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Prevalence of culturable and non-culturable airborne fungi in a grain store in Delhi

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Abstract

Fungal spores are important aeroallergens and proper knowledge of their qualitative and quantitative prevalence in indoor and outdoor environments is of paramount importance in the study of allergic disorders. The present investigation was aimed at the study of seasonal and annual prevalence of fungi inside a large grain storage facility. Sampling was carried out from September 1989 to August 1991. Although fungal spores occurred throughout the year there was seasonal variation. *Aspergillus flavus, Cladosporium* spp. *Epicoccum nigrum* and basidiospores had a definite seasonal pattern. *Aspergillus flavus* and *Ustilago* (smuts) were the most predominant fungi in the facility. Their concentration was significantly higher ($P < 0.05$) than in outside air. The need to reduce fungal concentration in grain storage is emphasised.

Keywords: Culturable; Non-culturable; Airborne fungi; Granary

1. Introduction

The incidence of respiratory symptoms among granary workers caused by grain dust has been known for a long time (Ramazzini, 1713; Harris, 1939; Lacey, 1980; Magan and Lacey, 1988; Mishra et al., 1992). These range from wheezing, breathlessness, rhinitis, coughing and conjunctivitis to allergic dermatitis. Grain dust is a complex material composed of plant fragments, fungi, actinomycetes, insect debris, mites, etc. and causes occupational lung diseases (Cohen and Osgood, 1953; Lacey, 1990; O'Neill et al., 1991). The components of grain dust that cause these symptoms often involve hypersensitivity to some of the spores of fungi. These fungal spores also cause rapid deterioration of grain, their nutritive value, germination and produce mycotoxins. Thus, a proper knowledge of the qualitative and quantitative prevalence of culturable and non-culturable moulds is of great importance, not only in the diagnosis and treatment of respiratory allergic patients, but also in grain management. In India only scant information is available on the types of fungi prevailing in grain storage. The present study was therefore aimed at investigating the qualitative and quantitative prevalence of different airborne fungi in a large grain store in the Delhi metropolis.

2. Materials and methods

The Central Warehouse Corporation, a large storage facility, located in Delhi was selected for the purposes of this study. The survey was carried out from September 1989 to August 1991, for a period of 2 consecutive years. Air samples were collected three times a month, at 10-day regular intervals, using an Andersen six-stage volumetric sampler (Andersen, 1958) to study the colony forming units (CFUs). A Burkard personal slide sampler (Burkard Manufacturing Co., UK) was used to measure non-culturable fungal spore loads, in addition to culturable fungi. Petri dishes containing Sabouraud's agar medium with Rose Bengal dye, and slides smeared with safranine stained glycerine jelly, were exposed for 10 min each time between 11–12 h in Andersen and Burkard samplers, respectively. The period of sampling

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was standardised after repeated sampling experiments. Two samples, and occasionally three, were collected from different sections and the average was considered representative of the grain storage facility at each time. Similarly, samples were also collected just outside the granary each time for the same duration to act as controls. The exposed Petri dishes were incubated at 27 ± 2°C for 2–3 days. Different colony forming units were isolated and identified to the lowest generic or specific level possible and the identities of some fungal forms were further confirmed by the International Mycological Institute (IMI), London (UK). The correction factor was used (Andersen, 1958) to find the exact number of colonies and were expressed as CFUs m^{-3} of air.

Slides exposed in the Burkard personal slide sampler were mounted in a drop of molten glycerine jelly. The exposed slides were examined visually under a Zeiss Binocular Axioscope (10×, 40×; 10 × 100×, in oil) microscope. On some occasions smaller spores were confirmed with 100× magnification. Identified spores of *Ustilago* were grouped together under the heading smuts. The spore counts were expressed as number of spores m^{-3} of air sampled.

To find the statistically significant variations in the concentrations of total colony forming units and total spore counts, and of 10 dominant types from those of outside air, analysis of variance (ANOVA) was used.

3. Results

3.1. Floral range

A total of 97 fungal forms, both culturable and non-culturable, were identified from the granary. The percentages contributed by the predominant fungal types to the total concentrations are presented in Table 1. The observations revealed that *Aspergillus flavus* Link, *A. niger* van Tieghem, *Cladosporium* spp. Link, *Penicillium* spp. Link, *Alternaria* spp. Nees, *Rhizopus* spp. Ehrenberg, *Ustilago* (smuts) and *Epicoccum nigrum* Link, were important contributors to the air inside the granary. However, *Aspergillus flavus* was the chief fungal species contributing 68.8% of the average annual concentration inside the granary, as compared to only 14.5% in the control air.

When the concentrations as recorded from slide exposure, were analysed for their average annual concentration, it was observed that 42% of the catch was for *Ustilago*, although the concentration in the control air, just outside the store was higher (56.7%). Aspergilli/penicilli constituted 39.6% of the total spore load.

The percentage occurrences of the dominant fungal forms in the 70 samples studied, both as CFUs and

Table 1
Annual average percent contribution to total colony forming units m^{-3} and spore concentration m^{-3} recorded from inside and outside a grain storage facility in Delhi

	Store		Outside	
	No.	Percentage	No.	Percentage
CFUs m^{-3}				
Aspergillus flavus	8829	68.8	1080	14.5
Cladosporium spp.	2380	18.5	4067	54.8
Aspergillus niger	645	5.0	635	8.5
Penicillium spp.	474	3.6	581	7.8
Alternaria alternata	139	1.0	353	4.7
Rhizopus nigricans	87	0.6	64	0.8
Aspergillus versicolor	59	0.4	126	1.6
Aspergillus fumigatus	41	0.3	101	1.3
Epicoccum nigrum	15	0.1	29	0.3
Curvularia spp.	51	0.007	6	0.08
Others	144	1.1	377	5.0
Spores m^{-3}				
Smut spores	29 348	42.9	17 281	56.7
Aspergilli/penicilli	27 103	39.6	4534	14.8
Alternaria	4088	5.9	1944	6.3
Cladosporium	3901	5.7	3359	11.0
Epicoccum	728	1.0	324	1.0
Curvularia	492	0.7	416	1.3
Nigrospora	421	0.6	416	1.3
Periconia	409	0.5	289	0.9
Basidiospores	344	0.4	248	0.8
Drechslera	318	0.4	204	0.6
Others	1893	2.7	1898	6.2

Table 2
Percent occurrence of dominant taxa in totals number of samples analysed during 1989–1991

	Store		Outside	
	No.	Percentage	No.	Percentage
CFUs m^{-3}				
Aspergillus flavus	67	95.7	65	92.8
Cladosporium spp.	60	85.7	64	91.4
Aspergillus niger	58	82.8	64	91.4
Penicillium spp.	43	61.4	52	74.2
Alternaria alternata	26	37.1	33	47.1
Rhizopus nigricans	57	81.4	40	57.1
Aspergillus versicolor	20	28.5	42	60.0
Aspergillus fumigatus	17	24.2	21	30.0
Epicoccum nigrum	10	14.2	9	12.8
Curvularia spp.	1	1.4	2	2.8
Spores m^{-3}				
Smut spores	62	98.4	61	96.8
Aspergilli/penicilli	61	96.8	61	96.8
Alternaria	61	96.8	61	96.8
Cladosporium	60	95.2	59	93.6
Epicoccum	51	80.9	33	52.3
Curvularia	48	76.1	48	76.1
Nigrospora	55	87.3	54	85.7
Periconia	49	77.7	44	69.8
Basidiospores	38	60.3	35	55.5
Drechslera	48	76.1	45	71.4

spores, are presented in Table 2. Colonies of *Aspergillus flavus, A. niger* and *Cladosporium* spp. were observed in more than 90% of the samples analysed. The other colony forming units were trapped in 2.8–74.0% of the samples analysed (Table 2). Others, comprising 87 types altogether, contributed 1.1% CFUs m^{-3} and 2.7% spores m^{-3} in the grain store.

3.2. Monthly concentration of different airborne fungi

3.2.1. Colony concentration. The colony counts (CFUs m^{-3}) for all types and the 10 dominant types were analysed for their average monthly concentration. The colony concentrations for all fungal types showed two distinct seasons: September to December, and March to June. The colony concentration was significantly higher

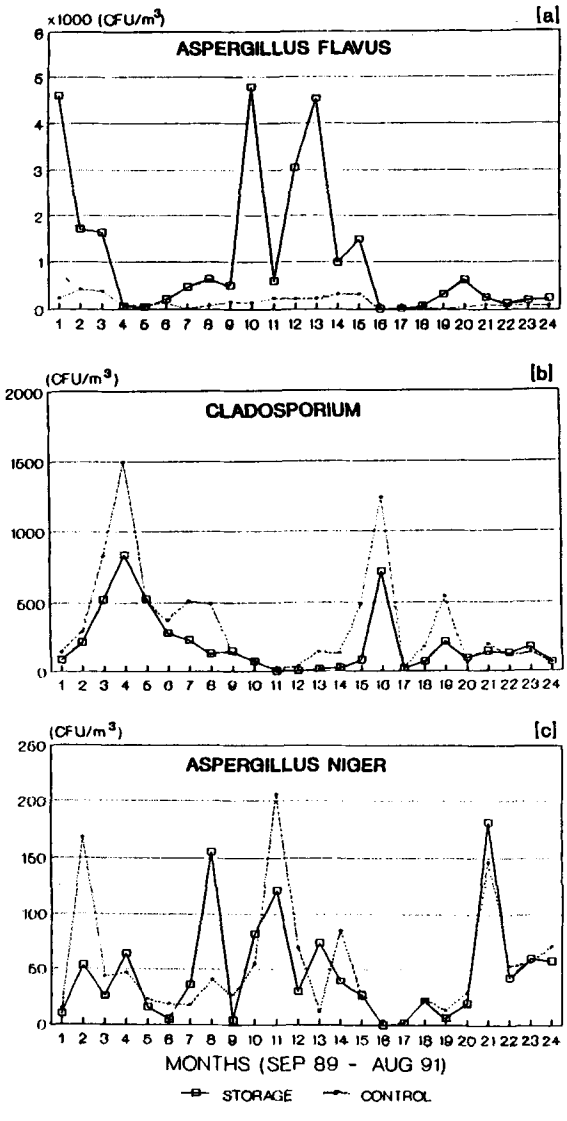

Fig. 1. Colony concentration (CFUs m^{-3}) of dominant fungi in different months during 1989–1990 and 1990–1991 inside and outside the granary. (a) *Aspergillus flavus,* (b) *Cladosporium* spp., (c) *Aspergillus niger.*

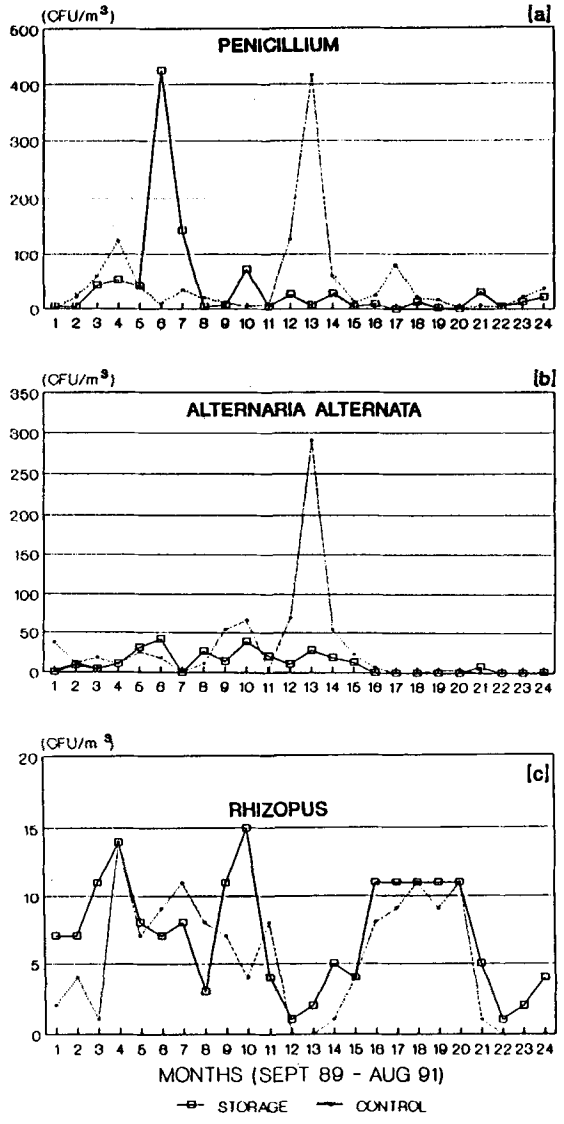

Fig. 2. Monthly colony concentration (CFUs m^{-3}) of dominant fungi (1989–1990 and 1990–1991) inside and outside the granary. (a) *Penicillium* spp., (b) *Alternaria alternata,* (c) *Rhizopus* spp.

inside the storage facility than in outside air in different months ($P < 0.01$). The highest total CFUs m^{-3} (13 802 CFUs m^{-3}) was observed in the month of June in 1990 inside the store while the lowest (24 CFUs m^{-3}) was recorded in the month of January 1991.

Aspergillus flavus, the most dominant species showed two distinct seasons, September to November and May to August (Fig. 1a). *Cladosporium* spp. were prevalent in the winter months (November to March) with their highest concentration (1497 CFUs m^{-3}) in the month of December 1991 (Fig. 1b). *Aspergillus niger* did not reveal any particular pattern, but peaks in April (1989–1990) and May (1990–1991) were observed (Fig. 1c).

Penicillium spp., *Alternaria* spp. and *Rhizopus* spp. had variable patterns of occurrence (Fig. 2a–c). In the

year 1989–1990 the colony concentrations of *Penicillium* spp. and *Rhizopus* spp. were observed to be higher in storage, while *Alternaria* spp. were predominant in control air. The different species of *Aspergillus* such as *A. versicolor* (Vuillemin) Tiraboschi, *A. japonicus* Saito, *A. fumigatus* Fresenius and *A. sydowi* (Bainier and Sartory) Thom and Church, had no specific seasonal occurrence and were characterised by higher concentrations in outside samples.

3.2.2. Total score concentration. The data on spore concentration, for total fungi and for certain dominant forms, for both years were also analysed for their monthly prevalence. Particular attention was paid to smuts and other basidiomycetes which were important components of wheat storage facilities.

The total spore concentration in the store was significantly high ($P < 0.01$) compared to control air as assessed by ANOVA, with a seasonal rise from November to July. The concentration inside the store was always higher than outside each month. It is interesting to note that in January 1991 the concentration reached as high as 26 925 spores m^{-3} inside the grain store, where as only 1863 spore m^{-3} were recorded in control air.

The average monthly concentrations of smuts and *Aspergillus/Penicillium* are illustrated in Fig. 3. Higher concentrations of smuts were recorded from March to May (Fig. 3a). Spores of the aspergilli/penicilli were higher during 1990–1991, with the highest concentration (20 190 m^{-3}) recorded in January 1991 (Fig. 3b). A variable concentration of *Alternaria* spp. was recorded in each month with a seasonal rise from March to June (Fig. 4a). The highest peak (993 m^{-3}) was observed in January of 1991. *Cladosporium* spp. were observed in each month but in increased concentrations from October to January; the highest catch (823 m^{-3}) was observed in October 1989 in the store (Fig. 4b). *Epicoccum nigrum* presented an irregular pattern, but the concentration of its spores was considerably higher from October to January inside the granary as compared to outside (Fig. 5a), while basidiospores showed a seasonal peak from July to September (Fig. 5b). The spores of *Drechslera* spp. (Fig. 5c), *Nigrospora* spp., *Curvularia*, and *Penicillium* spp. also showed variable monthly concentrations from 1 to 260 spores m^{-3}.

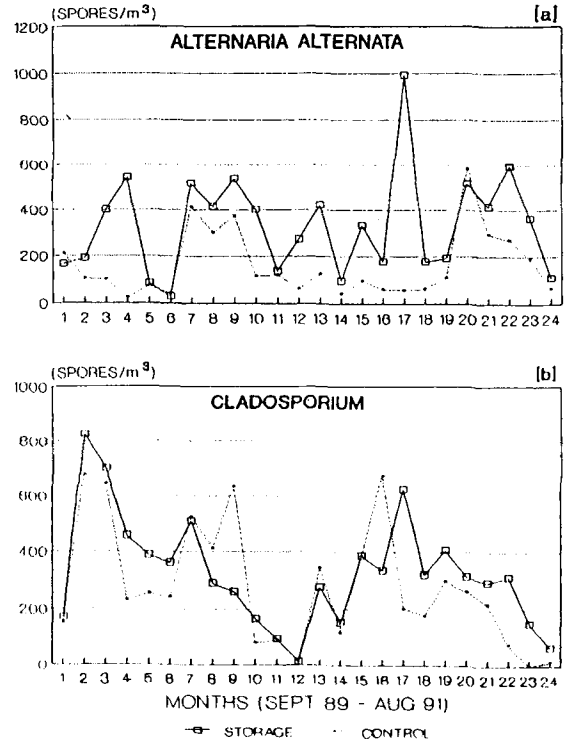

Fig. 4. Spore concentration (spores m^{-3}) of dominant fungi in different months from September 1989 to August 1991 inside and outside the granary. (a) *Alternaria* spp., (b) *Cladosporium* spp.

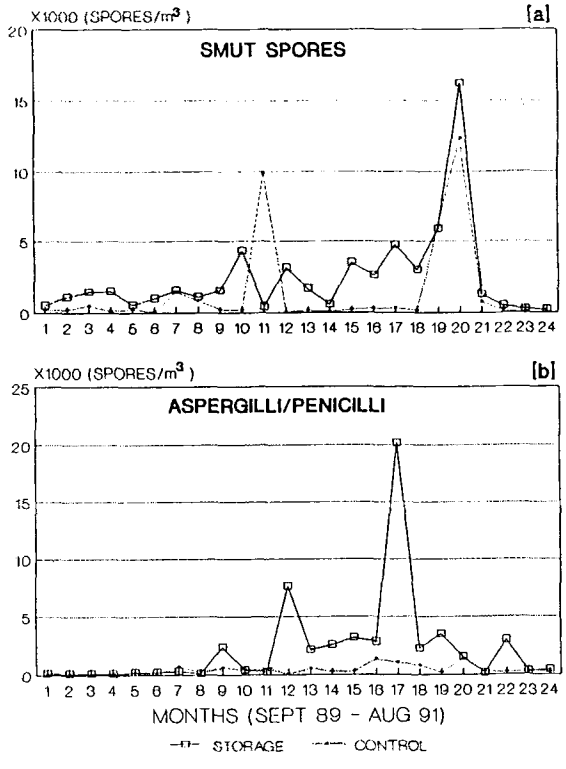

Fig. 3. Spore concentration (spores m^{-3}) of dominant fungi in different months from September 1989 to August 1991 inside and outside the granary. (a) Smut spores, (b) aspergilli/penicilli.

3.3. Annual concentration

The annual concentrations of total and dominant CFUs and spore counts are shown in Fig. 6. The total annual colony concentration was 14 055 (CFUs m^{-3}) and 11 574 (CFUs m^{-3}) in the store in 1989–1990 and 1990–1991, respectively; the concentrations in control air were nearly half these with only 8006 and 6832 (CFUs m^{-3}) in corresponding years (Fig. 6a). In-

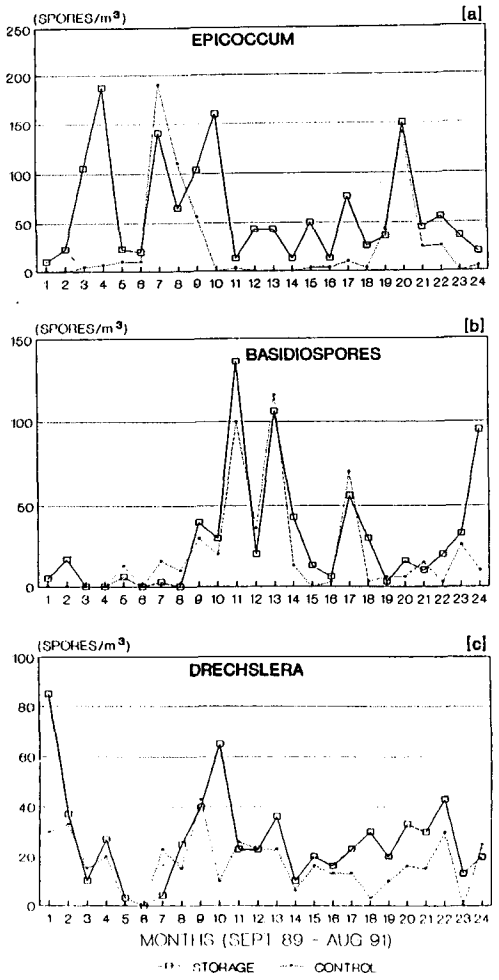

Fig. 5. Monthly concentration of spores (spores m^{-3}) of dominant fungi from September 1989 to August 1991 in and outside grain storage facility in Delhi. (a) *Epicoccum nigrum*, (b) basidiospores, (c) *Drechslera*.

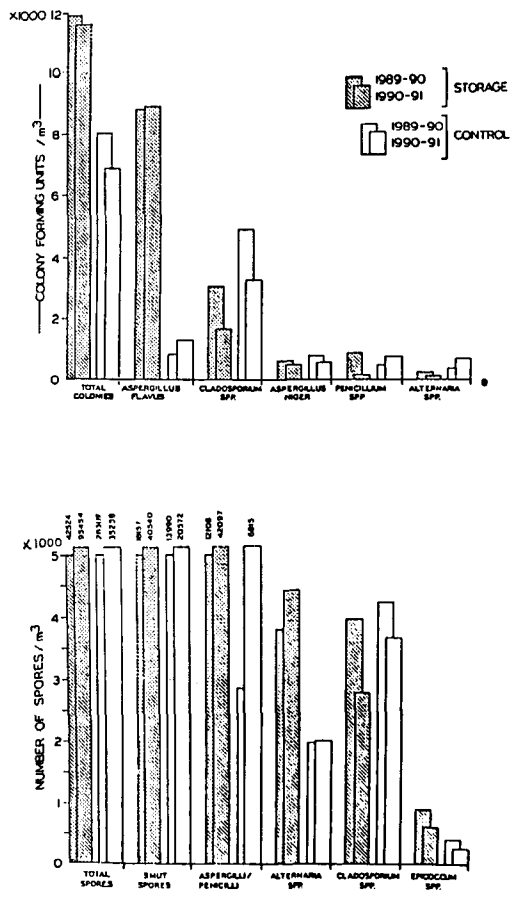

Fig. 6. Annual concentration of total and dominant fungal colonies (CFUs m^{-3}) and spores (spores m^{-3}) in the granary.

dividually, *Aspergillus flavus* (8840 CFUs m^{-3}) in 1990–1991 was highest followed by *Cladosporium* spp. (3024 CFUs m^{-3}) in 1989–1990. The other dominant types such as *Aspergillus niger*, *Penicillium* spp. and *Alternaria* spp. were higher in control air.

Compared to colony concentration the total spore concentration was considerably higher with 95 454 spores m^{-3} in 1990–1991 and 42 454 spores m^{-3} in 1989–1990 (Fig. 6b) in the grain store. Compared to this, the spore concentration in outside air was much lower with 26 308 spores m^{-3} and 35,258 spores m^{-3} in 1989–1990 and 1990–1991, respectively. The smut spores (*Ustilago* spp.) and those of *Aspergillus/ Penicillium*, were higher in storage while *Cladosporium* spp. and *Nigrospora* were higher in outside air.

4. Discussion

A rich fungal flora comprising 97 airborne types has been isolated from this grain storage facility in Delhi. In order to obtain a complete picture of the fungal airspora a combination of plate culture and slide exposure techniques was adopted. The use of the Andersen sampler provided visible colony forming units, while the Burkard personal slide sampler provided the total spore load.

It was interesting to record that both total CFUs and total spore concentrations inside the storage facility were significantly higher ($P < 0.01$) than outside as analysed using ANOVA. Although the predominant airborne fungi inside the store were *Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, Alternaria* spp., *Cladosporium, Mucor* and *Penicillium, Aspergillus flavus* was the chief contributor with more than 68% of the average annual concentration and was significantly higher ($P < 0.01$) inside the storage facility.

Among the spore types, smuts (*Ustilago cynodontis*) contributed as much as 42% of the total spore catch inside the store, followed by the aspergilli/penicilli group with 39.6%. The qualitative occurrence of these fungi conform with those observed by Lacey in the UK (1980, 1990).

Some of the fungal species showed distinct seasonal patterns of occurrence, e.g. *Aspergillus flavus* was predominant during September to November, while, *Cladosporium* was high in the winter months (November to February). Most of the airborne species were observed throughout the year but with seasonal peaks.

The richness of the microflora in the grain store depends chiefly on water content, the degree of spontaneous heating and aeration of the bulk grain (Magan and Lacey, 1990). Colonies of *Aspergillus flavus, Aspergillus niger* and *Cladosporium* were the frequently occurring types as they were recorded in more than 90% of the samples studied, while spores of smuts, aspergilli/penicilli and *Alternaria* were prevalent in 96% of the air samples analysed from the facility. Thus, these types not only contributed extensively to the indoor air mycoflora but were also consistently present throughout the year and provided a continuous source for inhalation by workers. Grain dust produced immediately after harvest consists of bacteria, actinomycetes, mites, pesticide residues, etc., in addition to fungi (Darke et al., 1976; Lacey, 1990). All workers handling grains are, therefore, exposed to concentrations of *Aspergillus flavus* and smut spores, for example, which are considerably higher than those in outdoor air. Similar observations were made by Drake et al. (1976) who suggested that inhalation should be minimised by preventing moulding and by the use of suitable mechanical devices and respirators as an avoidance strategy.

Besides causing IgE and IgG mediated respiratory allergies among workers, *Aspergillus flavus* is also known to produce mycotoxins (Lacey and Crook, 1988). It is important to collect regular information on fungal concentration in grain storage facilities from time-to-time to avoid occupational hazards. It is, therefore, suggested that proper storage conditions should be provided to reduce the growth of fungi inside the storage facility.

5. Conclusions

The data collected lead to several conclusions.

(1) *Aspergillus flavus* and *Ustilago* (smuts) are the two most predominant fungi in the wheat storage facility in Delhi which was studied; other important fungi are *Rhizopus* spp., aspergilli/penicilli, *Drechslera* spp., *Periconia* spp. and *Cladosporium* spp.

(2) The total colony and spore concentration is significantly higher ($P < 0.01$) in the grain store as compared to control air. *Aspergillus flavus, Cladosporium* spp. and smuts were observed to be significantly higher ($P < 0.05$) in the grain store as compared to outside air.

(3) *Aspergillus flavus, Cladosporium* spp., *Epicoccum nigrum,* basidiospores and *Nigrospora* exhibited a definite seasonality.

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