

Basic Study

## Pulmonary effects of intermittent, seasonal exposure to high concentrations of cotton dust

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### Abstract

**AIM:** To quantify the exposure levels and to assess pulmonary reactions associated with exposure to cotton dust and its biological contaminants.

**METHODS:** All employees (51 male workers) of a ginning industry as well as 51 referent unexposed subjects from clerical staff of an educational center were investigated. Atmospheric concentrations of cotton dust and bioaerosols were measured. Furthermore, bacterial and fungal genera and species were identified by an expert microbiologist and an experienced mycologist. A standard respiratory symptom questionnaire was filled out for the subjects and they underwent multiple spirometry tests, at the beginning and at the end of work season as well as prior to (pre-exposure base line values) and at end of the first shift of workweek (post exposure).

**RESULTS:** Gram negative bacteria including *Enterobacter agglomerans* and *Pseudomonas spp.* were found to be the dominant bacterial species and genera, respectively. Similarly, dominant fungi were identified to

be *Mucor* sp. *Rhizopus* sp. and *Aspergillus niger*. Mean atmospheric concentrations of cotton dust in ginning and outdoor areas were found to be 35.2 and 6.8 mg/m<sup>3</sup>, respectively. The prevalence rates of cough, phlegm, wheezing, dyspnea and grade 1/2 byssinosis among the exposed subjects were significantly higher than their corresponding values for the unexposed employees ( $P < 0.05$ ). Additionally, significant differences were noted in the mean baseline value (preshift) of vital capacity, forced expiratory volume in the first second (FEV1) and FEV1/forced vital capacity ratio of the exposed subjects when compared with those of their referent counterparts. Similarly, significant cross shift decrements were noted in most parameters of pulmonary function of the exposed subjects.

**CONCLUSION:** Seasonal exposure to cotton dust induces both acute, partially reversible, and chronic irreversible decrements in the lungs' functional capacities as well as increased prevalence of respiratory symptoms.

**Key words:** Cotton dust; Bioaerosols; Byssinosis; Lungs' functional impairments; Respiratory symptoms

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**Core tip:** It is not known whether long term seasonal exposure to high concentrations of cotton dust for a few months per year followed by several months of exposure free period in ginning industry is associated with any pulmonary effects. Findings of the present study indicate that even seasonal exposure to high concentrations of this organic dust is a risk factor for byssinosis manifested by acute partially reversible and chronic irreversible significant decrements in lungs' functional capacities and increased prevalence of respiratory symptoms.

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## INTRODUCTION

Cotton dust-induced asthma was first described about 300 years ago. Harvested cotton consists of a mixture of plant materials including leaves, bracts and stems, fiber, bacteria, fungi, and other contaminants<sup>[1]</sup>. Primarily, lint within unopened cotton bolls is free from any contamination<sup>[2]</sup>. However, it quickly becomes contaminated with different germs after the bolls open<sup>[3]</sup>. Endotoxins which originate from gram negative bacteria have been implicated as causal factors in the pathogenesis of byssinosis. Findings of a number of epidemiological and toxicological studies have shown

that exposure to bioaerosols has been associated with inflammatory pathogenic pulmonary effects<sup>[4-6]</sup>. Drummond and Hamlin<sup>[7]</sup> believe that soil is a major source from which cotton dust bacteria originate. Other potential sources include the seeds, insects, airborne microorganisms and germs which are deposited on the plants by the cultivation. Although it seems that more than one of these could be the reservoir, it won't be possible to ascertain where exactly the organisms which colonize the lint come from before the normal flora of the lint are known. Inhalation of cotton dust from textile mills (and also flax dust and soft hemp dust) produces gradual awareness of chest tightness or difficulty getting air into the chest. This generally occurs three or four hours after entering the cotton textile working area. It is accompanied by shortness of breath during periods of exertion and frequently by cough, usually without phlegm<sup>[8,9]</sup>.

From a clinical point of view, byssinosis is initially manifested by complaints described as chest tightness which is sometimes associated with a continual cough, dyspnea, and wheezing. These symptoms normally occur on the first day of the work week. At early stages of the disease, symptoms occur only occasionally and most often when the humidity is very high (grade 1/2 byssinosis). If exposure continues, symptoms progress to grade I byssinosis. At this stage patients complain from chest tightness on most workdays or at least on all first workdays of the week. Several years later, symptoms may progress to grade II byssinosis. At this stage, symptoms are present on days other than Monday and they still are generally worse at the beginning of the week, with many patients noting some degree of improvement at the end of the week. Improvement of the symptoms as the week progresses differentiates byssinosis from nonspecific airway reactivity in which symptoms actually worsen as the work week progresses. Symptoms of grade II byssinosis are reversible, provided that exposure to dust is entirely eliminated or significantly reduced. Otherwise, they progress to grade III byssinosis. Grade III byssinosis continues to worsen to the point where it is clinically irreversible. At this stage, significant chronic airway obstruction has developed<sup>[10]</sup>.

Significant variations exist in the prevalence rates of byssinosis in different parts of the world, ranging from about 1% to 50%<sup>[11-15]</sup>. It is known that washing and steaming of raw cotton significantly decrease the population of bioaerosols and eliminate water soluble chemicals responsible for acute byssinosis. This practice, which significantly reduces the biological activity of byssinosis, explains the low prevalence rate of byssinosis (1.1%) in some countries such as Australia<sup>[1]</sup>.

Additionally, other factors such as differences in the levels and duration of exposure to cotton dust, smoking habits of workers, presence or absence of local exhaust ventilation system in the workplace, and whether employees wear respiratory protective devices<sup>[14,16]</sup> also may explain, at least in part, why

byssinosis does not have a uniform distribution in the world. While byssinosis has shown a descending trend in recent two decades<sup>[11,17,18]</sup>, it has remained high in developing countries<sup>[14,18,19]</sup>. The precise mechanisms by which exposure to cotton dust induces byssinosis are not known. However, it has been proposed that bacterial endotoxins, immune-mediated IgE stimulation, non-immunological release of histamine and fungal (*Alternaria tenuis*, *Aspergillus niger* and *Fusarium solani*)<sup>[1]</sup> proteolytic enzymes may play a role in the pathogenesis of the disease<sup>[8,20-23]</sup>.

Higher prevalence of byssinosis and respiratory symptoms as well as reduced lung functional capacities have been reported in workers who are continuously exposed to high concentrations of cotton dust<sup>[15,24-28]</sup>. However, it is not known whether the same is true for intermittent, seasonal exposure to cotton dust. Ginning process is a seasonal activity which takes place for a few months per year followed by several months of exposure free period. Whether this long-term intermittent seasonal exposure to cotton dust is associated with any acute and/or chronic changes in the parameters of pulmonary function and the prevalence of respiratory symptoms, is not known. Additionally, it is not clear whether bioaerosols contaminating the cotton are similar in different parts of the world. Darab city in Fars province, south of Iran, is a place where cotton is cultivated and harvested at a relatively large scale and then it is processed in a local ginning industry. To date, no study has been carried out to evaluate respiratory health of the subjects exposed to cotton dust in this local plant, the level of exposure of employees of this industry to cotton dust and its bioaerosol contaminants is not known and the types of the cotton bioaerosols have not been determined.

This study was, therefore, undertaken to address these issues and identify employees with different grades of byssinosis, if any, particularly, those with reversible grades (1/2 and 1) whose progression could be prevented by appropriate interventions.

## MATERIALS AND METHODS

### Studied population

This cross sectional study was carried out in a local ginning industry in Darab (latitude and longitude 28.7519° N and 54.5444° E, respectively) city in Fars Province, south of Iran. Darab has a generally warm climate reaching 45 Celsius degrees in the summer. Darab's major agricultural products include wheat, citrus fruit, cotton, maize and palm. Cotton is cultivated and harvested at a relatively large scale and then it is processed in a local ginning industry. The studied population consisted of all employees (51 male subjects) of the plant as well as a group of 51 unexposed referent subjects from clerical staff of an educational center. The plant consisted of three separate sections, of feed, ginning and seed separator. The workers were equally distributed in these sections and frequently traveled between them, without wearing any respiratory pro-

TECTIVE equipment, to perform their duties. In the feed section, cotton is manually fed by the workers to the gin machines where cotton is transferred to a dryer to reduce its moisture and then passes through cleaning equipment for its foreign materials to be removed. In the seed separator section, revolving circular saws pull the lint through closely spaced ribs that prevent the seed from passing through. The lint is removed from the saw teeth by air blasts or rotating brushes, and then compressed into bales. Finally, cotton is stored in a warehouse until it is transferred to a textile mill.

### Prevalence of respiratory symptoms

Standard respiratory symptom questionnaires, as suggested by the American Thoracic Society<sup>[29]</sup>, with some additional specific questions for classification of different grades of byssinosis<sup>[10]</sup>, were filled out for both groups. The questionnaire contained questions regarding the employees' job, work history, symptoms and signs of respiratory diseases and smoking habits.

### Pulmonary function tests

The parameters of pulmonary function including vital capacity, forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and FEV1/FVC ratio were measured at the beginning of work season (November), at the end of work season (January), prior to first shift of workweek (pre-exposure base line values) and at end of the first shift of workweek (post exposure). Spirometry was performed with a calibrated Vitalograph (Spiro Analyzer ST-150, Japan) according to the standard method<sup>[30]</sup>, details of which are to be found elsewhere<sup>[31]</sup>. The mean percentage predicted value was based on the subjects' age, weight, standing height, sex and ethnic group as calculated and adjusted by spirometer device.

### Measurement of cotton dust concentrations, fungi and bacteria

In order to determine the atmospheric concentrations of cotton dust, several samples were collected from different parts of the plant and the mean concentrations were expressed in mg/m<sup>3</sup>. Samples were collected by SKC personal air sampling pumps equipped with PVC filters (5 µm pores). Pretest experiments showed that the appropriate sampling time and flow rate to avoid overloading of the filters were about 30 min and 2.4 L/min, respectively. To assess the extent to which the workers were exposed to bioaerosols, atmospheric concentrations of bacteria and fungi were determined according to NIOSH method 0800, by a single-stage Anderson sampler using Sabouraud Dextrose agar and blood agar as culture media. Flow rate of sampling pump was 28.3 L/min. Pretest experiments showed that the appropriate sampling time was about 6 min. Bacterial and fungal genera and species were identified by an expert microbiologist and an experienced mycologist. Air temperature, pressure and humidity were also measured during air sampling by a dry bulb

**Table 1** Demographic characteristics, smoking habits, and exposure levels of the studied subjects

Variables	Exposed workers (n = 51)	Unexposed subjects (n = 51)
Age (yr) <sup>1</sup>	40.7 ± 11.5 <sup>4</sup>	40.2 ± 12.1
Height (cm) <sup>1</sup>	169 ± 4.8 <sup>4</sup>	170.3 ± 4.8
Weight (kg) <sup>1</sup>	70.1 ± 9.7 <sup>4</sup>	73.5 ± 12.1
Duration of exposure or employment (yr) <sup>1</sup>	10.7 ± 7.8 <sup>4</sup>	9.5 ± 5.2
Marital status <sup>2</sup>		
Single	5 (10%) <sup>4</sup>	9 (18%)
Married	46 (90%) <sup>4</sup>	42 (82%)
Smoking <sup>2</sup>		
Yes	31 (61%) <sup>4</sup>	28 (55%)
No	20 (39%) <sup>4</sup>	23 (45%)
Light <sup>3</sup>	21 (41%) <sup>4</sup>	19 (37%)
Heavy	10 (20%) <sup>4</sup>	9 (18%)
Cotton exposure (mg/m <sup>3</sup> )		
Ginning section	35.2 ± 11.6	NA
Feed section	14.9 ± 6.2	NA
Seed separator area	18.5 ± 10.1	NA
Outdoor	6.8 ± 4.2	NA

<sup>1</sup>Independent sample T test; <sup>2</sup> $\chi^2$  or Fisher's exact test; <sup>3</sup>Light: < 5 cigarettes per day, heavy:  $\geq$  5 cigarettes per day; <sup>4</sup>No significant differences exist between the exposed and unexposed subjects ( $P > 0.05$ ). NA: Not available.

thermometer, a digital barometer and a whirling hygrometer, respectively.

### Statistical analysis

Data were statistically analyzed using  $t$ ; and  $\chi^2$ ; or Fisher's exact test, where applicable (with a preset probability of  $P < 0.05$ ). Additionally, using multiple linear regression analysis, the simultaneous effects of confounding variables on the prevalence of respiratory symptoms and changes in the parameters of pulmonary function were evaluated. Mean concentrations of total dust and bioaerosols in different parts of the mill were compared using ANOVA test. The statistical methods of this study were reviewed by a biostatistician and a clinical epidemiologist.

## RESULTS

The averages (mean  $\pm$  SD) of age (year), weight (kg), height (cm), duration of exposure (length of employment for the referent subjects), marital status and smoking habits of the studied population are presented in Table 1. As shown, there were no significant differences between both groups as far as demographic variables and smoking habits were concerned. None of the exposed subjects had a past medical or family history of respiratory illness or any other chest operations or injuries. Likewise, none of the referent subjects had been exposed to cotton dust or other chemicals known to cause respiratory symptoms or ventilatory disorders during the course of their employment or prior to it. The mean concentrations of cotton dust from 17 area air samples collected from different parts of the

**Table 2** Frequency (%) of respiratory symptoms in the exposed and unexposed subjects<sup>1</sup> n (%)

Symptoms	Exposed (n = 51)	Unexposed (n = 51)
Cough	19 (37) <sup>b</sup>	2 (4%)
Phlegm	19 (37) <sup>b</sup>	4 (8%)
Productive cough	4 (8)	0
Wheezing during a cold	21 (41) <sup>b</sup>	5 (10%)
Wheezing apart from colds	14 (27) <sup>b</sup>	3 (6%)
Wheezing accompanied by	7 (14) <sup>d</sup>	0
Shortness of breath		
Shortness of breath	8 (16) <sup>d</sup>	1 (2%)
Byssinosis grade 1/2	9 (17.6) <sup>b</sup>	0
Byssinosis grade I	3 (6)	0
Byssinosis grade II	2 (4)	0

<sup>1</sup> $\chi^2$  or Fisher's exact test; <sup>b</sup> $P < 0.001$ ; <sup>d</sup> $P < 0.01$ .

plant including ginning, seed separator and feed areas were calculated to be  $35.2 \pm 11.6$ ,  $14.9 \pm 6.2$ , and  $18.5 \pm 10.1$  mg/m<sup>3</sup>, respectively. The mean outdoor concentration of cotton dust (17 air samples) was  $6.8 \pm 4.2$  mg/m<sup>3</sup>. The mean temperature and relative humidity in different parts of the plant were recorded to be as follows: 22.8 °C and 40.8%, 21.5 °C and 34.7%, 21 °C and 34.7%, and 20.7 °C and 33.7% for ginning section, seed separator area, feed section and outdoor air, respectively.

Isolated bacteria were gram negatives including *Enterobacter agglomerans*, *Pseudomonas spp.*, *Citrobacter freundii*, and *Enterobacter aerogenes*. *Enterobacter agglomerans* and *Pseudomonas spp.* were the dominant species and genera, respectively. Additionally, isolated fungi included *Penicillium*, *Mucor*, *Rhizopus*, *Aspergillus niger*, and *Aspergillus fumigatus* from which *Mucor*, *Rhizopus* and *Aspergillus niger* were the dominant genera and species. Bacterial count indicated that the atmospheric concentrations of bacteria in ginning section, seed separator area, feed section and outdoor were  $30045 \pm 8117$ ,  $6495 \pm 1594$ ,  $3103 \pm 883$ , and  $464 \pm 288$  CFU/m<sup>3</sup> respectively. Additionally, the concentrations of fungi in the ginning section, seed separator area, feed section, and outdoor were  $587 \pm 210$ ,  $58 \pm 21$ ,  $393 \pm 94$  and  $33 \pm 16$  CFU/m<sup>3</sup>, respectively. Statistically significant differences were noted among the mean concentrations of bacteria and fungi in different parts of the plant and at the outdoor environment ( $P < 0.05$ ). There were positive correlations between dust concentration and number of bacterial ( $r = 0.786$ ) and fungal ( $r = 0.718$ ) colonies in the work place ( $P \leq 0.0005$ ).

Table 2 shows the frequency of abnormal respiratory findings. As shown, the prevalence of most respiratory symptoms (cough, phlegm, wheezing, shortness of breath, wheezing accompanied by shortness of breath and grade 1/2 byssinosis) in the exposed subjects was significantly higher than those of the referent subjects ( $P < 0.05$ ). Table 3 exhibits the results of pulmonary function tests (PFTs) before the start of the work season



**Table 3** Changes in pulmonary function test parameters of the exposed and unexposed subjects

Parameters	Exposed workers ( <i>n</i> = 51)					Referent group ( <i>n</i> = 51)
	Preshift <sup>1</sup>	Postshift <sup>1</sup>	End of season <sup>2</sup>	End of shift end of workweek <sup>3</sup>	After 48 h of exposure free period <sup>4</sup>	
VC	78.9 ± 16.3 <sup>b</sup>	76.6 ± 17 <sup>d</sup>	73.2 ± 16.6 <sup>f</sup>	78.8 ± 16.3 <sup>b</sup>	76.1 ± 16.3	85.5 ± 13.5
FVC	84.8 ± 12.6 <sup>b</sup>	85.2 ± 12.9	86.2 ± 16.5	85.5 ± 12.8	84.8 ± 12.6	81 ± 4.4
FEV1	79.7 ± 13.7 <sup>b</sup>	78.3 ± 12.4 <sup>d</sup>	74.6 ± 13.1 <sup>f</sup>	76.7 ± 11.7 <sup>b</sup>	79.1 ± 13.7	90.1 ± 10.5
FEV1/FVC	94.1 ± 14.5 <sup>b</sup>	89.3 ± 20.2	89.3 ± 20.2	90.6 ± 12 <sup>h</sup>	94.1 ± 14.5	107.6 ± 14.5

<sup>1</sup>At the beginning of work season (prior to and end of first shift of workweek); <sup>2</sup>At end of the work season; <sup>3</sup>At end of shift at end of workweek; <sup>4</sup>48 h after exposure ceased; <sup>b</sup>*P* < 0.05 *vs* the referent group; <sup>d</sup>*P* < 0.05 *vs* preshift; <sup>f</sup>*P* < 0.05 *vs* preshift; <sup>h</sup>*P* < 0.05 *vs* after 48 h of exposure free period. VC: Vital capacity; FVC: Forced vital capacity; FEV: Forced expiratory volume.

**Table 4** Association between exposure to cotton dust and duration of exposure with changes in the pulmonary function test parameters<sup>1</sup> (*n* = 51)

Period of time	Parameters	Independent variable	Adjusted R <sup>2</sup>	β
At the beginning of work season	VC <sup>b</sup>	Duration of exposure	0.23	-0.5
	FVC <sup>b</sup>		0.4	-0.65
	FEV <sub>1</sub> <sup>b</sup>		0.21	-0.48
	FEV <sub>1</sub> /FVC <sup>d</sup>		0.16	0.42
During the work shift	FVC <sup>a</sup>	Duration of exposure	0.06	-0.28
During the work shift	FEV <sub>1</sub> <sup>d</sup>	Dust concentration	0.14	0.4
During the work season	VC <sup>d</sup>	Dust concentration	0.1	0.4
	FVC <sup>d</sup>		0.1	0.32
	FEV <sub>1</sub> <sup>a</sup>		0.06	0.28

<sup>1</sup>Multiple linear regression analysis; <sup>b</sup>*P* < 0.001; <sup>d</sup>*P* < 0.01; <sup>a</sup>*P* < 0.05. VC: Vital capacity; FVC: Forced vital capacity; FEV: Forced expiratory volume.

**Table 5** Comparison of the subjects with normal spirogram, possibly restrictive, obstructive or mixed pattern for both groups<sup>1</sup>

Period of time		Spirogram pattern <i>n</i> (%)			
		Normal	Restrictive	Obstructive	Mixed
Before exposure, at the start of the work season	Exposed	40 (87.4)	6 (11.8)	5 (9.8)	0
	Unexposed	45 (88.2)	4 (7.8)	2 (3.9)	0
After exposure, at the start of the work season	Exposed	39 (76.5)	4 (7.8)	8 (15.7)	0
	Unexposed	45 (88.2)	4 (7.8)	2 (3.9)	0
At the end of the work season <sup>2</sup>	Exposed	27 (53)	6 (11.8)	18 (35.3)	0
	Unexposed	45 (88.2)	4 (7.8)	2 (3.9)	0

<sup>1</sup>χ<sup>2</sup> or Fisher's exact test; <sup>2</sup>significant difference exists between the exposed and unexposed groups (*P* < 0.001).

(following an eight-month exposure-free period), cross shift and seasonal changes as well as changes in PFTs after a temporary short time (48 h) exposure free period. As shown, base line values of PFTs of the exposed subjects were significantly lower than those of the referent subjects. Additionally, further significant cross shift and seasonal decrements observed after exposure to cotton dust. However, a relative and partial recovery was also noted after a brief, 48 h, exposure free period. Similarly, cross shift changes showed that a significant number of the workers (51%) experienced 5% or more decline in FEV1 value. Stratification for smoking yielded similar results (for the sake of clarity data were not shown).

Association between cotton dust concentration and duration of exposure with the changes in the parameters of pulmonary function is displayed in Table 4. Multiple linear regression analysis including variables of age, weight, height, smoking habits, and marital status

in the model showed that after adjusting for these important confounders, there were statistically significant associations between exposure to cotton dust and duration of exposure with lung function parameters at the beginning of the season, during the work shift and during the work season. Table 5 shows the proportion of the subjects with normal and impaired spirometry results. As shown, at the end of the work season, a significantly higher proportion of the exposed subjects had abnormal spirometry results.

## DISCUSSION

This study was conducted to ascertain whether seasonal intermittent exposure to cotton dust and its bioaerosol contaminants for a couple of months per year, followed by several months of exposure free period, is associated with any symptoms of respiratory disease and/or any acute and /or chronic ventilatory disorders, over years.

Additionally, it aimed to assess the extent to which employees were exposed to cotton dust and bioaerosols and identify and characterize the predominant germs.

Given the data provided, it was evident that seasonal exposure to high concentrations of cotton dust increases the prevalence rates of respiratory symptoms and grade 1/2 byssinosis when compared with the corresponding values of the unexposed referent group (Table 2). Similarly, the base line values of all parameters of pulmonary function of the exposed employees (values at the beginning of work season prior to exposure) were shown to be significantly lower than those of the referent subjects (Table 3) indicating that, under the exposure scenario explained in this study, even seasonal exposure to cotton dust, over years, may induce chronic irreversible ventilatory disorders. Moreover, additional significant cross shift and end of season decrements were noted in most parameters of pulmonary function of the exposed employees. However, a relative, but significant, recovery was also evident in the spiograms of the exposed employees following a short (48 h) exposure-free period (Table 3), indicating that exposure to cotton dust is also associated with acute partially reversible changes in the parameters of pulmonary function. Similar findings have been reported by other investigators where acute airway obstruction has been shown as a result of short-term exposure to cotton dust<sup>[32,33]</sup>.

For employees who are covered by OSHA's Cotton Standard (29 CFR 1910.1043), the exposure limits are as follows: 200  $\mu\text{g}/\text{m}^3$  of cotton dust for yarn manufacturing; 500  $\mu\text{g}/\text{m}^3$  for textile waste houses; 750  $\mu\text{g}/\text{m}^3$  for slashing and weaving operations; and 1000  $\mu\text{g}/\text{m}^3$  for waste recycling and ginning. Operations such as cotton gins and non-textile processing are covered by a different standard (29 CFR 1910.1000). In this standard the PEL is 1  $\text{mg}/\text{m}^3$  measured over an eight-hour workday<sup>[34]</sup>. Dust concentrations in different parts of this plant were much higher than those of similar studies such as those of Jiang *et al.*<sup>[11]</sup>, Zuskin *et al.*<sup>[15]</sup>, Glindmeyer *et al.*<sup>[16]</sup>, Alemu *et al.*<sup>[25]</sup>, Fox *et al.*<sup>[35]</sup>, Molyneux *et al.*<sup>[36]</sup>, and Fishwick *et al.*<sup>[37]</sup>.

No significant differences existed between both groups as far as major confounding variables of weight, height, length of employment, number of smokers, and severity of smoking were concerned. Additionally, the subjects were free from past medical or family history of respiratory illnesses or any other chest operations or injuries. Therefore, the findings of the study could not be attributed to these confounding variables, and particularly, to that of the most important potential confounder, smoking. This conclusion is further supported by the results of multiple linear regression analysis where after adjusting for the effects of potential confounders significant associations were present between exposure to cotton dust and changes in lung function parameters (Table 4).

In this study, prevalence of byssinosis was found to be high (27%); this is consistent with the findings

of Alemu *et al.*<sup>[25]</sup> (43%), Molyneux *et al.*<sup>[36]</sup> (39%), El Batawi *et al.*<sup>[26]</sup> (52.6%), Awad el Karim *et al.*<sup>[38]</sup> (46%), Woldeyohannes *et al.*<sup>[39]</sup> (43%), Memon *et al.*<sup>[40]</sup> (35.6%), and Nafees *et al.*<sup>[41]</sup> (10.5%). Similarly, the prevalence of respiratory symptoms such as cough (37%), phlegm (37%), productive cough (9%), wheezing (27%), shortness of breath (16%) and wheezing associated with shortness of breath (14%) was quantitatively similar to those reported by others<sup>[20,25,42]</sup>. Likewise, significant declines in FEV<sub>1</sub> values noted in this study were in accord with the observations of Wang *et al.*<sup>[20]</sup>, Jiang *et al.*<sup>[11]</sup>, Zuskin *et al.*<sup>[15]</sup>, Fox *et al.*<sup>[35]</sup>, Kamat *et al.*<sup>[42]</sup>, and Christiani *et al.*<sup>[43]</sup>.

The rate of FEV<sub>1</sub> decrement has been proposed as an appropriate parameter to evaluate the effects of exposure to cotton dust. For clinicians, it is essential to determine the association between exposure to cotton dust and decline in FEV<sub>1</sub> and FVC values. The bronchospasm (which is typically reversible at early stages), seen with byssinosis, can be demonstrated by spirometry performed prior to the start of a working shift and again 5 to 6 h later to determine if there has been any diminution in FEV<sub>1</sub> value. A 10% decline in FEV<sub>1</sub> is generally considered to be sufficient evidence that a worker is significantly reactive to cotton dust. It is important to note that OSHA considers FEV<sub>1</sub> declines of as little as 5% to be clinically significant. In these cases, OSHA requires that such individuals be placed on a program of increased surveillance<sup>[10]</sup>. In the present study, a significant number of the workers (51%) experienced 5% or more decline in FEV<sub>1</sub>.

More than one third (35.3%) of the employees, at the end of the work season, had a spirometric pattern consistent with that of obstructive ventilatory disorders (Table 5) because in obstructive ventilatory disorders FVC is either normal or increased, but the hallmark is a decreased FEV<sub>1</sub>. Therefore, the ratio of FEV<sub>1</sub>/FVC is characteristically decreased<sup>[44]</sup>. This is consistent with the mechanism of respiratory effects of exposure to cotton dust<sup>[11,20,43]</sup>. Given the reversible nature of byssinosis and other respiratory disorders and symptoms at the early stages, susceptible individuals should be identified and placed under increased surveillance and their exposure to cotton dust should be eliminated or significantly reduced. Moreover, the high prevalence of respiratory symptoms and disorders noted in this study deserve serious attention.

Different genera and species of gram negative bacteria (*Enterobacter agglomerans*, *Pseudomonas spp.*, *Citrobacter freundii*, and *Enterobacter aerogenes*) and fungi (*Penicillium*, *Mucor*, *Rhizopus*, *Aspergillus niger* and *Aspergillus fumigatus*) were isolated and identified. While some investigators have suggested that similar organisms are harbored on lint from cotton grown and harvested from different parts of United States, and other countries<sup>[45]</sup>, others<sup>[46]</sup> believe that these may vary from one sample to another, and a large number of samples would be required to establish a valid generalization regarding the types of

bacteria commonly present. Rylander and Lundholm<sup>[47]</sup> examined the bacterial contamination of various parts of the cotton plant as well as the baled cotton in several textile mills. They found that the predominant bacterial species were *Enterobacter agglomerans*, *Pseudomonas syringae* and *Agrobacterium spp.* which were found in about 60% of the cotton samples. They reported that waste cotton from carding machines contained up to 10<sup>8</sup> bacteria/g and that occasionally up to 50% of them were gram positive. They also examined raw cotton plant parts and cotton from blending machines and isolated gram-negative microorganisms such as *Enterobacter agglomerans*, *Pseudomonas syringae*, *Agrobacterium*, and occasionally klebsiella, *Enterobacter cloacae*, *Acinetobacter*, *Flavobacterium*, and other *Pseudomonas*. Furthermore, the most widely isolated fungi were several species of *Aspergillus*<sup>[47]</sup>. Air borne respirable fungal species of cotton mills were listed by Fischer<sup>[48]</sup>. *Aspergillus* species including niger, glaucus, and versicolor, *Penicillium*, *Hormodendrum*, *Fusarium*, *Alternaria*, and occasionally *Rhizopus* were the most isolated germs.

Our findings are qualitatively compatible with those of other studies in which most contaminants of cotton dust were reported to be gram negative bacteria<sup>[2,3,47]</sup>. However, quantitatively, airborne concentrations of bacteria and fungi in this study were lower than those of Cinkotai *et al.*<sup>[49]</sup>, Lacey *et al.*<sup>[50]</sup>, and Tuffnell *et al.*<sup>[51]</sup>. This is presumably due to unfavorable climate conditions for rapid growth of bacteria and fungi (lower ambient temperatures and relative humidity in our studied plant over the course of study in the winter). Additionally, fungal and bacterial contaminants of the cotton dust at species level were not necessarily similar to those reported from elsewhere of the world.

Inherent limitations of cross sectional studies such as the present study do not allow a cause and effect relationship to be established. Therefore, it may be argued that significant increases in the prevalence of respiratory symptoms and decrements in the lungs' functional capacities could not necessarily be linked with exposure to cotton dust. While true, it should none the less be noted that a few lines of circumstantial evidence indicate that these are very likely to be the direct consequence of exposure to cotton dust and its biological contaminants. First, none of the exposed employees had any history of respiratory disorders or preexisting medical conditions at the beginning of their employment in the plant or prior to it. Second, the exposed individuals, apart from cotton dust, did not have any exposure to chemical agents known to be pulmonotoxic during the course of their employment in the plant or prior to it. Third, the prevalence of respiratory symptoms was significantly higher in the exposed workers than in the unexposed population. Fourth, although the exposed subjects performed better in their pre-shift spirometry test (the test was conducted after a 48 h exposure free period), the difference between the exposed and unexposed groups remained

statistically significant. Fifth, after adjusting for potential confounders significant associations were noted between exposure and reduced lungs' functional capacities and increased prevalence of respiratory symptoms. Sixth, base line, end of shift and end of season values of PFTs of the exposed subjects were both significantly lower than those of referent individuals.

In conclusion, the findings of the present study collectively indicate that unprotected long term intermittent seasonal occupational exposure to high concentrations of cotton dust and its contaminating bioaerosols, even for a couple of months per year, can be a risk factor for byssinosis manifested by acute, partially reversible, and chronic irreversible significant decrements in the lungs' functional capacities and increased prevalence of respiratory symptoms. Additionally, while cotton dust was contaminated with gram negative bacteria and fungi, they were not exactly similar to those reported from elsewhere in the world, *Enterobacter*, *Alternaria tenuis*, *Aspergillus niger* and *Fusarium Solani*<sup>[1]</sup>. Thus, engineering control measures (replacing of old machines by new ones, work rotation, and installation of local exhaust ventilation systems), administrative measures (work rotation and changing the job of susceptible and reactive individuals), and the use of appropriate respirators are recommended to eliminate or reduce the workers' exposure to this organic dust. Additionally, the exposed workers should be instructed to quit smoking. Active rather than common periodic examinations are recommended to identify reactive workers to cotton dust prior to developing permanent irreversible respiratory disorders. Further, longitudinal, follow up cohort studies with larger sample sizes are required to further substantiate our findings and provide corroborative evidence in favor of the notion that long-term seasonal exposure to high concentrations of cotton dust in ginning industry is associated with byssinosis, similar to continuous exposure to this organic dust in textile industries.

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## COMMENTS

### Background

Some studies in the textile industry have shown that the prevalence rates of byssinosis and respiratory symptoms in workers who are continuously exposed to cotton dust are very high. Similarly, significant associations between exposure to cotton dust and reduced lung functional capacities have been demonstrated. However, it is not known whether the same is true for intermittent, seasonal exposure to cotton dust. Ginning process is a seasonal activity which takes place for a few months per year followed by several months of exposure free period. Whether this long-term intermittent seasonal exposure to cotton dust is associated with any acute and/or chronic changes in the parameters of pulmonary function and increased prevalence of respiratory symptoms, is not known. Additionally, it is not clear whether bioaerosols

contaminating the cotton are similar in different parts of the world. This study was undertaken to further address these issues.

### Innovations and breakthroughs

Interestingly, the findings of our study demonstrate that even seasonal exposure to cotton dust, over years, may induce chronic irreversible ventilatory disorders. Moreover, additional significant cross shift and end of season decrements were noted in most parameters of pulmonary function of the exposed employees. However, a relative, but significant, recovery was also evident in the spiograms of the exposed employees following a short (48 h) exposure-free period, indicating that exposure to cotton dust is also associated with acute partially reversible changes in the parameters of pulmonary function.

### Applications

Similar to textile industries, in ginning industries also active rather than common periodic examinations are recommended to identify reactive workers to cotton dust prior to developing permanent irreversible respiratory disorders.

### Peer-review

The manuscript is well written. The aims are well spelt out and the methodology is sound. The results are also well tabulated and described followed by adequate interpretations of the findings in the Discussion.

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