



## Airborne fungal spores in a sawmill environment in Palakkad District, Kerala, India

Padmanabhan S. Jothish & Themath Soman Nayar\*

Tropical Botanic Garden and Research Institute, Karimancode PO, Palode, Thiruvananthapuram – 695 562 Kerala, India

(\*author for correspondence, e-mail: tsnayarthemath@yahoo.co.in; fax: 91-(0)472 2869646)

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### Abstract

Concentration of airborne fungal spores in indoor and outdoor environments of a sawmill in Palakkad district of Kerala, India was studied with Burkard Personal Slide Sampler from January to December 1997. Total spore concentration in the indoor and outdoor showed a 3:2 ratio. Higher spore count was observed in indoor in January and in outdoor in October. Thirty three fungal spore types were identified from the indoor and twenty six from the outdoor. *Aspergillus/Penicillium*, *Cladosporium*, *Nigrospora*, *Ganoderma*, 'other basidiospores' and ascospores were the dominant components of the airspora. *Aspergillus/Penicillium*, the most dominant spore type in the indoor contributed 51.19% and *Cladosporium*, the most dominant spore type in the outdoor contributed 44.75% of the total spores. The study revealed high prevalence of predominantly allergenic fungal spores in the sawmill environment.

### 1. Introduction

Airborne fungal spores are one of the major biopollutants capable of causing allergic disorders (Burge, 1989; Lacey and Dutkiewicz, 1994; Verhoeff and Burge, 1997). People who are constantly exposed to an indoor environment where works on organic substances and their byproducts are dealt with, may often develop respiratory disorders. A qualitative and quantitative knowledge of fungal spore types encountered in such working environments is an essential prerequisite for the effective diagnosis and treatment of allergic disorders.

Kerala, which occupies an area of 38,863 square km, is one of the states in India with the highest number of sawmills (Wealth of India, 1971). There are about 1400 registered sawmills in Kerala (Muraleedharan and Bhat, 1989) providing direct employment to about 15,000 people. The sawmilling industry in the state, which processes about 70% of the total round wood, including imported wood, available, plays a vital role in the economy of Kerala. Though airborne fungi occurring in different indoor environments such

as libraries, markets, flour mills, poultry farms, hospital wards, bakeries etc., have been reported from different parts of India (Narayan et al., 1982; Vittal and Glory, 1985; Misra and Jamil, 1991; Singh and Singh, 1994; Surekha et al., 1996; Sharma and Datta, 2001) no such study has been conducted so far with reference to year round airborne fungi of sawmill environment in India. Reports of such studies are available from Scandinavian (Jappinen et al., 1987), French (Simeray et al., 1997), Canadian (Duchaine et al., 2000) and Polish (Dutkiewicz et al., 2001a) sawmills. The present one year study records prevalence of airborne fungal spores of a sawmill in Palakkad district in Kerala and is the first report of its kind from India.

### 2. Materials and methods

#### 2.1 Study site

The sawmill subjected to sampling has a 23 cent compound fenced with wooden planks. The sawing

machine was housed in a wooden shed thatched with tar sheets. Processed wooden planks and chips were piled up inside the shed. Huge wooden logs were heaped for sizing and chopping in the compound, which was mostly covered with sawdust and defoliated barks. Eight people worked in the mill as daily wagers.

## 2.2 Sampling procedure

Sampling was conducted using a Burkard Personal Slide Sampler (Burkard Manufacturing co, Rickmansworth, UK) for one year at 15 day intervals from January to December 1997. Exposures were made for 10 minutes at human height between 9.00 and 11.00 am which recorded, as per our standardization trial, maximum concentration of airborne spores. The sampler sucks 10 litres of air per minute. The area inside the shed was treated as an indoor environment and a site selected 100 metres away from the sawmill, the outdoor environment.

Exposed slides were mounted in glycerin jelly stained with basic fuschin. All fungal spores from exposed slides were examined, counted and identified under 40X and 100X objectives of a Nikon Labophot-2 optical microscope. Results are expressed as total spores  $m^{-3}$ . Spores of *Aspergillus* and *Penicillium*, which could not be distinguished separately under optical microscope, are categorized under one group. Basidiospores other than *Ganoderma* and spores of rusts and smuts are grouped under 'other basidiospores'.

## 2.3 Climate

Kerala experiences wet (June to November; 21 °C–30 °C) dry (December to February; 18 °C–33 °C) and hot (March to May; 23 °C–37 °C) seasons. Temperature, relative humidity and wind velocity were recorded at both indoor and outdoor sampling sites.

## 3. Results

Fungal spores were present throughout the year. They were found more in the indoor air than in the outdoor. In the indoor, the highest spore count was observed in January (5500 spores  $m^{-3}$ ) and the lowest in August (1090 spores  $m^{-3}$ ). In the outdoor the highest count was seen in October (3650 spores  $m^{-3}$ ) and the lowest in February (340 spores  $m^{-3}$ ). In August, both the indoor

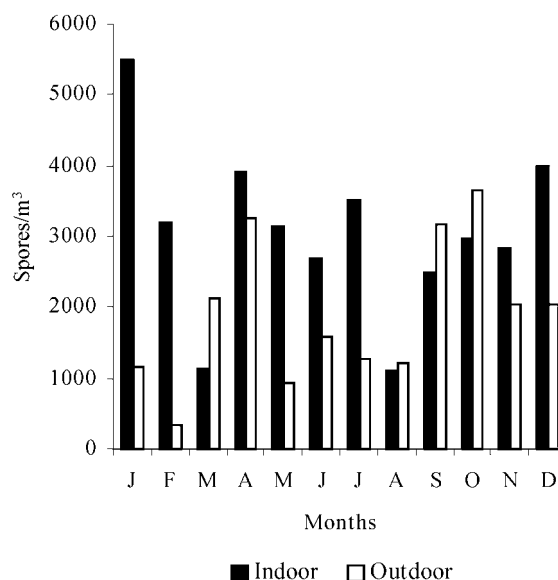


Figure 1. Monthly total spore distribution in the indoor and outdoor of sawmill environment.

and outdoor fungal concentrations remained more or less at the same level (Figure 1).

### 3.1 Indoor airspora

Thirty three fungal spore types were observed in the sawmill atmosphere. *Aspergillus/Penicillium*, *Cladosporium*, *Nigrospora* and 'other basidiospores' were the dominant component of the airspora. They were present throughout the year and they together constituted 89.48% of the total airspora. Rest of the spore types which were not present throughout the year collectively constituted 10.1% (see Table 1). Four fungal spore types that constituted 0.43% could not be identified.

*Aspergillus/Penicillium* was the dominant component in the indoor air with a total concentration of 18,650 spores  $m^{-3}$ . Its highest concentration was recorded in December (3250 spores  $m^{-3}$ ) and the lowest in March (250 spores  $m^{-3}$ ). *Cladosporium*, which constituted 12000 spores  $m^{-3}$ , peaked in January (3360 spores  $m^{-3}$ ) recording the lowest concentration in December (210 spores  $m^{-3}$ ). 'Other basidiospores' which comprised 2.58% of the total airborne spores registered the highest concentration in July and November (210 spores  $m^{-3}$  each) and the lowest in March (10 spores  $m^{-3}$ ). *Nigrospora*, like *Aspergillus/Penicillium*, showed the highest concen-

Table 1. Total spore concentration and percentage contribution of spores present in indoor and outdoor of sawmill.

| Spore types                    | Indoor                       |            | Outdoor                      |            |
|--------------------------------|------------------------------|------------|------------------------------|------------|
|                                | Total spores m <sup>-3</sup> | Percentage | Total spores m <sup>-3</sup> | Percentage |
| <i>Alternaria</i>              | 390                          | 1.07       | 240                          | 1.05       |
| Ascospores                     | 570                          | 1.56       | 740                          | 3.25       |
| <i>Aspergillus/Penicillium</i> | 18650                        | 51.19      | 7190                         | 31.6       |
| <i>Beltrania</i>               | 10                           | 0.03       | –                            | –          |
| <i>Bispora</i>                 | 130                          | 0.36       | –                            | –          |
| <i>Botriodiploidea</i>         | 330                          | 0.9        | 50                           | 0.22       |
| <i>Cercospora</i>              | –                            | –          | 10                           | 0.04       |
| <i>Chaetomium</i>              | 20                           | 0.05       | 30                           | 0.13       |
| <i>Choanephora</i>             | 10                           | 0.03       | –                            | –          |
| <i>Cladosporium</i>            | 12000                        | 32.94      | 10180                        | 44.75      |
| <i>Corynespora</i>             | 60                           | 0.16       | –                            | –          |
| <i>Curvularia</i>              | 220                          | 0.6        | 150                          | 0.66       |
| <i>Dendriphiella</i>           | 10                           | 0.03       | –                            | –          |
| <i>Dictyoarthrinium</i>        | 30                           | 0.08       | –                            | –          |
| <i>Drechslera</i>              | 90                           | 0.25       | 130                          | 0.57       |
| <i>Epicoccum</i>               | 60                           | 0.16       | –                            | –          |
| <i>Ganoderma</i>               | 370                          | 1.01       | 800                          | 3.51       |
| <i>Helminthosporium</i>        | 10                           | 0.03       | –                            | –          |
| <i>Melanographium</i>          | 10                           | 0.03       | –                            | –          |
| <i>Nigrospora</i>              | 1010                         | 2.77       | 810                          | 3.56       |
| Other basidiospores            | 940                          | 2.58       | 1140                         | 5.01       |
| <i>Periconia</i>               | 250                          | 0.69       | 50                           | 0.22       |
| <i>Pestalotiopsis</i>          | 60                           | 0.16       | 30                           | 0.13       |
| <i>Pithomyces</i>              | 200                          | 0.54       | 220                          | 0.96       |
| Rusts/smuts                    | 500                          | 1.37       | 430                          | 1.89       |
| <i>Sporidesmium</i>            | 40                           | 0.11       | 30                           | 0.13       |
| <i>Tetraploa</i>               | 10                           | 0.03       | –                            | –          |
| <i>Thermomyces</i>             | 10                           | 0.03       | 10                           | 0.04       |
| <i>Torula</i>                  | 200                          | 0.54       | 220                          | 0.96       |
| <i>Trichoconis</i>             | 80                           | 0.22       | 20                           | 0.09       |
| Unidentified                   | 160                          | 0.43       | 270                          | 1.18       |

tration in December (230 spores m<sup>-3</sup>) and the lowest in May (20 spores m<sup>-3</sup>) (Figure 2).

Ascospores (570 spores m<sup>-3</sup>) were absent in March and April, spores of rusts and smuts (500 spores m<sup>-3</sup>) in March, May, June and August, *Ganoderma* (370 spores m<sup>-3</sup>) in February, March, June and July and *Alternaria* (390 spores m<sup>-3</sup>) in April, July, September, October and December. Other spore types occurred in very small concentrations (Table 1) and were found sporadically.

### 3.2 Outdoor airspora

A total of 26 spore types were captured from the outdoor air. *Cladosporium* and *Aspergillus/Penicillium* were the most dominant types. They were found throughout the year and together they constituted 76.35% of the total airspora. Rest of the identified spore types comprised 22.47%. Six spore types that constituted 1.18% of the airspora could not be identified. Spores of *Nigrospora*, *Ganoderma*, *Alternaria*, 'other basidiospores', ascospores and spores of rusts and smuts were not recorded throughout the year though they individually contributed more than 1%

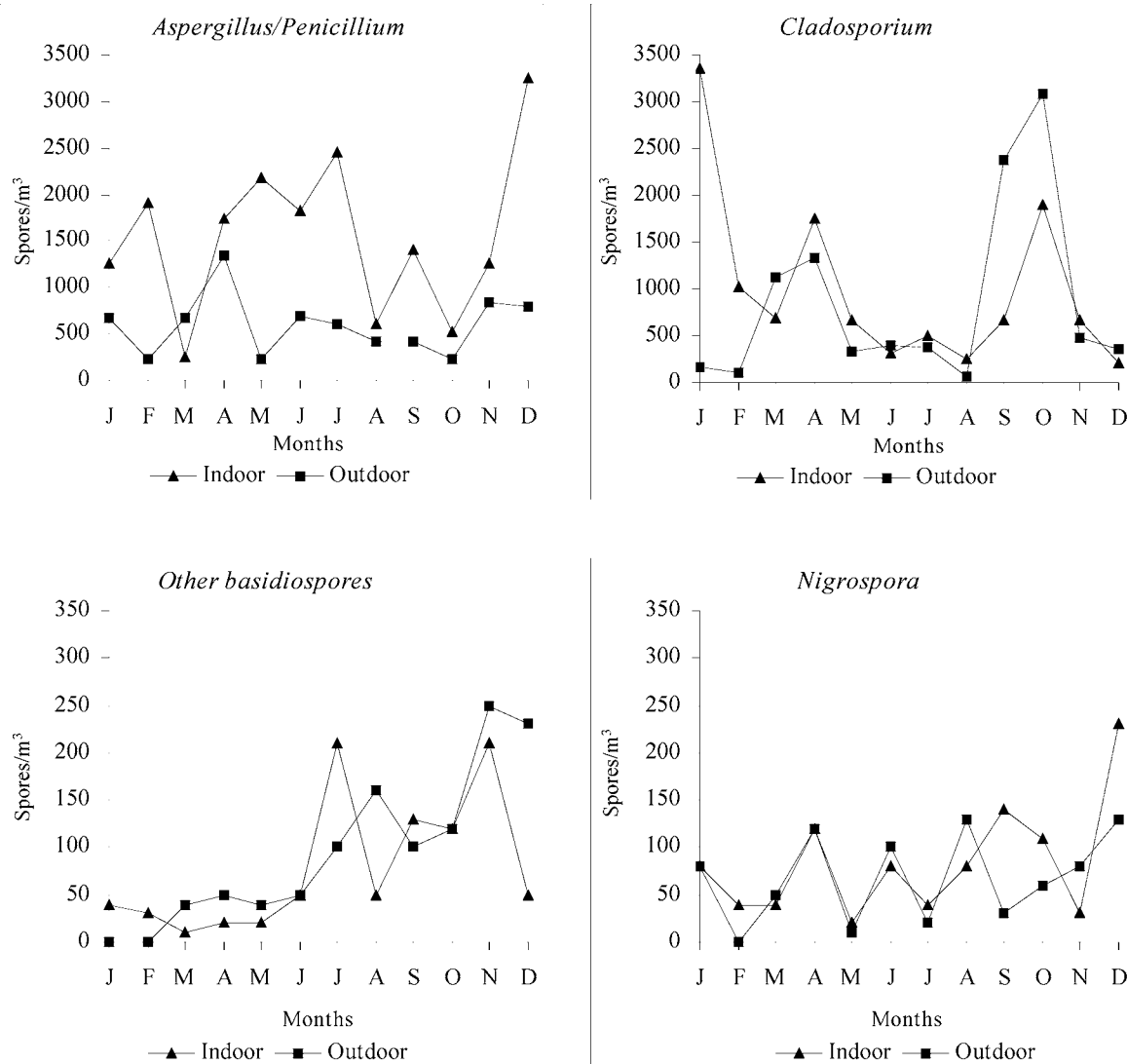


Figure 2. Monthly spore distribution of *Aspergillus/Penicillium*, *Cladosporium*, 'other basidiospore' and *Nigrospora* in indoor and outdoor of sawmill environment.

of the total spore concentration to the outdoor air (Table 1).

*Cladosporium*, the dominant and major spore type constituted 10,180 spores  $m^{-3}$  of the total airspora. It showed the highest spore incidence in October (3080 spores  $m^{-3}$ ) and the least in August (60 spores  $m^{-3}$ ). *Aspergillus/Penicillium* contributed 7190 spores  $m^{-3}$  to the total airspora in the outdoor air. Its maximum concentration was observed in April (1350 spores  $m^{-3}$ ) and minimum in February (230 spores  $m^{-3}$ ) (Figure 2).

'Other basidiospores' and *Ganoderma* which comprised 1140 spores  $m^{-3}$  and 800 spores  $m^{-3}$  respectively were absent in January and February. *Nigrospora* (810 spores  $m^{-3}$ ) and ascospores (740 spores  $m^{-3}$ ) were not recorded in February. Spores of rusts and smuts (430 spores  $m^{-3}$ ) were absent in January, February and April while *Alternaria* (240 spores  $m^{-3}$ ) was absent from February to April and August to October. 'Other basidiospores' showed the highest concentration in November (250 spores  $m^{-3}$ ), *Ganoderma* in August (230 spores  $m^{-3}$ ), ascospores

in December ( $240 \text{ spores m}^{-3}$ ) and *Nigrospora* in August and December ( $120 \text{ spores m}^{-3}$  each).

When monthly total airspora concentration and dominant spore types such as *Aspergillus/Penicillium*, *Cladosporium*, 'other basidiospores', *Ganoderma*, *Nigrospora* and ascospores in indoor and outdoor were tested for any significant differences using ANOVA at  $p\text{-value} < 0.05$ , it was found that only monthly total airspores and *Aspergillus/Penicillium* showed significant difference.

#### 4. Discussion

Sawmills and wood working shops let out large quantities of fungal spores along with sawdust into the air. The present survey demonstrated that the indoor environment registered more total fungal spore concentration and spore types than the outdoor environment with an indoor/outdoor ratio of 3:2. Monthly spore incidence showed that all the eight months except March, August, September and October showed high concentration in the indoor (Figure 1). The highest spore incidence in the indoor occurred in January and in the outdoor in October. In January and February indoor showed much higher spore concentration. Higher temperature during these months might have reduced the growth rate of fungi in the outdoor. The indoor environment, on the otherhand, provided suitable growth substrates such as wood and wood dust for the growth of fungi. The study conducted by Simeray et al. (1997) in French sawmills showed that the number of colony forming units per cubic meter of indoor air was always above or equal to that observed from outdoor, an observation which draws similarity with the findings of the present work.

*Aspergillus/Penicillium* was the dominant and most abundant spore type inside the sawmill environment while *Cladosporium* was so in the outdoor. Dutkiewicz et al. (2001a, b) also observed *Aspergillus* and *Penicillium* as the dominant genera in Polish sawmills and fiberboard and chipboard factories whereas Simeray et al. (1997) detected *Penicillium* as the predominant form in French sawmills. Fungal genera that are normally not abundant in the outdoor but are associated with the indoor contamination include *Aspergillus* and *Penicillium* (Burge, 1989; Levetin et al., 1995). This is due to the availability of sources in the indoor sites. Grant et al. (1989) expressed the opinion that mold species appear on a substrate in a succession according to their moisture requirements.

Accordingly, the primary colonisers are species of *Aspergillus* and *Penicillium* which can grow even on temporarily wet surfaces and sporulate. Thus these fungi can colonize on any wet surfaces like wet wood logs, planks, chips, and sawdust which are available in plenty in sawmills. Presence of prolonged rainy season in Kerala (June to November) provides more favourable conditions. Predominance of *Aspergillus/Penicillium* has also been reported from other indoor environments such as a library (Vittal and Glory, 1985), a flour mill (Misra and Jamil, 1991), a bakery (Singh and Singh, 1994) and schools (Levetin et al., 1995).

*Cladosporium* was the second dominant spore type. *Aspergillus/Penicillium* and *Cladosporium* showed a ratio of 3:2 and 2:3 in indoor and outdoor environments respectively. The peak concentration of total spores in indoor in January and outdoor in October was due to the abundance of *Cladosporium* spores (cf. Figures 1 and 2). But Simeray et al. (1997) reported *Cladosporium* as the most dominant spore type in French sawmills. They were more in sawmills which processed coniferous wood. Studies conducted in Polish sawmills also showed *Cladosporium* as a significant constituent in the airspora (Dutkiewicz et al., 2001a). Air sampling conducted in the extramural environments of India and elsewhere in tropical countries (Janakibai and Subbareddi, 1981; Vittal and Krishnamoorthi, 1988; Lim et al., 1998; Majumdar and Bhattacharya, 2000) have also shown dominance of *Cladosporium* in the outdoor environment.

Most of the studies conducted in sawmills were confined to the indoor environments representing total microorganisms including bacteria, actinomycetes and fungal spores (Jappinen et al., 1987; Kolmodin-Hedman et al., 1987; Halpin et al., 1994; Dutkiewicz et al., 2001a). The present study which sampled indoor and outdoor but confined to fungal spores alone recorded a mean total of  $1.52 \times 10^3 \text{ spores m}^{-3}$  in the indoor. Simeray et al. (1997) observed a mean total of  $1.5 \times 10^3$ , almost equal to what we observed, in the indoor of French sawmills which processed conifer wood and  $2.11 \times 10^3$  in sawmills which processed hardwood. In Polish sawmills Dutkiewicz et al. (2001a) found total fungal spore concentration at  $4.2 \times 10^3$  and at  $3.9 \times 10^3$ , the former processing coniferous wood and later processing deciduous wood. Jappinen et al. (1987) and Kolmodin-Hedman et al. (1987) found large quantity of fungal spores ranging from  $10^2$  to  $10^8 \text{ CFU m}^{-3}$  in the studies conducted by them in Scandinavian sawmills.

The present study detected remarkable diversity in spore types. Thirty three spore types were recorded from the sawmill environment. Simeray et al. (1997) recorded 30 genera of fungi in French sawmills and Dutkiewicz et al. (2001a, b) observed 21 types in Polish sawmills and 16 types in fiberboard and chipboard factories. As far as the number of spore types are concerned, our observation draws a close comparison with that of French sawmills. The spore concentration and diversity of spore types appear to be varying depending on the type and nature of wood processed, wood processing cycle and environmental condition of sawmill (Jappinen et al., 1987; Kolmodin-Hedman et al., 1987; Halpin et al., 1994; Simeray et al., 1997; Dutkiewicz et al., 2001a, b). Since this study was conducted in a sawmill where all types of wood were being processed, the specific effect of a particular wood on spore concentration and on spore types could not be assessed.

*Ganoderma*, 'other basidiospores', ascospores and *Nigrospora* showed double or more total spore concentration in the outdoor (see Table 1). Lack of suitable growth substrates might be the main reason for less incidence of these fungi inside the sawmill. Their presence in the indoor could also be attributed to their free flow from the outdoor air. *Ganoderma* and 'other basidiospores' were found abundant during the rainy months (July to November). Lacey (1990) observed abundance of basidiospores during rainy period in tropical countries. Rainy season accelerates development of sporocarps and release of more spores because of high humidity and water availability. Other spore types which intermittently occurred in the sawmill belonging to hyphomycetes (*Curvularia*, *Drechslera*, *Torula*, *Corynespora*, *Trichoconis*, *Periconia* etc.). They are mostly parasitic and saprophytic forms, being liberated from infected wood stored in forests, lumber yards and sawmill compounds.

The main source of biopollution in sawmill atmosphere is from primary or secondary infection of timber and wood logs by microorganisms and from sawdust (Dutkiewicz et al., 2001a). Primary infection develops in timber logs that are stored in forests and lumber yards whilst secondary infection proceeds on chopped wood and sawdust. In sawmill environments of Kerala, sources are abundant for fungal growth, as most of the wood logs and chopped wood are kept for prolonged periods in open air. Timber during such periods of storage is attacked by various fungi. Moreover, wood logs brought to the sawmills from forests and lumber yards would have already

be encolonised by moulds. Waste wood chips, poles, defoliated barks and sawdust spreadout in the sawmill compounds make the environment all the more suitable for fungal growth. These fungal spore reservoirs are disturbed and spores are released into the air when wood logs and planks are handled by workers.

## 5. Conclusion

The study showed that there were abundant and diverse mycoflora in the sawmill atmosphere. *Aspergillus/Penicillium*, *Cladosporium*, 'other basidiospores', *Nigrospora* and *Ganoderma* constituted the dominant components in the airspora. Some of these spore types are hazardous because they are allergenic. Eventhough threshold concentrations of most of these spores eliciting allergic symptoms are unknown, in sensitive individuals even small quantities may elicit allergic symptoms.

Since moulds enter the sawmill environment along with the wood logs brought in, it is difficult to avoid the entry of fungal spores into sawmills. Hence, it is essential that workers who handle wood, wood logs and saw dust take precautionary measures to avoid allergic diseases. Modernisation of sawmills with advanced technology such as usage of band or laser guided saws with high performance particle aspirator system would certainly reduce the rate of fungal spore concentration in sawmill atmosphere.

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