

## Aerometric study on thermophilous fungi in a farm house, Chennai

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A survey on airborne thermophilous fungi at a farmhouse in Chennai, India was made using an Andersen 2-stage viable sampler for the period from January 1997 to December 1997 at fortnight intervals. A total of 582 colonies belonging to 17 species were recorded. The species *Emericella nidulans* was dominant with an average of 60.2 CFU/m<sup>3</sup> of air followed by *Aspergillus fumigatus* and *Thermomyces lanuginosus* with 34.7 CFU/m<sup>3</sup> of air and 32.2 CFU/m<sup>3</sup> of air, respectively. The total respirable fraction recorded was 58.4 %.

**Key words:** airborne fungi, Andersen 2-stage sampler, occupational environment, respirable fraction, India.

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Termofilní mikroskopické houby vyskytující se v podobě konidií ve vzduchu byly studovány na statku ve městě Chennai v Indii za pomoci dvoustupňového Andersenova aeroskopu, a sice v intervalu 14 dnů od ledna 1997 do prosince roku 1997. Celkem bylo izolováno 582 kolonií patřících 17 druhům hub. Dominantním druhem byla *Emericella nidulans* s průměrným počtem 60.2 CFU/m<sup>3</sup> vzduchu; za ní následovaly druhy *Aspergillus fumigatus* a *Thermomyces lanuginosus* s 34.7 CFU/m<sup>3</sup>, resp. 32.2 CFU/m<sup>3</sup> vzduchu. Celková respirabilní frakce činila 58.4 %.

### INTRODUCTION

The term thermophilous fungi includes both thermophilic and thermotolerant fungi, terms that have been widely used by different authors (Apinis and Pugh 1967, Evans 1972, Hudson 1973, Kuthubutheen and Pugh 1977, Sandhu et al. 1980, Sandhu and Singh 1985). However, Hedger (1974) stressed that any discussion on thermophilic fungi must first underline the adapted definition of thermophilism (Mouchacca 1985). Hence, in the present study the fungi which have an ability to produce colonies at 50 °C are termed as thermophilous fungi. Thermophilous fungi have been isolated from many sources including air (Abdel-Fattah and Swelim 1982, Evans 1972, Hudson 1973, Hughes and Crosier 1973, Jones and Cookson 1985, Rippon et al. 1980). In India only few reports are available regarding airborne thermophilous fungi (Deshmukh and Shukla 1984, Sandhu and Singh 1985). Thakur (1977) dealt with airborne thermophilic fungi near the

farmhouse in Bombay. However, there was no report from Chennai regarding airborne thermophilous fungi. Hence, an aerometric study on thermophilous fungi at a farmhouse in Chennai was conducted.

#### MATERIALS AND METHODS

**The sampler:** The 2-stage Andersen microbial air sampler is a portable sampler using a 12V battery (Andersen Samplers, Inc., Atlanta, Georgia). The air inflow rate of the sampler is 0.028 m<sup>3</sup>/minute. The sampler is made of aluminium with 200 holes arranged in a radial pattern on each stage. The 50 % effective cut-off diameter is 8  $\mu$ m. Thus, the microbial particles on stage 1 are large particle fractions and those on stage 2, the small particle fraction, includes the vast majority of respirable particles, i.e. those less than 5  $\mu$ m in aerodynamic diameter which are deposited in human tracheobronchial and alveolar regions (Jones and Cookson 1983).

**Sampling site:** The sampling site in Chennai (Madras renamed as Chennai, situated at 13°8' N and 80°19' E on the east coast of India) is a farmhouse located opposite the Basinbridge bus station in the northern part of the city. The length of the farmhouse is about 80 m and the width is 64 m. The samples were taken at the central part within the farmhouse. Nearly 400 people are residing in the environment and the number of dairy animal exceeds 300 at the site.

**Sampling procedure:** The portable sampler was disinfected by wiping with 70 % alcohol dipped cotton swabs and then loaded with 2 Petri dishes containing YpSs medium (Cooney and Emerson 1964; yeast extract - 4.0 g, K<sub>2</sub>PO<sub>4</sub> - 1.0 g, MgSO<sub>4</sub> - 0.5 g, soluble starch - 15.0 g and agar - 20.0 g). Streptomycin was added to the medium to arrest the bacterial growth. The sampler was placed at a height of 1 meter and was operated for 5 minutes duration at each sampling. The samples were taken between 10 and 11 o'clock in the morning hours. This was repeated at fortnight intervals starting from January 1997 to December 1997. However, in November only one sample was taken due to flood. After the sampling, the plates were brought to the laboratory and incubated at 50 °C in an incubator. A trough of water was placed within the incubator to avoid dehydration of the media. The developing colonies were counted, isolated and identified after 5 days of incubation.

**Data analysis:** The data received were analysed and presented as average CFU/m<sup>3</sup> of air, relative contribution, isolation frequency and respirable fractions, as follows.

The colonies isolated were converted to Colony Forming Units (CFU)/m<sup>3</sup> of air as follows:

$$X = \frac{y_1 + y_2}{(0.1415)}$$

Whereby  $X$  = no. of CFU/m<sup>3</sup>  
 $y_1$  = number of colonies on plate 1  
 $y_2$  = number of colonies on plate 2  
 0.1415 = amount of sampled air (m<sup>3</sup>)

$$\text{Average CFU/m}^3 \text{ of an individual species} = \frac{\text{Total CFU/m}^3 \text{ of a species}}{\text{Total number of samplings (23)}}$$

$$\text{Relative contribution} = \frac{\text{Total CFU/m}^3 \text{ of an individual species}}{\text{Total number of CFU/m}^3 \text{ of all species}} \times 100$$

$$\text{Isolation frequency} = \frac{\text{No. of samplings in which the species was isolated}}{\text{Total number of samplings (23)}} \times 100$$

$$\text{Respirable fraction} = \frac{\text{Total no. of colonies recorded on plate 2 of the sampler}}{\text{Total no. of colonies recorded on both plates}} \times 100$$

## RESULTS

During the study period (January 1997 to December 1997) 582 colonies of thermophilous fungi belonging to 17 species were recorded. Among the fungi isolated *Emericella nidulans*, *Aspergillus fumigatus* and *Thermomyces lanuginosus* occupied the first, second and third position with 60.1, 34.7 and 32.2 CFU/m<sup>3</sup> of air, respectively, out of a total 178.3 CFU/m<sup>3</sup> of air on average. *Emericella nidulans* contributed to 39.1 % and *Aspergillus fumigatus* contributed to 22.6 % of the total composition.

The isolation frequency of *Emericella nidulans* and *Aspergillus fumigatus* was nearly 70 % and that of *Thermomyces lanuginosus* was 43.5 % of the samplings. The fungi *Aspergillus terreus* and *Myceliophthora thermophila* were isolated from 21.7 % of the samplings. The average CFU/m<sup>3</sup>, percent contribution and isolation frequency per species are given in Table 1.

The maximum amount of CFU/m<sup>3</sup> of air was obtained during the month of August followed by the month of March. In February and July a more or less equal amount of CFU was recorded and similarity was also seen among the months of April, June and December. However, there were no thermophilous fungi recorded in November (Fig. 1).

Species such as *Aspergillus fumigatus*, *Aspergillus terreus* and *Rhizomucor pusillus* had respirable fractions of more than 80 % and the fungi *Emericella*

**Table 1.** List of thermophilous fungi isolated from a farmhouse in Chennai, their average CFU/m<sup>3</sup> of air, relative contribution and isolation frequency.

No.	Species	Average CFU/m <sup>3</sup>	Relative contribution %	Isolation frequency
01	<i>Absidia corymbifera</i>	1.53	0.86	17.39
02	<i>Acremonium</i> sp.	0.30	0.17	4.34
03	<i>Aspergillus fumigatus</i>	34.68	19.58	69.56
04	<i>Aspergillus terreus</i>	11.35	6.41	21.73
05	<i>Chaetomium thermophilum</i> var. <i>coprophilum</i>	0.30	0.17	4.34
06	<i>Chaetomium thermophilum</i> var. <i>dissitum</i>	0.60	0.34	4.34
07	<i>Emericella nidulans</i>	60.16	33.96	73.91
08	<i>Humicola grisea</i> var. <i>thermoidea</i>	2.76	1.55	13.04
09	<i>Humicola insolens</i>	0.30	0.17	4.34
10	<i>Malbranchea cinnamomea</i>	1.22	0.69	17.39
11	<i>Myceliophthora thermophila</i>	14.73	8.31	21.73
12	<i>Rhizomucor pusillus</i>	10.43	5.89	13.04
13	<i>Rhizopus stolonifer</i>	0.30	0.17	4.34
14	<i>Paecilomyces variotii</i>	6.44	3.63	4.34
15	<i>Penicillium dupontii</i>	0.60	0.34	4.34
16	<i>Thermoascus aurantiacus</i>	0.60	0.34	8.69
17	<i>Thermomyces lanuginosus</i>	32.23	18.19	43.47

*nidulans* and *Myceliophthora thermophila* nearly 65 %. The total respirable fraction is given in Fig. 2.

#### DISCUSSION

In farmhouses in general, isolation of thermophilous fungi in higher concentrations depends on the availability of source material within the environment. The huge accumulation of cattle dung, hay material and the urine of animals result in a self-heated pile, which is favourable for the proliferation of thermophilous fungi. Andersen and Coe (1974) reported that moist, sun heated piles of herbivore dung can maintain a temperature suitable for growth of thermophilic fungi. The recovery of *Emericella nidulans* and *Aspergillus fumigatus* in large amounts of CFU/m<sup>3</sup> of air in Chennai is due to their thermotolerant nature and their ability to tolerate wide range of temperatures (Hudson 1973).

The dissemination theory explained by Maheshwari (1997) explains how spores get into hay, wood chips and agricultural produce through air. It explains their presence in dung of herbivores – the spores of thermophilic fungi present in fodder are eaten by the herbivores and are discharged in dung, which heats up when accumulated in mass. The isolation of 17 species from the aerial environment of the farmhouse confirms the dissemination of spores of thermophilous fungi by means of air.

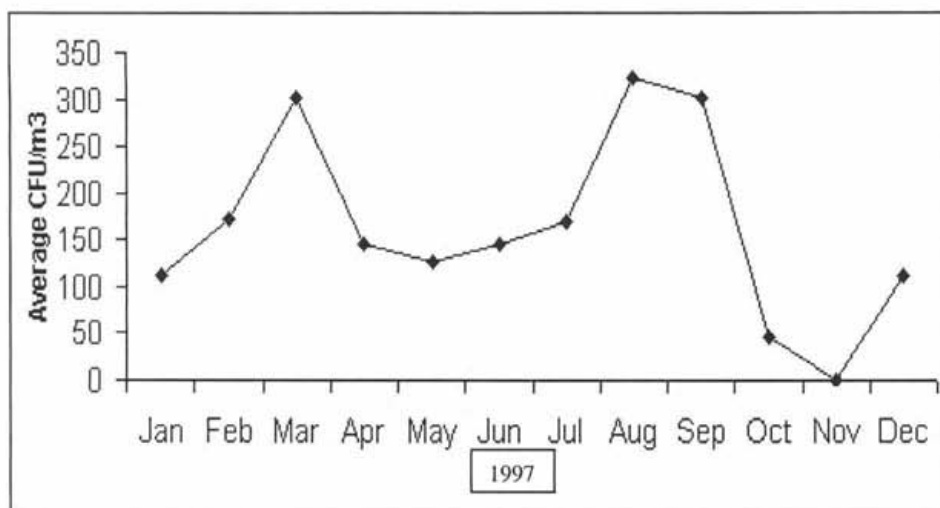


Fig. 1. Seasonal distribution of thermophilous fungi in the atmosphere of the farmhouse.

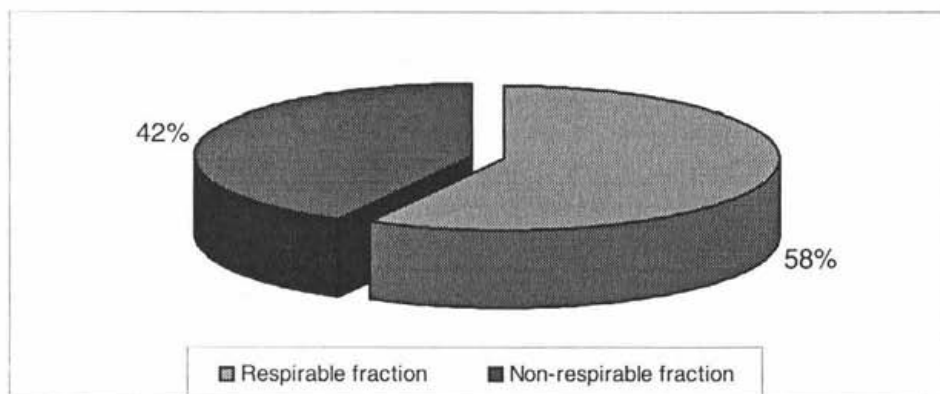


Fig. 2. Total respirable fraction recorded from the farmhouse.

In our study the occurrence of thermophilous fungi reached a peak during the month of August followed by March. The occurrence of the peak in August and the double maxima was already reported by other authors (Evans 1972, Hudson 1973, Sandhu and Singh 1985).

Our study provides year round data on the presence of thermophilous fungi in a farmhouse environment with high human activity. Species such as *Absidia corymbifera*, *Aspergillus fumigatus*, *Rhizomucor pusillus* and *Thermomyces lanuginosus* were already reported as opportunistic pathogens (Hughes and Crosier

1973). Thus, the people and the animals in the environment are prone to exposure to such airborne thermophilous fungi which act as a source of antigens for respiratory hypersensitive syndromes such as Farmer's Lung Disease and Allergic Broncho-Pulmonary Aspergillosis (Gregory and Lacey 1963, Lacey and Lacey 1964, Hughes and Crosier 1973, Tansey and Brock 1978). Hence, further study is required to determine the role of thermophilous fungi in relation to human diseases.

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