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An aeromycological survey of different Bus stand environments in Puducherry District

Bijaya Kumar Nayak

**Department of Botany, K. M. Centre for P.G. Studies (Autonomous)
Lawspet, Puducherry-605008, India**

Abstract: An aeromycological study at five different bus stands environments in Puducherry District was carried out during January to March 2015 by volumetric Burkard's sampler on agar plates. During the study period, total number of 3440 fungal colony forming units (CFUs) were isolated from all the five sites, among which Gorimedu bus stand contributed the maximum of (30%) followed by the Thanthaiyeriyar bus stand (22%), New bus stand (11%), Villianur bus stand (18%) and old bus stand (11%). Among the recorded taxa, members of *Aspergillus* spp, were predominant in their occurrence, followed by the members of *Cladosporium* spp. In qualitative analysis, altogether 15 fungal species under 9 genera were isolated from all the sites. Data on fungal occurrence revealed that the *Aspergillus* sp, *Cladosporium* sp. and *Penicillium* sp. were the highest in all the five sites chosen for study. Fungal species like, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium*, *Curvularia palescens*, Pink sterile mycelia, *Penicillium chrysogenum*, *Penicillium citrinum* were predominant in the bus stand areas. Besides, the dominant ones, contributing substantially to the total fungal CFUs, a large number of fungal types recorded incidentally one or three times during the study period contributing thus very negligibly.

Keywords: Aeromycological survey, Bus stand, Burkard's sampler, Fungal CFUs, Taxa.

Introduction

Air contains a variety of microorganism mainly fungal spores which are considered to be very dangerous, causing many health ailments to humans, plants and animals. The study of microbes, particularly fungal spores in the aerial environments is defined as aeromycology. Fungal spores are part and parcel of air and their quality and quantity depends on time of day, weather, seasons and climatic conditions of the surrounding area and presence of local source of spores¹. Identification of airborne fungal spores is must for a number of reasons including assessing the air quality, detection of pathogenic organisms, epidemiological aspects and the human health hazards. Before sampling for airborne fungal spores, it is important to have clear objectives and the data that would meet those objectives. Some of these objectives require counting and identification of the airborne fungal particulates. Temperature and relative humidity play an important role in controlling fungal population in the environment². Monitoring for airborne microorganisms has traditionally focused on the recovery of fungal spores, using spore count and culture techniques³. Concentrations of airborne fungi depend on several factors like seasonal and daily variations in meteorological conditions viz., temperature, humidity, wind speed, rainfall, solar radiation, vegetation, air pollution, agricultural, industrial and other human activities⁴. Some spores of the fungi are responsible for allergy. Fungal propagules in the ambient air are regularly and continuously inhaled by human beings and the spores are inhaled and deposited on sensitive mucosa⁵. It causes allergic human diseases such as asthma, rhinitis and a range of cardio-respiratory diseases are attributed to inhalation of airborne fungal spores and pollen grains⁶. Airborne fungi are sometimes associated with respiratory diseases, such as chronic bronchitis, asthma, allergies, hypersensitive pneumonitis

and infectious diseases such as aspergillosis. The present study was carried out to analyze the composition and concentration of airborne allergenic/ pathogenic fungi in bus stands in and around Puducherry District area. It will give the information about the fungal spore concentrations and composition, which enables people to avoid exposure at certain hours and days during the fungal spore season in the concern areas.⁷

Materials and Methods

Puducherry district is the biggest one in the state of Puducherry (UT) situated 160 km away from Chennai on the south and it is in the coromandal coast of Bay of Bengal basically ruled by the French. Puducherry is located in between 11 degree 46° and 12 degree 30 ° of north latitude and between 79 degree 36 ° and 9 degree 52° of east longitude. The layout of Puducherry is located within Tamil Nadu presents a peculiar picture of territorial jurisdiction, perhaps the only one of its kind in the world. The historical reasons, which prompted the French and the British to limit their territories in the intermingling manner, are not known. The city area is not completely free from industrial set-up and other pollution, but unhygienic way of living is the catastrophe behind the aero-biopollution. The city has little above five lakh population and has a number of bus stands which shatter the needs of the people to travel from one place to another easily with a capacity of nearly one lakh people per day.

An aeromycological study was carried out in the extramural sites of five different bus stands of the Puducherry district viz., 1) Old bus stand (Uppalam), 2) New bus stand (Maraimalai Adigal Salai), 3) Villianur bus stand (Villianur), 4) Gorimedu bus stand (Gorimedu), 5) Thanthaiperiyar bus stand (Veerampatinam) during January to April 2015. Bus stands are one of the most frequently visiting places for all the people and it also serves the needs of the common people in Puducherry city. The Bus stands were selected for our study based on their situation all around the district and all are surrounded on all sides by market complexes, schools, colleges, residential quarters etc.

Air Sampling

The Burkard's Volumetric Air Sampler on agar plates was used in the present study. The air quality was analyzed by collecting airborne fungal bioaerosols directly onto the petriplates in the outdoor sites of the bus stand environments. The Volumetric Air Sampler is designed for short-term sampling in domestic or industrial environments particularly where no power supplies are available. The Burkard's Volumetric Air Sampler is a perfect air quality monitor used in culture rooms, clean rooms, laminar flow cabinets and all outdoor environments for collecting fungi and bacteria directly onto the plates for microscopic observation on growth. It was designed to record the total number of bioaerosols per cubic meter of air in the sampling sites.

Air samples for culturing fungi were collected by the Petri Plates supplemented with SDA medium (Sabouraud's Dextrose Agar) in the operating samplers. The sampler was run at the height (1.5-2m) above the ground just to the breathing level based on the substrates available in the Bus stand. After operation, the Petri Plates were brought to the laboratory in the Pre-sterilized polythene bags and incubated at 25±3 °C for 3-7 days. After three days of incubation, the fungal colonies were counted for individual species and the total number CFUs were calculated. Microscopic slides stained with lactophenol cotton blue were prepared from each CFUs and observed microscopically under the light microscope to identify directly them up to species level. The colony forming units (CFUs) that could not be identified directly from plates were sub cultured in PDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa^{8,9,10}. Cultured fungi on agar plates of different hotel sites and the identified fungal taxa up to their species level are given in tables. Percentage occurrence of individual fungus were determined and plotted in the form of tables and figures.

Calculation of Percentage contribution of an individual fungus:

% occurrence of the fungus =	Total CFUs recorded by the individual fungus	x 100
	Total CFUs recorded by total number of fungi	

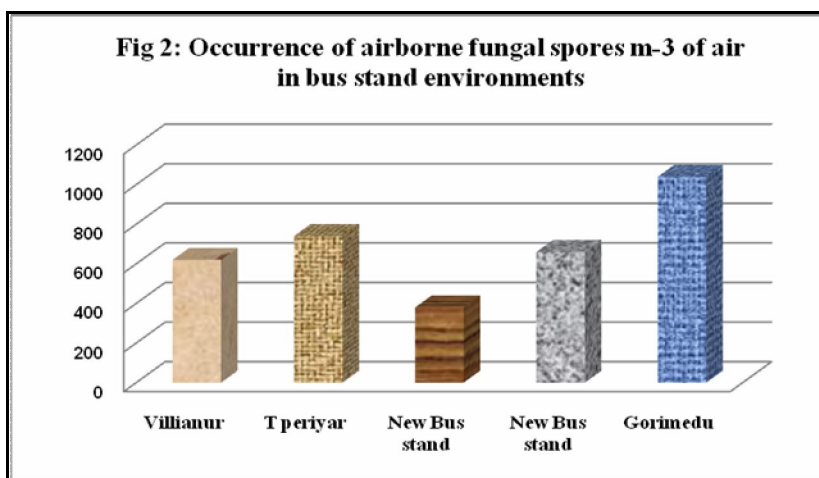
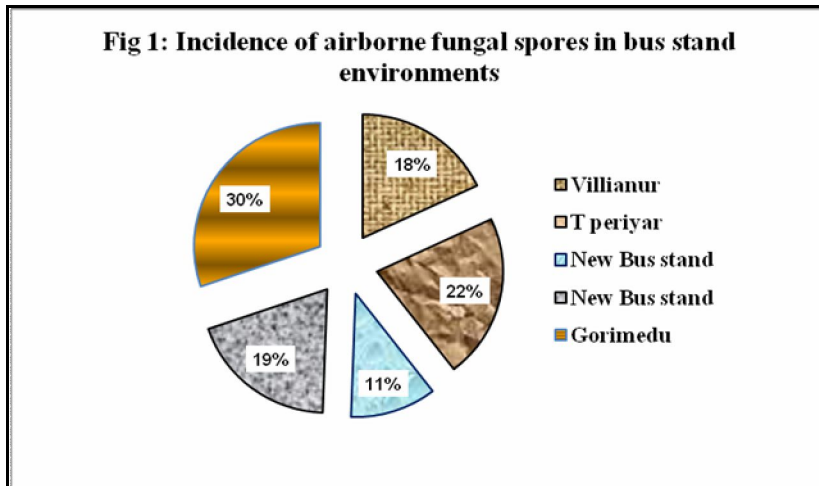
Results

During the study period, a total number of 3440 fungal colony forming units (CFUs) m^{-3} of air were isolated from outdoor environments of five bus stands. In fungal composition and concentration, more fungal spores were recorded in Gorimedu bus stand in comparison to all other bus stands. Percentage incidence of each fungal species and their abundance is given in Table 1. Among the recorded fungal species, members of Deuteromycotina were found as the dominant group followed by the members Zygomycotina. In qualitative analysis, altogether 15 fungal species under 9 genera were observed. The total numbers of isolated fungal species were *Aspergillus flavus*, *Aspergillus flavipes*, *Aspergillus niger*, *Aspergillus tamarii*, *Cladosporium* sp., *Curvularia palescens*, *Fusarium oxysporum*, Gray sterile mycelia, Pink sterile mycelia, *Penicillium chrysogenum*, *Penicillium oxalicum*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Trichoderma* sp. and White sterile mycelia.

Table 1: Percentage of occurrence of air borne fungal spores m^{-3} of air in the bus stand environments in Puducherry district.

Sl. No.	Fungi	Villianur Bus stand	Thanthai Periyar Bus stand	New Bus stand	Old Bus stand	Gorimedu Bus stand
1	<i>Aspergillus flavus</i>	3.22	-	10.52	15.15	3.84
2	<i>Aspergillus flavipes</i>	-	27.02	21.05	3.303	-
3	<i>Aspergillus niger</i>	19.35	37.83	10.52	33.33	3.84
4	<i>Aspergillus awamori</i>	-	2.70	-	-	1.92
5	<i>Cladosporium</i> sp.	-	2.70	-	-	1.92
6	<i>Curvularia palescens</i>	22.58	-	-	12.12	42.30
7	<i>Fusarium oxysporum</i>	12.90	-	-	-	9.61
8	Grey sterile mycelia	-	-	-	-	11.53
9	Pink sterile mycelia	-	-	5.26	3.03	1.92
10	<i>Penicillium chrysogenum</i>	6.45	2.70	5.26	3.03	1.92
11	<i>Penicillium oxalicum</i>	22.58	8.10	10.52	6.06	5.76
12	<i>Penicillium citrinum</i>	9.67	2.70	5.26	9.09	1.92
13	<i>Rhizopus stolonifer</i>	3.22	5.40	5.26	3.03	5.76
14	<i>Trichoderma harzianum</i>	-	5.40	10.52	3.03	1.92
15	White sterile mycelia	-	5.40	10.52	3.03	1.92

Among the entire bus stands studied, Gorimedu bus stand was dominated in harboring the maximum fungal species followed by Thanthaiperiyar bus stand, New bus stand, Villianur bus stand and old bus stand (Fig 1 and 2). Predominantly occurred fungal species were *Aspergillus niger*, *Cladosporium* sp., *Penicillium chrysogenum* and *Aspergillus flavipes*. *Aspergillus niger* was presented the maximum numbers in the Gorimedu Bus Stand i.e., 15 out of 14 Species and least number of species were occurred in the New Bus Stand. In these five Bus stand environment, mostly *Aspergillus niger* were dominant and found in Thanthaiperiyar bus stand and followed by their *Aspergillus flavipes* occurred in this Thanthaiperiyar bus stand. *Aspergillus niger*, *Penicillium citrinum*, *Penicillium chrysogenum* and *Rhizopus stolonifer* were isolated from all five bus stand environments. In our present observation, imperfect fungi which could not show the fructification or lost their sexual reproduction were categorized as Pink sterile mycelia, white sterile mycelia and grey sterile mycelia.



Discussion

Studies on aeromycoflora of five bus stand areas in Puducherry district were carried out for three consecutive months during 2015 by means of volumetric sampling technique. A more or less uniform spore concentration were observed during the early part of the days, but later on the number of spore concentration were increased and peak during the noon. The dominant genera isolated were *Aspergillus*, *Curvularia*, *Penicillium*, *Cladosporium* and *Penicillium*. *Aspergilli* appeared in high concentrations from bus stand areas. The present report was agreed with the previous workers elsewhere (Naim and Chakraverty 1995; Singh (1994). *Aspergillus*, *Curvularia* and *Penicillium* occurred regularly throughout the study period in the extramural environments, which was corroborated with the previous workers done in various working environments¹¹. *Aspergillus flavus*, a well known fungus for production of mycotoxin (aflatoxin), cause asthma in workers of food processing plants¹². Aflatoxin, a very important carcinogenic agent, causes liver cancer¹³ and Reye's syndrome, which is an acute pediatric syndrome of high mortality¹⁴. The high concentration of *A. flavus*, the casual agent of "aflatoxicosis" in birds, needs a detailed investigation in view of its role in Farmer's lung disease¹⁵ besides, type I hypersensitivity disorders¹⁶. *Aspergillus fumigatus* causes respiratory infection leading to broncho pulmonary aspergillosis¹⁷. It was also noted that *Penicillium* sp. predominated in air samplings next to *Aspergillus* spp in the coastal environments¹⁸. In tropical environments these fungi are the dominant ones¹⁹ and are known for allergenicity²⁰. They are well known for the cause of allergic alveolitis²¹. *Rhizopus*, *Curvularia* are saprophytic fungi and weak pathogen, were reported in the present study. Besides these prominent human allergens, a number of other fungi were also recorded.

Conclusion

The analysis of data indicated that concentrations of airborne fungi were very high in few bus stands and quite variable depending on the climatic conditions and substrate availability. The allergenic spores of

Aspergillus and *Penicillium* were found to be predominant, probably due to their wide host range, substrate adaptability and opportunistic nature. The variation of the fungal types was highly related to the availability of the vegetation nearby needed for the fungal growth. The occupational variations and climatic alteration had positive/negative influence on occurrence of aeromycoflora in the outdoor environments of the bus stands studied herewith. The occurrence of microfungi in the environment emphasizes both allergists and mycologists with an interest in health and environment bio pollution to solve the sufferings of the people who have allergenic disorders in the said environments. Particular remedy would be taken care to minimize the fungal load in these environments to protect common people from atopic allergic dysfunctions.

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