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RESEARCH ARTICLE

Indoor and outdoor aeromycospora studies in coastal village

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SUMMARY : Studies on the prevalence of airborne fungal spores with their seasonal periodicity in the indoors and outdoors of the houses in Kalapet, a coastal village of Pondicherry were carried out by implementing Petri plate sedimentation method from October, 2008 to September, 2009. Composition and concentration of fungal spores considerably varied from indoors to outdoors as well as from season to season. Outdoor air harboured maximum fungal spores (53%) in comparison to indoor air (47%). Occurrence of fungal species was predominated with more number of propagules during mid winter (December) and early rainy (July) periods in comparison to other months. In qualitative analysis, out of the total species recorded, *Aspergillus* was found with the highest frequency and had eleven members *i.e.*, *A. awamori, A. fumigatus, A. niger, A. flavus, A. flavipes, A. nidulans, A. ochraceous, A. japonicus, A. terreus, A. versicolor* and *A. wentii,* but quantitatively, *Penicillium* was isolated highest in its contribution to total CFUs followed by *Aspergillus*. Out of the 33 isolated fungal taxa, *Aspergillus fumigatus, A. awamori, A. niger*, *Rhizopus stolonifer* and *Alternaria alternata* were the predominant aeroallergens, which cause different types of respiratory/lung diseases in atopic human beings. In seasonal periodicity, winter contributed the maximum spore load (41%) followed by rainy (33%), summer was found with the least (26%) in harboring the spore mass in the indoors and outdoors in the village environment. *Alternaria alternata,* which is accounted as a human allergen for sporosis inducer and an agent for hay fever and other pathologies, was also intermittently recorded. In addition to the above aero allergenic fungi, a few plant pathogenic, saprophytic, field and storage fungi were also recorded during the study period.

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Incidence of micro fungi in the environment has traditionally been a matter of concern for both allergologists and biologists with an interest in health and environmental pollution problems. Such concern has fostered studies aimed at the qualitative and quantitative characterization of the aeromycoflora in many villages throughout the world. Fungi, on account of their biological features and ease of dispersion of their spores both indoors and outdoors can contaminate of any type of substrate. Because of their ubiquity, fungi generally cause major diseases in plants, animals and in human beings (Nayak *et al.,* 1998).

Outdoor aero-allergens are an important part of the exposures that lead to allergic diseases (Salvaggio and Aukrust, 1981). Primary sources for outdoor allergens include vascular plants (pollen, fern spores, soy dust), and fungi (spores, hyphae). Non-vascular plants, algae, and arthropods contribute small numbers of allergen-bearing particles. Particles are released from sources into the air by wind, rain, mechanical disturbance or active discharge mechanisms. Once airborne, they follow the physical laws that apply to all airborne particles. Although some outdoor allergens penetrate indoor spaces and exposure occurs mostly outdoors. Even short-term peak outdoor exposures can be important in eliciting acute symptoms. Centrally located monitoring stations give regional-scale measurements for aeroallergen levels. Pollen and fungal spore exposures have both been implicated in acute exacerbations of asthma and sensitivity to some fungal spores predicts the existence of asthma. Synergism and/or antagonism probably

occur with other outdoor air particles and gases. Control involves avoidance of exposure (staying indoors, preventing entry of outdoor aerosols) as well as immunotherapy, which is effective for pollen but of limited effect for spores. Outdoor allergens have been the subject of only limited studies with respect to the epidemiology of asthma. Much remains to be studied with respect to prevalence patterns, exposure and disease relationships, and control.

Till date, a number of authors have reported indoor habitats to feature characteristics fungal flora that differ from those found in outdoors (Dupont *et al.,* 1967; Lumpkins and Corbit, 1976; Hirsch and Sosman, 1976; Solomon *et al.,* 1978; Gravesen, 1979; Infante *et al.,* 1992). In outdoor air, fungal spores are almost always present. Usually they number 10^3 to 10^4 spores m^{-3} air but sometimes up to 10^6 spores m^{-3} can occur. Most of the Indian houses are constructed merely for meeting the accommodation without any consideration to health, hygiene and sanitation. The patterns of houses are such that prevents adequate ventilation and facilities, which cannot contribute to the health and efficiency of the occupants. Due to unhygienic condition of the rural and urban environment and prevailing aero-biopollutants, the people suffer from a large number of allergic diseases mainly asthma, hay fever, dermatitis, rhinitis and conjunctivitis because the environment that plays an important role in precipitation of allergic symptoms.

The seasonal periodicities (Miquel, 1883) of a variety of airborne fungal spores have been demonstrated by a number of workers in various countries (Nayak *et al.,* 1998; Gonzalenz *et al.,* 1993). They found the availability of different fungi in air varied from place to place depending on the prevailing weather conditions (Nanda *et al.,* 2000). Seasonal periodicity of aero-allergenic micro fungi was studied out by Nanda and her coworkers (2000) in different dwellings of south Orissa. Works carried out at different dwellings recorded, majority of the spore types follow certain seasonal and diurnal periodicities and their peak concentrations occur during the season at particular time/times of the day (Mishra and Srivastava, 1971; Jankyn and Banfeld, 1973; Smith and Crosby, 1973). It has already been conclusively proved that the meteorological parameters *i.e.,* relative humidity, temperature, wind velocity and rainfall directly regulate the airborne fungal spores (Nayak *et al.,* 1998; Panda and Behera, 1991; Ganguly, 1992; Nayak and Behera, 1996). Since the middle of the present century, consistent efforts have been put to increase the horizon of knowledge in aerobiology on different fields, but Gregory (1973) categorically emphasized the need of aerobiological research of special environments.

Aeromycoflora studies of various dwellings described fungal spores occur in indoors as a result of penetrating dwellings from the outdoors through ventilation or colonizing the variety of substrates found in them, which supports the assumption that dwellings may have their own characteristics mycoflora (Infante *et al.,* 1992). The dispersal of fungal spores in indoor environment is less frequent, that makes high concentration of spores in air of limited flora particularly growing over the substrates stored inside the houses (Andersen, 1985). Chakraverty and Sinha (1985) also described the substrates (Vegetables, fruits and cereal grains etc.) present in the above mentioned site, particularly in house habitats, play as the source of the fungal spores, where they grow saprophytically and produce spores profusely, thereby increasing the concentration in the indoor environments. Many of these micro fungi are strong or weak pathogens of live stocks, human beings and cattle. Micro-fungi of house habitats are the sources of the most potent allergens (Maunsell, 1971; Andersen, 1985).

An analysis of the indoor micro fungi, obtained from house recorded, the presence of species of *Aspergillus, Cladosporium, Penicillium, Alternaria, Rhizopus* and *Mucor* which were most common (Santra and Chanda, 1989).

The present investigation is an attempt to study the prevalence and seasonality of airborne micro fungi in indoors and outdoors of the house of a costal village in Pondicherry region. In India, the problems of houses and its environments (outdoors and indoors) presently demand the planning for ecologically sound for better living, since one third of our life time is spent in dwellings. The indoor environments of the houses are not anthropogenically polluted but it lacks a hygienic condition of living due to the lack of scientific information's on aeromycology in the country like India.

EXPERIMENTAL METHODOLOGY

The present study was carried out in indoors and outdoors of a house in a coastal village, Kalapet (11^0 46" and 12^0 30" N latitudes and 79^0 36" and 79^0 53" E longitudes) of Pondicherry region for constant one year from October, 2008 to September, 2009.

Air samplings:

Air samplings were taken for continuous one year from October, 2008 to September, 2009, at monthly intervals, between 11 to 11.30 A.M. from indoors (living room) and outdoors (20 feet away from house) exposing media plates at 5ft height from the floor in the indoors and at 10ft height from the ground (outdoors). Three replicates of media plates (θ= 9cm) containing Potato Dextrose Agar (PDA) medium with streptomycin/ penicillin (50 mg^{-1}) were carried to the study sites with sterilized container and exposed to the air for five minutes to receive the sedimentation of the air borne fungal spores on the media plates. Altogether 72 Petri plates were exposed in the indoors and outdoors of the house. The exposure time was standardized to get 10 - 45 number of fungal colonies/colony

forming units (CFUs) per plate. After exposed, each set of plates were brought with utmost care and incubated in culture room at 25±3^{0}C upside down for 15 days with constant observation after 3-4 days of incubation. Fungal colonies developed in plates were counted for individual species and to get the total number CFUs. Microscopic slides stained with lacto phenol cotton blue were prepared from each CFUs and observed microscopically to identify them up to species level. The colony forming units (CFUs) that could not be identified directly from plates were sub cultured in PDA medium again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa (Barnett and Hunter, 1972; Gilman, 1957; Ellis 1971; Ellis, 1976; Ellis and Ellis, 1985; Onions *et al.,* 1986). Annual and monthly percentage occurrence of individual fungus was determined. Pearson's correlation analysis was made between indoors' and outdoors' fungal spores and meteorological parameters like temperature, relative humidity and rain fall.

EXPERIMENTAL FINDINGS AND DISCUSSION

During the study period, a total number of 1042 fungal CFUs were isolated from both indoors and outdoors house of the coastal village, of which outdoor environment contributed (53%) followed by indoor environment (47%). Incidence of airborne fungal species, their CFUs contribution and annual occurrence recorded in each environment of the house are given on Table 1 and 2. Among the recorded taxa, member of Deuteromycotina were most prominent in their occurrences followed by the members of Zygomycotina.

In qualitative analysis, altogether 33 fungal species were isolated comprising of 20 genera from both outdoors and indoors. Among these, 29 fungal species out of 18 genera were isolated from outdoors of the house but from indoors, 30 fungal species of 20 genera were recorded. Among the total number of isolated fungal species, *Aspergillus* contributed 11 species followed by *Penicillium* 2 species, *Cladosporium* (2) species and *Mucor* (2) only. Other genera were found one or two in their contributions.

Comparative analysis of the occurrence of dominant fungal species with their contribution in indoors and outdoors of the house is given in Fig. 8. *Aspergillus* sp. contributed maximum in both indoor and outdoor followed by *Penicillium*, *Absidia* and *Cladosporium* (Fig. 8).

Based on the annual occurrences, *Aspergillus niger* was found to be the highest (18.7%) in outdoors, (13.7%) in outdoors , *Absidia spinosa* (11.9%) in indoor (6.47) in outdoor, *Cladosporium herbarum* (10.52%) in indoor and (10.25%) in outdoor, *Aspergillus fumigatus* (14.81%) and (1.43%) and white sterile mycelia (10.52%) in indoor and outdoor (10.43%) etc. (Table 1 and 2).

Monthly incidence of fungal spores with the total rainfall in indoors and outdoors showed a clear view of the distribution of fungi (Fig. 1.) The month of December contributed the maximum spores in the indoors. The month of July contributed the maximum spores in the outdoors. Other months, especially November to February were recorded more. Less number of fungal spores are recorded in month of March to June (Fig. 1 and 2).

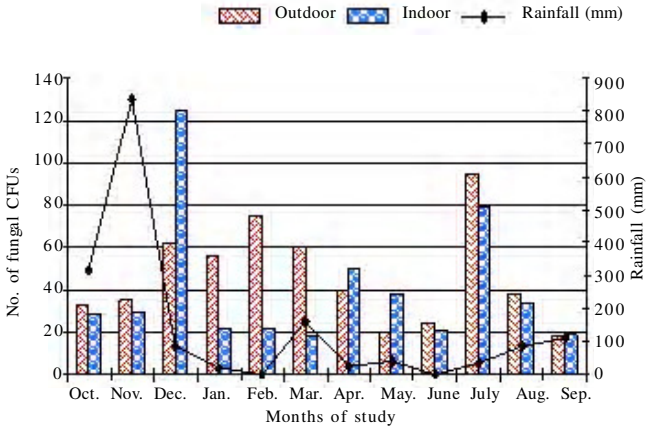


Fig. 1: Monthly incidence of fungal CFUs in indoors and outdoors of the house with recroded total rainfall

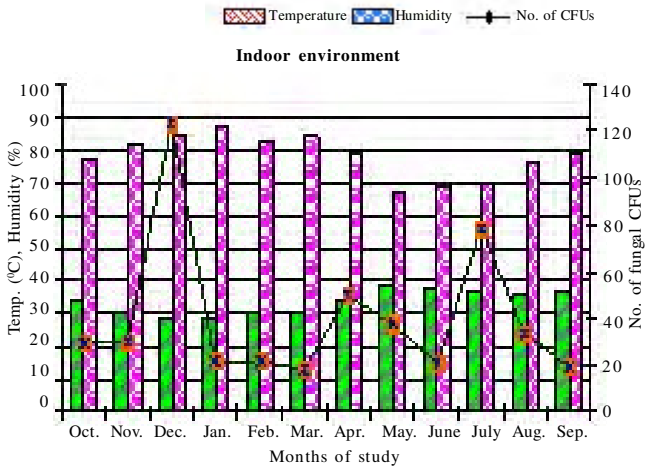


Fig. 2: Comparison of temperature and humidity with monthly isolated fungal CPUs

Seasonal occurrence of fungal spores in indoors and outdoors is given in Fig. 3, which shows the winter season contributed the maximum (42 %) followed by rainy (33%) and summer (26%) in outdoors but in indoors, it was (41%) in winter (33%) in rainy and (26%) in summer (Fig. 3). Over all, winter contributed the maximum spores due to the abundant occurrence of *Penicillium citrinum* in the month of December after a heavy rain fall.

Table 1: Monthly and annual incidences of air borne fungal (CFU/m³) recorded in indoor of Kalapet coastal village houses

Sr. No.	Fungi	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	% occurrence
1.	*Absidia spinosa*	17.24	13.3	2.41	..	5	..	82	..	9.52	..	..	10.52	11.9
2.	*Alternaria alternata*	..	..	..	..	..	..	14	..	..	..	..	..	1.44
3.	*Aspergillus awamori*	3.44	..	0.80	..	..	5.55	..	..	9.52	..	..	5.26	1.23
4.	*A. flavipes*	..	..	1.61	2.09	..	..	..	..	..	..	2.94	..	1.32
5.	*A.flavus*	6.89	..	..	..	..	11.11	..	13.15	..	3.79	..	5.26	2.67
6.	*A.fumigatus*	10.34	6.66	..	..	..	5.55	..	..	..	10.14	..	8.82	14.81
7.	*A. niger*	17.24	6.66	3.22	..	..	16.66	..	47.36	33.33	13.65	32.35	42.10	13.1
8.	*A. nidulans*	..	..	..	..	..	..	..	5.26	..	..	..	5.26	0.61
9.	*A. terreus*	..	..	..	..	..	..	..	2.63	..	..	..	..	0.20
10.	*A.japonicus*	..	..	..	..	9.09	..	..	..	..	..	..	..	0.41
11.	*A. versicolor*	..	..	..	..	..	..	..	..	9.52	..	..	..	0.41
12.	*Acrocalcidium pallidans*	..	..	..	..	..	5.55	..	..	..	..	..	..	0.20
13.	*Candida sp.*	..	..	..	..	..	..	..	..	..	1.26	..	..	0.20
14.	*Cladosporium herbarum*	..	10	3.22	..	36.36	..	..	..	..	..	..	10.5	3.49
15.	*C. sphaerospermum*	..	10	..	4.54	..	..	..	..	..	..	..	..	0.82
16.	*Curvularia lunata*	10.34	13.3	..	2.09	9.09	22.22	..	2.63	9.52	1.26	17.64	..	3.14
17.	*Drechslera sp.*	..	..	..	..	..	16.66	..	..	..	..	..	5.26	0.82
18.	*Grey sterile mycelia*	6.89	..	..	4.54	13.63	..	4	..	..	..	..	5.26	1.64
19.	*Fusarium oxysporum*	..	..	..	..	9.09	..	..	..	..	..	..	5.26	0.61
20.	*Helminthosporium*	3.44	..	..	..	..	..	..	..	..	..	..	..	0.20
21.	*Mucor sp.*	..	20	..	..	..	16.66	..	7.89	..	..	..	..	2.45

Table 2: Monthly aeromycospora incidences of air borne fungal (CFU/m³) recorded in outdoor of Kalappet coastal village house.

Sr. No.	Fungi	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	% occurrence
1.	*Alssaria spinosa*	3.03	..	1.61	..	1.33	..	77.5	..	..	..	..	11.1	6.47
2.	*Alternaria alternata*	..	..	..	..	..	..	12.5	..	..	..	..	..	0.89
3.	*Aspergillus awamori*	3.03	2.85	3.22	5.35	..	8.33	..	..	4.16	20	..	..	5.7
4.	*A. flavipes*	6.06	8.57	..	..	..	..	..	5	8.33	1.05	2.63	..	1.7
5.	*A.flavus*	..	5.71	17.74	..	..	11.67	..	5	8.33	10.52	7.89	..	6.47
6.	*A.funisynnus*	6.06	..	..	..	..	..	..	..	..	3.15	7.89	..	1.43
7.	*A. niger*	24.24	2.85	3.22	5.35	2.66	10	..	10	37.5	60	26.31	22.2	18.1
8.	*A. nidulans*	..	..	..	..	..	..	..	5	..	..	..	..	0.17
9.	*A. terreus*	3.03	..	..	..	..	..	..	5	..	..	..	..	0.35
10.	*A.japonicus*	..	..	..	..	2.66	6.67	..	..	..	..	..	..	1.01
11.	*A. versicolor*	..	..	..	..	..	3.33	..	..	..	..	..	..	0.35
12.	*Auerbasidium pullulans*	6.06	..	..	..	..	..	..	..	..	4.21	..	..	1.01
13.	*Candida sp.*	..	..	..	..	..	3.33	10	..	..	..	..	..	1.01
14.	*Cladosporium herbarum*	..	..	..	..	2.66	..	..	..	..	..	..	..	0.35
15.	*C. sphaerospermum*	..	..	..	46.42	34.56	..	..	..	..	..	..	22.1	10.25
16.	*Curvularia lunata*	9.09	11.42	..	7.14	..	..	..	15	12.5	1.05	..	..	3.39
17.	*Drechslera sp.*	6.06	..	..	..	..	..	..	..	..	..	5.26	..	0.89
18.	*Grey sterile mycelia*	6.06	22.85	..	6.67	..	..	..	5	12.5	..	7.89	..	0.35
19.	*Fusarium oxysporum*	..	..	..	..	2.66	6.67	..	..	..	..	..	5.55	4.31
20.	*Helminthosporium*	6.06	..	..	..	..	..	..	..	..	..	..	..	1.25
21.	*Mucor sp.*	..	..	..	..	..	6.67	..	..	..	..	5.26	..	0.71
22.	*Mucor plumbeus*	..	..	..	..	..	..	..	..	12.5	..	..	..	0.71
23.	*Neurospora sp.*	..	..	..	..	..	..	..	..	..	..	7.89	..	0.53

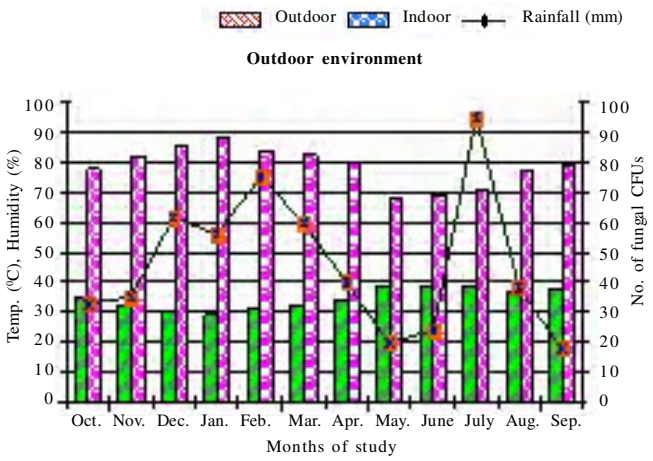


Fig. 3: Comparison of temperature and humidity with monthly isolated fungal CPUs

Comparison co-efficient between temperature, humidity and rainfall with fungal CFUs collected during the sampling time showed a significant at different levels (Fig. 6). It was found that the meteorological parameters had direct effect on air borne fungal spores in the outdoor indoor environments. But rainfall had effect only in outdoors.

Among the recorded fungal taxa *Candida* sp, *Cladosporium sphaerospermum, Monilia sitophila* and *Penicillium citrinum* were isolated absolutely from Indoor of the house only. But *A. wentii*, *A. ochraceous*, were isolated from outdoor only.

Pearson's correlation of co-efficient analysis between fungal spores of indoors and outdoors and meteorological parameters are given in Fig. 6 and 7. It showed positive and negative correlation between the independent and dependent variables. All the correlations were found significance at 0.05 'p' level, which showed there is a close relationship between the variables.

Aeromycospora studies employ a number of sampling techniques of which, gravity settling of spores on culture media is one of the widely used technique by different workers (Nanda *et al.,* 2000; Nayak *et al.,* 1998) both in indoor and outdoor environments but its use in indoors is more appropriate as the sedimentation of spores its less affected by wind turbulence (Infante *et al.,* 1992). The settled viable spores germinated, grow the mycelia and sporulate on the

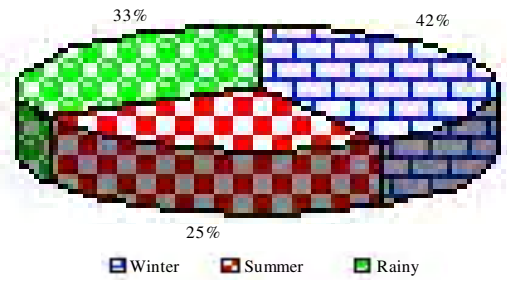


Fig. 4: Seasonal occurrence of fungal spores in outdoor environment

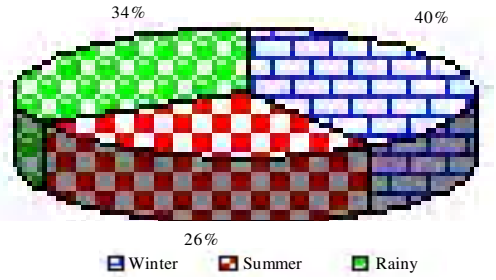
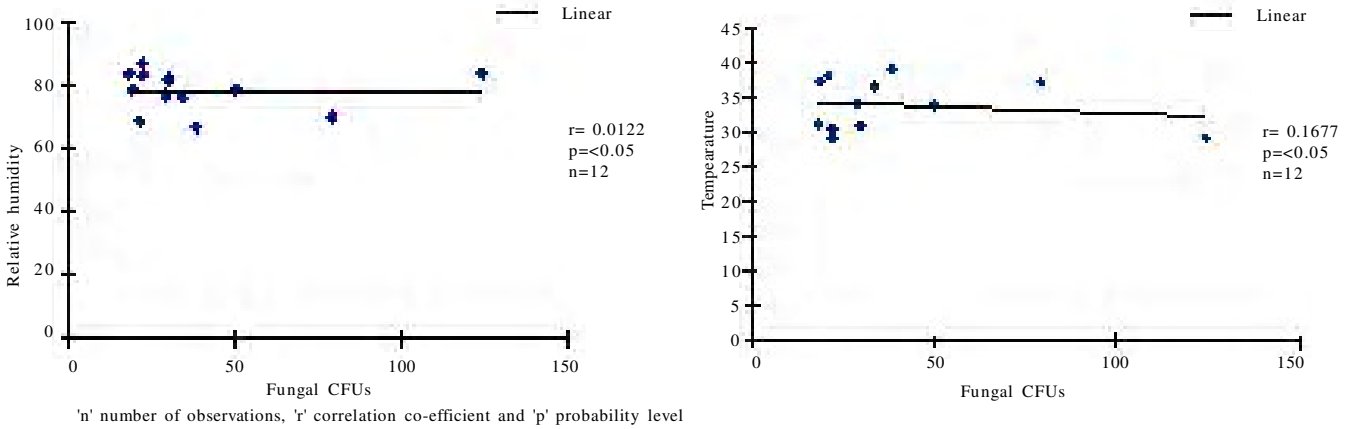


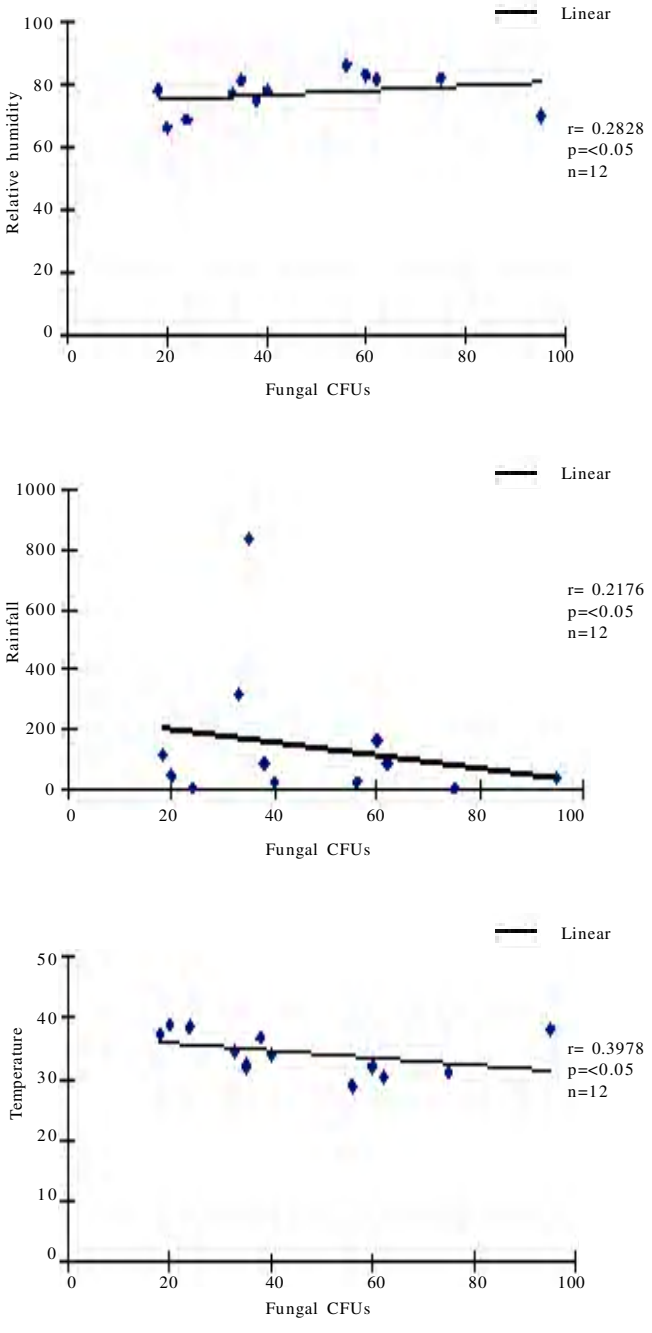
Fig. 5: Seasonal occurrence of fungal spores in indoor environment



'n' number of observations, 'r' correlation co-efficient and 'p' probability level

Fig. 6: Pearson's co-efficient of correlation of fungal CFUs recorded from indoors vs. temperature (°C) and relative humidity (%)

broad spectrum media. It facilitates the microscopic study of the colony forming units (CFUs) and enables the identification of the species. It is trend suitable for qualitative study but the result neither would nor be set forth quantitatively as it was not possible to express them as a unit of air volume. The present study rightly used this technique expressing the results only qualitatively.



'n' number of observations, **'r'** correlation co-efficient and **'p'** Probability level

Fig. 7 : Pearson's co-efficient of correlation of fungal CFUs recorded from outdoors vs. temperature (°C), rainfall (mm) and relative humidity (%).

Out of the isolated fungal species, most of them belonged to the members of Deuteromycotina followed by members of Zygomycotina, which found both in indoors and outdoors of the house as reported by previous authors (Li *et al.,* 1995; Infante *et al.*, 1992). Outdoor environment contributed more spore profile in comparison to indoor environment of the dwelling (Nanda *et al.,* 2000). Lumpkins *et al.* (1973) described the group of Deuteromycotina fungi were reported from both indoors and outdoors and he also reported that there is a common reservoir pool for both indoor and outdoor mycobiota from where they represent in the ambient air.

Among the members of fungal species, *Penicillium citrinum* contributed the maximum both in indoors and outdoors, but its concentration was more in outdoors in comparison to indoors, which was found unique in its occurrences in Pondicherry region.

Microfungi in outdoor and indoor air of 14 houses were observed in Cordoba city of Spain (Infante *et al.,* 1992). They reported significant quantitative differences between indoor and outdoor air. Air borne species were numerous outside the residences than inside the residences.

Based on the species distribution, *Aspergillus* comprised of 10 species, via., *A. awamori*, *A. flavipes*, *A. flavus*, A. *fumigatus*, *A. terrus*, *A. versicolor* and *A. wentii* were recorded from indoor and outdoors of the village environment in agreement with the findings of many other workers (Nanda *et al.,* 2000).

Three seasons in a year *viz.,* winter, summer and rainy exhibited marked differences in population density of fungal spores in air; winter was found to contribute maximum spore had (41%) of the total isolated followed by rainy (33%) and summer (26%) in both indoors and outdoors, with contributing fungal CFUs, the seasonal concentration of spores was found in order of winter-rainy-summer.

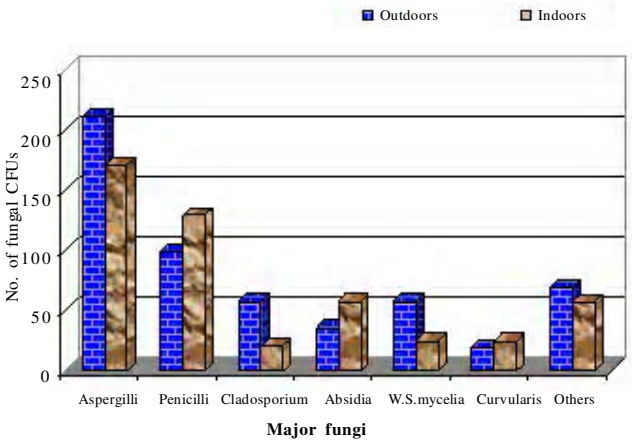


Fig. 8 : Distribution of major group of airborne fungi isolated from indoors and outdoors of coastal village

In tropical countries, particularly the southern India, winter season is highly congenial for growth, sporulation and dispersal of spores. In winter months the average temperature of the study sites ranges in between 20-25^{0}C, the retreating monsoon caused moderate rain during November to February and the ambient Relative humidity were the main causes for higher spore concentration in air. High concentration of spores in air of indoor and outdoor during winter months have been confirmed by a number of workers (Nayak *et al.,* 1998; Tiwari, 1984).

It was confirmed from the Pearson's correlation of coefficient analysis (Fig. 6 and 7) that the variable (fungal CFUs) is dependant on the independent variables like temperature, humidity and rainfall (Nayak and Behera, 1996). In outdoors, mostly rainfall controlled the abundance of fungal spores, but in indoors and outdoors, temperature and relative humidity acted as major factors in controlling the airborne microfungi (Nayak *et al.,* 1998; Nanda *et al.,* 2000).

Attention has been focused on the fungi in relation to human diseases, especially in sick building syndrome (Li *et al.,* 1995). However, the presence of fungi in building does not necessarily imply a cause and effect relationship with illness, but should alert physicians and healthcare professionals to do more vigorous environmental testing.

Correlation of co-efficient analysis between airborne fungal spores and meteorological parameters were found significance at 0.05 'p' level, which showed there was a close relationship between the two variables.

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