

A preliminary study of airborne fungal spores in few temples of Pondicherry

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Abstract: Fungal spores are unanimous in distribution i.e., present is both outdoors and indoors of different environment and constitute the major portion of the suspended bioparticulate matter in the air. They are dispersed in a very high concentration and hence remain in the air for an extended period of time. An aeromycological survey of present study, indoors and outdoors of various temples in Pondicherry city was carried out by employing Burkard's volumetric sampler on agar plates. Air samplings were made at the intervals of one week isolating the prevalent fungi from the study sites in between 10 AM to 11 AM from January to March (2015). Altogether, 12 fungal species under 8 genera were isolated, among which *Cladosporium spp* were recorded as the dominant followed by *Aspergillus spp* and *Penicillium spp*. The prevalence of spores was found more inside the temple in comparison to outside environment. Besides these fungi, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma* and sterile mycelia were also recorded. The analysis of data indicated that concentration of airborne fungi in the two sites of indoor and outdoor of the temple is moderate and quite variable depending on the climatic conditions and substrate availability.

Keywords: Airborne fungal spores, Temple, Burkard's sampler on agar plates.

Introduction

A temple is a structure reserved for spiritual activities, such as prayer and sacrifice or analogous rites. A temple constituted a sacred precinct as defined by a priest, or augur. It has the same root as the word "template," a plan in preparation of the building that was marked out on the ground by the augur. It is also associated with the dwelling places of a god or gods. Despite the specific set of meanings associated with the religion of the ancient Rome, the word has now become quite widely used to describe a house of worship for any number of religions and is even used for time periods prior to the Romans. It is associated with an environment, where people of all religion gather for the same motivation and remain for long time to fulfill their wishes by the deities in the temple. Moreover, due to different regions, the indoor environment of the temples are quite polluted due to the occurrence of airborne fungi and bacteria, and no work pertaining to this aeromycological research was done so far in India. This project was carried out for the analysis of airborne fungi in indoors and outdoors of the few temple environments of Pondicherry city. The Burkard's Air Sampler monitors air quality by collecting airborne bioaerosols on Agar dishes both in indoor and outdoor environments¹. The Portable Air Sampler is designed for short-term sampling in domestic or industrial environments, particularly where no power supplies are available². In the present work, the Burkard's Sampler was applied in order to find out the quantitative assessment of fungal propagules of these sites, viz., indoors and the outdoors of the five different temples in Pondicherry City. The vernacular name of the temple in Pondicherry is Kovil.

Materials and Methods

Pondicherry is the capital city of Puducherry state 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. Pondicherry 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 Pondicherry is located within Tamil Nadu which presents a peculiar picture of territorial jurisdiction perhaps the only one of its kind in the world.

An aeromycological study was carried out at weekly interval in the indoors and outdoors of five temples in Pondicherry city viz., 1) Perumal Kovil, 2) Sengazhuneer Amman Kovil, 3) Iyyanar Kovil, 4) Siddhananda Kovil, 5) Kamatchi Amman Kovil during January to April 2015. Temple is one of the most frequently visiting places for all the people and it also serves the needs of the common people in any place for getting supernatural power from their deities. The temples were selected for the present study based on their situation all around the city area which 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 Indoor and outdoor sites of the temple environment. 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 domestic and working environments for collecting culturable fungi and other particles directly onto a petriplate for microscopic observation. 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 petriplates 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 at the breathing level based on the sampling sites. 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 [3,4,5,6,7]. Cultured fungi on agar plates of different temple 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, altogether 12 species under 9 genera were isolated from the two sites of the temples. Out of which, 12 fungal species of 7 genera were recorded from the indoors and 11 species within 7 genera were represented from outdoors. Moreover indoors of the temple atmosphere was found to be more in concentration than the outdoors (Fig 1 & 2). Incidence and percentage occurrence of air borne fungal spores m⁻³ of air indoor and outdoor environments of various temples in Pondicherry city is given in Table 1 and 2 respectively. On a total of 7040 fungal CFUs, indoor covered 54% of fungal spores and indoor had 46% of fungal spores (Fig 1).

Among the recorded fungal flora, members of Deuteromycotina were most prominent in their occurrence followed by the members of Zygomycotina inside and outside of the temple premises. Among all, *Aspergillus* species was found to be the dominant one in indoors followed by *Cladosporium* sp. and *Penicillium*

sp. The fungal spores of aspergilli were the dominant followed by *Cladosporium sp.*, *Fusarium oxysporum* and *Penicillium citrinum*. Besides these *Aspergillus awamori*, *Aspergillus flavipes*, *Aspergillus niger*, *Aspergillus tamari* and other *Aspergillus sp.* were recorded frequently from the temple environments.

All together, four species of *Aspergillus* were isolated i.e., *A. awamori*, *A. flavipes*, *A. niger*, and *A. tamarii*. Two species from *Penicillium* i.e., *P. chrysogenum* and *P. citrinum* were isolated from both indoors and outdoors. Among the isolated fungal taxa, *Aspergillus niger*, *Penicillium sp.*, *Rhizopus sp.* were predominant aeroallergens that can cause different type of respiratory/lung diseases in atopic human beings. The fungal spores are recorded with the maximum in indoors due to the abundance of *Aspergilli*, but *Cladosporium* were found in two sites of the five different of temple environments. In Perumal temple, more spores were found and lesser in Iyyanar Temple. *Aspergillus flavipes* and other fungi like *Aspergillus sp* were moderately occurred like *Penicillium citrinum* in the indoor temple environment. In Sengazhuneer Amman Temple, more species were found and lesser in Siddhananda Temple. *Aspergillus sp.* and *Cladosporium sp.* were mostly present in the outdoor temple environment and moderately occurred by *A. flavipes* and *A. niger* in the outdoor environment.

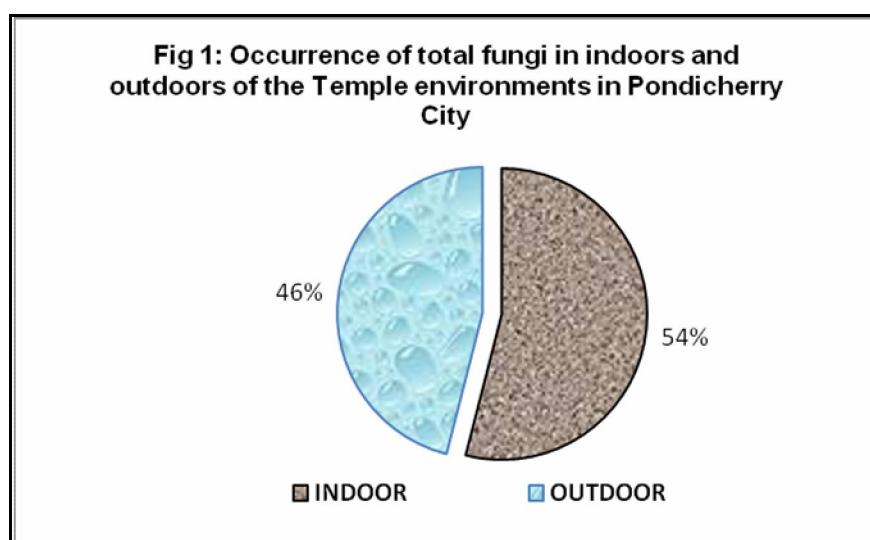
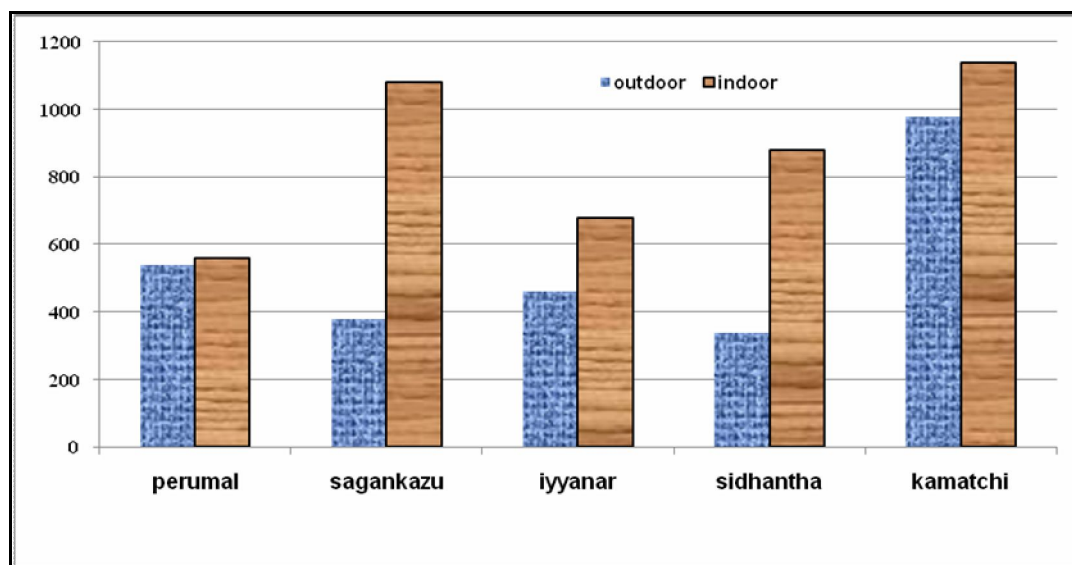


Table 1: Percentage occurrence of air borne fungal spores m^{-3} of air indoors of temple environment in Pondicherry city.

Sl. No.	Name of the Fungi	Temple 1	Temple 2	Temple 3	Temple 4	Temple 5
1	<i>Aspergillus awamori</i>	3.70	-	5.88	-	-
2	<i>Aspergillus flavipes</i>	-	44.44	29.41	63.6	-
3	<i>Aspergillus niger</i>	-	3.70	-	13.6	1.75
4	<i>Aspergillus tamarii</i>	3.70	-	-	-	-
5	<i>Aspergillus sp.</i>	-	16.66	-	20.45	87.71
6	<i>Cladosporium sp.</i>	11.11	5.55	50.88	-	1.75
7	<i>Fusarium oxysporum</i>	7.40	-	8.82	-	3.50
8	<i>Mucor sp.</i>	-	-	-	-	-
9	<i>Penicillium chrysogenum</i>	3.70	1.85	-	-	-
10	<i>Penicillium citrinum</i>	71.4	27.77	-	2.27	5.26
11	<i>Rhizopus sp.</i>	-	-	5.88	-	-
12	<i>Trichoderma sp.</i>	-	-	-	2.27	-

Table 2: Percentage occurrence of air borne fungal spores m^{-3} of air outdoors of temple environment in Pondicherry city.

Sl. No.	Name of the Fungi	Temple 1	Temple 2	Temple 3	Temple 4	Temple 5
1	<i>Aspergillus awamori</i>	7.14	-	4.34	-	-
2	<i>Aspergillus flavipes</i>	-	26.31	-	58.82	-
3	<i>Aspergillus niger</i>	3.57	15.78	-	5.88	4.80
4	<i>Aspergillus</i> sp.	-	-	-	35.29	61.2
5	<i>Cladosporium</i> sp.	70.3	26.31	73.91	-	2.88
6	<i>Fusarium oxysporum</i>	7.14	5.26	8.69	-	2.88
7	<i>Mucor</i> sp.	-	5.26	-	-	-
8	<i>Penicillium chrysogenum</i>	7.14	15.78	-	-	-
9	<i>Penicillium citrinum</i>	-	5.26	-	-	-
10	<i>Rhizopus</i> sp.	3.57	-	-	-	-
11	<i>Trichoderma</i> sp.	-	-	13.04	-	-

Fig 2: Occurrence of fungal spores m^{-3} of air in the indoor and outdoor of temple environments.

Discussion

In our present study, we employed Burkard's personal sampler on agar plates, which is considered as volumetric sampler and used purposefully to evaluate the real quantitative data of the bioaerosols per meter⁻³ of air^{8,9}. Aeromycological study employs a number of sampling methods of which, gravity settling of spores on culture medium is the one widely used by workers^{10,11,12,13} both in indoor and outdoor environment. Its use in indoor environment is more appropriate as the deposition of spores is less affected by wind turbulence¹⁴ but not for outdoor use^{1,2,9}.

In the present study, fungal spores were observed both from indoors and outdoors of the temples that reflect an overall trend in coastal area pattern. It was clear that in comparison with domestic conditions, the fungal spore content is high in temples¹⁰. One reason may be attributed that temples employ high quantities of water for religious purposes and also biogenic materials like flowers and leaves all of which may attract fungal colony proliferation¹⁵. Another point may be considered that the temples remain dark for a good time of the day also attract fungi to grow in high numbers forming a good source of fungal colonization⁹.

The point to be noted here is that among the retrieved fungal spores, five species (all from *Aspergillus*) could be allergenic to human beings causing respiratory issues. In addition, some of them could also cause skin problems. This signifies the main output of the present work and the presence of allergens in temple premises.

where human beings gather often in large numbers. It will be interesting to note such incidences from hospital records of the town. It remains to be seen if the proximity of the sea to the studied sites could be a cause of high spore content. One more point is the humidity in the town. Pondicherry is known to be a place with high humidity and this could be a reason why fungal spores remain suspended in the air and settle down eventually. This in effect exaggerates the possible incidences of allergenic issues. Medical studies indicate that allergens in the air, coupled with high humidity, could cause bronchitis-like respiratory problems. These could be higher during monsoon seasons and during rainy days. During festive occasions, temples also offer lot of edible items to the devotees and very often the remains of the food items are not disposed of properly. They attract not only fungi but also other pathogens. The places where the washed water was gutting out of the main sanctum seemed to contain higher fungal content. In fact this place was the main part of the temple where indoor samples were collected. In addition, the water also comes outdoors and allow the growth of fungal colonies could have contributed to the high percentages of spores. The constructions of the temple structures (Gopuram) contain lot of crevices and dark spaces very often forming dwelling places for birds and bats. They could possibly provide substratum for the growth of fungi. It is also observed that the temple constructions are mainly done with granite materials, usually remaining cooler than the surrounding city conditions. This temperature factor could be one reason that may have favored fungal growth. These are all only the possible factors which could have contributed to the presence of fungal spores in the studies areas.

Conclusion

The incidence of fungal spores in the temple premises relies on the reasons given in the present work those dark conditions, presence of biogenic materials, high content of food materials, moisture contents and bird droppings, all of which could attract fungal colonies in temple premises. The alarming issue is that a few of these fungi are potentially allergenic and pathogenic, highlighting the importance of containing them to within manageable levels. The temple premises should be frequently wiped out in order to remove excess water and should be exposed to sunlight for a longer duration of the day. Stricter discipline should be imposed on the visitors and devotees regarding usage of edibles and their disposal. The premises must also be cleaned frequently by temple authorities. Special attention must be paid especially during rainy seasons when the fungal content coupled with humidity could be potentially dangerous.

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