

Causes of hypersensitivity reactions in flour mill workers in Sudan

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A study of 237 male workers at two flour mills and 71 controls was carried out in Sudan in order to investigate the main possible causes of hypersensitivity reactions to flour dust. Statistical analysis was carried out for the skin test results of wheat, mixed moulds (A13), grain dust, and mixed moulds (group MH) allergen extracts. The results showed that there was no statistical significance between the two exposed groups namely allergy complaining vs. allergy non-complaining except for wheat results in one of the two flour mills which was very slightly significant. But there were highly significant differences between the exposed compared with the control group in all allergens tested.

There was no significant correlation, between allergic symptoms and precipitins to wheat or grain dust, nor between precipitins and positive skin tests.

The results of respirable flour dust concentrations at different work locations had exceeded the threshold limit values which were taken as 10 mg/m^3 .

Aspergillus spp. and *Rhizopus niger* were the predominant fungi in the wheat grains and products which were collected from the two mills.

INTRODUCTION

Flour mill workers in Sudan complain of health problems arising from exposure to wheat and flour dust. Physicians have from time-to-time noticed a high incidence of respiratory and allergic symptoms among men who handle grain. Some clinical and epidemiological surveys in other parts of the world have been carried out in the grain industry¹⁻⁴. These studies on grain handler population had shown a prevalence of symptoms.

The purpose of this study was to identify the possible main causes of hypersensitivity in flour mill workers of Sudan. Emphasis was directed to check the possibility of a hypersensitivity reaction to grain dust as an underlying cause. Also to determine the levels of respirable flour dust concentrations and the main types of fungal contaminants which might have an effective role in producing the signs and symptoms.

MATERIALS AND METHODS

This study was done on 237 males workers from two flour mills. Seventy-one controls were included (*Table 1*). These groups were of the same ethnic origin and geographical area (ie Central Region, Sudan). All workers are on permanent job service. The system of mechanical ventilation in both mills was not functioning at its full capacity due to lack of spare parts.

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FLOUR DUST EXPOSED GROUPS

Blue Nile Flour Mill Company, Wad Medani

There were 131 workers, 18 were on vacation or outside work, another 2 were excluded due to illness or incomplete data, so 111 were included.

Gezira Flour Mill Company

There were 142 workers, 12 were on vacation or outside work, and 4 were excluded due to illness or incomplete data. So 126 people were included.

CONTROL GROUP

The members of this group were the Engineer's Office workers, University of Gezira. They had not come in contact with flour dust in their life. The number in this category were 110. Thirty-nine were on vacation or outside duties. So the number taken for examination was 71.

ALLERGIC SYMPTOMS

Each included individual was interviewed using a questionnaire specially designed for this study. This questionnaire was first tested in a group of Sudanese flour mill workers and then arranged and corrected according to that pilot study. The main questions were

Table 1. Mean age and job duration in the surveyed Sudanese Flour Mill Workers

Workplace	No. of workers	Mean age	Mean job duration (years)
Blue Nile Flour Mill Company	111	38.4 ± 11.9 (20–60)	10.2 ± 4.8 (0.1–16)
Gezira Flour Mill Company	126	31.8 ± 11.3 (18–64)	6.8 ± 5.7 (0.2–16)
Control group Engineer's Office workers Gezira University	71	33.7 ± 11.5 (18–61)	8.9 ± 3.0 (0.1–17)

related to the history of occupation and the presence of allergy-related symptoms including cough, chest tightness, wheeze, headache, rhinitis, conjunctivitis and skin itch. Then these symptoms were checked by a medical examination.

SKIN TEST

We applied a number of commercially available allergens which were wheat, housedust, mixed beans, mixed moulds A 13, grain dust, mixed moulds group MH, peanut and whole egg. Positive and negative controls were used.

These extracts were provided by Bencard Allergy Laboratories Mississauga, Ontario, Canada. There were some difficulties in preparing specific allergen extracts locally due to shortage of required reagents. We used the extracts originating from Canada, because the imported wheat in that season used by both mills in the survey was from Canada. Further points are put in the discussion. Prick skin tests were performed on both right and left flexor side of the forearms. At no time was the observer or the tested person allowed to know the nature of the allergen which was being tested. The wheal diameter was read by a gauge made by Bencard Allergy Laboratories, 20 min after the application of the test.

PRECIPITIN TEST

Serum was examined for precipitins against flour dust using the double diffusion method (gel diffusion of Outchterlony) as follows.

Petri dishes of 4 cm diameter were used; 1 per cent iron agar No. 1, (Oxoid) was poured on the dishes which were put on a levelling table and allowed to set. The wells were cut in them according to a specifying template in which there is a central and six peripheral wells.

Each serum was tested against flour dust. The antigen was put in the central well and the sera in the peripheral wells. These were then incubated in a humid chamber at 26 °C for 48 hours. The plates were then put in the washing buffer for 48 hours to remove excess serum antigen and any non-specific precipitin line. The washing buffer was made of the following: 4 g sodium tetraborate, 4 g sodium chloride and 1 litre distilled water.

Then the agar was transferred to a clean labelling slide. A clean filter paper was put on top of the agar. The slides were allowed to dry at 37 °C incubation. Then they were stained. The stain was made of the following: 0.5 g naphthalein black, 500 ml methanol, 100 ml glacial acetic acid and 400 ml distilled water.

The slides were kept in the stain for 10 min. Then they were transferred to the differentiator for 3 min which has the same composition as the stain but without the naphthalein black.

The slides were put in air to dry. Precipitin lines were looked for using a viewing box.

The flour dust antigen used for the test was prepared from samples collected from the two surveyed flour mills. These samples were mixed thoroughly before starting the preparation. The collected dust was prepared as described by Sheldon *et al.*⁵

The extract material was prepared using the following: 100 g dust, 25 ml glycerine, 2 ml toluene, 50 ml buffered saline and 325 ml distilled water. The extract was concentrated to 1/10 original volume in a Cellophane tube. Then the extract solution was sterilized by filtration through a Seitz filter⁶.

RESPIRABLE FLOUR DUST MEASUREMENTS

Respirable flour dust samples were taken in the two surveyed mills. Dust particles are carried with the air stream into the lung during inhalation. The majority of them being either exhaled or eliminated by means of the lung clearance mechanism. A small number of these particles may be deposited in the lung, depending on their size, and in accordance with the physical laws governing impaction, sedimentation and Brownian movement. Medical research has shown that particles of 0.1–5 µm can remain in the alveolar passages (respirable dust)⁷.

A Bendix super sampler (BDX44) pump with respirable dust labeled sampling head was used for the collection of the flour dust samples. This sampler has a motor which is driven by small batteries. Air from the breathing zone of the subject is sucked through a tube. The respirable dust particles are shunted to settle on a filter paper which is fixed inside a special plastic container. The difference in weight of the filter paper and the container before and after exposure is the weight of the respirable dust to which that person was exposed in the time taken. The volume of sucked air by the sampler is recorded by a meter fixed to the equipment. Then the concentration of respirable flour dust in air was calculated quantitatively.

TEST FOR FUNGI ASSOCIATED WITH WHEAT GRAINS AND FLOUR MILL PRODUCTS

Five composite samples of wheat grain, flour, bran, impurities and waste powder were collected from both Blue Nile and Gezira Flour mills.

The objective of the study was to qualify the degree of fungal contamination as a possible factor contributing to flour dust hypersensitivity.

Methodology

Evaluation of grain contamination, unlike wheat products was qualitative. Blotter method was used for the test⁸. Unwashed seeds were plated in sterilized Petri dishes on moistened blotters. A total of 30 grains in each dish were incubated at 30 °C in a cooled incubator under continuous light. The plates were examined after 7 days and fungi associated with the grains were identified and the total number of contaminated grains was recorded.

A dilution plates method was used for bioassay of all samples of wheat products; 0.2 g of each sample was suspended in 10 ml sterile distilled water to which a trace of Tween 80 had been added and shaken intermittently for 5 minutes. Then a series of dilutions were made from each suspension; 0.1 ml of each suspension was plated in casein hydrolysate + bronopal + penicillin medium (4 g casein hydrolysate (Osoid); 10 g dextrose; 1 g KH PO₄; 0.7 g MgSO₄; 0.5 g H₂O₂;

1 litre sterile distilled water; 40 ppm bronopal; 40 ppm penicillin). The plates were incubated at 25 °C for 5 days before identification and counting of colony numbers.

RESULTS

Prevalence of allergy

The prevalence of flour dust allergy by complaint is shown in Table 2. It showed that 28.7 per cent of the flour mill workers complain of allergy compared to 0 per cent of the controls.

Allergy skin test

As shown in Table 3 there was a response to wheat extract in the two work sites but the response to mixed moulds and grain dust was greater in both exposed groups, ie allergy complaining and allergy non-complaining groups. In statistical analysis difference of proportion was applied. The results of this analysis are shown in Table 4. These results showed that there were no statistical differences between allergy complaining and allergy non-complaining groups, who are equally exposed to the flour dust, except for wheat in the Blue Nile Flour Mill. But there were highly significant differences between the exposed groups (both allergy complaining and allergy non-complaining) versus the control group.

Precipitins

Precipitins to flour dust extract were demonstrated by double diffusion method in 8 people (7.2 per cent) at the Blue Nile Flour Mill Company; 12 people (9.5 per

Table 2. Prevalence of flour dust allergy by complaint in subjects included in the survey

Occupational group	No. of workers	Flour dust hypersensitivity	
		No.	%
Blue Nile Flour Mill Company	111	43	38.7
Gezira Flour Mill Company	126	25	19.8
Total	237	68	28.7
Control group Engineer's Office workers, University of Gezira	71	0.0	0.0

Table 3. Response by prick test to different allergen extracts, including wheat, in examined workers

No.	Allergen extract	Group	Blue Nile Flour Mill				Gezira Flour Mill				Control group Engineer's Office workers Gezira University			
			-ve		+ve		-ve		+ve		-ve		+ve	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	Wheat	A	29	67	14	33	18	72.0	7	28.0	—	—	—	—
		N-A	55	80.9	13	19.1	76	75.2	25	24.8	71	100	0	00.0
2	House dust	A	38	88.4	5	11.6	19	76.0	6	24.0	—	—	—	—
		N-A	60	88.2	8	11.8	81	80.2	20	19.8	64	90.1	7	9.9
3	Mixed beans	A	39	90.7	4	9.3	21	84.0	4	16.0	—	—	—	—
		N-A	65	95.6	3	4.4	91	90.1	10	9.9	67	94.4	4	5.6
4	Mixed moulds (A13)	A	26	60.5	17	39.5	16	64.0	7	36	—	—	—	—
		N-A	44	64.7	24	35.3	75	74.3	26	24.7	67	94.4	4	5.6
5	Grain dust	A	23	53.5	20	46.5	14	56.0	11	44.0	—	—	—	—
		N-A	49	72.1	19	27.9	67	66.3	34	33.7	63	88.7	8	11.3
6	Mixed moulds group M.H.	A	28	65.1	15	34.9	16	64.0	9	36.0	—	—	—	—
		N-A	50	73.5	18	26.5	69	68.3	32	31.7	65	91.5	6	8.5
7	Peanut	A	30	69.8	13	30.2	23	92.0	2	8.0	—	—	—	—
		N-A	57	83.8	11	16.2	91	90.1	10	9.9	65	91.5	6	8.5
8	Whole egg	A	38	88.4	5	11.6	25	100.0	0	00.0	—	—	—	—
		N-A	68	100.0	0	00.0	99	98.0	2	20	67	99.4	4	5.6

A = Allergy complaining group: n = 43 at Blue Nile Mill and n = 25 at Gezira Mill.

N-A = Non-allergy complaining group: n = 68 at Blue Nile Mill and n = 101 at Gezira Mill.

Control group workers had not been exposed to flour dust in their life (n = 71).

Table 4. Differences in proportion at $\alpha = 0.05$ (T. 95), the critical value of $Z = 1.645$, for prick tests at the two mills

Group	Allergen extract	Z	p
Blue Nile Flour Mill			
Allergy complaining vs. Allergy non-complaining	Wheat	1.645 < 1.69 (S)	= 0.0455
	Mixed moulds (A13)	1.645 > 0.53 (NS)	= 0.2981
	Grain dust	1.645 > 1.32 (NS)	= 0.0934
Exposed vs. control	Mixed moulds (group M.H.)	1.645 > 1.02 (NS)	= 0.1539
	Wheat	1.645 < 4.44 (S)	< 0.0001
	Mixed moulds (A13)	1.645 < 4.70 (S)	< 0.0001
	Grain dust	1.645 < 3.60 (S)	= 0.0002
	Mixed moulds	1.645 < 3.39 (S)	= 0.0003
Gezira Flour Mill			
Allergy complaining vs. Allergy non-complaining	Wheat	1.645 > 0.31 (NS)	= 0.3783
	Mixed moulds (A13)	1.645 > 0.20 (NS)	= 0.4443
	Grain dust	1.645 > 0.933 (NS)	= 0.1762
Exposed vs. control	Mixed moulds (group M.H.)	1.645 > 0.381 (NS)	= 0.3520
	Wheat	1.645 < 3.85 (S)	= 0.0001
	Mixed moulds (A13)	1.645 < 3.40 (S)	= 0.0003
	Grain dust	1.645 < 3.78 (S)	= 0.0001
	Mixed moulds (group M.H.)	1.645 < 3.97 (S)	< 0.0001

* Both groups are exposed to flour dust during work.

S = Significant.

NS = Not Significant.

cent) at the Gezira Flour Mill Company, and 3 people (4.2 per cent) of the control group.

A single regression test was used. There were no significant correlations between presence of precipitins versus allergy complaint ($p > 0.05$), nor results of precipitins versus allergy complaint ($p > 0.05$), nor results of precipitins versus positive skin reactions to wheat or grain dust ($p > 0.05$).

Respirable flour dust

The threshold limit values for grain nuisance dust is considered⁹ as 10 mg/m³. The results of respirable flour

dust concentration at different work locations in the Blue Nile Flour Mill Company and Gezira Flour Mill Company are shown in Table 5. Dust concentration at different workplaces exceed the threshold limit values for grain dust.

Fungi in wheat and flour mill products

As seen in Table 6 *Aspergillus spp.* and *Rhizopus niger* were the predominant fungi associated with wheat grains at the Blue Nile and Gezira Flour Mill Companies. Nearly the same fungal species predominated the bioassay of wheat products as seen in Table 7.

Table 5. Respirable flour dust concentration

Sample no.	Work location	Concentration (mg/m ³)	
		Blue Nile Flour Mill Company	Gezira Flour Mill Company
1	Rollers	90	40
2	Wheat Cleaning	160	50
3	Seives	25	20
4	Plantsifters	30	50
5	Bran Section	45	50
6	Packing	100	90
7	Storage	25	20

DISCUSSION

There is no doubt that health hazards due to exposure to wheat and flour dust have aroused the enthusiasm of many investigators and several extensive studies have been carried out in different parts of the world.

The percentage of the prevalence of allergy by complaint in the Blue Nile Flour Mill Company is greater than the Gezira Flour Mill Company, ie 38.7 and 19.8 per cent respectively compared to 0 per cent in the control group.

Table 6. Fungi associated with wheat grains at Blue Nile and Gezira Flour Mill Companies

Source	Fungal isolates (%)				
	<i>A. falvus</i>	<i>Aspergillus spp</i> <i>A. Niger</i>	Others	<i>Rhizopus niger</i>	<i>Fusarium spp*</i>
Blue Nile Flour Mill					
raw grains	18.3	4.2	19.2	50.2	3.3
cleaned grains	12.0	6.3	8.3	23.5	2.1
Gezira Flour Mill					
raw grains	19.5	0.7	16.0	55.0	2.1
cleaned grains	9.1	3.5	7.2	31.0	2.8

* *F. mogniliformi*; *F. solani*; *F. accuminatum*.

Table 7. Fungi associated with wheat products at Blue Nile and Gezira Flour Mill Companies. Figures expressed as number of colonies (10^3 /g product)

Product	Location	Actual	Total Transformed* (Log)	Aspergillus spp		Others	Rhizopus niger	Others†
				A. falvus	A. Niger			
Flour	B. Nile	13.0	1.03	0.3	0.3	9.7	0.0	3.0
	Gezira	47.0	1.66	3.5	3.5	28.5	11.5	0.0
Bran	B. Nile	5.7	0.54	0.3	0.7	2.7	0.0	2.0
	Gezira	10.5	1.96	0.0	0.0	103.5	0.0	0.0
Impurities	B. Nile	55.6	1.75	31.7	11.0	9.3	3.6	0.0
	Gezira	73.5	1.83	18.5	8.5	35.0	11.5	0.0
Waste powder	B. Nile	18.3	1.32	10.0	5.0	0.3	3.0	0.0
	Gezira	10.0	0.99	0.0	0.0	10.3	0.0	0.0

* SE $\pm$ 0.12† Mainly *Penicillium* spp. and *Fusarium* spp.

We have used in our survey commercially prepared Canadian wheat, grain dust, mixed moulds and other extracts for skin testing. It should be noticed here that the Canadian wheat was routinely processed in the two mills surveyed. So the tested mill worker groups were exposed during the work to the allergens which could be found in the Canadian grain. There was a difficulty in preparing mixed mould and some other used extracts in our local laboratory. This was due to shortage of required reagents. All extracts used in this survey were prepared by Bencard Allergy Laboratories, Canada, this should help to standardize the method of preparation of the reagents.

From our results it could be shown that the response to mixed moulds and grain dust extract had a greater percentage response compared to the wheat extract alone. Grain dust is a complex mixture consisting of husk particles, cellulose hairs and spikes, starch granules, spores of fungi, insect debris, pollens, rat hair, and approximately 5 per cent mineral particles¹⁰. Most of these are known allergens and are expected to have a major effect in the positive prick test results.

Pure wheat extract does not cause a high percentage of skin response compared to that of moulds and grain dust. The control group had a low response rate compared to the exposed groups. Those who responded from the control group mainly to house dust, grain dust, and mixed moulds, might have met these substances before in their houses and/or farms of the Gezira Agricultural Area.

It is interesting to find that in the statistical tests, difference in proportion, which was carried out in the prick test results for wheat, mixed moulds (A 13), grain dust and mixed moulds (group MH) (Table 4), there was no statistical significance between exposed individuals with and without allergic symptoms, except for wheat which was slightly significant in the Blue Nile Flour Mill. The above results could be explained by all individuals in both groups ((A) allergy complaining, (B) allergy non-complaining), being exposed to the flour dust and having the same work conditions. Some workers may not complain or try to deny any complaints because they are afraid that complaining might affect the future of their jobs. So when the skin tests were carried out positive results appeared in those who were in fact allergic, but did not complain. This would

explain why there was no statistical significance between the two exposed groups. But the real highly statistical differences were between all exposed workers (both allergy complaining plus allergy non-complaining) versus the non-exposed group which was the control.

The results of skin prick tests suggest that the other different constituents of wheat or flour dust are of major importance in giving the signs and symptoms of ill health.

The presence of serum precipitin extracts to flour dust in only 8 people (7.2 per cent) at the blue Nile Flour Mill Company and 12 people (9.5 per cent) at the Gezira Flour Mill Company and their lack of correlation with the symptoms or positive prick skin tests to wheat and grain dust shows that the immunopathogenetic factor might not be of great effect in the development of symptoms.

This might be in agreement with the study done by Chan-Yeung *et al.*¹¹ for serum precipitin, where the precipitating antibodies were not detected against any antigen tested in the groups studied.

In our survey the mean particle size of the airborne dust may be less than 5 μ m, regardless of their organic nature¹². Particles of this size can potentially cause small airway and alveolar reactions, as well as upper airway injury. The level of the respirable dust concentration in examined sections of the two surveyed mills was more than the threshold limit values which was 10 mg/m³. This might be due to lack of efficient mechanical ventilation. The natural ventilation depended on many uncontrollable factors and was not always reliable. This could be considered as an effective factor in giving the high prevalence of allergy by complaint as was shown in Table 2. The effective element might be mechanical factors due to dust particles affecting mainly the respiratory system and skin.

In this study the presence of different fungi species in the wheat and flour dust, might be one of the main causes of allergies in flour mills. The results from the skin tests agree with these findings.

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