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A study of fungi in air in selected areas of Visakhapatnam city, India

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ABSTRACT

Studies on air borne fungi have received much attention due to increase in asthma and allergic rhinitis. It is necessary to have the knowledge of filamentous fungi present in air in a region for proper diagnosis and specific treatment towards allergic symptoms induced by inhaled allergens. In this study, the variability of air borne fungi was identified and assessed on a monthly basis in fruit juice locations of five different areas of Visakhapatnam. In these selected five areas more people travel regularly and are exposed to air. In the present study the airborne fungi in the selected five areas are high and can cause allergic symptoms. The study was carried out for 24 months (November 2009-October 2011) by means of gravity settling method through Petri plate using Rose Bengal Agar medium. In this study seasonal variation among the fungi was observed. A total of 3840 samples were collected. Thirty fungal species were identified from these sampling sites. The most dominating species were Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Rhizopus and Penicillium. The spores of Alternaria, Aspergillus and Cladosporium are prone to causing allergies and in the present study area these were found at high alarming rate. The fungi ranged from $1.3x10^2$ to $4.3x10^6$. The results of the present study reveal the significance of air as a major source of microbial contamination in four areas and it is necessary to encourage the hygienic conditions in and surrounding of the four areas to maintain safety of the local consumers.

Key words: Fungi, asthma, *Aspergillus*, Visakhapatnam.

INTRODUCTION

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Fungi are heterotrophic eukaryotes that are usually filamentous, devoid of chlorophyll and chitinous cell wall and produces spores. They are found in the soil, water, air, on vegetation, on humans and every where in the environment.[1] Fungi mostly present in the air and causes allergy are called aeroallergens. Fungal density in the air varies in accordance with geographical regions and seasons. Besides, climatic parameters such as wind, humidity, temperature, precipitation, altitude and flora combination may also affect the type and amount of fungi in the air [1],[2],[3]. The shape and size of conidia of fungi along with the meteorological factors determines its speed and dispersal [4]. Fruit juice shops contains large number of fungal spores as some of them are carried from harvested places, other due to storage for the long period of time. These fungi are associated with a number of allergic diseases in humans. Certain airborne fungi can cause severe respiratory diseases in children and adults [5],[6]. The prevalence of respiratory allergy to fungi was estimated at 20% to 30% among atopic individuals and up to 6% in the general population. The major allergic manifestations induced by fungi are asthma, rhinitis allergic

bronchopulmonary mycoses and hypersensitivity pneumonitis. These diseases can result from exposure to spores, vegetative cells or metabolites of the fungi [7],[8]. Some fungi like *Alternaria, Aspergillus, Cladosporium* and *Penicillium* are generally considered to be important causes of both allergic rhinitis and allergic asthma [7],[9]. *Cladosporium* and *Alternaria* exist more commonly in the atmosphere in periods of warm air while *Aspergillus* and *Penicillium* exist more intensively in cool periods [2],[10],[11]. The present paper deals in determining the types, prevalence and mostly distributed aeroallergens in and around fruit juices shops of Visakhapatnam city.

MATERIALS AND METHODS

1.1 SAMPLING LOCATIONS:
Five different fruit juice shops were selected showing different environmental conditions in around Visakhapatnam.viz; Jagadamba, NAD Jn, Kancharapalem, Gajuwaka and Narshimhanagar area.

1.2 SAMPLING METHODS
Air sampling was done daily twice in a week on a monthly basis (from November 2009-October 2011). Samples were collected on seasonal variation (winter, summer, and monsoon). Aeroallergens are collected from air through gravity Petri dish methods. Timings were selected in such a way, where more people were exposed to air, during day time from 10a.m-12p.m and 6p.m-8p.m in the evenings. At each sampling site four sterilized Petri plates with Rose Bengal agar were horizontally placed above the ground level which traps the spores. After exposing for 5 minutes the Petri plates were closed and brought to the laboratory keeping the closed Petri plates in a sterile container and incubated at 25^0C for 5 days. The Fungus developed after fives days was stained with cotton blue and morphology was studied under compound microscope by using pictorial atlas of soil and seed fungi [9] .

RESULTS AND DISSCUSSION

In the survey of 24 months a total of 7300 fungal species were identified from 1920 Petri plates. The air borne fungi collected from all five areas in the present study viz… Jagadamba, NAD Jn, Kancharapalem, Gajuwaka and Narshimhanagar area. Fungal population ranged from$1.3x10^2$ to $4.3x10^6$ CFU. (Table-3) Thirty fungal species were isolated belonging to 18 genera. These are *Alternaria alternate, Alternaria solani, Aspergillus candidus, Aspergillus flavus. Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus niger, Aspergillus terrus, Aspergillus versicolor, Botrytis. Cephelosporium, Cercospora, Cladosporium, Colletotrichum, Curvularia affinis, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, Helminthosporium sp Mortierella zonata, Mucor microsporus, Mucor racemosus, Penicillium sp Rhizopus oryzae, Rhizopus stolonifera, Stachybotrys sp, Trichoderma sp, Trichothecium sp, Verticillium sp,* and *Yeast cells.* In this work, it was revealed that *Aspergillu*s is predominated in all the sampling sites as shown in fig: 1 and 2. During winter season (Nov-Feb) *Aspergillus flavus* was found at high alarming rate in Jagdamba, NAD Jn and Gajuwaka. Among the airborne fungi that spread air spores at high rate from different sources, and these are important aeroallergens of the world are *Aspergillus, Alternaria, Cladosporium, Penicillium*.[4],[10] in our study these species were found in all sampling sites as shown in table:1.

According to Lacey and Dutkiewiez [11], *Rhizopus* is known to cause organic dust syndrome. In the present study, spores of *Rhizopus* were abundantly found in fruit juice shops. The concentration of mycoflora was recorded highest from the month of August to January (during monsoon and winter season) and gradually declined towards May (summer season). Our study reveals the presence of fungal spores in the month of November, which is in accordance with the study of Devi et.al [12], who reported that certain species like *Aspergillus, Cladosporium,* and *Alternaria* were predominant in the month of November.

According to Lee et.al [13] *Aspergillus* and *Alternaria* are showing high prevalence in air in store houses. In the present study these two species were found in the fruit juice shops. Spores of *Alternaria, Penicillium* and *Cladosporium* play a significant role in causing allergic asthma[2]. In our survey these species are showing moderate to high occurrence. Many fungus spores can survive in difficult conditions like low temperatures in winter and high temperatures in summer and can be transported by air. The five sampling sites shown high moisture conditions in surrounding areas which might be one of the reasons for high prevalence of fungi. Fungal density in the air reaches the highest level in monsoon and winter compared to summer. The increase in fungal density in October-January plays a significant role in seasonal distribution and highest fungus isolation was observed in this month [2]. In our study similar seasonal variation was observed. Some species of *Aspergillus (A.flavus, A.parasiticus*) causes

aminotoxicity in humans [2]. In the present study *Aspergillus* were found in all sampling sites posing a severe threat to cause allergy.

Table: 1 Spatial variation of fungal species in different Months for 2010-2011

Months	Mean	N	S.D	F-value	P-value	Decision
May	18.1558	30	7.08894	27.958	0.000	S
June	17.9577	30	4.24655			
July	14.2700	30	6.35520			
August	18.1558	30	7.08894			
September	16.2842	30	8.24874			
October	22.6083	30	10.32337			
November	27.8259	30	10.45857			
December	35.9517	30	12.18732			
January	34.6995	30	16.09143			
February	19.8633	30	10.44897			
March	14.3025	30	7.92301			
April	4.0908	30	2.85485			

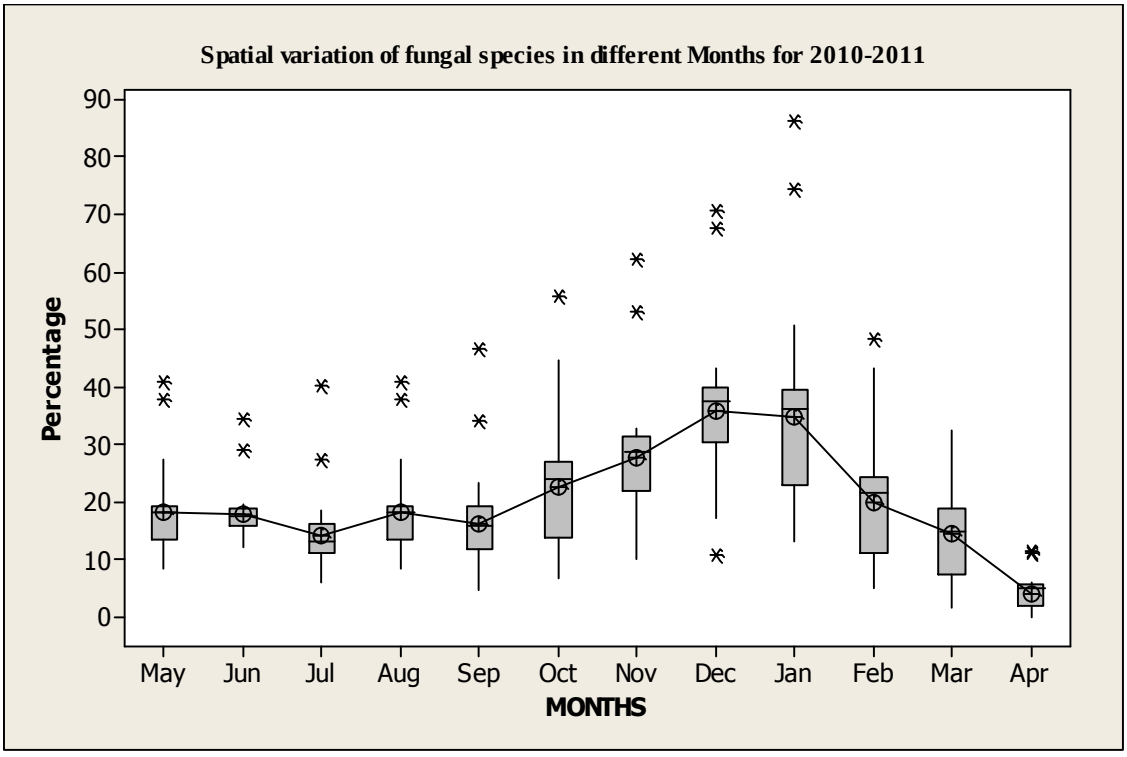

Table: 2 Spatial variation of fungal species in different Months for 2009-2010

Months	Mean	N	S.D	F-value	P-value	Decision
May	3.6275	30	3.96382	51.508	.000	
June	14.2433	30	5.29985			
July	12.9633	30	5.65719			
August	13.8600	30	5.15410			
September	11.5142	30	10.18061			
October	12.5608	30	10.18531			
November	19.2400	30	10.60589			
December	31.9546	30	10.94254			
January	39.3253	30	10.94932			
February	38.7317	30	10.91386			
March	27.4800	30	10.22301			
April	12.2275	30	8.20316			

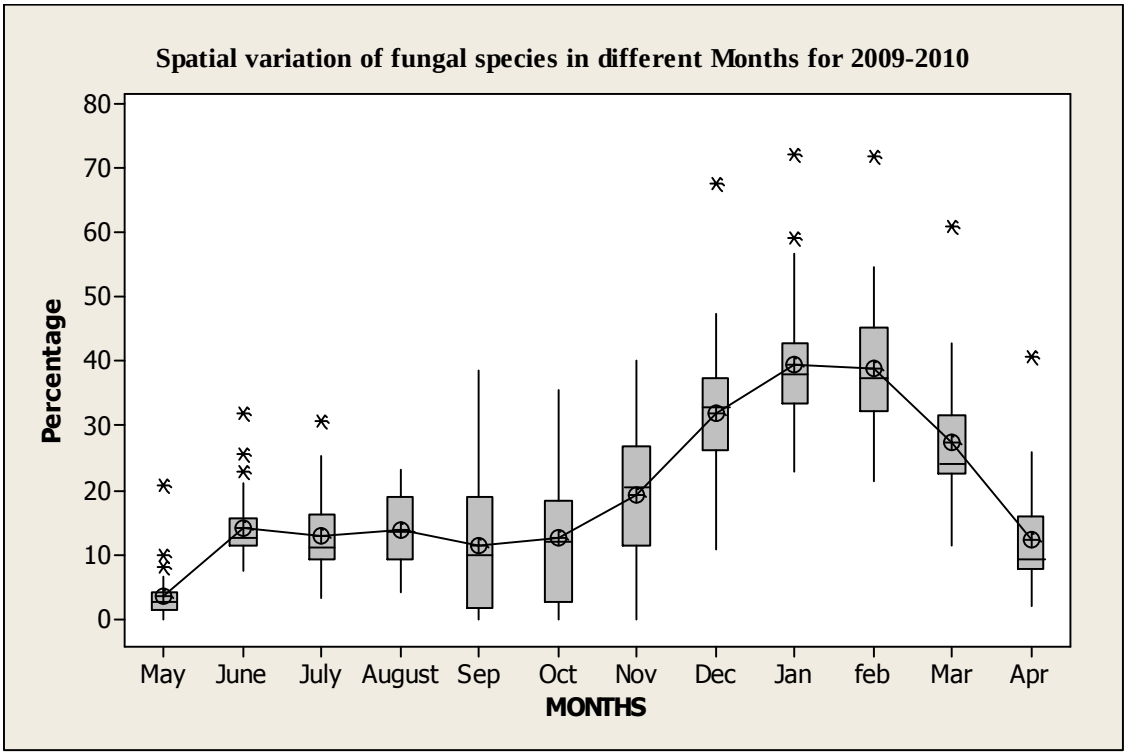

Table: 3 Colony forming units of Fungi

S. No.	Sampling area	Fungi		
		Winter	Summer	Monsoon
1	Jagdamba	$6.5x10^5$	$3.2x10^2$	$4.2x10^3$
2	NAD junction	$7.5x10^4$	$4.2x10^2$	$5.8x10^3$
3	Kancherlapalem	$4.3x10^6$	$2.7x10^3$	$3.7x10^4$
4	Gajuwaka	$4.7x10^5$	$2.9x10^2$	$2.7x10^4$
5	Narshimahanagar	$5.6x10^2$	$1.4x10^1$	$2.4x10^2$

CONCLUSION

There are certain agencies like BEI (Biological Exposure Indices) and ACGIH (American Conference of Government Industrial Hygienists) which give guidelines to hygienists to control health hazards in work place. In most of the developing countries, the knowledge to exposure to molds and their effect on health is not wide spread. To the best of our knowledge OEL (Occupational Exposure Limit) for airborne fungi have not been introduced in our country. For easy diagnosis and effective treatment OEL and BEI values should be introduced for every area of Visakhapatnam and in other parts of India which could be used as a guide to control health hazards.

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