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OCCUPATIONAL EXPOSURE TO AIRBORNE FUNGI AMONG RICE MILL WORKERS WITH SPECIAL REFERENCE TO AFLATOXIN PRODUCING *A. FLAVUS* STRAINS

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Abstract: A study was undertaken on environmental mycoflora of rice mills situated in Bawla town, Ahmedabad district. The airborne fungal communities were isolated and identified quantitatively by using Andersen-6-stage viable sampler, midget impinger and high volume samplers (Cone and Hexhlet for total and respirable dusts respectively). Of all the isolates, genus *Aspergillus* was predominant and among the *Aspergillus* species, *A. flavus* was the common isolate, irrespective of the method applied for sample collection. Number of isolates recovered from the working place was significantly greater ($p < 0.01$) compared to control. Total percentage of aflatoxin positive strains of *A. flavus* was 8%. These aflatoxin producing strains were identified on various media, such as Czapek agar (Cz) with 0.05% anisaldehyde, APA and CAM. Surface morphology of aflatoxin positive strains was studied by SEM. Highly significant total and respirable dust concentrations were found in the work place ($p < 0.01$) whereas in the store, only the total dust concentration was significantly higher ($p < 0.05$) than the control site. The study indicates that the rice mill workers are occupationally exposed to airborne aflatoxin producing strains of *A. flavus*. Thus, they require protective mask for their safety.

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INTRODUCTION

Numerous respiratory diseases of workers exposed to grain dusts have been described in the literature [11], particularly in relation to farmer's lung [2], but only a few reports are available on the effects of occupational exposure to grain dusts in the grain transformation industry [8, 10, 7]. In addition to other organic and inorganic matters, grain dusts also harbour microorganisms, their toxins and other bioactive materials [8, 17]. A large quantity of dust is generated into the environment of grain processing industries when agricultural commodities are converted into an edible form for human consumption, thus causing a potential health risk to workers due to inhalation of vegetable dusts. There are few reports which deal with exposure to airborne aflatoxin through inhalation [6, 9, 15]. Residue levels of airborne aflatoxin were detected in agricultural dust. It has been reported that the respiratory system's pneumocytes are capable of metabolizing aflatoxin B1 (AFB1) to its ultimate carcinogenic form [4]. Cases of pulmonary interstitial fibrosis have been reported after occupational exposure to aflatoxin [4]. The present study deals with occupational exposure to aflatoxin producing strains of *A. flavus* among rice mill workers.

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MATERIALS AND METHODS

The study was carried out in rice mills located at Bawla town in Ahmedabad district (India). Samples were collected from three sites - work place, store and office (control). Samples were taken from the breathing zone of the workers.

Collection and processing of environmental samples. Environmental fungal communities were isolated quantitively by using Andersen-6-stage viable sampler (Andersen Sampler Inc. Atlanta, CA.), midget impinger and high volume gravimetric samplers (Cone and Hexhlet). Five samples from each site were taken by each instrument after their sterilization as per manufacturer's recommendation. Air samples were collected at a flow rate of 28.3 1/min. as described by Andersen [1] and results were expressed as cfu/m^3. In the case of the midget impinger, sterile normal saline (10 ml) was used as impinging medium. Samples were collected at the flow rate of 12 l/min for 10 minutes and taken immediately to the laboratory. Original sample and its serial dilutions (up to 10^6) were inoculated on Czapek agar (Cz) plate. Airborne total dusts were collected on sterile filter paper (Whatman No. 41) using Cone sampler at the flow rate of 28 l/min for two hours. In the case of the Hexhlet, Sartorious 8 µm sterile filters were used to collect respirable dusts at the flow rate of 45 l/min for two hours. Afterwards, the filters were washed with sterile distilled water and plated with 0.1 ml suspension of original sample and its dilutions (up to 10^6) on Cz agar plate. All plates were incubated at 22ºC for at least one week.

Identification of mycoflora. Colonies were identified on the basis of colony morphology, microscopy, slide culture, Scotch tape technique, etc. as described elsewhere [16, 18, 13]. Different species of *Aspergillus* were identified according to Raper and Fennell [12]. Qualitative media were used for identification of aflatoxigenic strains. The colonies grown on *Aspergillus flavus* - parasiticus Agar (AFPA) medium were used for inoculum on the following media:

(a) Cz agar with 0.05% anisaldehyde [13] - toxigenic strains produced pink conidia on the medium (at 30ºC after 7 days).

(b) Fluoroscence agar media:

(i) Aflatoxin producing ability media (APA) [16] - through 7^{th}–10^{th} day of incubation (at 28ºC in dark), toxigenic strains produced blue fluorescence surrounding the colony under UV (366 nm).

(ii) Coconut agar medium (CAM) [18] - after 2–5 days incubation (at 20ºC upside down) toxigenic strain produced bluish-green fluorescence surrounding the colony under UV, and orange yellow visual pigmentation on the reverse side.

Surface morphology study of *A. flavus* strains by scanning electron microscope (SEM). A small piece of agar block with sporulated strains of *A. flavus* was fixed,

Table 1. Total and respirable dust concentrations at various sites of rice mill environment.

Sites	Environmental dust concentration (mg/m^3)	
	Total dust	Respirable dust
	Mean ± S.D.	Mean ± S.D.
Work place	80.71* ± 20.70	1.92** ± 0.50
Store	1.75* ± 0.48	0.58 ± 0.12
Control	0.82 ± 0.26	0.31 ± 0.16

* $p < 0.05$, ** $p < 0.01$ (compared to control).

Table 2. Total number of fungi isolated from various sites of rice mill by Andersen-6-stage viable sampler.

Sites	cfu × 10^3/m^3 of air		
	Total No. of fungi	Non-respirable fraction	Respirable fraction
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Work place	48.42 ± 11.48	7.98 ± 1.91	39.98 ± 9.57
Store	13.71 ± 3.45	2.28 ± 0.25	11.29 ± 3.20
Control	9.42 ± 4.39	1.49 ± 0.70	7.84 ± 3.69

Table 3. Total number of fungi isolated from various sites of rice mill environment by midget impinger.

Sites	cfu × 10^3/m^3 of air
	Mean ± S.D.
Work place	18.64 ± 6.27
Store	11.90 ± 4.75
Control	3.47 ± 1.05

Table 4. Isolation and identification of different types of fungi from rice mill environment by midget impinger (Mean ± S.D).

Types of fungi	Sites (cfu × 10^3/m^3 of air)		
	Work place	Store	Control
Aspergillus	55.55± 17.40	33.33 ± 12.54	2.77 ± 0.89
Cladosporium	44.44± 15.30	22.22 ± 7.97	5.55 ± 1.54
Penicillium	11.11± 4.10	13.88 ± 3.55	2.77 ± 0.76
Aureobasidium	2.77± 1.30	-	-
Mucor	-	2.77 ± 0.95	-
Rhizopus	-	2.77 ± 0.74	2.77 ± 1.02
Alternaria	5.55 ± 2.30	-	-
Others	11.10 ± 3.80	8.32 ± 2.40	-

Table 5. Total number of fungi isolated from various sites of rice mill environment by gravimetric samplers.

Sites	Total No. of fungi × 10^3/m^3 of air	
	Total dust	Respirable dust
	Mean ± S.D.	Mean ± S.D.
Work place	30.04 ± 8.05	13.65 ± 5.30
Store	14.19 ± 4.57	8.10 ± 3.39
Control	3.70 ± 1.97	2.59 ± 0.83

dehydrated and mounted on the stab which was subsequently coated with a thin layer of gold in a vacuum coating sputter by thermal evaporation, and then observed under a Cambridge sterio-scan S4-10 SEM.

RESULTS AND DISCUSSION

Gravimetric determinations of airborne total and respirable dust concentrations are given in Table 1 for various sites of the rice mill. Highest dust level was observed in the work place (80.7 mg/m^3 and 1.92 mg/m^3 for total and respirable dust respectively). Control site yielded very low concentrations for both the types of dusts. Both total and respirable dusts concentrations were significantly greater ($p < 0.01$) at the work place sites in comparison to control site. High concentration of airborne total dust was also found in the store in comparison to control ($p < 0.05$).

Total number of fungi (cfu/m^3 of air) recovered by Andersen-6-stage viable sampler was always higher than that obtained by the midget impinger (Tab. 2 & 3). Quantitative evaluation of environmental fungal communities using these instruments shows that the trend of recovery was the same, irrespective of the type of instruments used. Table 2 also shows that the total number of cfu recovered from the respirable fraction (>80%) was greater compared to the non-respirable fraction. A total of 7 different genera were isolated from the rice mills by using midget impinger (Tab. 4).

Table 6. Isolation and identification of various types of fungi from rice mill environment by Cone sampler (from total dust) (mean ± S.D.).

Types of fungi	Sites (cfu × 10^3/m^3 of air)		
	Work place	Store	Control
Aspergillus	166.60 ± 42.62	31.48 ± 8.20	1.85 ± 0.55
Cladosporium	38.88 ± 7.40	27.77 ± 11.50	5.55 ± 2.40
Penicillium	24.07 ± 4.80	14.81 ± 4.10	5.55 ± 2.30
Fusarium	11.11 ± 3.10	-	-
Aureobasidium	7.40 ± 2.09	-	-
Curvularia	1.85 ± 0.07	1.85 ± 0.70	-
Rhizopus	3.70 ± 1.80	1.85 ± 0.60	-
Others	16.66 ± 3.01	3.40 ± 2.30	5.60 ± 2.10

Table 7. Isolation and identification of various types of fungi from rice mill environment by Hexhlet sampler (from respirable dust) (mean ± S.D.).

Types of fungi	Sites (cfu × 10^3/m^3 of air)		
	Work place	Store	Control
Aspergillus	75.91 ± 21.30	20.37 ± 6.50	1.85 ± 0.52
Cladosporium	11.11 ± 3.30	12.96 ± 4.40	3.70 ± 1.70
Penicillium	7.40 ± 2.10	9.25 ± 2.70	1.85 ± 0.40
Fusarium	3.70 ± 1.30	1.85 ± 0.88	-
Aureobasidium	3.70 ± 1.10	1.85 ± 0.29	1.85 ± 0.74
Others	7.40 ± 2.70	18.50 ± 5.60	3.70 ± 0.90

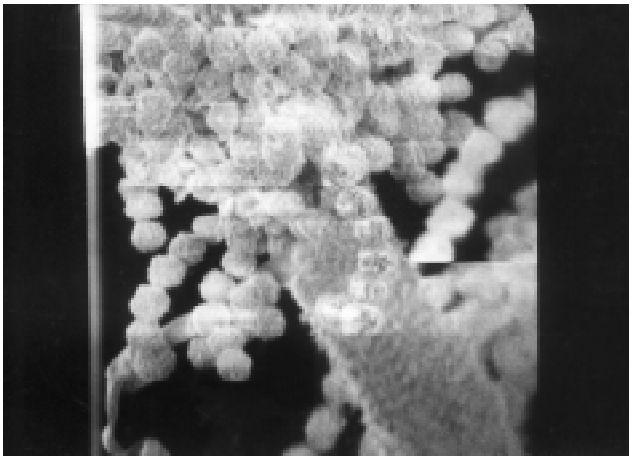

Figure 1. Scanning electron micrograph of a toxigenic strain of *A. flavus* showing rough conidia and conidiophore wall, × 910.

Maximum number of isolates belonged to genus *Aspergillus* (40.3%) and the strains of *A. flavus* were recovered from all the sites of the rice mills except from control site.

Gravimetric samplers yielded maximum numbers of recovery of fungi (cfu/m^3 of air) from total and respirable dust samples (Tables 5–7). These tables show that genus *Aspergillus* was the dominant in mycoflora from total (53.4%) and respirable dust (52.4%).

A total of 19 aflatoxin producing strains of *A. flavus* was recovered (8%) from the work place. These strains were differentiated initially on Cz agar containing 0.05% anisaldehyde; the strains produced pink colouration of the conidia after 7 days incubation at 30ºC. These strains were further confirmed for their aflatoxin producing ability on other flourescence media, such as Aflatoxin Producing Ability (APA) and Coconut Agar Media (CAM). Thus, the results obtained on these media were reproducible and correlated well with different media, as reported by Wei *et al.* [19].

Detail surface morphology of *A. flavus* strains was studied under SEM. Non-toxigenic strains had smooth conidia conidiophore and uniseriate sterigmate, while the aflatoxigenic strains of *A. flavus* had uniseriate sterigmata, rough conidia (2–6 nm) and conidiophore. The shape of the conidial heads was globose to subglobose, reaching a diameter of 20–55 µm (Fig. 1).

From this study it can be inferred that the workers in rice mills are exposed to the aflatoxin producing strains of *A. flavus* as these strains have been recovered from the work environment of the mill. Therefore, the workers who work for more than 8 hours daily in this type of mill are at high risk of inhaling the spores or fragments of mycelium of *A. flavus* strains containing aflatoxin [4, 3, 14]. Although aflatoxins are well known as a potent hepatotoxin and hepatocarcinogen, recent study has revealed their possible role through the respiratory route [4]. Therefore, the workers should be advised to wear protective mask and practice proper personal hygiene, besides the installation of well-planned exhaust system in the department.

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