Studies on airborne fungal spores in the fish market environment of Puducherry city

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Abstract: Studies on airborne micro fungi with their seasonal distribution in the working environment (Fish market) of Pondicherry city was carried out by gravitation method from January 2011 to December 2011. Incidence of fungal spores considerably varied from indoors to outdoors as well as from season to season. The air of the market harbored maximum fungal species which was predominated with more number of propagules during the months of December and July in comparison to other months. Altogether, 296 fungal isolates were recorded from the fish market during of the study period. To the total population of the fish market major fungal groups like *Aspergillus, Cladosporium* and *Penicillium* contributed 27.71%, 27.37% and 12.50% respectively. Out of 18 fungal numbers the genus *Aspergillus* had 5 members, *Penicillium* had 3 members, *Fusarium* and *Cladosporium* had 2 species each, *Aureobasidium pullulans, Curvularia lunata, Monilia sitophilla* and *Rhizopus stolonifer* had 1 species each. In seasonal periodicity, winter contributed the maximum spore load (47%) followed by rainy (28%), summer was found with the least (25%) in harboring the spore mass in the indoors and outdoors. *Alternaria alternata,* which is accounted as a human allergen for ear infection and an agent for hay fever and other parasites, was also intermittently recorded.

Key words: Fish market, Fungal spores, Mean, Variance, Correlation coefficient.

Introduction

Fish market is characterized as a human activity enriched site and also a highly trafficked site. People in these areas are actively engaged in handling of different fishes and crabs originated from different harbors, sea sides and nearby fish ponds which was exposed to large quantities of aerosolized microorganism. The bio-aerosols generated in due course of mechanical disturbance, contain many organic compounds enable to sensitize vital respiratory organs of local inhabitants. Inhalation of airborne microorganisms can expose workers to risks from infection, toxicosis and allergy. The presence of fungal spores in air due to the mechanical areosolization indicates higher rate of dissemination of pollutants in these occupation associated areas. The aerosolized spore-forming fungi are able to survive in air for a long duration and get easily transported to different places. The situation becomes worse when these microorganisms are able to multiply in these environments[1]. Infectious microorganisms must be viable to cause infections, but infectious as well as non-infectious microorganisms may pose other health hazards even if they are dead and disintegrated. Inhalation of noninfectious microorganisms and their constituents can cause inflammation of the respiratory system, while antigens and allergens may activate the immune system and cause allergic and immunotoxic effects[2,3]. This comprehensive study has been made in order to evaluate the quantity and quality of potentially hazardous culturable fungi of variable types represented in the air of fish market with special reference to aeroallergenic forms in this environment. The effects of environmental factors on the total airborne fungal load were also analyzed by using correlation analysis and a regression model for the purpose of prediction was prepared. The present aero-mycological investigation included enumeration, identification and numerical analysis of different types of culturable airborne fungi in the fish market of the city of Pondicherry in order to measure the degree of

aerobiopollution for this environment. The aeromycological samplings were done at monthly intervals for a period of one year from January 2011 to December 2011.

Materials and methods

Pondicherry is the capital city of Puducherry state situated in the coromandal coast of Bay of Bengal and 160 km away from Chennai metropolis in the south. The present study was carried out inside the occupational working environment, Fish Market of Pondicherry city for constant one year from January 2011 to December 2011. The Fish Market comprises of 350 shops adjacent to the vegetable market in the same premises. There are about 500 fish sellers, 400 females and 100 males who sell sea food such as fish, prawn, crabs, etc. who get fishes from Pondicherry Sea Harbor and from local fisheries in Solai Nagar, Vaithikuppam, Kuruchikuppam, Vambakeerapalayam, Veerampattinam, Chinna Veerampattinam, Periya Veerampattinam, Nathamedu and Kalapet. There are almost 60 to 70 varieties of fishes available in the fish market and at least 25 tons of fish sold every week. There are totally 51 sanitary workers work every day to dispose the fish waste and vegetable waste from Puducherry Municipality who works 8hrs a day and they dispose ½ to 1 tonnes fish waste.The market is situated near to the coastal area (500m away) in the main city and surrounded by a other markets and local plant communities. The study site lies within 11^0 46'' and 12^0 30'' N latitudes and 79^0 36' and 79^0 53'' E longitudes.

Air Samplings

Air samplings were taken for continuous one year from January 2011 to December 2011, at monthly intervals, between 10 to 11 A.M. from indoors exposing media plates at 5ft height from the floor in indoors. Three replicated media plates (θ=9cm) containing Potato Dextrose Agar (PDA) medium with streptomycin/ penicillin ($50mg^{-1}$) were carried to the study sites, fish market 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 36 Petri plates were exposed in the indoors of the market. The exposure time was standardized to get countable number of fungal colonies/colony forming units (CFUs) per plate. After exposed, each set of plates were brought separately to the Microbiology Laboratory, Department of Botany, KMCPGS (Autonomous), Pondicherry with utmost care and incubated in culture room at 25 ± 3^0c 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 CFU 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/SDA/CDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa. Annual and monthly percentage occurrence of individual fungus was determined. Mean, media and variance with Pearson's correlation analysis were made between fungal spores and the meteorological parameters like temperature and relative humidity.

Results

Quantitative analysis

Monthly distribution and percentage incidence of fungal species recorded in Fish market and the total number of isolated fungal colonies per month in the environment during the year 2011 are given in the Table 1. Altogether, 296 fungal isolates were recorded from the fish market complex of the vegetable market during of the study period. To the total population of the fish market major fungal groups like *Aspergillus, Cladosporium* and *Penicillium* contributed 27.71%, 27.37% and 12.50% respectively (Table 1). Although members of aspergilli together dominantly represented the population, individually *Cladosporium cladosporioides* was the dominant one and it contributed 16.50% to the total populations of the fish market. It was followed by *Aspergillus niger* (16.78%) and *Cladosporium herbarum* (11.48%) and others. Monthly variations in spore concentration as well as number of colony forming units (CFUs) recorded every month during 2011 are given in Fig 1. Maximum number of fungal colonies was isolated in the months of December and January but the least number of fungal spores were recorded during July, August and March, April months. Monthly mean, maximum and minimum temperature recorded during the study period during 2011 (Fig 2).
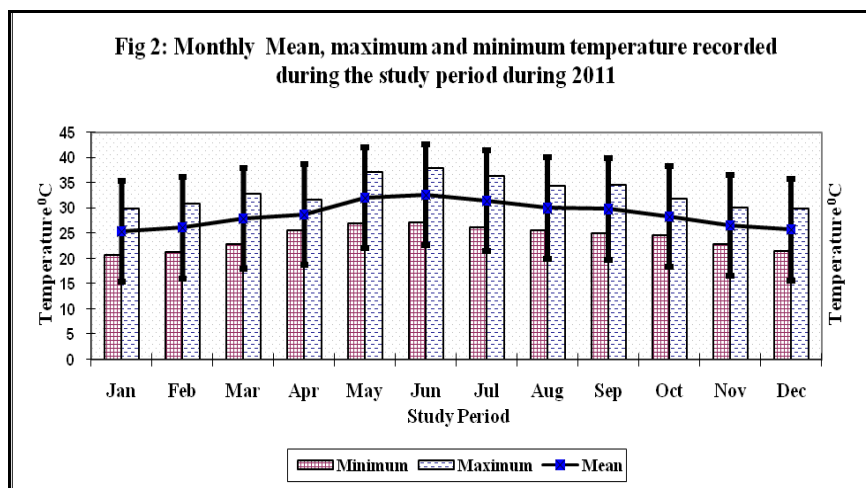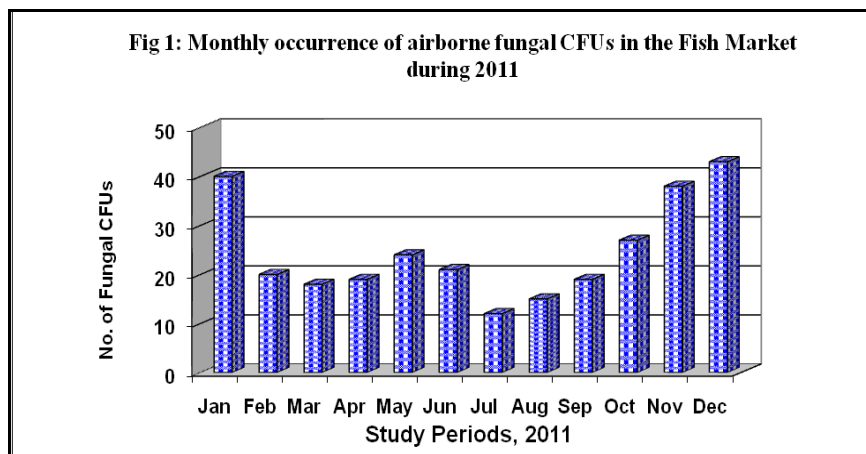
Fig 1: Monthly occurrence of airborne fungal CFUs in the Fish Market during 2011

Fig 2: Monthly Mean, maximum and minimum temperature recorded during the study period during 2011

Table 1: Monthly percentage incidence of airborne fungal spores in Fish market during 2011.

Sl. No.	Name of fungi	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	% Occ.
1	*Aspergillus flavipes*	0.00	10.00	0.00	0.00	4.17	0.00	0.00	6.67	0.00	0.00	2.63	2.33	2.03
2	*Aspergillus flavus*	0.00	0.00	22.22	0.00	0.00	4.76	0.00	0.00	10.53	0.00	0.00	6.98	3.38
3	*Aspergillus fumigatus*	10.00	0.00	0.00	15.79	0.00	4.76	8.33	0.00	5.26	0.00	0.00	0.00	3.38
4	*Aspergillus niger*	20.00	30.00	0.00	0.00	8.33	9.52	8.33	20.00	0.00	33.33	15.79	18.60	15.20
5	*Aspergillus terreus*	5.00	0.00	16.67	0.00	4.17	0.00	8.33	0.00	10.53	3.70	2.63	0.00	3.72
6	*Aureobasidium pullulans*	2.50	0.00	0.00	0.00	12.50	9.52	8.33	0.00	10.53	3.70	7.89	9.30	5.74
7	*Cladosporium cladosporioides*	20.00	20.00	11.11	15.79	0.00	14.29	0.00	13.33	21.05	22.22	21.05	16.28	15.88
8	*Cladosporium herbarum*	10.00	20.00	0.00	10.53	16.67	9.52	0.00	33.33	0.00	7.41	13.16	13.95	11.49
9	*Curvularia lunata*	7.50	0.00	11.11	0.00	8.33	14.29	8.33	0.00	21.05	0.00	5.26	6.98	6.76
10	*Fusarium moniliforme*	0.00	10.00	0.00	15.79	0.00	0.00	16.67	0.00	0.00	11.11	10.53	0.00	4.73
11	*Fusarium oxysporum*	0.00	0.00	0.00	5.26	0.00	4.76	0.00	0.00	5.26	0.00	0.00	2.33	1.35
12	*Monilia sitophilla*	10.00	0.00	16.67	0.00	25.00	9.52	0.00	0.00	0.00	3.70	0.00	0.00	5.41
13	*Mucor racemosus*	2.50	0.00	0.00	5.26	0.00	4.76	0.00	13.33	0.00	0.00	0.00	0.00	1.69

14	*Penicillium fellutanum*	5.00	0.00	5.56	26.32	8.33	4.76	25.00	13.33	5.26	7.41	15.79	16.28	10.81
15	*Penicillium frequetans*	0.00	10.00	0.00	0.00	4.17	0.00	0.00	0.00	5.26	0.00	2.63	0.00	1.69
16	*Rhizopus stolonifer*	2.50	0.00	0.00	5.26	4.17	0.00	8.33	0.00	0.00	3.70	0.00	2.33	2.03
17	*Saccharomyces cerevisiae*	2.50	0.00	5.56	0.00	0.00	4.76	0.00	0.00	0.00	3.70	2.63	2.33	2.03
18	White sterile mycelia	2.50	0.00	11.11	0.00	4.17	4.76	8.33	0.00	5.26	0.00	0.00	2.33	2.70

Seasonal incidence

Seasonal variation in fungal spore distribution of the fish market atmosphere showed that the winter months harbored maximum fungal spores in the working environments. Number of colony forming units recorded during the winter months contributed 50% to the total annual spore load. Seasonality of spore profile in fish market exhibited the maximum number of spores (47%) during winter season, rainy season registered the spore numbers lesser than winter and the summer. They contributed 28% (summer) and 25% (rainy) respectively.

Qualitative analysis

Most of the fungal taxa isolated by gravity petriplate method were identified up to species level except a few (Table 1). Overall, 18 fungal species within 11 genera were recognized from outdoors and are listed in this table. Out of 18 fungal numbers the genus *Aspergillus* had 5 members, *Penicillium* had 3 members, *Fusarium* and *Cladosporium* had 2 species each, *Aureobasidium pullulans, Curvularia lunata, Monilia sitophilla* and *Rhizopus stolonifer* had 1 species each. Deuteromycetacious fungal members were recorded in more numbers followed by Zygomycotina and Ascomycotina. Spores of *A. niger* were frequent in their occurrence in the months of November, December and February. In contrast to *A. niger, Penicillium citrinum* was recorded in more numbers from outdoors than indoors and fish market but the November, December and January harbored the maximum penicilli than other months. *Cladosporium herbarum* was found as the maximum contributor in the market to the total spore load next to *P. citrinum*, but its distribution was more in the months of January, May and June during the study period.

Statistical analysis

The fungal isolates recorded from fish market were subjected to different statistical analysis and the values are sample mean: 24.67, median: 20.5, variance: 104.78, n:12 and df: 11. Pearson's co-efficient of correlation and regression values determined for the total fungal CFUs with temperature and relative humidity recorded from fish market showed that the positive correlations between fungal isolates and relative humidity ($r=0.641$, $p<0.01$) and negative correlation between fungal isolates and temperature ($r=-0.654$, $p<0.05$).

Discussion

In our present study, gravity petriplate method was used which is one of the widely used technique by different workers [1,4,5] both in indoor and outdoor environments but its use in indoors is more appropriate as the sedimentation of spores is less affected by wind turbulence[5]. The present study rightly used this technique expressing the results only qualitatively. Out of the isolated fungal species, most of them belonged to the members of Deuteromycetes followed by members of Zygomycetes, which found both in the indoors of the fish market. In the comparative analysis among members of fungal species, Aspergilli and Cladosporia contributed the maximum, but their concentration was more in indoors, which was unique in its occurrences in fish market environments. Based on the species distribution, *Aspergillus* comprised of 5 species, via., *A. flavipes, A. fumigatus, A. japonicas, A. niger,* and *A. sydowii* were recorded from the environment was in agreement with the findings of many others[6,7]. In our study winter was found to be the highest contributor of fungal propagules in fish market environment and it was corroborated with previous work made by Reddy [6] and his coworkers in Vishakhapatnam, India. In their finding more fungal species were present in which *Aspergillus* were dominating like our reports [6]. Record of least fungi in Fish market may be attributed to the regular splash of water drops over the area and fish coverage, made the spores to remain in their substrates without any dispersion in the air

for long time during also our sampling time too[8]. *Fusarium* and *Curvularia* are saprophytic fungi and weak pathogen of vegetables and crop plants, were reported in the present study. It was found that the meteorological parameters had direct effect on air borne fungal spores in the fish market environments.

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