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Health effects of exposure to inhalation of organic dust in hop farmers

Organic dusts and their biological components (bioaerosols), comprising potentially allergenic microorganisms (bacteria, fungi, actinomycetes) and bacterial endotoxin, are the main cause of occupational respiratory diseases among agricultural workers. Bioaerosols may evoke in exposed workers specific allergic reactions (4-8, 10, 12, 15, 18) and non-specific immunotoxic reactions (4, 11, 13, 19, 21). In contrast to other organic dusts (e.g. grain or flax dusts), little is known about the effects of hop (*Humulus lupulus*) dust to persons occupationally exposed to this dust. Alexandrov and Georgijev (1) found only low concentration of microorganisms in the air of hop processing plants in Russia and suggested that it was due to antimicrobial activity of hops. Langezaal et al. (9) found that essential oils and extracts of hops showed antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and the fungus *Trichophyton mentagrophytes*, but not against Gram-negative bacterium *Escherichia coli* and the yeast *Candida albicans*.

The above results may suggest that the exposure to hop dust is not as harmful as to other organic dusts. However, work-related health problems that occur among hop processing workers while sorting and drying, do not confirm this conclusion. The exposure to hop dust and fumes may cause asthma, conjunctivitis, rhinitis and dermatitis, due to allergic and/or toxic mechanisms (14,16,22). Hop dust showed a strong contractile activity on isolated tracheal smooth muscle (17).

The aim of the present work was to assess the effects of the exposure to hop dust in the population of farmers from eastern Poland engaged in cultivating, sorting and drying of this plant. It is noteworthy that until recently this occupational group has been the subject of only few investigations (22).

MATERIAL AND METHODS

Examined population. A group of 23 hop growing farmers (13 males and 10 females) aged 51.13 ± 14.06 (mean ± S.D.) were examined. The farmers lived in the village S., located on the territory of the Łęczna district, Lublin region, eastern Poland. The study was conducted on individual farms while harvesting, sorting and transporting hop cones. As a reference group, 50 office workers and students living in the city of Lublin who were not exposed to organic dust were examined (Tab. 1).

Table 1. Demographics of the study population

	Hop farmers	Reference group
Number of examined persons (N)	23	50
Males	13 (56.5%)	24 (48%)
Females	10 (43.5%)	26 (52%)
Mean age (years, x ± S D)	51.13 ± 14.06	37.36 ± 11.95
Persons reporting work-related symptoms	5 (21.7%)	0
Persons reporting symptoms of chronic obturative pulmonary disease (COPD)	8 (34.8%)	0
Current smokers	5 (21.7%)	19 (38%)
Ex-smokers	5 (21.7%)	6 (12%)
Never smoked	13 (56.5%)	25 (50%)

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 (3) for the study of work-related symptoms among persons exposed to organic dust. Next, the examined persons were subjected to routine physical examinations and to lung function examinations with the use of spirometer and pickflowmeter produced by MES (Kraków, Poland). The spirometric values of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC (%), and peak expiratory flow (PEF) were determined.

Determination of cytokines concentration. The concentrations of interleukin–1α (IL–1 α), tumour necrosis factor α (TNF-α), and interleukin–6 (IL–6) were determined in the serum samples of hop farmers and referents. The tests were carried out by enzyme-linked immunosorbent assay (ELISA) with the use of ENDOGEN commercial sets (Endogen, Inc., Woburn, MA, USA).

Allergological tests. The following tests were performed: skin test, precipitation test, test for inhibition of leukocyte migration, and ELISA test for the detection of specific antibodies against *Aspergillus fumigatus*. In all tests (except for ELISA) lyophilised saline extracts of bacterial or fungal mass produced in the Department of Occupational Biohazards at the Institute of Agricultural Medicine in Lublin were used as antigens.

Skin tests were carried out by prick method with the antigens (allergens) of the following microorganisms: *Pantoea agglomerans* (syn.: *Erwinia herbicola, Enterobacter agglomerans*), *Saccharopolyspora rectivirgula* (syn.: *Micropolyspora faeni, Faenia rectivirgula*), *Streptomyces albus, Aspergillus fumigatus,* and the mixture of two hop extracts "A" and "B" in the proportion 1 : 1: "A" in 0.5 % phenol, "B" in 10 % glycerine (in the case of hop farmers). The antigens were dissolved in saline (P.B.S., Biomed, Kraków, Poland) at the concentration of 5 mg/ml, sterilized by filtering through Minisart NML 0.45 μm filter, and thereafter 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 after 20 min. The wheal and/or erythema reactions of 3 mm or more in diameter were regarded as positive (18).

The agar-gel precipitation test was carried out by double diffusion method in purified 1.5% Difco agar, using staining with azocarmine B (15). The test was performed with serum samples of hop farmers and referents and antigens of the twelve following microorganisms: *Acinetobacter calcoaceticus, Alcaligenes faecalis, Arthrobacter globiformis, Bacillus subtilis, Pantoea agglomerans, Saccharopolyspora rectivirgula, Streptomyces albus, Thermoactinomyces vulgaris,*

Alternaria alternata, Aspergillus candidus, Aspergillus fumigatus, Penicillium citrinum, and the mixture of two hop extracts "A" and "B" in the proportion 1 : 1: "A" in 0.5 % phenol, "B" in 10 % glycerine (in the case of hops farmers). All antigens were dissolved in PBS. at the concentration of 30 mg/ml.

A 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. (2) with the antigens of *Arthrobacter globiformis, Pantoea agglomerans, Saccharopolyspora rectivirgula,* and *Aspergillus fumigatus,* which were dissolved in PBS at the concentration of 100 μg/ml. The tests were considered as positive at the migration index (MI) equal to 0.79 or lower. Moreover, an ELISA test was also performed for the determination of the concentration of specific IgG antibodies against *Aspergillus fumigatus.*

RESULTS

Five (21.7%) out of 23 interviewed farmers reported occurrence of work–related symptoms (Tab. 2). Hop farmers described their work as an easy and pleasant job. The most common complaint was eye itching reported by four people, followed by body itching reported by three persons and skin rash, blocking of the nose, dry cough each reported by two farmers. Eight persons (34.8%) reported symptoms characteristic of chronic bronchitis according to the ATS questionnaire. In two farmers (8.7%) auscultation revealed coarse rales over lungs. Among five persons having work-related symptoms, two persons reported three symptoms, and one person each – one symptom, two symptoms, and five symptoms. None of the members of the reference group reported the occurrence of work-related symptoms.

Table 2. Prevalence of work-related symptoms in hops farmers (N=23)

Work-related symptoms	Workers reporting symptoms (number, percent)
Dry cough	2 (8.7%)
Productive cough	0
Dyspnoea	1 (4.3%)
Chest tightness	0
Blocking of the nose	2 (8.7%)
Wheezing	0
Hoarseness	0
Fever	0
Shivering	0
Nausea	0
Vomiting	0
Headache	0
Malaise	0
Sweating	0
Joint and muscle aching	0
Body aching	0
Fatigue	1 (4.3%)
Body itching	3 (13.0%)
Skin rash	2 (8.7%)
Eyes itching	4 (17.4%)
Total symptomatic workers	5 (21.7%)

Table 3. Mean spirometric values in hop farmers and referents

Spirometric values	Number of examined persons	Mean ± S D	Percent of predicted value (%, x ± S D)
VC (litres)	8	4.07 ± 1.04	96.2 ± 17.5
FEV_1 (litres)	8	3.12 ± 1.1	89.73 ± 23.75
FEV_1%VC	8	75.85 ± 11.91	
PEF (litres/sec)	23	5.92 ± 1.97	84.63 ± 14.06
Reference group			
PEF (litres/sec)	50	5.95 ± 2.31	87.29 ± 18.21

Mean spirometric values did not show significant differences compared to the reference group and were within normal ranges (Tab. 3). The levels of the three examined cytokines (IL–1, IL–6, TNF-α) in blood serum of hop farmers and referents were within the normal ranges. The frequency of positive skin response of the farmers to microbial allergens associated with hop dust was low and did not show any significant difference compared to the reference group. One person each (4.3%) reacted positively to the allergens of *Pantoea agglomerans*, *Aspergillus fumigatus* and hop extract (Tab. 4). The frequency of positive precipitin reactions of hop farmers to microbial allergens associated with hop dust was within the range of 8.7–43.5%. It is noteworthy that positive reactions in farmers were found with the antigens of Gram-negative bacteria *Pantoea*

Table 4. Frequency of positive skin reactions in hop farmers and referents (%)

Allergens	*Pantoea agglomerans*	*Saccharopolyspora rectivirgula*	*Streptomyces albus*	*Aspergillus fumigatus*	Hop extract	Control (PBS)
Hops farmers N=23	4.3	0	0	4.3	4.3	0
Reference group N=50	2.0	0	2.0	2.0	ND	0

ND = not determined

Table 5. Frequency of positive precipitin reactions in hop farmers and referents (%)

Antigens	Hop farmers	Reference group
Acinetobacter calcoaceticus	8.7	6.0
Alcaligenes faecalis	0	8.0
Arthrobacter globiformis	0	0
Bacillus subtilis	0	0
Pantoea agglomerans	43.5**	12.0
Saccharopolyspora rectivirgula	0	0
Streptomyces albus	0	0
Thermoactinomyces vulgaris	0	0
Alternaria alternata	0	0
Aspergillus candidus	0	0
Aspergillus fumigatus	0	8.0
Penicillium citrinum.	0	0
Hops extract	0	ND

*** Significantly greater compared to the reference group; *p < 0.05, ** p < 0.01
ND = not determined

Table 6. Results of the test for the inhibition of leukocyte migration in the presence of specific antigen in hop farmers and referents

Antigen	Migration Index – MI (mean ± S E), percent of positive results (%)			
	Arthrobacter globiformis	*Pantoea agglomerans*	*Saccharopolyspora rectivirgula*	*Aspergillus fumigatus*
Hop farmers N=23	0.9794 ± 0.1036 4.3%	0.9338 ± 0.1762 13.0%	0.9463 ± 0.0744 4.3%	0.9634 ± 0.1312 4.3%
Reference group N=50	1.0152 ± 0.0978 0	0.9947 ± 0.1045 0	1.0008 ± 0.0098 0	0.9866 ± 0.0963 0

agglomerans and *Acinetobacter calcoaceticus*. Frequency of positive precipitin reactions with the antigen of *Pantoea agglomerans* was significantly higher ($p<0.01$) in the examined group of hop farmers compared to the reference group (Tab. 5). The frequency of positive response in the test for specific inhibition of leukocyte migration was within the range of 4.3–13.0%. The highest frequency of positive results occurred with the antigen of *Pantoea agglomerans* (13%), being significantly higher ($p<0.05$) than in the reference group (Tab. 6). In the ELISA test for the presence of specific IgG antibodies against *Aspergillus fumigatus*, a positive result was found in seven farmers (30.4%).

DISCUSSION

The results indicate that hop growing farmers, who are exposed to hop dust and associated airborne microbial allergens while harvesting, sorting and drying of hop cones, are under increased risk of work-related allergic and immunotoxic pulmonary disorders. This presumption is supported by the occurrence of the work-related symptoms in hop farmers like: eye itching, body itching, rash and blocking of the nose. These symptoms are similar to those described by Raith and Jäger (16) in a 43-year-old laboratory worker with hop allergy, who had conjunctivitis, contact dermatitis and blocking of the nose after contact with hop. According to Newmark (14) and Schachter et al. (17), these disorders are caused by allergic and immunotoxic reactions to hop constituents.

The results of the present study demonstrate that besides hop components, also certain microorganisms associated with plant dust should be considered as potential causative agents of the disorders due to exposure to hops dust. The greatest risk represents an epiphytic Gram-negative bacterium *Pantoea agglomerans* (syn.: *Erwinia herbicola, Enterobacter agglomerans*), which reveals strong allergenic properties and produces a biologically potent endotoxin (4–6, 8, 10–12, 19). The hop farmers revealed the increased humoral and cellular reactivity to the antigen of *Pantoea agglomerans*. This was shown by the results of the agar-gel precipitation test and the test for inhibition of leukocyte migration, which demonstrated a significantly greater frequency of positive reactions in hop farmers compared to the reference group. Besides *Pantoea agglomerans*, the examined hop farmers showed the presence of precipitins only to *Acinetobacter calcoaceticus*, another Gram-negative species associated with organic dust which possesses allergenic and endotoxic properties (20, 21). There was described antiseptic activity of hop extract against Gram-positive bacteria and lack of such activity against Gram-negative bacteria (9). This might contribute to some extent to the fact that in the present work a high frequency of positive allergic reactions was observed only with the antigens of Gram-negative bacteria. Although positive results of allergological tests are not equivalent with the presence of a disease, the stated hypersensitivity to work-related allergens increases the risk of occupational disease in the case of further exposure to hops dust (4, 5, 8, 10–12, 15).

Mean spirometric values were within normal ranges, but in two persons pathologic symptoms were found by lung auscultation. The frequency of reported symptoms of chronic bronchitis

symptoms was very high (34.8%). It corresponds with other studies which showed the occurrence of respiratory system disorders after a contact with hop cones (7, 14, 16, 17). Godnic-Cvar et al. (7) examined the population of brewery workers exposed to organic dust from hop, barley, and baker's yeasts. This study showed that work in a brewery can lead to impairment of the respiratory system and significantly higher frequency of chronic diseases of the respiratory tract. The frequency of the positive skin prick test reactions to allergens of hop, barley and moulds was significantly higher in brewery workers than in the reference group.

CONCLUSION

Hop growing farmers represent a group at elevated professional risk because of incidence of work related-symptoms and high frequency of positive reactions to bacterial allergens associated with organic dust.

REFERENCES

1. Aleksandrov L. A., Georgijev W. G.: Voprosy gigeny truda khmelevodov pri ruchnoy uborke i sushke khmela. Gig. Truda Prof. Zabol., 12, 46, 1977.
2. Bowszyc J. et al.: Test zahamowania migracji leukocytów w mikrohodowlach z pełnej krwi w ocenie odporności komórkowej w gruźlicy. Gruźlica, 43, 369, 1975.
3. Dutkiewicz J. et al.: Zapobieganie chorobom wywoływanym przez pyły organiczne w rolnictwie i przemyśle rolnym. Instytut Medycyny Wsi, Lublin 2000.
4. Dutkiewicz J., Jabłoński L.: Biologiczne Szkodliwości Zawodowe. PZWL, Warszawa 1989.
5. Dutkiewicz J.: Exposure to dust-borne bacteria in agriculture. II. Immunological survey. Arch. Environ. Health, 33, 260, 1978.
6. Dutkiewicz J.: Studies on endotoxins of *Erwinia herbicola* and their biological activity. Zbl. Bakt. Hyg. I. Abt. Orig. A., 236, 487, 1976.
7. Godnic-Cvar J. et al.: Respiratory and immunological findings in brewery workers. Am. J. Ind. Med., 35, 68, 1999.
8. Kuś L.: Alergiczne zapalenie pęcherzyków płucnych w wyniku ekspozycji na antygeny wystepujące w pyle zbożowym w świetle własnych badań klinicznych i doświadczalnych. Med. Wiejska, 15, 73, 1980.
9. Langezaal C.R. et al.: Antimicrobal screening of essential oils and extracts of some Humulus lupulus L. cultivars. Pharm. Weekbl. Sci., 14, 353, 1992.
10. Milanowski J. et al.: Allergic alveolitis among agricultural workers in eastern Poland. Ann. Agric. Environ., Med., 5, 31, 1998.
11. Milanowski J.: Effects of *Pantoea agglomerans* on the respiratory system. Part II. Studies in vivo. Ann. Agric. Environ., Med., 1, 44, 1994.
12. Milanowski J.: Immunological investigation in Polish farm workers with extrinsic allergic alveolitis. Ann. Allergy, 68, 89, 1992.
13. Milanowski J.: Zespół toksyczny wywołany pyłem organicznym (ODTS). Pneumonol. Alergol. Pol., 62, 5, 1994.
14. Newmark F.M.: Hops allergy and terpene sensitivity: an occupational disease. Ann. Allergy, 41, 311, 1978.
15. Pepys J., Jenkins P.A.: Precipitin (F.L.H.) test in farmer's lung. Thorax, 20, 21, 1965.
16. Raith L., Jäger K.: Hop allergy. Contact Dermatitits, 11, 53, 1984.

17. Schachter E.N. et al.: Pharmacologic properties of brewery dust extracts in vitro. Chest, 119, 1870, 2001.
18. Skórska C. et al.: Effects of exposure to grain dust in Polish farmers: work-related symptoms and immunologic response to microbial antigens associated with dust. Ann. Agric. Environ. Med., 5, 147, 1998.
19. Skórska C. et al.: Endotoksyny bakteryjne *Alcaligenes faecalis* i *Erwinia herbicola* czynnikami narażenia zawodowego w rolnictwie. Pneumonol. Alergol. Pol., 64, 9, 1996.
20. Skórska C.: Badania nad aktywnością biologiczną endotoksyn *Acinetobacter calcoaceticus*. Med. Wiejska, 23, 203, 1988
21. Skórska C.: Ocena skutków inhalacji zwierząt doświadczalnych alergenem otrzymanym z bakterii *Acinetobacter calcoaceticus*, dokonana metodami immunologicznymi. Med. Wiejska, 26, 140, 1991.
22. Śpiewak R. et al.: Work-related skin symptoms and type I allergy among eastern-Polish farmers growing hops and other crops. Ann. Agric. Environ. Med., 8, 51, 2001.

SUMMARY

Medical examinations were performed in a group of 23 hops farmers exposed to organic dust from hop (*Humulus lupulus*). The examinations took place in individual farms during harvesting, sorting and transporting of hop cones. As a reference group, 50 urban dwellers not exposed to organic dust were examined. There were conducted physical examinations, interviews concerning the occurrence of respiratory disorders and work-related symptoms, lung function tests, determination of cytokines concentrations, and allergological tests comprising skin prick test with 4 microbial antigens associated with organic dust, precipitin test with 12 microbial antigens, and a test for inhibition of leukocyte migration. Five farmers (21.7%) reported occurrence of work-related symptoms, including dry cough and dyspnoea. Eight farmers (34.8%) reported symptoms of chronic bronchitis. Mean spirometric values were within normal ranges. The farmers showed positive responses in precipitin test and test for inhibition of leukocyte migration to antigens of environmental microbes, mainly to the antigen of Gram–negative bacterium *Pantoea agglomerans*. The results showed a potential risk of occupational respiratory diseases in the population of hop farmers.

Występowanie objawów alergii w wyniku ekspozycji na pył organiczny u hodowców chmielu

Badania zostały przeprowadzone u 23 plantatorów chmielu, u których udowodniono ekspozycję na pyły organiczne pochodzące z chmielu (*Humulus lupulus*). Badanie miało miejsce w indywidualnych gospodarstwach podczas zbioru, sortowania i transportu szyszek chmielowych. Grupę kontrolną stanowiło 50 mieszkańców miasta, którzy nie mieli ekspozycji na pyły organiczne. Dane zostały zebrane na podstawie badania fizykalnego, wywiadu dotyczącego przede wszystkim pojawiania się objawów chorób układu oddechowego i objawów związanych z wykonywaną pracą, testów czynnościowych płuc, oznaczenia stężenia cytokin w surowicy krwi, testów skórnych z 4 antygenami mikrobiologicznymi związanymi z pyłami organicznymi, testu precypitacji z 12 antygenami mikrobiologicznymi oraz testu zahamowania migracji leukocytów. Pięciu rolników (21,7%) zgłaszało pojawianie się objawów związanych z pracą: suchego kaszlu i duszności. U ośmiu plantatorów chmielu (34,8%) występowały objawy przewlekłego zapalenia oskrzeli. Średnie wartości testów czynnościowych płuc pozostawały w granicach normy. Natomiast wynik testu precypitacji i testu zahamowania migracji leukocytów dla antygenów mikrobiologicznych środowiska pracy, zwłaszcza dla antygenów gram-ujemnej bakterii *Pantoea agglomerans*, był pozytywny. Otrzymane wyniki wskazują na zwiększone ryzyko wystąpienia chorób układu oddechowego u osób uprawiających chmiel.