EFFECTS OF EXPOSURE TO FLAX DUST IN POLISH FARMERS: WORK-RELATED SYMPTOMS AND IMMUNOLOGIC RESPONSE TO MICROBIAL ANTIGENS ASSOCIATED WITH DUST

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Abstract: Medical examinations were performed in a group of 51 Polish farmers heavily exposed to flax dust during harvesting and scutching (threshing) and in a group of 50 healthy urban dwellers not exposed to organic dusts (controls). The examinations included: interview concerning the occurrence of respiratory disorders and work-related symptoms, physical examination, X-ray examination of chest, lung function tests, oxymetric examinations, determination of the concentration of cytokines (IL-1α, IL-6, TNFα) in blood serum and allergological tests with microbial antigens associated with organic dust, comprising: skin prick test with 4 antigens, agar-gel precipitation test with 12 antigens and test for specific inhibition of leukocyte migration with 4 antigens. As many as 32 farmers (62.7%) reported the occurrence of work-related symptoms during harvesting, transporting and scutching of flax. The most common complaint was general weakness reported by 15 farmers (29.4%), followed by headache reported by 14 (27.5%), blocking of the nose - by 11 (21.6%), dry cough, shivering, and eyes itching - each by 10 (19.8%), chest tightness and hoarseness - each by 9 (17.6%). No control subjects reported these work-related symptoms. The mean spirometric values in the examined group of farmers were within a normal range and did not show a significant post-shift decline. In contrast, a significant post-shift decline of oxymetric values was found among flax farmers. The farmers showed a frequency of the positive early skin reactions to environmental allergens in the range of 0–19.6%, a frequency of positive precipitin reactions in a range of 0–56.9%, and frequency of positive reactions of leukocyte migration inhibition in a range of 7.8–21.6%. The members of the control group responded to the majority of allergens with a significantly lower frequency of positive results compared to the farmers. Elevated concentrations of IL-1α and IL-6, but not TNFα, were found in blood sera of flax farmers. In conclusion, farmers engaged in harvesting and scutching of flax represent a group of elevated professional risk because of high incidence of work-related symptoms and high frequency of allergic reactions to bacteria and fungi associated with organic dust.

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Key words: farmers, flax, harvesting, scutching, organic dust, occupational exposure, health effects, work-related symptoms, allergy, microbial antigens, cytokines.

INTRODUCTION

It has been known for many years that the inhalation of dust released during handling of plant fibres from flax, cotton, jute and hemp may cause respiratory disorders in exposed workers [1, 11, 15, 23, 28, 37, 44]. Among the workers handling flax (Linum usitatissimum), the best known disorder is byssinosis, a condition widely studied...
MATERIALS AND METHODS

Examined population. Demographics of the study population are shown in Table 1. A group of 51 farmers growing flax (farm owners and their relatives) living in the Lublin region (eastern Poland) was examined. The farmers were examined when harvesting, unloading and scutching flax. As a control group, 50 office workers not exposed to organic dusts were examined. Human subjects protocols were approved by the Ethics Commission of the Institute of Agricultural Medicine and all subjects gave formal consent.

Medical examinations. Farmers were interviewed with the American Thoracic Society (ATS) standard questionnaire and with the questionnaire developed in the Institute of Agricultural Medicine in Lublin for the study of work-related symptoms. They were next subjected to routine physical examinations, X-ray examinations of chest, and to lung function examinations with the LUNGTEST 500 spirometer produced by MES (Kraków, Poland). The spirometric values of the forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC (%), and peak expiratory flow (PEF) were determined. Oxymetric examinations were also carried out with the use of the Pulsox-6 oximeter (Hoyer, USA).

Allergological tests. Skin tests, agar-gel precipitation tests and tests for specific inhibition of leukocyte migration were applied to the groups of flax farmers and controls using the antigens of microorganisms, occurring commonly in the agricultural working environment of eastern Poland. In all tests lyophilised saline extracts of bacterial or fungal mass, produced in the Institute of Agricultural Medicine in Lublin, were used as antigens. In the case of mesophilic, non-branching bacteria the mass was harvested from nutrient agar cultures, while in the case of actinomycetes and fungi the mass was harvested from sugar broth cultures. Then, the mass was homogenised and extracted in saline (0.85% NaCl) in the proportion 1 : 2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwars, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.1–0.15 of previous volume and lyophilised [26, 43].

Skin tests were carried out by prick method with the antigens of Pantoea agglomerans (syn.: Erwinia herbicola, Enterobacter agglomerans), Streptomyces albus, Saccharopolyspora rectivirgula (syn.: Micropolyspora faeni, Faenia rectivirgula) and Aspergillus fumigatus. The antigens were dissolved in saline (P.B.S., Biomed,
Effects of exposure to flax dust in Polish farmers

Kraków, Poland) at the concentration of 5 mg/ml, sterilised by filtering and checked for sterility and lack of toxicity. The test was performed on the forearm with the antigenic extracts and P.B.S. as a control. The test sites were observed at 20 min. The wheal and/or erythema reactions of 3 mm or more in diameter were regarded as positive [43].

The agar-gel precipitation test was performed by Ouchterlony double diffusion method in purified 1.5% Difco agar with the antigens of Acinetobacter calcoaceticus, Alcaligenes faecalis, Arthrobacter globiformis, Bacillus subtilis, Pantoea agglomerans, Streptomyces albus, Saccharopolyspora rectivirgula, Thermoaotinomyces vulgaris, Alternaria alternata, Aspergillus candidus, Aspergillus fumigatus, and Penicillium citrinum. The undiluted serum separated from the circulatory blood sample of each examined subject was placed in the central well, and antigens - at the concentration of 30 mg/ml - in the peripheral wells. The plates were incubated for 6 days at room temperature, then washed in saline and 5% sodium citrate solution (to prevent false positive reactions), and stained with azocarmine B [30, 43].

Test for inhibition of leukocyte migration in the presence of specific antigen was performed by the whole blood capillary microculture method according to Bowszyc et al. [3] with the antigens of Arthrobacter globiformis, Pantoea agglomerans, Saccharopolyspora rectivirgula and Aspergillus fumigatus. Patient’s blood and Parker’s culture medium were added in the volumes of 0.5 ml and 0.12 ml, respectively, to two silicon test tubes. Then, 0.12 ml of the antigen solution in the concentration of 100 µg/ml was added to one tube, while to the other 0.12 ml of the diluent (P.B.S.) as a control. Both suspensions were incubated for 30 min at room temperature and thereafter distributed to heparinised glass capillars 75 × 1 mm. Capillars were sealed at both ends with a 4:1 mixture of paraffin and vaseline, centrifuged for 10 min at 1,500 rev/min and fastened tangentially on microscopic slides with sticky tape at an angle of 10°. The microcultures obtained were incubated for 4 hrs at 37°C in a humid chamber. The leukocyte migration distances, visible as distinct white zones, were measured under a binocular microscope. The results were expressed as a migration index (MI), e.g. the ratio of the mean migration distance of leukocytes in microcultures with antigen, to the analogical distance in microcultures without antigen. The test was considered as positive at the MI equal to 0.790 or lower.

Determination of cytokine concentrations. The concentrations of interleukin-1α (IL-1 α), interleukin-6 (IL-6) and tumour necrosis factor α (TNF α) were determined in the serum samples of flax farmers and controls with enzyme-linked immunosorbent assay (ELISA) using ENDOGEN commercial sets (Endogen, Inc., Woburn, MA, USA). Absorbance was measured on an ELISA reader at 450 nm and 550 nm. A result was considered as positive if absorbance was greater than cut-off evaluated according to the instruction.

Statistical analysis. The obtained results were analysed by the Student’s t-test and by Pearson’s test for correlation.

RESULTS

Occurrence of work-related symptoms in grain handling farmers. Thirty-two out of 51 interviewed farmers (62.7%) reported the occurrence of work-related, general and respiratory symptoms during handling of flax.
The most common complaint was general weakness reported by 15 farmers (29.4%), followed by headache reported by 14 (27.5%), blocking of the nose - by 11 (21.6%), dry cough, shivering, and eyes itching - each by 10 (19.6%), chest tightness and hoarseness - each by 9 (17.6%) (Tab. 2). Among 32 farmers having work-related symptoms, eight persons reported only one symptom, eight - two symptoms, two - three symptoms, five - four symptoms, one - five symptoms, two - six symptoms, three - seven symptoms, two - eight symptoms, and one - as many as nine symptoms.

Five out 51 examined farmers (9.8%) reported symptoms characteristic for chronic bronchitis according to the ATS questionnaire (Tab. 1). Physical examination by auscultation found crepitations and rales in 2 persons (3.2%). X-ray examinations of chest were normal in all persons.

None of the members of the control group reported the occurrence of work-related symptoms or symptoms characteristic for chronic bronchitis.

### Lung function changes

The mean baseline spirometric values in the groups of farmers and controls did not show significant differences compared to the normal values [33]. A slight post-shift decrease of the mean values of FVC, FEV₁ and PEF were noted in flax farmers; these differences, however, were not significant and remained within a normal range (Tab. 3). No differences between the spirometric values before and after work were found in control subjects.

### Oxymetric changes

A significant post-shift decline of blood saturation with oxygen was found in flax farmers (p<0.001), while no significant difference occurred in the control group (Tab. 4). Nevertheless, in both groups the oxymetric values remained constant within a normal range.

### Table 3. Mean spirometric values in farmers engaged in harvesting and scutching of flax and in control subjects.

<table>
<thead>
<tr>
<th>Spirometric values</th>
<th>Flax farmers (n=51)</th>
<th>Controls (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Percent of predicted value (%)</td>
</tr>
<tr>
<td>FVC I (ml)</td>
<td>4340 ± 1360</td>
<td>103.8</td>
</tr>
<tr>
<td>FVC II (ml)</td>
<td>4000 ± 1210</td>
<td>94.8</td>
</tr>
<tr>
<td>FEV₁ I (ml)</td>
<td>3410 ± 1090</td>
<td>98.1</td>
</tr>
<tr>
<td>FEV₁ II (ml)</td>
<td>3130 ± 0.960</td>
<td>90.4</td>
</tr>
<tr>
<td>FEV₁/FVC I (%)</td>
<td>75.64 ± 12.84</td>
<td>92.5</td>
</tr>
<tr>
<td>FEV₁/FVC II (%)</td>
<td>77.51 ± 9.52</td>
<td>93.1</td>
</tr>
<tr>
<td>PEF I (l/sec)</td>
<td>7.12 ± 2.36</td>
<td>88.6</td>
</tr>
<tr>
<td>PEF II (l/sec)</td>
<td>6.65 ± 2.18</td>
<td>82.0</td>
</tr>
</tbody>
</table>

I - before work, II - after work. The predicted value for each individual has been corrected for age, height, sex and smoking status [33].

### Table 4. Mean oximetric values in farmers engaged in harvesting and scutching of flax and in control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Flax farmers (n=51)</th>
<th>Controls (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Saturation O₂ I (%)</td>
<td>97.42 ± 0.9</td>
<td>98.12 ± 0.71</td>
</tr>
<tr>
<td>Saturation O₂ II (%)</td>
<td>95.11 ± 0.79***</td>
<td>97.80 ± 0.73</td>
</tr>
</tbody>
</table>

I - before work, II - after work; * - *** a significant cross-workshift decline; **p<0.001.

### Table 5. Frequency of positive precipitin reactions in grain farmers and controls (%).

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Flax farmers (n=51)</th>
<th>Control group (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter calcoaceticus</td>
<td>9.8</td>
<td>6.0</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>25.4*</td>
<td>8.0</td>
</tr>
<tr>
<td>Pantoea agglomerans</td>
<td>56.9***</td>
<td>12.0</td>
</tr>
<tr>
<td>Arthrobacter globiformis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomyces albus</td>
<td>21.6***</td>
<td>0</td>
</tr>
<tr>
<td>Saccharopolyspora rectivirpula</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Thermoactinomyces vulgaris</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus candidus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>35.3**</td>
<td>8.0</td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>3.9</td>
<td>0</td>
</tr>
</tbody>
</table>

* - ***: value significantly higher compared to control group. *p < 0.05, **p < 0.01, ***p < 0.001.
Allergic reactions. None of the examined farmers showed a positive skin reaction to *Streptomyces albus*, whereas the frequency of positive skin response to other three microbial allergens associated with organic dust was within a range of 7.8–19.6%. Ten farmers (19.6%) showed immediate positive skin reactions to *Saccharopolyspora rectivirgula*, 8 (15.7%) to *Aspergillus fumigatus*, and 4 (7.8%) to *Pantoea agglomerans* (Tab. 6). The frequency of positive skin response to the same allergens in the control group was within a range 0–2.0% and in the case of *S. rectivirgula* and *A. fumigatus* was significantly lower compared to farmers (p < 0.01 and p < 0.05, respectively).

The frequency of positive precipitin reactions to 12 microbial allergens associated with organic dust was within a range of 0–56.9% in the group of flax farmers, and within a range of 0–12.0% in the control group (Tab. 5). In the group of farmers, the incidence of positive precipitin reactions with the antigens of *Pantoea agglomerans*, *Aspergillus fumigatus*, *Alcaligenes faecalis*, and *Streptomyces albus*, was distinctly higher compared to other antigens (56.9%, 35.3%, 25.4%, and 21.6%, respectively), and occurred significantly more frequently than in the control group (p < 0.05). The frequency of positive reactions to 4 other bacterial and fungal allergens (*Bacillus subtilis, Saccharopolyspora rectivirgula, Penicillium citrinum, Acinetobacter calcoaceticus*) was much lower, being in the range of 2.0–9.2%, while with remaining 4 antigens (*Arthrobacter globiformis, Thermoactinomyces vulgaris, Aspergillus candidus, Alternaria alternata*) no positive reactions were found.

In the test for inhibition of leukocyte migration in the presence of specific antigen, the frequency of positive reactions with 4 antigens ranged from 7.8–21.6% in the group of flax farmers. In all cases it was significantly higher compared to control group (p < 0.05) where all results were negative (Tab. 7). The positive cellular response was highest to *Aspergillus fumigatus* (21.6%), followed by *Pantoea agglomerans* (17.6%), *Arthrobacter globiformis* (7.8%), and *Saccharopolyspora rectivirgula* (7.8%).

Thirty one out 32 farmers reporting work-related symptoms showed the positive response to the examined allergens, at least to one allergen in one test. This response rate (96.9%) was significantly greater (p < 0.05) compared to farmers not reporting symptoms (68.4%). The response rates to particular allergens were usually greater in symptomatic farmers than in asymptomatic ones, but did not attain the significance level except for skin response to *Aspergillus fumigatus* (25.0% vs 0, p < 0.05). The response rates in the test for inhibition of leukocyte migration were distinctly greater in symptomatic farmers but only in the case of *Pantoea agglomerans* where a tendency towards a significant difference (25.0% vs 5.2%, 0.05 < p < 0.1) was observed.

Cytokine concentrations. The blood serum concentrations of interleukin-1α (IL-1α) and interleukin-6 (IL-6) were...
significantly elevated in 7.8% and 17.6% of flax farmers, being significantly higher compared to control group (p < 0.05) where all results were within normal range. No significant elevation of tumour necrosis factor (TNFα) was found, either in the farmers’ or control group.

**DISCUSSION**

The frequency of work-related symptoms among farmers exposed to flax dust was high (62.7%), distinctly higher compared to those exposed to grain dust (44.7%) examined in an earlier study [43]. Moreover, the structure of reported symptoms in both groups was totally different. While among grain handlers there prevailed typical, ODTD-like respiratory symptoms (dry cough, followed by dyspnoea) [43], among flax handlers the most common were general symptoms: general weakness, followed by headache.

The common occurrence of work-related symptoms among flax handlers was not accompanied by changes in lung function. The spirometric values were within a normal range and did not show a significant post-shift decline. Similar results were obtained by Zejda et al. [46] among grain farmers. In contrast, oxymetric values showed a significant post-shift decline in flax farmers. However, this finding should be interpreted with caution, as the post-shift oxymetric values did not fall beyond the normal range. The incidence of the clinically diagnosed chronic respiratory illnesses among examined workers exposed to flax dust (9.8%) was lower compared to the data reported by Malenkii [23], Noweir et al. [28] and Zaritskaia [44], similar to those given by Cinkotai et al. [5] and higher than figures reported by Baskakova [1] for the workers of large flax processing factory. To summarize, the obtained results suggest that work-related symptoms observed in this study among the rather young population of flax farmers had mostly a mild character, corresponding to the initial stages of organic dust toxic syndrome (ODTS, toxic pneumonitis) and/or byssinosis. Nevertheless, as - according to earlier authors [23, 28] - the severe occupational disease in flax farmers may still appear after a long job duration (20-30 years), the common occurrence of work-related symptoms found in this study should be considered as a significant health problem in this occupational group.

The occupational risk in the examined group of flax farmers is increased by the common occurrence of allergic reactions to the microbial antigens associated with organic dust. Of particular significance are high frequencies of positive reactions found in the test for inhibition of leukocyte migration in the presence of specific antigen, which is recognised as reliable in detecting the specific agents causing cell-mediated inflammation [13, 26, 27]. Antigens of *Aspergillus fumigatus* and *Pantoea agglomerans* showed the highest prevalence of positive reactions in this test and, considering also the results of other tests, should be regarded as major risk factors for the flax handling workers.

The overall results of the tests seem to indicate a filamentous fungus *Aspergillus fumigatus* as the most important occupational health hazard for flax farmers. The development of this species and other fungi on flax is enhanced by wetting of flax by farmers for better separation of fibres during scutching [39]. *Aspergillus fumigatus* is a well-known occupational pathogen, causing allergic alveolitis, asthma, aspergillosis and most probably also immunotoxic disorders [7, 17, 21, 22]. In the present study, the frequency of positive reactions to *A. fumigatus* in all tests was significantly greater than in controls. The potentially pathogenic properties of fungi associated with flax were recently confirmed by Pieckova and Jesenska [31] who found that 29% of the strains of filamentous microfungi isolated from flax were toxic for chick tracheal cultures.

*Pantoea agglomerans* appeared as a second important hazard for flax farmers. This epiphytic Gram-negative bacterium, common in air polluted with flax dust [18] is widely distributed in nature. It produces strong endotoxin [8, 24, 25] and allergens [9, 10, 19, 24, 25, 42, 43] and represents a major cause of allergic alveolitis in eastern Poland [19, 26]. In the present study, the antigenic extract of this bacterium gave the highest rate of positive precipitin reactions and the second high rate of positive reactions in the test for inhibition of leukocyte migration.

**Table 8.** Proportion of farmers showing elevated levels of cytokines in blood serum after exposure to flax dust, compared to controls.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Flax farmers (n=51)</th>
<th>Control group (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-1α</td>
<td>Persons with significant elevation (%)</td>
<td>4 (7.8%)*</td>
</tr>
<tr>
<td></td>
<td>Range of elevated concentrations (pg/ml)</td>
<td>2.92–9.14</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Persons with significant elevation (%)</td>
<td>9 (17.6%)**</td>
</tr>
<tr>
<td></td>
<td>Range of elevated concentrations (pg/ml)</td>
<td>2.12–90.19</td>
</tr>
<tr>
<td>TNFα</td>
<td>Persons with significant elevation (%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Range of elevated concentrations (pg/ml)</td>
<td>0</td>
</tr>
</tbody>
</table>

*a*significant elevation was considered at 2.0 pg/ml.  
**,**: value significantly higher compared to control group, *p < 0.05, **p < 0.01.
Among other examined microbial species, some may also play a role as potential occupational allergens. The coryneform bacterium *Arthrobacter globiformis*, described as a cause of allergic alveolitis in eastern Poland [26], gave 7.8% positive reactions in the test for inhibition of leukocyte migration, significantly more than in controls. The thermophilic actinomycete *Saccharopolyspora rectivirgula*, the best known causative agent of farmer’s lung [21], showed a significant proportion of positive reactions in the prick test and test for inhibition of leukocyte migration. In the precipitation test, rather unexpectedly, the prevalence of positive reactions to this antigen was low. In contrast, the mesophilic actinomycete *Streptomyces albus*, described also as a cause of allergic alveolitis [16], showed a high frequency of precipitin reactions. A high frequency of positive precipitin reactions was also found with the antigen of Gram-negative bacterium *Alcaligenes faecalis*, similar to the study by Kuś et al. [20] in a group of Polish herb processing workers exposed to the inhalation of large quantities of these bacteria, and in our earlier study in grain farmers [43]. Another allergenic Gram-negative species, *Acinetobacter calcoaceticus* [41], in spite of common occurrence in air polluted with flax dust [18], gave a lower proportion of precipitin reactions (9.8%).

To the best of our knowledge, no tests with the microbial antigens associated with organic dusts have yet been performed among flax handling workers, so that any comparisons with the allergological tests carried out by earlier authors have a limited value because of the differences in allergens and tests used. Using intradermal injections of flax dust extracts, Russian authors obtained high frequency of positive results in the workers of flax factories (50.3–57.3%) [2, 11]. The results were even higher (71–93.4%) in the subgroups of workers with respiratory disorders [2, 11, 44]. According to recent views, cytokines (mostly IL-1, IL-6, IL-8, TNFα,) play an important role as mediators of inflammatory reactions caused by the inhalation of organic dusts and their constituents, mostly endotoxins [4, 6, 34, 37, 40]. The elevation of the concentrations of IL-1α and IL-6 found in this study in the sera of examined farmers suggests a possible role of these interleukins in the pathogenesis of the disorders due to inhalation of flax dust. No changes in the concentrations of TNFα, a cytokine associated with endotoxin exposure [4], was found, which suggests, that in spite of high pollution of work environment with endotoxin [18], other agents are also probably involved in the pathogenic processes.

CONCLUSION

Farmers engaged in harvesting and sifting of flax represent a group at elevated professional risk because of the high incidence of work-related symptoms and high frequency of allergic reactions to bacteria and fungi associated with organic dust.

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