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HEALTH EFFECTS OF EXPOSURE TO HERB DUST IN VALERIAN GROWING FARMERS

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Abstract: The aim of the present study was to determine the health status of farmers cultivating valerian (*Valeriana officinalis* L.) and occupationally exposed to dust from this plant. A group of 75 valerian growing farmers were examined. As a reference group, 50 urban dwellers, not exposed to any kind of organic dust were examined. All people were interviewed for the presence of work-related symptoms and subjected to physical and spirometric examinations. Skin prick tests were conducted with 4 microbial antigens associated with organic dust and 3 herbal extracts, precipitin tests with 12 microbial antigens and 4 herbal extracts and tests for specific inhibition of leukocyte migration with 4 microbial antigens. 30.7% of the valerian farmers reported occurrence of work–related symptoms. No significant differences were found between the spirometric values in the group of valerian farmers and the reference group. Valerian farmers showed a low frequency of positive skin reactions to all tested antigens (0-4.0%), not significantly greater compared to reference group. The frequency of positive precipitin reactions to the antigen of Gram-negative bacterium *Pantoea agglomerans* was very high in valerian farmers (45.5%) with 3-fold concentrated sera and significantly greater compared to the reference group ($p<0.001$). The positive precipitin response of valerian farmers to other microbial and herbal antigens was much lower or absent and did not show any difference compared to reference group. In the test for specific inhibition of leukocyte migration, the highest frequencies of positive reactions in valerian farmers were noted with *Pantoea agglomerans* and *Saccharopolyspora rectivirgula* (15.0% each), in both cases significantly greater compared to reference group ($p<0.05$). In conclusion, the farmers growing valerian showed a moderate frequency of work-related symptoms and low reactivity to most microbial and herbal allergens. They exhibited an increased immunologic response to Gram-negative bacterium *Pantoea agglomerans* which appears to be the most important risk factor associated with valerian dust.

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INTRODUCTION

Herbal medicines are ubiquitous and their use vigorously increases - only within the years 1985–1995 the world phytopharmaceutical market grew at an annual rate of over 5% [14]. Only in Poland are herbs cultivated on about 20,000 farms [15]. The increase in the use of herbs results in a great number of people occupationally exposed to dust from herbs which may contain allergens and toxins originated from the herbs themselves and from

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microorganisms associated with herbs [9, 12, 17, 30]. In the populations of herb growing farmers and herb processing workers the frequent incidence of work-related symptoms, decrease of spirometric values and increased immunologic reactivity to microorganisms associated with herbs were demonstrated [9, 10, 11, 12, 13]. Occupational exposure to herb dust could be a cause of occupational asthma [17], allergic conjunctivitis [27], allergic alveolitis [19], and airborne contact dermatitis [28].

As many as 3,000 herbal products are registered in Poland [15]. One of the most popular herbs, the extract of which, consisting neuroactive flavonoids [20], is used as a sedative, is valerian (*Valeriana officinalis* L.). This perennial plant belongs to the family Valerianaceae and its roots have been used in medicine for thousands of years. 2-year-old roots are harvested in the autumn and utilized in dried or fresh form [3, 4]. Skórska *et al.* [26] found that farmers cultivating valerian could be exposed during processing of valerian roots (including cleaning, drying and packing) to airborne microorganisms, dust and endotoxin posing a risk of work-related respiratory disease.

The aim of the present study was to assess the health status of farmers occupationally exposed to valerian dust and to determine the degree of immunologic response to dust-borne antigens.

MATERIALS AND METHODS

Examined population. A group of 75 people occupationally exposed to dust from valerian were examined. The group comprised 36 males and 39 females, aged 39.24 ± 14.35 yrs (range 17-75 yrs). In the examined group 56.0% (42 persons) were tobacco smokers, and 44.0% (33 persons) were non-smokers (no ex-smokers were recorded).

As a reference group, 50 urban dwellers not exposed to any kind of organic dust were examined. The group consisted of 24 males and 26 females, aged 37.4 ± 12.1 yrs (range 20-65 yrs). In the reference group, 32.0% (16 persons) were tobacco smokers, 12.0% (6 persons) ex-smokers and 56.0% (28 persons) were non-smokers.

All subjects gave formal consent to participate in the study. The Ethics Commission of the Institute of Agricultural Medicine approved human subjects protocols.

Physical examination. A routine physical examination consisted of auscultation of the chest with the use of a stethoscope was conducted.

Lung function changes. The examinations were performed in the groups of valerian farmers and referents with the use of LUNGTEST 500 spirometer, produced by MES (Kraków, Poland). Vital capacity (VC), forced expiratory volume in the first second (FEV_1) and FEV_1/VC (%) were measured. The lung function testing was performed in accordance with European Respiratory Society guidelines [23].

Questionnaire examination. All people were interviewed by use of the American Thoracic Society Standard Questionnaire compiled by Ferris [1], and by the questionnaire developed at the Institute of Agricultural Medicine in Lublin for the examination of work-related symptoms caused by organic dusts [7].

Allergological tests. Skin tests, agar-gel precipitation tests and tests for specific inhibition of leukocyte migration were applied in the groups of valerian farmers and controls using the antigens of microorganisms associated with organic dusts, and antigens of herbs. Antigens of the following microorganisms, occurring commonly in the agricultural working environment of eastern Poland and reported as causative agents of work-related respiratory disorders, were used for the production of antigens (allergens):

- Gram-negative bacteria of the species *Acinetobacter calcoaceticus*, *Alcaligenes faecalis* and *Pantoea agglomerans* (syn.: *Erwinia herbicola*, *Enterobacter agglomerans*);
- Gram-positive non-branching bacteria of the species *Arthrobacter globiformis* and *Bacillus subtilis*;
- Actinomycetes of the species *Streptomyces albus*, *Saccharopolyspora rectivirgula* (syn.: *Micropolyspora faeni*, *Faenia rectivirgula*) and *Thermoactinomyces vulgaris*;
- Fungi of the species *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus* and *Penicillium citrinum*.
- Sage (*Salvia officinalis*), peppermint (*Mentha piperita*), chamomile (*Matricaria recutita*), and valerian (*Valeriana officinalis*) extracts were used as herbal antigens (allergens).

In all tests, lyophilised saline extracts of bacterial or fungal mass, produced at 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. The mass was then homogenised and extracted in saline (0.85% NaCl) in the proportion 1:2 for 48 hrs at 4ºC, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, 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 [21, 24, 25].

Herbal antigens were obtained by extraction of minced and defatted herbs (sage leaves, peppermint and chamomile flowers, and valerian roots) in Coca's solution in the proportion 1:10 (w/v) [29], dialysis against distilled water and lyophilisation.

Skin tests were carried out by prick method with the microbial antigens of *Pantoea agglomerans*, *Saccharopolyspora rectivirgula*, *Streptomyces albus*, and *Aspergillus fumigatus*, as well as with herbal extracts of chamomile, sage and peppermint. The antigens were dissolved in saline (PBS, Biomed, Kraków, Poland) at the concentration of 5 mg/ml. The diluted allergens were

sterilised by filtering (using Minisart 0.45 µm filters, Sartorius, Göttingen, Germany) and checked for sterility and lack of toxicity. The test was performed on the forearm with the antigenic extracts, PBS as a negative control, and histamine dihydrochloride (Allergopharma, Reinbek, Germany) as a positive 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 [25].

The agar-gel precipitation test was performed by Ouchterlony double diffusion method in purified 1.5% *Difco* agar with the microbial antigens of *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Arthrobacter globiformis*, *Bacillus subtilis*, *Pantoea agglomerans*, *Saccharopolyspora rectivirgula*, *Streptomyces albus*, *Thermoactinomyces vulgaris*, *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus* and *Penicillium citrinum*; as well as with herbal extracts of chamomile, sage, peppermint and valerian. The examined serum was placed in the central well and antigens in the peripheral wells. The antigens were dissolved in saline at the concentration of 30 mg/ml. Each serum was tested twice: not concentrated, and 3-fold concentrated, for the detection of low levels of precipitins. 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 [22, 24, 25].

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*. Patient's blood and Parker's culture medium were added in the volumes of 0.5 ml and 0.12 ml, respectively, to 2 silicon test tubes. Then, 0.12 ml of the antigen solution in the concentration of 100 µg/ml was added to 1 tube, while to the other 0.12 ml of the diluent (PBS) as a control. Both suspensions were incubated for 30 min at room temperature and thereafter distributed to heparinised glass capillary tubes 75 × 1 mm. Capillary tubes were sealed at both ends with the 4:1 mixture of paraffin and vaseline, centrifuged for 10 min at 1,500 rev/min and fastened tangentially on microscopic slides with a sticky tape at an angle of 10º. The microcultures obtained were incubated for 4 hrs at 37ºC 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), i.e., 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. Because of technical reasons, only 15 farmers were examined in this test.

Statistical analysis. Statistical analysis was performed with the use of non-parametric tests: Wilcoxon test, Mann-Whitney U test and Spearman test for correlation. For the analysis of discrete variables the chi-square test was used. The $p<0.05$ level was considered significant. Statistical analysis was carried out with the use of Statistica™ ver. 4.5 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

RESULTS

Occurrence of work-related symptoms in valerian farmers. 30.7% of the farmers exposed to valerian dust reported occurrence of work-related symptoms (Tab. 1). The most common complaints were conjunctivitis (20.0%) and blocking of the nose (9.3%). Dyspnoea, body itching and rash were reported by 5.3% of the persons from the exposed group.

None of the members of the reference group reported the occurrence of work-related symptoms.

Chest auscultation. On chest auscultation, pathologic symptoms were found in 2 valerian farmers. These comprised crepitation in 1 case and wheezing also in 1 case. No pathologic symptoms were found by chest auscultation in the reference group.

Lung function changes. The mean baseline spirometric values in the study and reference groups did not show significant differences compared to the normal values. No significant differences were found between the spirometric values in the group of valerian farmers and the reference group (Fig. 1). The spirometric values (VC, FEV_1) decreased together with the age of valerian farmers (data not shown) and the correlation proved to be

Table 1. Prevalence of work-related symptoms in farmers occupationally exposed to valerian dust (N=75).

Work-related symptoms	Farmers reporting symptoms	
	number	percent
Dry cough	1	1.3
Productive cough	3	4.0
Dyspnoea	4	5.3
Chest tightness	0	0
Blocking of the nose	7	9.3
Sore throat	0	0
Hoarseness	1	1.3
Fever	0	0
Shivering	0	0
Nausea	0	0
Vomiting	0	0
Headache	0	0
Increased perspiration	0	0
Joint and muscle aching	0	0
Body pain	0	0
Fatigue	1	1.3
Body itching	4	5.3
Rash	4	5.3
Conjunctivitis	15	20.0
Total number of farmers reporting work-related symptoms	23	30.7

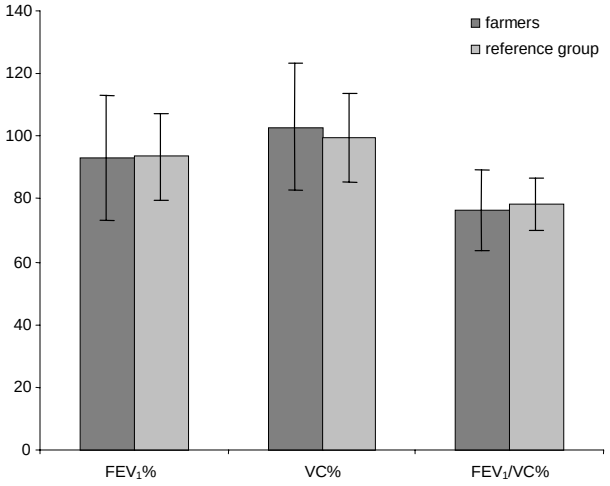

Figure 1. Mean spirometric values in the groups of valerian farmers (N=75) and referents (N=50).

significant ($p<0.01$, Spearman's correlation coefficient r = - 0.42 for FEV_1 values and r = - 0.33 for VC values).

Skin prick test. Valerian farmers showed positive reactions to all tested microbial antigens at a low frequency of 1.3–4.0% that was not significantly greater compared to reference group (Tab. 2). The highest frequencies of positive skin response were noted to the antigens of actinomycetes: *Streptomyces albus* (4.0%) and *Saccharopolyspora rectivirgula* (2.7%). Only 2 positive reactions to herbal extracts were noted in the group of farmers: 1 to chamomile extract and 1 to peppermint extract (Tab. 2).

Agar gel precipitation test. The prevalence of positive precipitin reactions is presented in Table 3. A very high frequency of positive reactions to the antigen of Gram-negative bacterium *Pantoea agglomerans* was noted in valerian farmers with 3-fold concentrated sera (45.5%). This value was significantly greater compared to the reference group ($p<0.001$).

Relatively high frequencies of positive precipitin reactions to antigens of other Gram-negative bacteria *Alcaligenes faecalis* and *Acinetobacter calcoaceticus* were found in valerian farmers with 3-fold concentrated sera (17.3% and 9.3% respectively), but these values were not significantly greater compared to the reference group ($p>0.05$). The positive precipitin response to other microbial antigens and to herbal extracts was low or absent.

Test for specific inhibition of leukocyte migration. The mean values of migration index (MI) in the presence of all antigens were significantly smaller in valerian farmers compared to reference group ($p<0.05$ – $p<0.01$) (Tab. 4). The frequency of positive reactions in valerian farmers was within the range 6.7%–15.0% while no positive reactions were observed in the reference group. The highest frequencies of positive reactions in valerian farmers were noted with *Pantoea agglomerans* and *Saccharopolyspora rectivirgula* (15.0% each). In both cases, the differences versus reference group were significant ($p<0.05$) (Tab. 4).

Table 2. Frequency of positive skin reactions to antigens associated with herb dust in valerian farmers and referents.

Antigen	Persons showing positive reactions (number, percent)	
	Valerian farmers N=75	Reference group# N=50
Pantoea agglomerans	1 (1.3%)	1 (2.0%)
Saccharopolyspora rectivirgula	2 (2.7%)	0
Streptomyces albus	3 (4.0%)	1 (2.0%)
Aspergillus fumigatus	1 (1.3%)	1 (2.0%)
Chamomile extract (*Matricaria recutita*)	1 (1.3%)	0
Sage extract (*Salvia officinalis*)	0	0
Peppermint extract (*Mentha piperita*)	1 (1.3%)	N.t.

With chamomile and sage extracts 30 referents were tested; N.t. = not tested.

Table 3. Frequency of positive precipitin reactions to antigens associated with herb dust in valerian farmers and referents (A - sera not concentrated, B - sera 3-fold concentrated).

Antigen	Persons showing positive reactions (number, percent)			
	Valerian farmers N=75		Reference group N=50	
	A	B	A	B
Acinetobacter calcoaceticus	0	7 (9.3%)	3 (6.0%)	3 (6.0%)
Alcaligenes faecalis	4 (5.3%)	13 (17.3%)	4 (8.0%)	4 (8.0%)
Arthrobacter globiformis	0	0	0	0
Bacillus subtilis	0	0	0	0
Pantoea agglomerans	14 (18.7%)	34 (45.5%)***	6 (12.0%)	6 (12.0%)
Saccharopolyspora rectivirgula	0	0	0	0
Streptomyces albus	0	2 (2.7%)	0	0
Thermoactinomyces vulgaris	0	4 (5.3%)	0	0
Aspergillus candidus	0	0	0	0
Aspergillus fumigatus	0	0	4 (8.0%)	4 (8.0%)
Penicillium citrinum	0	0	0	0
Alternaria alternata	0	0	0	0
Chamomile extract (*Matricaria recutita*)	0	0	N.t.	N.t.
Sage extract (*Salvia officinalis*)	0	0	N.t.	N.t.
Peppermint extract (*Mentha piperita*)	2 (2.7%)	5 (6.7%)	N.t.	N.t.
Valerian extract (*Valeriana officinalis*)	0	0	N.t.	N.t.

*-*** significantly greater compared to reference group: *p<0.05, **p<0.01, ***p<0.001; N.t. = not tested.

Table 4. Results of the test for inhibition of leukocyte migration in the presence of specific antigen in valerian farmers and referents.

Antigen	Value	Valerian farmers N=15	Reference group N=50
Arthrobacter globiformis	MI (mean ± SD)	0.9021 ± 0.0753$^{\#}$	1.0152 ± 0.0978
	Positive reactions (number, percent)	1 (6.7%)	0
Pantoea agglomerans	MI (mean ± SD)	0.8978 ± 0.0794$^{\#}$	0.9947 ± 0.1045
	Positive reactions (number, percent)	3 (15.0%)*	0
Saccharopolyspora rectivirgula	MI (mean ± SD)	0.8958 ± 0.0800$^{\#\#}$	1.0008 ± 0.0098
	Positive reactions (number, percent)	3 (15.0%)*	0
Aspergillus fumigatus	MI (mean ± SD)	0.8715 ± 0.0665$^{\#}$	0.9866 ± 0.0963
	Positive reactions (number, percent)	2 (13.3%)	0

MI = migration index; $^{\#\text{-}\#\#\#}$ significantly smaller compared to reference group: $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$. *-*** significantly greater compared to reference group: *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

The frequency of work–related symptoms in the farmers growing valerian (30.7%) was much lower compared to values found in the earlier studies of our group in farmers growing thyme (63.8%), sage (68.8%) and chamomile (80.6%), as well as in workers of a herb processing facility (76.5%) [9, 10, 11]. It was also lower than in people occupationally exposed to other organic dusts: flax dust (62.7%) [25], grain dust (44.7%) [24], and dust occurring in pig farms (58.5%) [18].

The performed lung function tests did not reveal any significant differences in spirometric values between the valerian farmers and the reference group, similar to an earlier study on herb growing and processing workers [13].

The prevalence of positive skin prick reactions to microbial and herbal allergens was low, on an average similar to that noted by Golec *et al.* [12] in the groups of chamomile and sage farmers, but lower compared to that found in thyme farmers and herb industry workers. The response to microbial allergens was also low compared to that found by Skórska *et al.* in farmers exposed to grain [24] and flax [25].

The high frequency of positive precipitin reactions to antigen of *Pantoea agglomerans* found in valerian farmers is in accordance with the results obtained by Dutkiewicz *et al.* [9] and Golec *et al.* [12], who found a high precipitin response to this bacterium in herb processing workers and herb growing farmers, significantly greater compared to reference groups. The percentages of positive precipitin reactions to other microbial antigens were low, in most cases lower compared to those recorded by the above-mentioned authors [9, 12].

The strongest immunologic response of valerian farmers to work-related microbial antigens was noted in the test for specific inhibition of leukocyte migration, similar to the earlier study by Golec *et al.* [12]. This was evidenced by the significant decrease of migration index with all antigens and by a significantly greater, compared to reference group, prevalence of positive reactions to *Pantoea agglomerans* and *Saccharopolyspora rectivirgula*. This finding supports further evidence for important role of *P. agglomerans* as an occupational risk factor for valerian growing farmers.

Pantoea agglomerans (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) is a common epiphytic, Gram-negative bacterium that has been isolated in large quantities from air contaminated with dust from various plant materials, such as grain and herbs [6, 8], recently also from the air contaminated with valerian dust [26]. *Pantoea agglomerans* produces a potent endotoxin [5] and was identified as a cause of allergic alveolitis in grain and herb workers [19, 21]

Summarizing, valerian growing farmers show less work-related symptoms and lower immunologic response to dust-borne allergens compared to people handling other herbs. This may be due, at least in part, to the fact that the median level of airborne microorganisms associated with valerian dust was lower compared to other herbs [8, 16] in spite of incidental peaks [26]. The greatest occupational risk is presented by *Pantoea agglomerans* which exhibits strong allergenic and endotoxic properties.

CONCLUSION

Farmers growing valerian showed a moderate frequency of work-related symptoms and low reactivity to most microbial and herbal allergens. They exhibited an increased immunologic response to the Gram-negative bacterium *Pantoea agglomerans* which appears to be the most important risk factor associated with valerian dust.

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