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RESPONSE OF HERB PROCESSING WORKERS TO WORK-RELATED AIRBORNE ALLERGENS*

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Abstract: A group of 51 herb processing workers employed in a big herb processing facility located in eastern Poland were examined by the skin and precipitin tests with, respectively, 4 and 17 extracts of microorganisms associated with organic dusts. Out of this number, 32 workers were examined by the skin test with 7 extracts of selected herbs processed in the facility. All the subjects were asked about the occurrence of work-related symptoms. 32 healthy office workers were examined with microbial extracts as a reference group. The herb processing workers showed a high proportion of early skin reactions (after 20 min) to the extract of Gram-negative bacterium *Alcaligenes faecalis* (41.2%), significantly higher compared to the reference group ($p<0.01$). At all time intervals (20 min, 8 hrs, 24 hrs), the workers responded with a high frequency to the extract of *Bacillus subtilis* (respectively 72.5%, 64.7%, and 15.7%), significantly greater compared to the reference group (respectively $p<0.001$, $p<0.001$, and $p<0.05$). No significant differences were found between the groups of herb processing workers and referents in skin response to the extracts of *Streptomyces albus* and *Alternaria alternata* and, except for the extract of *Pantoea agglomerans*, in the frequency of positive precipitin reactions to microbial antigens. In the skin test with herb extracts, the highest response among workers were caused by the extracts of chamomile flowers and nettle leaves which evoked 40-65% of positive skin reactions at all time intervals. 39 out of 51 interviewed herb processing workers (76.5%) reported the occurrence of work-related general, respiratory and skin symptoms. The positive skin reactions occurred more frequently among symptomatic workers which suggests that the specific immunologic response might be implicated in etiopathogenesis of work-related symptoms in examined workers. However, in most cases the differences did not attain a significance level which indicates that there is no direct relationship between a positive immunologic response and the appearance of symptoms caused by occupational exposure to herb dust, and that most probably a considerable part of these symptoms might be also due to non-specific immunologic and/or toxic mechanisms.

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Key words: herb processing workers, sensitization, allergens, organic dust, bacteria, fungi, herbs, intradermal test, precipitation test, work-related symptoms.

INTRODUCTION

The workers of agricultural industry exposed to the inhalation of organic dust during the handling of various types of vegetable matter are under risk of various work-related respiratory disorders of an allergic and/or immunotoxic background, such as: allergic alveolitis, asthma, organic dust toxic syndrome (ODTS), mucous

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*This article is dedicated to the memory of the late Professor Leszek Kuś, MD, Clinic of Lung Diseases of the Medical Academy in Lublin, a pioneer in clinical studies on respiratory diseases caused by occupational exposure to bioaerosols in Poland. The premature death of Professor Kuś in 1998 brought an untimely end to his important study on the clinical analysis of work-related respiratory diseases among herb processing workers.

membrane irritation (MMI), and rhinitis [3, 9, 21, 31, 38-40]. The disorders may be caused by substances produced by bacteria and fungi associated with vegetable dusts (protein and glycoprotein allergens, bacterial endotoxin, fungal glucans, volatile organic compounds) or by substances of plant origin (protein allergens, volatile oils, alkaloids, glycosides) [10, 14, 16, 22, 28].

Medical examinations of workers employed in grain mills and stores, malthouses, and the tobacco industry revealed a common occurrence of work-related symptoms and allergic reactions to bacteria and fungi occurring in respirable dust at work sites [5, 9, 26, 37]. So far, little is known about occupational allergy and work-related diseases in herb processing workers who are exposed to dusts from a large variety of plants defined as "herbs" and widely used as medications, food additives or components of cosmetics. Due to growing interest in herbal therapy, the number of workers in the herb processing industry is increasing worldwide. At present, circa 2,500 persons are employed in this industry branch in Poland.

It is known that herbs may exert adverse respiratory, dermal and general effects due to allergic and/or toxic properties [16, 28] but so far little is known about occupational disorders caused by various herbs. Recently, cases of asthma [23], allergic alveolitis [25] and airborne contact dermatitis [36] due to occupational exposure to dust from thyme (*Thymus vulgaris*) have been described. According to Benito *et al.* [1] thyme and related herbs of Lamiaceae (Labiatae) family (sage, mint, lavender, marjoram, basil, hyssop, oregano) share a common allergen and show cross-sensitivity. Occupational asthma may also be caused by exposure to chicory [29], rosemary, and bay leaf [23], while allergic dermatitis may be evoked by St. John's wort, rose, yarrow, peppermint and celandine [28]. Some herbs reveal toxic properties due to the presence of volatile oils (sage, mint, wormwood) or alkaloids (celandine) [16].

The aim of this work was to determine the immunological reactivity of herb processing workers to a wide spectrum of microbial and plant allergens associated with dust from various herbs, in order to assess a potential risk of an occupational allergic disease in this professional group. Allergens for this study were selected on the basis of an earlier microbiological analysis of the air in the examined facility [13] and questionnaire examination of the workers.

MATERIALS AND METHODS

Examined population. A group of 51 herb processing workers (2 males + 49 females) aged 38.6 ± 11.3 yrs (mean ± S.D.) were examined. They worked in a big herb processing facility located in eastern Poland in which a total of 90 workers were employed. The production process comprised cleaning, cutting or grinding, sorting and/or sieving, and final packing of over 25 kinds of herbs in portions suitable for use as medicines, spices, or ingredients of cosmetics. Previously, microbiological studies of the air for selecting the antigens for allergological examination had been performed [6, 13].

Thirty two healthy office workers living in a city and not exposed to organic dusts were examined as a reference group. This group comprised 11 males and 21 females, aged 36.4 ± 8.6 yrs (mean ± S.D.).

All herb processing workers and members of the reference group were examined by the skin and precipitin tests with saline extracts of the cultures of microorganisms isolated from the airborne dust. In addition, 32 workers were examined by the skin test with the saline extracts of selected herbs processed in the facility. All the subjects were also interviewed with a questionnaire developed at the Institute of Agricultural Medicine in Lublin [11] for the study of work-related symptoms. Human subjects protocols were approved by the Ethics Commission of the Institute of Agricultural Medicine and all subjects gave informed consent.

Preparation of allergens. The antigens of the following 17 microorganisms, associated with organic dusts and found in the air of the examined facility [13] were used in the study:

- Gram negative bacteria: *Acinetobacter calcoaceticus, Alcaligenes faecalis, Pantoea agglomerans* (syn. *Erwinia herbicola, Enterobacter agglomerans*);
- Gram-positive bacteria: *Arthrobacter globiformis, Bacillus subtilis, Brevibacterium linens, Corynebacterium xerosis, Corynebacterium* sp.;
- Actinomycetes: *Rhodococcus* sp., *Saccharopolyspora rectivirgula* (syn. *Micropolyspora faeni, Faenia rectivirgula*), *Streptomyces albus, Thermoactinomyces vulgaris*;
- Fungi: *Alternaria alternata, Aspergillus candidus, Aspergillus clavatus, Aspergillus fumigatus, Penicillium citrinum.*

All these antigens were used in the agar-gel precipitation test. For the skin test, on the basis of the common occurrence in the air of the examined facility and potential pathogenic properties, the following four antigens were selected: *Alcaligenes faecalis, Bacillus subtilis, Streptomyces albus,* and *Alternaria alternata.* The antigens of *Alcaligenes faecalis* and *Corynebacterium xerosis* were prepared from the strains isolated in the examined herb processing plants, while the other antigens were prepared from the reference strains used for the research and diagnostic purposes in the Institute of Agricultural Medicine in Lublin [5, 12].

Both in skin and agar-gel precipitation 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 Gram-negative and Gram-positive bacteria the mass was harvested from nutrient agar cultures while in the case of actinomycetes and fungi - 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^{o}C, with intermittent disruption of cells by 10-fold freezing and thawing.

Table 1. Skin response of herb processing workers to the extracts of microoorganisms associated with herb dusts.

Extract, group	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Alcaligenes faecalis												
Herb processing workers N = 51	21** (41.2%)	10* (19.6%)	0	0	48 (94.1%)	46 (90.2%)	21** (41.2%)	1 (2.0%)	50 (98.0%)	47 (92.2%)	37*** (72.5%)	5 (9.8%)
Reference group N = 32	4 (12.5%)	1 (3.1%)	1 (3.1%)	0	28 (87.5%)	25 (78.1%)	3 (9.4%)	0	28 (87.5%)	26 (81.3%)	11 (34.4%)	0
Bacillus subtilis												
Herb processing workers N = 51	37*** (72.5%)	28*** (54.9%)	2 (3.9%)	0	33*** (64.7%)	12** (23.5%)	1 (2.0%)	0	8* (15.7%)	3 (5.9%)	1 (2.0%)	0
Reference group N = 32	8 (25.0%)	2 (6.3%)	1 (3.1%)	0	7 (21.9%)	0	0	0	0	0	0	0
Streptomyces albus												
Herb processing workers N = 51	35 (68.6%)	24 (47.1%)	8 (15.7%)	2 (3.9%)	48 (94.1%)	41 (80.4%)	17 (33.3%)	0	48 (94.1%)	48 (94.1%)	43*** (84.3%)	7* (13.7%)
Reference group N = 32	16 (50.0%)	10 (31.2%)	3 (9.4%)	0	28 (87.5%)	24 (75.0%)	7 (21.9%)	0	30 (93.7%)	27 (84.4%)	16 (50.0%)	0
Alternaria alternata												
Herb processing workers N = 51	11 (21.6%)	7 (13.7%)	2 (3.9%)	0	5 (9.8%)	1 (2.0%)	0	0	2 (3.9%)	1 (2.0%)	1 (2.0%)	1 (2.0%)
Reference group N = 32	3 (9.4%)	2 (6.3%)	1 (3.1%)	0	1 (3.1%)	0	0	0	1 (3.1%)	1 (3.1%)	0	0

Grade 1 = weakly positive reactions, diameter ≥ 5 mm. Grade 2 = positive reactions, diameter ≥ 10 mm. Grade 3 = strongly positive reactions, diameter ≥ 20 mm. Grade 4 = very strongly positive reactions, diameter ≥ 40 mm. * - ***: significantly greater compared to reference group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

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. In skin test, the antigens were used at the concentration of 1 mg/ml and in agar-gel precipitation test at the concentration of 30 mg/ml [27, 34].

Samples of seven herbs, reported by the workers as the most common cause of work-related symptoms were collected in the facility for preparation of the allergenic extracts for skin tests. The herbs were the following:

Sage leaves (*Salvia officinalis* L.), caraway seeds (*Carum carvi* L.), mint leaves (*Mentha pulegium* L.), chamomile flowers (*Matricaria chamomilla* L.), St. John's wort herb (*Hypericum perforatum* L.), stinging nettle leaves (*Urtica dioica* L.), and horsetail herb (*Equisetum arvense* L.).

The herbs were extracted in Coca's solution [35] and used for the skin tests in the dilution of 1:100 (v/v), using saline (0.85% NaCl) as a diluent. Because of technical and organisational problems, only 34 persons out of the total group of 51 herb processing workers could be tested with herb extracts, and the members of the reference group were not tested with these extracts.

Skin test. The test was performed by the intradermal method. The antigens were dissolved or diluted in 0.85% NaCl, sterilised by filtering and checked for sterility and lack of toxicity. The test was performed by intracutaneously injecting 0.1 ml of the antigenic extracts and of saline (as a control) into the forearm skin of the subject. The test sites were observed at 20 min for immediate (early) reactions, at 8 hrs for delayed reactions and at 24 hrs for late reactions [5, 12, 27]. The wheal and/or erythema reactions of 5 mm or more in diameter (at negative control) were regarded as positive. The intensity of positive reactions was graded on the basis of diameter as follows: ≥ 5 mm - grade 1, ≥ 10 mm - grade 2, ≥ 20 mm - grade 3, ≥ 40 mm - grade 4 [12].

Agar-gel precipitation test. The test was performed by Ouchterlony double diffusion method in purified 1.5% *Difco* agar. The patient's serum was placed in the central well and antigens, dissolved in 0.85% NaCl, in the peripheral wells. Each serum was tested twice: not concentrated, and three-fold concentrated, for the detection of low levels of precipitins. The plates were incubated for six days at room temperature, then washed

Table 2. Skin response of herb processing workers (N = 34) to the extracts of various herbs.

Extract	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Sage leaves (*Salvia officinalis* L.)	14 (41.2%)	11 (32.4%)	10 (29.4%)	0	13 (38.2%)	6 (17.6%)	0	0	3 (8.8%)	1 (2.9%)	0	0
Caraway seeds (*Carum carvi* L.)	7 (20.6%)	5 (14.7%)	3 (8.8%)	0	10 (29.4%)	7 (20.6%)	1 (2.9%)	0	3 (8.8%)	1 (2.9%)	0	0
Mint leaves (*Mentha pulegium* L.)	17 (50.0%)	11 (32.4%)	6 (17.6%)	0	12 (35.3%)	3 (8.8%)	0	0	6 (17.6%)	4 (11.8%)	0	0
Chamomile flowers (*Matricaria chamomilla* L.)	21 (61.8%)	15 (44.1%)	8 (23.5%)	0	20 (58.8%)	11 (32.4%)	3 (8.8%)	0	22 (64.7%)	17 (50.0%)	5 (14.7%)	1 (2.9%)
St. John's wort herb (*Hypericum perforatum* L.)	7 (20.6%)	4 (11.8%)	1 (2.9%)	0	13 (38.2%)	7 (20.6%)	1 (2.9%)	0	4 (11.8%)	3 (8.8%)	0	0
Stinging nettle leaves (*Urtica dioica* L.)	22 (64.7%)	16 (47.1%)	7 (20.6%)	0	21 (61.8%)	14 (41.2%)	0	0	14 (41.2%)	7 (20.6%)	2 (5.9%)	0
Horsetail herb (*Equisetum arvense* L.)	20 (58.8%)	10 (29.4%)	3 (8.8%)	0	7 (20.6%)	0	0	0	3 (8.8%)	1 (2.9%)	1 (2.9%)	0

Grade 1 = weakly positive reactions, diameter ≥ 5 mm. Grade 2 = positive reactions, diameter ≥ 10 mm. Grade 3 = strongly positive reactions, diameter ≥ 20 mm. Grade 4 = very strongly positive reactions, diameter ≥ 40 mm.

in saline and in 5% sodium citrate solution (for preventing false positive reactions), and stained with azocarmine B [27, 30, 33, 34].

Statistical analysis. The obtained results were analysed by the Student's t-test, assuming $p < 0.05$ as a significance level.

The study was performed mostly during 1984-1986 and continued in the years 1995-2001. All the basic allergological examinations of the members of workers' group and reference group have been done in similar time in the first phase of the study (1984-1986). Preliminary results of this work have been reported elsewhere [6, 7, 8].

RESULTS

Skin reactions. The skin response of herb processing workers to the extracts of microorganisms associated with herb dust is presented in Table 1. The workers showed a high proportion of early skin reactions (after 20 min) to the extract of Gram-negative bacterium *Alcaligenes faecalis* (41.2%), significantly higher compared to the reference group ($p < 0.01$). The delayed (after 8 h) and late (after 24 h) skin response of the workers to *A. faecalis* extract was even higher but did not differ significantly from the response of the referents, except for strong (grade 3) reactions ($p < 0.01$).

At all time intervals (20 min, 8 hrs, 24 hrs), the herb processing workers responded with a high frequency to the extract of *Bacillus subtilis* (respectively 72.5%, 64.7%, and 15.7%), significantly greater compared to the reference group (respectively $p < 0.001$, $p < 0.001$, and $p < 0.05$) (Tab. 1). A similarly strong response was noted for the extract of *Streptomyces albus*, and the frequency of positive response was higher in herb workers than in referents. However, the differences were not statistically significant, except for strong and very strong (grade 3-4) late reactions (respectively $p < 0.001$, and $p < 0.05$). The workers responded with the lowest frequency to the extract of the fungus *Alternaria alternata*. The proportion of early and delayed skin reactions to this extract was higher compared to the reference group, but the differences were not statistically significant (Tab. 1).

The skin response of herb workers to the extracts of seven various herbs processed in the facility is shown in Table 2. As seen from the Table, the tested herb extracts may be divided into three groups depending on their sensitizing activity. The highest response among workers was caused by the extracts of chamomile flowers and nettle leaves which evoked 40–65% of positive skin reactions at all time intervals (20 min, 8 hrs, 24 hrs). A moderate response was caused by the extracts of horsetail herb, mint leaves, and sage leaves which elicited 41-59% of early positive reactions (after 20 min) but much less (9–38%) of delayed and late reactions (respectively after 8 h and 24 h). The lowest response was caused by the extracts of caraway seeds and St. John's wort herb which evoked 9–38% positive reactions at all time intervals (Tab. 2). Compared to the extracts of environmental microbes, the extracts of herbs evoked greater numbers of early strong reactions, but much smaller numbers of delayed and late strong reactions.

Precipitin reactions. The frequency of positive precipitin reactions to microbial antigens associated with

Table 3. Precipitin reactions of herb processing workers to microbial antigens occurring in the air of work environment.

Antigen, group	Persons showing positive reaction (number, percent)			
	Herb processing workers N = 51		Reference group N = 32	
	Sera not concentrated	Sera 3-fold concentrated	Sera not concentrated	Sera 3-fold concentrated
Gram-negative bacteria				
Acinetobacter calcoaceticus	1 (2.0%)	4 (7.8%)	5 (15.6%)	15 (46.9%)
Alcaligenes faecalis	15 (29.4%)	27 (52.9%)	4 (12.5%)	13 (40.6%)
Pantoea agglomerans	11 (21.6%)*	19 (37.3%)***	1 (3.1%)	1 (3.1%)
Gram-positive bacteria				
Arthrobacter globiformis	1 (2.0%)	6 (11.8%)	0	2 (6.2%)
Bacillus subtilis	0	1 (2.0%)	0	0
Brevibacterium linens	0	1 (2.0%)	0	0
Corynebacterium xerosis	0	1 (2.0%)	0	0
Corynebacterium sp.	4 (7.8%)	8 (15.7%)	1 (3.1%)	7 (21.9%)
Actinomycetes				
Rhodococcus sp.	0	0	0	1 (3.1%)
Saccharopolyspora rectivirgula	2 (3.9%)	8 (15.7%)	0	2 (6.2%)
Streptomyces albus	0	0	0	0
Thermoactinomyces vulgaris	0	0	0	0
Fungi				
Alternaria alternata	0	1 (2.0%)	0	0
Aspergillus candidus	0	1 (2.0%)	0	0
Aspergillus clavatus	0	0	0	0
Aspergillus fumigatus	0	0	0	0
Penicillium citrinum	1 (2.0%)	1 (2.0%)	0	5 (15.6%)

* - ***: significantly greater compared to reference group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

herb dust was low in most cases and no significant differences were found between the herb processing workers and reference group (Tab. 3). The highest percentages of positive reactions were noted with the antigens of Gram-negative bacteria (*Alcaligenes faecalis*, *Pantoea agglomerans*), but only in the case of the extract of *Pantoea agglomerans* was the frequency of positive response in herb workers significantly greater compared to the reference group ($p < 0.05$). The response of the workers to antigens of Gram-positive bacteria, actinomycetes and fungi was either low or no antibodies were detected at all (Tab. 3).

Relationship between the occurrence of work-related symptoms and allergic reactions. Thirty nine out of 51 interviewed herb processing workers (76.5%) reported the occurrence of work-related general, respiratory and skin symptoms. The most common complaint was dyspnoea reported by 26 workers (51.0%), followed by eye itching reported by 24 (47.1%), cough and headache - each by 22 (43.1%), body itching (pruritus) by 17 (33.3%), chest tightness and hoarseness - each by 15 (29.4%) (Tab. 4). Among 39 workers having work-related symptoms, eight persons reported five symptoms, six - three symptoms, five - four symptoms, four persons each - one and nine symptoms, three persons each - two and six symptoms, two persons each - seven and eight symptoms, and one person each - as many as 10 and 11 symptoms. Three workers reported onset of symptoms within 10 minutes of beginning work, 17 - between 10–30 minutes, seven - from 30 minutes to two hours, nine - between 2–6 hours, one - between 6–12 hours, and two - after 12 hours.

Responding to the question about which kinds of herbs processed in the facility release an adverse dust causing their symptoms, twenty out of 39 symptomatic workers (51.3%) indicated sage, 15 (38.5%) - St. John’s wort, 12 (23.5%) - horsetail, 10 (25.6%) - nettle, and nine each

Table 4. Prevalence of work-related symptoms in herb processing workers (N = 51).

Work-related symptoms	Workers reporting symptoms (number, percent)
Cough	22 (43.1%)
Dyspnoea	26 (51.0%)
Chest tightness	15 (29.4%)
Wheezing	2 (3.9%)
Sneezing	5 (9.8%)
Hoarseness	15 (29.4%)
Fever	3 (5.9%)
Shivering	3 (5.9%)
Headache	22 (43.1%)
General weakness	14 (27.5%)
Sweating	12 (23.5%)
Eye itching	24 (47.1%)
Body itching	17 (33.3%)
Skin rash	11 (21.6%)
Total symptomatic workers	39 (76.5%)

Table 5. Herbs indicated by workers reporting work-related symptoms (N = 39) as a source of adverse dust causing symptoms.

Herb	Workers reporting symptoms associated with exposure to this herb (number, percent)
Caraway (*Carum carvi* L.)	9 (23.1%)
Celandine (*Chelidonium maius* L.)	2 (5.1%)
Chamomile (*Matricaria chamomilla* L.)	7 (17.9%)
Coriander (*Coriandrum sativum* L.)	3 (7.7%)
Horsetail (*Equisetum arvense* L.)	12 (23.5%)
Lavender (*Lavandula officinalis* Chaix et Villars)	3 (5.9%)
Linden (*Tilia cordata* Miller)	3 (7.7%)
Marjoram (*Majorana hortensis* L.)	3 (7.7%)
Mint (*Mentha pulegium* L.)	9 (23.1%)
Nettle (*Urtica dioica* L.)	10 (25.6%)
Pansy (*Viola tricolor* L.)	4 (10.3%)
Rose (*Rosa canina* L.)	2 (5.1%)
Sage (*Salvia officinalis* L.)	20 (51.3%
St. John's wort (*Hypericum perforatum* L.)	15 (38.5%)
Valerian (*Valeriana officinalis* L.)	4 (10.3%)
Wormwood (*Artemisia absinthium* L.)	3 (7.7%)

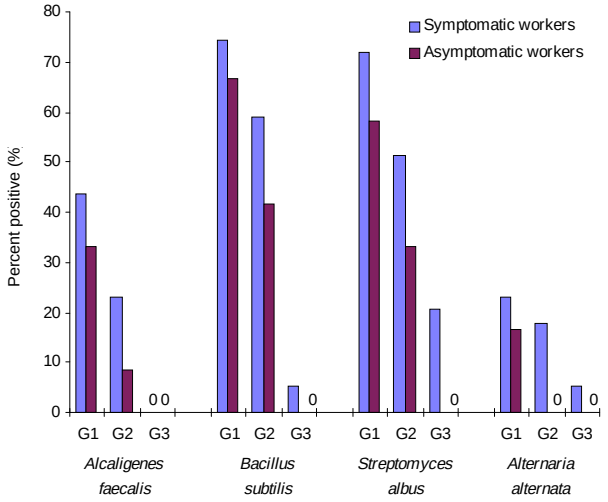

Figure 1. Frequency of positive early skin reactions to extracts of environmental microbes in the subgroups of symptomatic (N = 39) and asymptomatic (N = 12) herb processing workers. G1 = grade 1, G2 = grade 2, G3 = grade 3. Grade 4 reactions are not shown because their numbers in the subgroups were very low and the results were inconclusive.

(23.1%) - caraway and mint (Tab. 5). Altogether, 16 herbs were indicated by the workers as a source of adverse dust causing work-related symptoms. Seventeen workers indicated two kinds of herbs as a source of dust causing their symptoms, six - three herbs, five - one herb, four - four herbs, three - five herbs, four - six or more herbs.

The frequency of the positive skin and precipitin reactions in the subgroups of symptomatic and asymptomatic workers is presented in Figures 1-3. As seen in Figure 1, the workers reported symptoms showed in all cases a higher frequency of positive skin reactions to microbial allergens associated with herb dust. However, the differences were not statistically significant ($p>0.05$) which may be due, at least in part, to the fact that the number of asymptomatic workers was too small compared to symptomatic ones (12 *versus* 39) for a relevant statistical comparison. Similarly, the frequency of positive skin response to herb extracts in symptomatic workers in most cases was about twice as high as in asymptomatic ones (Fig. 2), but for the same reason the statistically significant difference was found only in the case of the extract of nettle leaves ($p < 0.05$). No clear-cut differences could be found between the frequency of positive precipitin reactions in symptomatic and asymptomatic workers (Fig. 3).

DISCUSSION

Workers employed in the herb processing facility showed a high frequency of positive intradermal reactions to the allergens of herbs and environmental microorganisms occurring in airborne dust in the work environment. The response rates were similar to those obtained by intradermal test with the extracts of

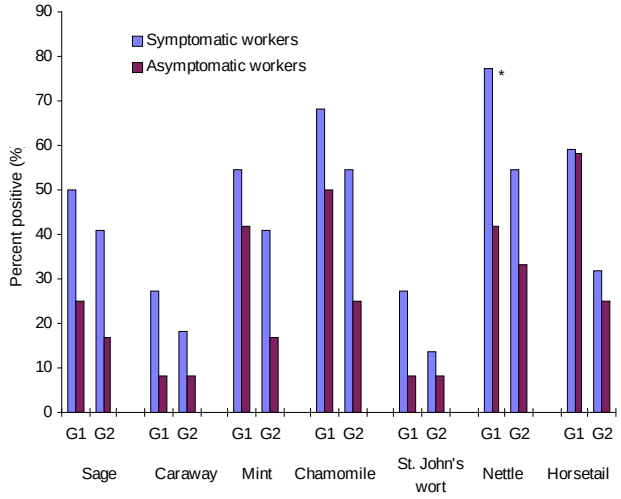

Figure 2. Frequency of positive early skin reactions to extracts of various herbs in the subgroups of symptomatic (N = 22) and asymptomatic (N = 12) herb processing workers. G1 = grade 1, G2 = grade 2. Grade 3-4 reactions are not shown because their numbers in the subgroups were very low and the results were inconclusive. *Significantly greater compared to asymptomatic workers, $p < 0.05$.

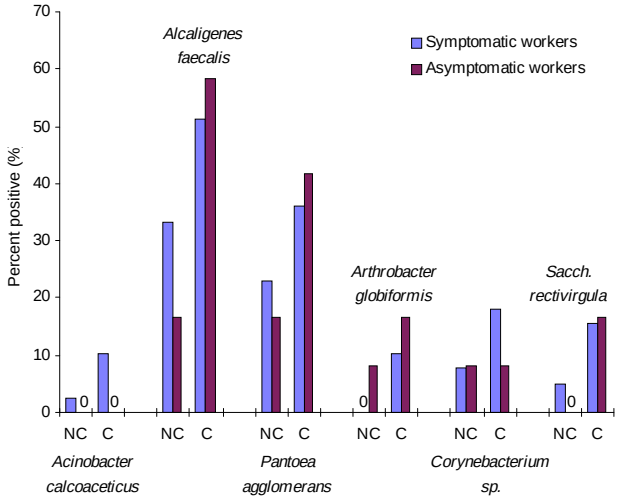

Figure 3. Frequency of positive precipitin reactions to extracts of environmental microbes in the subgroups of symptomatic (N = 39) and asymptomatic (N = 12) herb processing workers. NC = sera not concentrated, C = sera 3-fold concentrated. *Sacch.* = *Saccharopolyspora*. Results obtained with the remaining 11 antigens used in the test (*Bacillus subtilis, Brevibacterium linens, Corynebacterium xerosis, Rhodococcus* sp., *Streptomyces albus, Thermoactinomyces vulgaris, Alternaria alternata, Aspergillus candidus, Aspergillus clavatus, Aspergillus fumigatus, Penicillium citrinum*) are not shown because they were inconclusive. The numbers of positive reactions were either equal to zero or very low.

environmental microbes in the populations of farmers, grain elevator workers, maltworkers, and sawmill workers [4, 5, 8, 12, 37] and higher compared to those obtained by prick test, regarded as less sensitive, among grain and flax farmers, and pig breeders [24, 33, 34].

The skin response of herb processing workers to antigens derived from microorganisms associated with the airborne herb dust conformed to the results of an earlier aerobiological examination of the work environment [13]. A great frequency of early and delayed positive response to the extract of *Bacillus subtilis* (72.5% and 64.7%, respectively), highly significant compared to reference group ($p < 0.001$), was in accordance with the dominance of *B. subtilis* and related species in the air of the examined herb processing facility. *Bacillus* spp. prevailed at most of the examined work sites in this facility, occurring at these sites in concentrations up to 141.7-394.6 × 10^3 cfu/m^3 [13]. A frequency of early positive response to the extracts of *Streptomyces albus*, *Alcaligenes faecalis* and *Alternaria alternata* (68.6%, 41.2%, 21.6%, respectively) conformed to the maximal concentrations of these organisms found in the air of the examined plant, ranging within 18.9–296.3 × 10^3 cfu/m^3, 15.7–57.7 × 10^3 cfu/m^3, and 8.8–47.5 × 10^3 cfu/m^3, respectively. The relationship between the degree of exposure to *S. albus* or *A. faecalis* and skin response was best expressed by early reactions as the delayed and late reactions to the extracts of above-mentioned bacteria were, in most cases, non-specific, occurring with equally high frequency in herb processing workers and members of the reference group. It cannot be exluded that small quantities of endotoxin or other biologically active substances produced by *A. faecalis* or *S. albus* might contribute to non-specific irritation and false-positive reactions [10, 32].

It is noteworthy that *B. subtilis*, *S. albus*, and *A. faecalis* were reported as causative agents of allergic alveolitis [17, 18, 27] whereas *A. alternata* may be a cause of allergic rhinitis and asthma [21].

The frequency of positive antibody response of herb processing workers to the antigens of environmental microorganisms, as assessed by agar-gel precipitation test, was much lower compared to skin response and in most cases not be related to the exposure. The percentages of positive precipitin reactions with particular antigens were similar to or lower compared to earlier studies by our group which had been carried out in the occupational populations of farmers, grain elevator workers, maltworkers, and sawmill workers [5, 12, 24, 33, 34]. In the present study, similar to the earlier studies, the highest frequency of positive precipitin reactions was noted with the antigens of Gram-negative bacteria.

As many as 76.5% workers of the herb processing facility reported the occurrence of work-related symptoms. The prevalence of work-related symptoms in this occupational group was by 13-31% greater compared to various groups of Polish farmers [24, 33, 34].

Out of seven herbs reported by workers as the most common cause of work-related symptoms, the greatest frequency of positive skin reactions at all time intervals was given by the allergenic extracts of chamomile flowers and nettle leafs. Chamomile is known as a cause of allergic disorders of the skin and other organs [2, 28], and its sensitizing properties are attributed to the presence of nobilin, a potentially allergenic sesquiterpene lactone

[28]. Much less is known about the allergenic properties of nettle. The commonly known itching from the stinging hairs of nettle is ascribed to acetylcholine, histamine and 5-hydroxytryptamine [28]. The results of the present study suggest that appropriate preventive measures should be considered at industrial processing of chamomile and nettle. The early skin response to sage in this study (41.2%) was lower compared to 60% reported by Zuskin *et al.* [38].

The workers reporting work-related symptoms showed an increased rate of positive skin response to microbial and herbal antigens occurring in the airborne dust at the workplace. Most differences were not statistically significant; however, the results of the analyses might be biased by the aforementioned disproportion between the asymptomatic and symptomatic workers in the studied group. No differences could be found between the frequency of positive precipitin reactions in symptomatic and asymptomatic workers, which suggests that the precipitin-mediated mechanisms probably do not play an important role in the etiology of work-related symptoms in herb processing workers.

A limitation of the present study is that for technical reasons the intradermal test with herb extracts could not be performed in a reference group which affects the interpretation of the results obtained. Another limitation was that only seven out of 16 herbs reported by the workers as evoking work-related symptoms could be tested. Also, statistical comparisons between the groups of herb processing workers and healthy urban dwellers with respect to the results obtained with microbial antigens might be biased to some extent by the different gender proportion in both groups.

In spite of the above-mentioned reservations, the results of the present study clearly demonstrate that a large part of the workers employed at industrial cleaning, grinding, sieving, sorting and packing of various herbs are sensitized to antigens of herbs and microbes occurring in airborne dust at the workplace. Although a direct relationship between allergic reactions and work-related symptoms was not established, these reactions might be a predisposing factor for inducing work-related respiratory disorders. This supposition can be supported by a study of Kuś *et al.* [19, 20] who, on the basis of clinical examination done at the Clinic of Lung Diseases of Medical Academy in Lublin, diagnosed work-related pulmonary disorders in 23 female herb processing workers, selected out of a group of 51 workers examined in the present study. Twelve workers of this group (52.2%) showed a positive response in the inhalation challenge with the diluted extract of *Alcaligenes faecalis*, which had been suspected as a causative agent of the work-related respiratory disorders. X-ray examination of the chest revealed pathological interstitial changes in five persons (21.7%).

It is noteworthy, that not only the specific allergenic mechanisms might be involved in the etiopathogenesis of work-related respiratory disorders in herb processing workers, but also non-specific immunotoxic reactions to the constituents of microbial cell wall (e.g. endotoxin, peptidoglycan, glucan) or plant products (e.g. volatile oils).

Summarising, workers growing, collecting and processing herbs form worldwide a large occupational group which until now has not been thoroughly studied with respect to possible risk to work-related biohazards. The results of our studies on the exposure and reactivity of herb processing workers to work-related bioaerosols indicate that the risk to biohazards in this occupational group should not be underestimated and the workers should be protected against the potential effects of exposure to bioaerosols. Though consumption and application of the medicines, cosmetics and foods containing herbal constituents is safe, handling of raw herb materials may be associated with a risk of allergic and/or immunotoxic diseases. Taking into account an immense number of plants used worldwide as herbs, further studies on the risk associated with exposure to dust from various plant materials are needed.

CONCLUSIONS

• The workers of herb processing facility showed a high frequency of positive skin reactions to the extracts of particular herbs and microorganisms associated with herb dust but much lower frequency of positive precipitin reactions.

• The positive skin reactions occurred more frequently among the workers reporting work-related symptoms which suggests that the specific immunologic response might be implicated in a common occurrence of these symptoms among herb processing workers. However, for most microorganisms and herbs tested the differences did not attain a significance level which indicates that there is no direct relationship between a positive immunologic response and appearance of symptoms caused by occupational exposure to herb dust and most probably a considerable part of these symptoms might be also due to non-specific immunologic and/or toxic mechanisms.

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