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SELECTED PARAMETERS OF IMMUNOLOGICAL RESPONSE IN HOP GROWERS DURING THE PERIOD OF INTENSIVE APPLICATION OF PESTICIDES

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Abstract: The aim of the study was determination of selected parameters of immunological response among hop growers and farmers in conditions of intensive exposure to means of plant protection. Survey data was collected from 238 males aged 25-70 living in the area of Wilków near Puławy (Lublin Region). Control group were males from the area of Witoszyn (Lublin Region) - 53 people aged 25-70 occupied mainly with land cultivation. Based on an environmental survey conducted among hop growers and farmers, the respondents were divided into 3 age groups: 25-40, 41-55 and 56-70. Laboratory tests covered the determination of selected morphological parameters, phagocytic test, NBT test, and myeloperoxidasis (MPO) concentration in blood serum of hop growers and farmers.A significant decrease was noted in the number of platelets in the general population of hop growers and in individual age groups, compared to the control groups of farmers. Analysis of individual sub-populations of leukocytes showed a significantly higher number statistically of basophils and lymphocytes among hop growers, compared to farmers. A detailed analysis of the degree of phagocytic and bactericidal activity of neutrophils allowed us to presume that during the period of spraying there occured a mobilisation of the granulocytic system, manifested by the presence of over 90% of neutrophils of intensified phagocytic activity, and 20% of neutrophils of intensified bactericidal activity. The preparations prepared by the routine NBT test method were analysed with the use of LUCIA computer programme (version 4.51). The analysis of the level of MPO in blood serum in the populations examined showed the presence of statistically significant differences. In hop growers, the MPO level was significantly higher statistically (60.0 ng/ml), compared to the control group of farmers (43.4 ng/ml).

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INTRODUCTION

The permanence of means of plant protection applied in agriculture and their capability for endurance and dispersal in various elements of the farming environment may result in an increased exposure of rural inhabitants to contact with pesticides, compared to the general population. This concerns primarily people from the areas where crops requiring intensive plant protection are cultivated [4, 12]. These types of crops are horticulture and hop growing.

The Lublin Region is an area where 25% of Polish hops are produced. In this region, intensive chemical treatment is applied on plantations during the vegetation season - 10-14 sprayings, sometimes even 15-20.

Pesticides show a modulatory effect on the immunological system, most often immunosuppresive but sometimes stimulatory, according to the type of compound and its

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dose, as well as the type of antigen stimulus. Suppression of resistance is a phenomenon commonly noted with various types of xenobiotics. Immunosuppresion also caused an increased cancer risk - an increase in the number of cases has been observed recently among agricultural workers. Immunostimulatory effect is associated with an increase in the development of autoimmunological and allergic diseases [2, 9, 15, 17, 18].

Despite a large number of experimental data describing immunosuppresion induced by pesticides, it is not clear to what extent they may weaken the activity of the immunological system in people chronically exposed. One may talk about immunotoxicity of pesticides when they modulate immunological response at low concentrations [14]. The effect of pesticides expressed in their immunotoxicity, reflected in the modulation of the first systemic immunological barrier, is manifested by mobilization of neutrophils during the seasons of intensive chemical treatment [1, 21]. Immunocompetent cells, after direct contact with chemical compounds (pesticides), may alter their reactivity. Disorders in the functions of neutrophils may be an integral part of immunosuppression induced by pesticides, and consequently result in an increased susceptibility to diseases [3, 19]. Peripheral blood neutrophils in humans play an important role in the immunological response and show a great susceptibility to chemical compounds. Changes in the activity of neutrophils' enzymes precede the occurrence of severe toxic lesions of the hematopoietic system [10, 20]. The process of phagocytosis in which they participate, associated with the release of regulatory substances and free radicals, is an important part of the system of anti-bacterial and anti-cancer defence of the body [5].

MATERIAL AND METHODS

The area of Wilków near Puławy was selected for the study as a region of the greatest density of hop plantations. Field studies were conducted at the end of June and beginning of July - directly after the period of intensive application of pesticides on hop plantations and orchards. The area of Witoszyn (Lublin Region) was selected as an area of minimum use of pesticides.

Survey data was collected from 238 hops growers - males aged 25-70 from the region of Wilków near Puławy. Based on an environmental survey, the respondents were divided into 3 age groups: 25-40 (60 people), 41-55 (115), and 56-70 (62).

The control group were farmers - males from the area of Witoszyn (Lublin Region) aged 25-70. Based on an environmental survey which covered 53 people, the respondents were divided into 3 age groups: 25-40 (18), 41-55 (22) and 56-70 (13).

Blood for the determination of selected hematologic parameters was taken with syringes containing EDTA (ethylenediaminotetraacetic acid). Hematologic studies were performed with the use of Coulter STKS (Couter Corporation, Miami, Florida, USA). The number of erythrocytes, leukocytes and their sub-population, as well as the number of platelets, was estimated.

Blood for phagocytic test and NBT test was taken into heparinized test tubes.

Phagocytic test was performed from whole blood with the use of latex (Bacto-Latex 0.81, Difco, USA). While evaluating the phagocytic test, an analysis of 100 neutrophils was performed, and the percentage of cells which showed phagocytic activity was calculated based on the number of granulocytes containing at least 3 latex granules.

Nitratetrazolium blue reduction test (NBT) was performed according to the Park's method [13]. Blood smears were viewed through a light microscope in immerse magnification. While evaluating NBT test, 100 neutrophils were analysed. The neutrophils which contained formazan deposits of the size of at least 1 lobe of the nucleus were defined as positive. The specimens prepared by the routine NBT method were also analysed with the use of the LUCIA computer programme (version 4.51) for image analysis

Blood for determination of MPO concentration was taken into EDTA test tubes, centrifuged for 10 minutes at the temperature of 4ºC (3000 x g) within 6 hours of taking. The plasma was stored until determination at the temperature of -20ºC. The concentration of MPO in plasma was determined by immunoenzymatic test (BIOXYTEX® MPO Enzyme Immunoassay, OXIS International, Inc., USA).

RESULTS

The mean values concerning the number of erythrocytes in hop growers during the spraying season were slightly higher than those obtained from the control group (farmers); these values, however, were not statistically significant. An analysis within groups also did not show statistical significance.

The number of leukocytes in the group of hop growers was 7,860.1, whereas among farmers – 7,320.1. These differences were not statistically significant.

With respect to white morphotic elements of blood, only the mean values of the number of basophils and lymphocytes were significantly higher statistically, compared to the values obtained in the control group.

Mean values of the number of basophils were significantly higher statistically ($p<0.05$) in people growing hops (66.4), compared to farmers (35.4). An analysis of mean values of the number of lymphocytes showed a significant increase ($p<0.05$) among hop growers (2,666.7), compared to farmers (2,349.8). In all age groups analysed, mean values of the number of basophils and lymphocytes were higher among hop growers, compared to the values obtained in farmers, but statistically insignificant. Mean values of the number of platelets in hop growers (209.6) were significantly lower statistically ($p<0.01$) than in farmers (232.3) who were the control group.

An analysis conducted within the age groups showed a significantly lower number of platelets ($p<0.01$) among hop growers aged 41-55 (207.7), compared to the group of farmers within the same age interval (236.5). Also in the remaining age groups (25-40 and 56-70), the number of platelets in hop growers was lower, compared to farmers; however, these values were not statistically significant. Table 1 presents the results concerning the morphotic elements of blood.

The evaluation of phagocytic and bactericidal properties of neutrophils covered both the mean number of neutrophils engaged in the above-mentioned processes, and the percentage of cells showing phagocytic and bactericidal properties. Table 2 presents detailed results.

Mean values of the number of neutrophils of phagocytic properties among hop growers were higher (3835.2), compared to the control group of farmers (3551.7); these differences, however, were not significant statistically. Also, the mean values of the number of neutrophils with bactericidal properties were higher in hop growers (855.4) than in farmers (738.8). In this case, the differences were also not statistically significant.

In hop growers aged 25-40 and 41-55, the mean numbers of neutrophils with phagocytic and bactericidal properties were higher in hop growers, compared to farmers, but statistically insignificant. Mean numbers of phagocytic and bactericidal neutrophils in hop growers aged 56-70 were comparable to the values obtained in the control group of farmers.

While evaluating the percentage of phagocytic neutrophils, significantly higher statistical values with respect to phagocytic neutrophils were observed in hop growers (91.3%), compared to farmers (87.1%). Irrespective of the age group, the percentage of neutrophils participating in the process of phagocytosis was always higher in people engaged in the growing of hops, compared to farmers; however, only in the youngest age group (25-40) were these differences statistically significant ($p<0.05$).

The results of NBT test showed that the percentage of neutrophils with bactericidal properties was considerably higher in hop growers (20.4%) than in farmers (17.2%), these differences being statistically significant ($p<0.01$). The percentage of positive cells in NBT test in hop growers aged 25-40 was significantly higher statistically ($p<0.01$), compared to the control group of farmers at analogous age, and was 20.9% and 16.3% respectively. In the older age groups (41-55 and 56-70) in hop growers, the percentage of neutrophils participating in the bactericidal process was higher, compared to the control group in analogous age groups; these differences were not statistically significant.

An analysis of the surface of neutrophills nuclear lobuli and formazan deposits, conducted with the use of computer programme LUCIA (version 4.51), showed that the mean surface of nuclear lobuli in neutrophil cells was 11.7 μm^2 (minimum value noted - 8.3 μm^2; maximum value - 19.3 μm^2), while the mean surface of formazan occurring in neutrophil cells after nitrotetrazolium blue reduction was 14.0 μm^2 (minimum value noted - 5.5 μm^2; maximum value - 24.6 μm^2).

Mean density of nuclear lobuli of neutrophils was 0.7 (minimum value - 0.5, maximum value - 0.9), while the mean density of formazan deposits present in neutrophil cells after nitratetrazolium blue reduction was 1.7 (minimum value - 1.0; maximum value - 3.7).

In hop growers, the mean concentration of myeloperoxidase (MPO) in plasma was 60.9 ng/ml and was significantly higher statistically ($p<0.01$) than the values obtained in the control group of farmers (43.4 ng/ml) (Tab. 3).

The highest mean MPO values (64.2 ng/ml) were observed in hop growers of the medium age group (41-55). In an analogous age group of farmers, the level of MPO was 43.4 ng/ml, the differences being statistically significant ($p<0.05$). In the oldest group of hop growers

Table 1. Morphotic elements of blood examined in the study population.

Morphotic elements of blood	Hop growers		Farmers	
	N	x ± SE	N	x ± SE
Erythrocytes	238	5,370,000 ± 25,000	53	4,840,000 ± 33,000
Leukocytes	238	7,860.1 ± 1,800.0	53	7,320.1 ± 1,900.0
• Neutrophils	238	4,198.6 ± 1,391.6	53	4,061.6 ± 1,341.8
• Eosynophils	238	277.7 ± 160.6	53	289.8 ± 179.7
• Basophils	238	66.4 ± 6.4*	53	35.4 ± 2.6
• Monocytes	238	680.5 ± 90.5	53	638.0 ± 96.1
• Lymphocytes	238	2,666.7 ± 905.1*	53	2,349.8 ± 688.6
Platelets	238	209.6 ± 50.0**	53	232.3 ± 56.1

N - No. of examined people; *p<0.05, **p<0.01 compared to the control group.

Table 2. Mean values of neutrophils participating in phagocytic and bactericidal processes in the population examined during the spraying season.

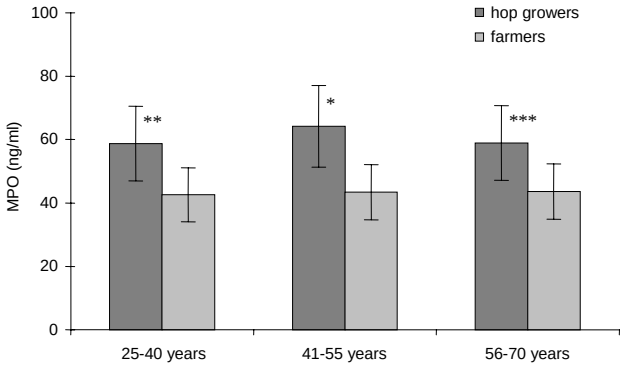
Neutrophils	Hop growers		Farmers	
	N	x ± SE	N	x ± SE
Phagocytic	238	3,835.2 ± 1,374.2	53	3,551.7 ± 1,344.2
Bactericidal	238	855.4 ± 546.0	53	738.8 ± 426.7
Phagocytic test (%)	238	91.3 ± 4.3*	53	87.1 ± 6.4
NBT test (%)	238	20.4 ± 7.5**	53	17.2 ± 5.8

N - No. of examined people; *p<0.05, **p<0.01 compared to the control group.

Table 3. Mean values of MPO level (ng/ml) in plasma in hop growers and farmers.

Examined groups	N	MPO (ng/ml)x ± SE
Hop growers	62	60.9 ± 11.9 *
Farmers	53	43.4 ± 8.6

N – No. of examined people; * p<0.01 compared to the control group.



*p<0.05, **p<0.01, ***p<0.001, I - standard deviation

Figure 1. Mean values of MPO level (ng/ml) in plasma in hop growers and farmers according to age groups.

(56-70), MPO level was significantly higher statistically (58.9 ng/ml, p<0.001), compared to farmers of an analogous age group (43.6 ng/ml).

In the youngest group of hop growers (25-40), the level of MPO was also significantly higher statistically (58.7 ng/ml, p<0.01), compared to the control group (42.6 ng/ml) (Fig. 1).

DISCUSSION

Intensive chemical protection of hops and fruit trees associated with the application of toxic substances such as pesticides causes risk for human health. For the general population, the main source of contact with pesticides is food, whereas for the inhabitants of regions of intensive chemical production the exposure is additionally connected with environment pollution, i.e. air, water and soil. An especially unfavourable phenomenon is the retention of this pollution for a certain period after the completion of plant protection treatment, penetration into ground water, evaporation from soil, and secondary contamination of the air [4, 18].

The presence of pesticides in the air of fruit-growing areas and inside lodgings in these regions, in soil and drinking water, indicates the possibility of contact with these substances, not only among people performing chemical treatment, but also all inhabitants in individual areas [12].

The results of own studies showed that the mean values of the number of erythrocytes in hop growers during the spraying season were insignificantly higher (5,370,000) than the values observed in the control group of farmers (4,840,000); the differences, however, were not significant statistically. A significant decrease was noted in the number of platelets, both in the general population of hop growers and in the individual age groups, compared to the control group of farmers. These results could suggest that thrombocytopenia may be associated with disturbed production of platelets due to the hypoplasia of bone marrow megacaryocytes, which may be the effect of pesticides. The results of the studies described differ from those obtained in the examinations of workers (males and females) engaged in production of organophosphorus pesticides, where a weakening of the activity of the erythrocytic system was observed. The contents of hemoglobin in the blood of females and the number of erythrocytes in males and females was lower, compared to the control group of people not exposed to organophosphorus compounds. Apart from ichemia, an increase in white morphotic elements was also noted in blood of the exposed workers. In addition to exposure to organophosphorus compounds, these workers were also exposed to the output products and indirect products of synthesis of the above-mentioned compounds, such as alcohols, xylene, cyclohexanon, etc. Changes in the erythrocytic system might have been connected with the effect of these substances on human body [7].

In people occupationally exposed to chronic effect of plant protection means, mainly organophosphorus compounds, an increase was observed in the number of white blood cells in peripheral blood [16].

In own studies, no differences in the absolute number of white morphotic elements were observed in the blood of hop growers, compared to the control group of farmers. An analysis of individual sub-populations of leukocytes, however, showed a significantly statistically higher number of basophils and leukocytes among hop growers, compared to farmers.

The degree of activity of the granulocyte system is assessed, among other things, by means of the phagocytic test and nitratetrazolium blue reduction tests, which may be tests for exposure to chemical agents in the environment. Phagocytic reactions are also used in the determination of acute exogenous poisonings [6, 11].

A detailed analysis of the degree of phagocytic and bactericidal activity of neutrophils conducted in own studies allowed us to presume that during the spraying season there occurs a mobilisation of the granular system, manifested by the presence of over 90% of neutrophils of intensified phagocytic activity, and 20% of neutrophils of intensified bactericidal activity. An increase in phagocytic and bactericidal activity of neutrophils in the spring-summer season, which was noted in the population examined, is probably associated with intensive chemical treatment carried out during this season.

Cytoenzymatic granulocytes tests conducted among males of repair teams employed in the “Organika-Azot” chemical plant in Jaworzno showed a significant decrease in the global value of alkaline phosphatase, but an intensification of the reaction for acid phosphatase and myeloperoxidase did not differ from the control group. The number of leukocytes in peripheral blood of workers of the repair teams was significantly higher (p<0.001) than the values noted in the control group. Considering the percentage distribution of white blood cells, only the percentage of monocytes was significantly lower (p<0.01), compared to the control group [8].

The studies of employees of facilities producing pesticides showed a different direction of changes in

enzymatic activity of granulocytes, most frequently the weakening of cytochemical reaction for MPO, but only in people employed in the section producing polychloric pesticides was the reaction for MPO intensified with a simultaneous weakening of reaction for acid phosphatase. Cytoenzymatic tests conducted in males employed in the production of organophosphorus pesticides showed a weakening of reaction to myeloperoxidase and increased reaction to acid phosphatase. A smaller reaction to myeloperoxidase may be the result of the direct effect of organophosphorus compounds to this enzyme [7, 20]. In own studies, the analysis of MPO in blood plasma among the population examined showed statistically significant differences. In hop growers, the level of MPO was significantly higher (60.9 ng/ml), compared to the control group of farmers (43.4 ng/ml).

Myeloperoxidase present in the granules of azurophilic (primary) neutrophils catalyses the production of hypochlorous acid of bactericidal properties. As a result of the reaction of hypochlorous acid with amines the most effective bactericidal agents are produced by neutrophils - chloramines [10]. An elevation of the level of MPO in plasma among hop growers, with a simultaneous high percentage of neutrophils in the test, may be evidence of an intensification of bactericidal processes in people exposed to intensive effect of pesticides.

CONCLUSIONS

1. Clear changes observed with respect to the hematologic parameters examined, such as: platelets, basophils, lymphocytes, may be evidence of potential exposure to pesticides.

2. An increase in phagocytic and bactericidal activity of neutrophils in people exposed to plant protection means may confirm an intensification of mechanisms of non-specific cellular resistance. Phagocytic test and nitratetrazolium blue reduction tests (NBT) may be the tests for exposure to chemical agents in the environment.

3. An elevated level of extracellular myeloperoxidase (MPO) in the blood plasma of hop growers, compared to the control group of farmers, may be evidence of increased enzymatic activity of granulocytes, and increased secretion of immunomodulating cytokines during the period of intensive application of plant protection means.

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