

Allergic potential of some *Aspergilli* on saw mill workers in Lucknow, India

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Abstract

Fifty fungal types were isolated from the indoor atmosphere of saw mills by exposing Petri plates containing Czapek-dox Agar, Potato-dextrose Agar and Sabouraud Agar media for 5 min. The fungal flora of the outdoor surroundings was also studied for comparison. Species of *Aspergilli* dominated in the saw mills, being represented by 16 species including one ascospore form. Other fungi were species of *Cladosporium*, *Alternaria*, *Curvularia*, *Penicillium*, *Fusarium*, etc. Variations in the fungal population in different months were also observed. Fungal spores recovered using the Rotorod Sampler were *Alternaria*, *Curvularia lunata*, *Curvularia tetramera*, *Cladosporium*, *Dreschlera* sp., *Epicoccum* sp., *Pithomyces* sp., *Nigrospora*, *Stemphylium* sp. and *Torula* sp. Mycelial fragments and unidentifiable spores were also seen in abundance. Varying allergic responses of patients were also recorded by testing intradermally, the antigens of nine *Aspergilli*, viz *Aspergillus flavus*, *A. fumigatus*, *A. japonicus*, *A. melleus*, *A. nidulans*, *A. niger*, *A. niveus*, *A. tamarii* and *A. terreus*. Copyright © 1996 Elsevier Science Ireland Ltd.

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1. Introduction

In India as industries develop both in size and complexity, occupational health will pose new and more difficult problems. The national government has recognized the need for protecting the health of workers. The directive principles of state policy in the Indian Constitution are important in this context.

Occupational health is essentially preventive medicine, the prevention of diseases and maintenance of the highest degree of physical, mental and social well-being of workers in all occupations. Many allergic respiratory diseases are known to be caused by airborne fungal spores because of their high concentration in the working environment (Prince et al., 1964; Agarwal et al., 1969; Gupta and Singh, 1983; Mehta and Sandhu, 1983; Blomquist et al., 1984; Shukla and

Misra, 1986). Hence, the present investigation has been carried out to examine the concentration, type and composition of different aeromycoflora of saw mills in Lucknow and also their possible role in causing allergic diseases among workers.

There are over 100 saw mills in and around Lucknow with size ranging from 30' × 35' × 10' to 85' × 70' × 15'. The actual sawing area is approximately 15' × 15' where the machines are installed and covered with tin shed. The rest of the area is used to store logs of wood. Ten saw mills of different sizes were selected and studies were carried out once a month in each of the saw mills for a period of 12 months.

2. Materials and methods

Air sampling was carried out using the gravity sedimentation method (Gregory, 1961). Czapek-dox agar medium was used with antibiotic (40 units/ml) to in-

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Table 1
Different types of wood cut in saw mills

Sr.no.	Common name	Botanical name	Frequency of sawing ^a	Common complaints experienced by the workers ^b
1.	Aam	<i>Mangifera indica</i>	High	Cough, chest stiffness
2.	Babul	<i>Acacia arabica</i>	Low	Itching in eyes
3.	Chir	<i>Pinus roxburghii</i>	High	Headache, sneezing
4.	Deodar	<i>Cedrus deodara</i>	High	Headache
5.	Jamun	<i>Syzynium cumini</i>	Low	Itching in eyes
6.	Mahua	<i>Madhuca indica</i>	Low	Itching in eyes
7.	Neem	<i>Azadirachta indica</i>	High	Cough, chest burning
8.	Sal	<i>Shorea robusta</i>	High	Cough, headache
9.	Sagwan	<i>Tectona grandis</i>	High	Cough, chest stiffness
10.	Shisham	<i>Dalbergia sisso</i>	High	Chest stiffness

^aHigh, 5–6 h per day; Low, 1–2 h per day. Normal working hours, 8–10 h per day.

^bAverage number of workers, 4–12. Age range, 17–65 years.

hibit bacterial growth. The Petri plates (9 cm diameter) were kept at the four corners of the sawing machine at a height of 150 cm and one Petri plate was placed in the middle of the machine at a height of 250 cm. The plates were exposed three times a day for 5 min, between 10:30 h and 16:00 h; Simultaneously, Petri dishes containing the same medium were exposed outside the mills to compare the concentration for indoor and outdoor environments. The exposed Petri plates were incubated in the laboratory at $27 \pm 1^\circ\text{C}$ and the colonies which appeared from the 3rd to 7th day were counted.

Daily temperatures (max. and min.), relative humidity (max. and min.) and monthly rainfall were obtained from the Regional Meteorological Office situated at Amousi Airport and monthly averages were calculated.

The Rotorod Air Sampler of Perkins (1957) modified by Harrington (1959) had two arms 4 cm in length and 0.159 cm wide set apart on the rotating axis with a diameter of 6 cm. The rotorod was kept near the sawing machine at a height of 2 m and was run for 30 min, 10 days a month for 12 months when the mill was under operation. The leading edges of the collecting arms were covered with clear tape and coated with glycerine jelly to retain spores. The exposed tapes were then mounted on clean glass slides with glycerine jelly. The slides were scanned and spores identified. The frequency of occurrence of spores was calculated using the formula:

$$\frac{\text{Total no. of specific fungal spores observed}}{\text{Total no. of fungal spores observed}} \times 100$$

Antigens were prepared by the technique outlined by Sheldon et al. (1967). The *Aspergilli* were cultured in sabouraud broth (pH 8.0) for 15–20 days at $27 \pm 1^\circ\text{C}$. The harvested mats were washed with distilled water, using Whatman filter paper, to remove the traces of culture medium and then lyophilized. The lyophilized spores were pulverized and then defatted using ether

(48 h at 4°C). Antigens were extracted in Cocas buffer, centrifuged at 5000 rev./min, filter sterilized and stored at 4°C . Allergy tests were performed on the upper and lower arms of patients by injecting 0.01 ml of the antigen (dilution 1:500) into the intradermal layer. Buffer saline was used as a control test (Misra et al., 1988). Reactions were recorded within 15–20 min of injection and were graded according to Agarwal et al. (1980) and indices calculated using the Voorhost formula (Voorhost, 1980).

$$\text{Voorhost index} = 1/2[(L + W)\text{Flare} + 3.7 \\ + 11.0\text{Log}(L + W)\text{Wheal}]$$

where L is length and W , width.

3. Results and discussion

General information regarding variety of wood being cut and details of workers is shown in Table 1. Based on personal interviews with the workers, we have tried to tabulate the common health related complaints experienced by them in the course of their working with specific varieties of wood.

Fifty fungal species were identified from the indoor air samples from the saw mills and 41 species from the outdoor environment near the mills. *Aspergilli* dominated the samples, both indoor and outdoor, being represented by 16 species including *Emmericella nidulans* var. lata. *Fusarium*, *Cladosporium*, *Curvularia*, *Alternaria*, *Penicillium* and *Phoma* were also represented. It is clear that seasons affect the phenology of fungi. During the rainy and winter seasons, higher numbers of species of fungi were isolated, the highest occurring in November (47 species); while in the hot summer months, few species were isolated (Table 2). Negative and significant correlation exists at the 5% level of probability between temperature and total fungal species isolated ($r = -0.62298$).

Table 2
Effect of seasons on fungal spores recovered

Season	Month	Temperature (°C)		Humidity	Rainfall (mm)	Fungal species isolated	<i>Aspergilli</i> (%)
		Max.	Min.				
Winter	Nov.	27	13	74	—	47	33
	Dec.	25	10	72	10	45	35
	Jan.	23	8	72	—	40	35
	Feb.	25	10	71	30	36	32
Summer	Mar.	34	15	51	10	35	32
	Apr.	37	20	38	—	21	62
	May	39	26	41	10	18	74
	Jun.	39	23	60	220	20	63
Rainy	Jul.	33	25	82	290	24	59
	Aug.	34	25	79	150	38	52
	Sep.	34	23	68	60	44	42
	Oct.	30	19	80	200	46	38

Values are monthly averages.

The spores of 10 different types were identified from the Rotorod Sampler caches (Table 3). Unidentified spores constituted a high percentage. The spores of *Aspergilli*, *Penicillium*, *Rhizopus* and *Mucor* have a similar structure. We are of the opinion that these genera constitute a major part of the unidentified spores, the rest being mycelial fragments and other unidentified spores. This argument may be confirmed by the fact that *Aspergilli* is the dominant genera isolated by the gravity sedimentation method.

Antigens prepared from species of *Aspergilli*, tested for their allergic responses, gave positive responses in 60–98% of the patients tested (Table 4).

4. Conclusion

Fifty species of fungi were identified from the saw mills compared to 41 from the outdoor environment. This agrees with the studies of Gregory (1961), who observed that fungi in the indoor environment occur in greater numbers than outdoors. The findings of Jayaprakash and Ramalingam (1981, 1983), Mallea et

al. (1982), Sinha et al. (1984) and Tilak and Saibaba (1984) also agree with our observations.

The genus *Aspergillus* is the dominant fungi recovered from the saw mill environments; the reports of Jayaprakash et al. (1978), Tilak and Chakre (1979), Santra and Chandra (1981) and Mehta and Sandhu (1983) have also recorded the dominance of *Aspergillus* in the indoor environment.

The presence of species of *Phoma* in the air of saw mills was probably because *Phoma* is known to inhabit wood materials. The subdominant species of *Alternaria*, *Cladosporium*, *Fusarium*, *Curvularia* and *Penicillium* are the most common fungal species recorded from the saw mill environment (Wimander and Belin, 1980). Occurrence of fungal species in higher numbers during cooler months was probably due to the lower temperatures favouring their growth, while during hotter months only a few species, mainly *Aspergillus*, are favoured.

All the fungal antigens tested on patients showed positive reactions, indicating that fungal components of the atmosphere in the saw mills have the potential to cause respiratory allergic diseases. Our results agree with those who have reported significant allergic re-

Table 3
Fungal spores structures recovered using Rotorod Sampler

<i>Alternarium</i> sp.	14%
<i>Cladosporium</i> sp.	12%
<i>Curvularia lunata</i>	11%
<i>Curvularia tetramera</i>	8%
<i>Drechslera</i> sp.	6%
<i>Epicoccum</i> sp.	4%
<i>Nigrospora</i> sp.	8%
<i>Pithomyces</i> sp.	2%
<i>Stemphylium</i> sp.	3%
<i>Torula</i> sp.	2%
Mycelial fragments and unidentified spores	30%

Table 4
Allergic potential of *Aspergilli*

Antigen	Patients tested	Positive cases (%)
<i>A. flavus</i>	62	82.25
<i>A. fumigatus</i>	40	90.00
<i>A. japonicus</i>	40	70.00
<i>A. melleus</i>	40	92.00
<i>A. nidulans</i>	40	60.00
<i>A. niger</i>	40	98.00
<i>A. niveus</i>	40	97.50
<i>A. tammarii</i>	40	80.00
<i>A. terreus</i>	62	90.35

sponses to *Aspergilli* among the human population (Wittich and Stakman, 1937; Darke et al., 1976; Mehta and Sandhu, 1983; Misra et al., 1988).

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