. June 2015-March 2016. In one of the examined schools a high prevalence of fungi was observed. Most of the dominating fungi belonged to genera: *Aspergillus, Cladosporium, Penicillium, Alternaria, Rhizopus and Curvularia. Aspergillus flavus* and *Cladosporium Cldosporiodes* were reported dominating in one of the play schools which show the possibility of allergy. One of the play schools reported more spores in indoor environment. The present study stated that the presence of air borne fungal concentration varied significantly in indoor play school. The study gives a data about allergic fungi in indoor play schools where the children are exposed to these fungal spores. A further detailed research is necessary to assess the exposure levels, influence of a climate conditions as well as the clinical aspects of the presence of allergic fungal spores.

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Keywords: Moulds, Play school, *Aspergillus*, *Penicillium*, Visakhapatnam

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1. Introduction

Moulds are found in nature for the breakdown of leaves, wood, and other plant and animal materials. The spores of these moulds can easily drifted by wind and travel distant places. Presence of these moulds in air can cause allergies to susceptible individuals [8]. The spores of fungi in air can cause respiratory disorders. The occupational diseases studied by authors [5] and [13] include hypersensitivity pneumonitis, allergic alveolitis, bronchopulmonary aspergillosis, asthma, coccidiodomycosis, blastomycosis.

Studies on the moulds and their growth on wood, dry wall, wall paper, fabric, ceiling tiles and carpeting are common in most of the European and North American countries. Some authors [6, 3, 10] reported different environmental conditions are responsible for growing allergic incidence in public in recent years. Higher frequencies of air borne fungal spores have been recorded from different such working environments [18, 20].

Some of the mould spores present in the air can cause mycoses and other allergic reactions. Environmental samples of *Aspergillus* species with clinical report stated the prevalence of asthma. *Aspergillus fumigatus* was identified by [12] as a potential air contaminant on the occurrence of respiratory and coetaneous mycoses.

Author [2] examined the relationships between the concentrations of ambient inhalable airborne fungi relating with temperature and relative humidity in Ohio USA. The temperature, seasonal variation and humidity have shown influence on the increase concentration of fungal spores. Suerdem TB and Yildirim I [22] determined the density of molds and their spores in the air varies in accordance with geographical regions and seasons. In addition, climatic conditions such as rainfall, speed of wind, height, and floral combination also influence the type of fungi present in the air.

The aim of the present study was to isolate and identify airborne fungi in different play schools with reference to variation in two seasons of Visakhapatnam city.

1.2 Experimental Analysis

1.2.1 *Study Area*

In our study two play schools were selected. One of them was very close to market area where every day tones of vegetables were loaded and unloaded. The other play school was chosen in the residential area. In the first play school, inside walls were damped on one side, as the overhead tank was leaking due to which the wall was wet and small mould growth was observed during the study. Where as in the second play school the walls were painted, no mould growth was seen.

1.2.2 *Sampling Procedure*

Air samples were collected once in a week from a height of 0.6m from ground level. Considering the height of the children present in the play school and inhaling position, height of the air sampling was fixed. The study was carried out for 10 months viz. June 2015 to March 2016. Open Petri-plate method was used to extract fungal spores from air. The Petri-plates consists of Potato dextrose agar and Sabouraud agar medium for trapping mould spores. These plates were exposed to air for 15 minutes to trap the spores. As less time period no proper growth of fungi was observed. More time of plates exposed to air overlap of spores was identified. After exposure to air the Petri-plates were closed and airtight with parafilm and placed in a sterile container and carried to the laboratory for isolation and identification of air borne fungi. The study was carried out in monsoon and winter seasons.

1.2.3 *Examination of mould flora*

The Petri-plates were incubated in an incubator for 5 days at 25°C. After incubation the Petri-plates were taken out and mycelium formed on plates were made into pure cultures on potato dextrose agar. These pure cultures were examined under compound microscope and identified using fungal keys provided by [9, 21].

1.3 Results and Discussions

During the ten months of the research study 3520 mould colonies of 21 genera were isolated from 320 Petri-plates. The air samples isolated from two different play schools viz: one play school close to market and the other from residential area. In each play school 20 children are present. Thirty five fungal species were isolated from 21 genera. However, some of the colonies were identified at the genus levels, e.g., *Penicillium sp, Stachybotrys sp, Trichoderma sp, Trichothecium sp, Verticillium sp.,* and others are marked unknown. The other species include *Alternaria alternata, Alternaria chlamydospora, Alternaria solani, Aspergillus flavus, Aspergillus candidus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Aspergillus versicolor, Aspergillus terreus, Botrytis, Cercospora, Cephalosporium, Cladosporium herbarum, Cladosporium cladosporioides, Curvularia lunata, Curvularia affinis, Fusarium solani, Fusarium moniliforme, Fusarium oxysporum, Helminthosporium sp, Mortierella zonata, Mucor racemosus, Mucor microsporus, Phoma herbarum, Rhizopus oryzae, Rhizopus stolonifera, Ulocladium botrytis, Yeast cells.* Figure: 1 represents the nine major genus of the study area, as they indicates three fourth of the total colony count from the entire study (Table-1).

The predominating genera of fungi (characterized by the highest colony counts) were as follows *Aspergillus* - 29.9%, *Cladosporium* (20.1%), *Penicillium* (17.7%), *Alternaria* (8.7%), *Fusarium* (6.4%), and *Curvularia* (3.4%). During the ten months of the study monsoon season showed less concentration of mould spores compared to winter season which can be seen in Table-1

Table:1 Total count of airborne moulds isolated from Play schools of Visakhapatnam city.

S.No	Mould species	Total Number of colonies	CFU/m^3	Colonies in Monsoon	Colonies in Winter
1.	*Alternaria alternata*	84	$4.2x10^3$	28	56
2.	*Alternaria chlamydospora*	18	$9.4x10^2$	07	11
3.	*Alternaria solani*	73	$3.8x10^3$	29	44
4.	*Aspergillus candidus*	56	$2.9x10^3$	26	30
5.	*Aspergillus flavus*	320	$1.6x10^4$	99	221
6.	*Aspergillus fumigatus*	121	$6.3x10^3$	35	86
7.	*Aspergillus niger*	119	$6.2x10^3$	40	79
8.	*Aspergillus parasiticus*	87	$4.5x10^3$	25	62
9.	*Aspergillus terreus*	34	$1.7x10^3$	10	24
10.	*Aspergillus versicolor*	22	$1.1x10^3$	10	12
11.	*Botrytis Sp*	31	$1.6x10^3$	08	23
12.	*Cephalosporium sp*	41	$2.1x10^3$	12	29
13.	*Cercospora Sp*	12	$6.2x10^2$	03	09
14.	*Cladosporium cladosporioides*	322	$1.6x10^4$	94	228
15.	*Cladosporium herbarum*	30	$1.5x10^3$	12	18
16.	*Curvularia affinis*	98	$5.1x10^3$	25	73
17.	*Curvularia lunata*	71	$3.7x10^3$	13	58
18.	*Fusarium moniliforme*	88	$4.6x10^3$	16	72
19.	*Fusarium oxysporum*	112	$5.8x10^3$	43	69
20.	*Fusarium solani*	69	$3.6x10^3$	21	48
21.	*Helminthosporium sp*	33	$1.7x10^3$	11	22
22.	*Mortierella zonata*	41	$2.1x10^3$	10	31
23.	*Mucor microsporus*	54	$2.8x10^3$	20	34
24.	*Mucor racemosus*	83	$4.3x10^3$	33	50
25.	*Penicillium sp*	314	$1.6x10^4$	87	227
26.	*Phoma herbarum*	74	$3.8x10^3$	23	51

27.	*Rhizopus oryzae*	95	$4.9x10^3$	31	64
28.	*Rhizopus stolonifera*	115	$6.0x10^3$	45	70
29.	*Stachybotrys sp*	120	$6.2x10^3$	51	69
30.	*Trichoderma sp*	88	$4.6x10^3$	34	54
31.	*Trichothecium sp*	91	$4.7x10^3$	32	59
32.	*Ulocladium botrytis*	23	$1.2x10^3$	05	18
33.	*Verticillium sp*	100	$5.2x10^3$	33	67
34.	*Unknown*	126	$6.5x10^3$	47	79
35.	*Yeast Cells*	355	$1.8x10^4$	121	234
	Total mould species	3520	$1.8x10^5$	1139	2381

Fig: 1 Major moulds in indoor air of playschools of Visakhapatnam city (total % colony count).

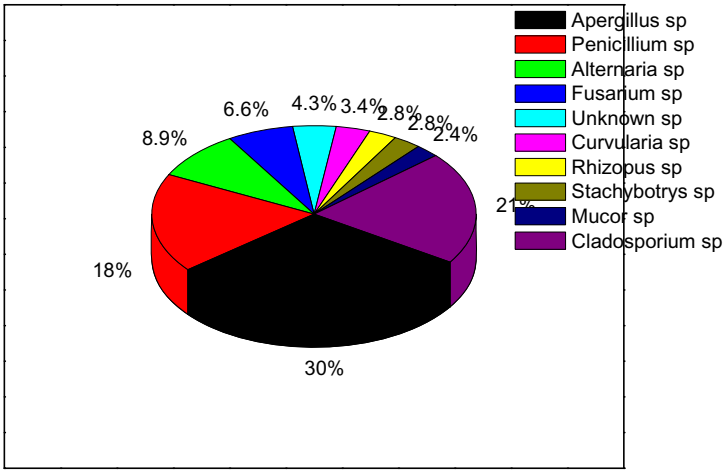

Table:2 Fungal species in the two play schools of ten months study

Moulds	Playschool-I [%]	Playschool-II [%]
Alternaria (A)	9.2	7.9
Aspergillus (As)	32.6	27.2
Cladosporium (C)	21.2	18.9
Curvularia (Cu)	5.9	1.3
Fusarium (F)	8.3	4.4
Mucor (M)	1.5	0.5
Penicillium (P)	18.9	15.9
Rhizopus (R)	1.1	2.5
Other species (Os)	1.0	5.5

Fig: 2 Graph showing variation in fungal species in two play schools of the study area.

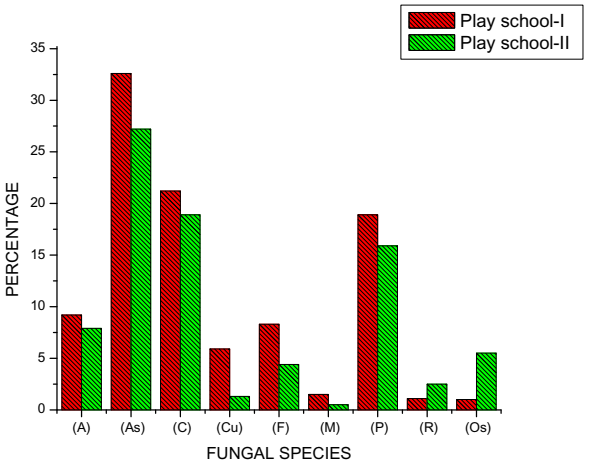

Figure: 2 showed high mould concentration in playschool-I compared to playschool-II in which *Aspergillus* species dominant with other species posing high percentage, this is followed by *Cladosporium, Penicillium, and Alternaria*. This could be due to improper maintenance of play school.

In the present work five moulds were found to be in dominance, these are *Aspergillus, Cladosporium, Penicillium, Alternaria, Fusarium* and *Rhizopus*. Levetin E et al [11] studied *Cladosporium* as abundantly found in indoor school environment along with deutromycota class of *Aspergillus* and *Penicillium*, considered as indoor contaminant. Similar species were identified in the present study area. According to author [15] *Penicillium*, *Aspergillus* and *Cladosporium* showed high prevalence in indoor air and one of the major causes for allergic symptoms in atopic children. Moreover these spores easily liberated to air as loading and unloading of vegetables in the market area could be one of the sources. In our study these species are showing their prevalence from moderate to high occurrence and similar environment was identified in playschool-I.

The examines of author [23] about different age groups of students and their sensitivities towards *Aspergillus, Alternaria, Botrytis, Cladosporium, Fusarium, Rhizopus* caused allergy noted through skin prick test. From our study these species were isolated at different concentrations as *Aspergillus*: 29.9%, *Alternaria*: 8.7%, *Botrytis*: 1.2%, *Cladosporium*: 20.1%, *Fusarium*: 8.7%, *Rhizopus*:2.8% .

Studies by authors [4, 16] stated that fungal concentration varied due to influence of meteorological factors. The presence of indoor moulds was due to outdoor environment, as these spores easily enter into indoors either through door or windows when they were opened. If they find the possible nutrient in the indoor environment like damp walls they start growing. This can cause allergies to the occupants. In one of our study area these damp walls was observed and physically molds were reported. Moreover poster attached to the wall, curtains to the windows is the suitable place for the growth of the molds [24]. Children handling these things during hours can disturb and disperse the spores in the room. High spore concentration of these types has been reported by many workers form similar working environments [7, 25].

According to Madureria et al [14] presence of fungi in air in primary schools and the health impacts of children due to exposure to contaminated air were analyzed and concluded that improper ventilation, unhygienic floor are reasons. Similar situations were observed in one of our study area. In the present study the fungi were present in the sampling site posing a potential risk of allergies.

During the study it was identified that fungal spores started increasing from the month of November, high peak

registered in the month of December and January and slowly getting reduced towards February. Our study correlates with that of [1, 17, 19].

1.4 Conclusions

As far as our knowledge concerned there is no data available about the mould growth and their impact in the form of allergies in play school children. So it is necessary for future investigations to study effect of moulds exposure and health problems. The present study provided information about the abundance of mould population in indoor play school environment, which is varied in species composition. Moreover, continuous seasonal assessment is required for proper diagnosis of mould for better treatment.

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