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BIODINAMINIŲ PREPARATŲ ĮTAKA DIRVOS SAVYBĖMS, DIDŽIŲJŲ MOLIŪGŲ VAISIŲ DERLIUI IR KOKYBEI

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INTRODUCTION

Relevance of the subject. Securing quality and safety of food raw materials is an important challenge for Lithuania. Like for all vegetables, the quality and yield of pumpkin fruit depend not only on the species, variety, soil properties, weather conditions but also on the cultivation technology applied.

The great pumpkin (*Cucurbita maxima* D.) is an annual, productive plant cultivated worldwide. According to Food and Agriculture Organization of United Nation (FAO), the world production of pumpkins in 2011 was estimated over 24.3 million tons harvested from 1.7 million hectares (FAOSTAT, 2013). The popularity of pumpkins in Lithuania has been increasing recently, because according to their nutritive value and technological characteristics they surpass many vegetables. Pumpkin fruit does not accumulate high concentrations of nitrates and other pollutants, they are low in calories and rich in various antioxidants (carotenoids, phenols, anthocyanins and others) (Danilčenko et al., 2014).

In Lithuania, the consumption of fresh fruit and vegetables has been on the increase recently; however, the daily intake is still insufficient and amounts to as little as 260 g instead of 400 g recommended by the World Health Organisation (LR Ministry of Agriculture, 2014). The shortage of antioxidants in food increases the risk of malignant tumours and cardiovascular diseases (Grabauskas et al., 2013).

Our research aimed to reveal the effect of biodynamic preparations on the quality of the great pumpkin fruit. The above mentioned preparations are used in the farms involved in the biodynamic agricultural production in Europe. Biodynamic agriculture not only produces safe and high-quality food products but also contributes to the development of sustainable, environment-friendly farming. An advocate of the biodynamic agriculture and a member of the *Demeter International* association Ms. Brigette Szezinski suggests that "what is important in this production system is not only economically and ecologically sustainable farming but also harmonious interaction between man and nature". Research done by foreign scientists has indicated that various plants whose cultivation technology included biodynamic preparations exhibited higher quality and better sensory properties and accumulated higher concentrations of antioxidant compounds. However, we found no research evidence on the effects of biodynamic preparations on the improvement of pumpkin fruit quality and on increasing of biologically active compounds content.

Research hypothesis. Biodynamic preparations used in the great pumpkin agrotechnology activate the physiological processes, reactions of metabolism that promote the synthesis of biologically active compounds and accumulation in plants and fruits.

Research objective. The study was aimed to explore and assess the effects of biodynamic preparations on the soil agrochemical composition, on the quality of the great pumpkin fruits.

Research tasks:

1. To investigate and assess the effects of biodynamic preparations on soil agrochemical composition and enzymatic activity.
2. To establish the impact of biodynamic preparations on the development of pumpkin plants.
3. To compare various great pumpkin varieties in terms of the quality of morphological parts of fruit – peel, flesh and seeds.
4. To determine the effect of biodynamic preparations on the content of biologically active compounds in the fruits of great pumpkin.

Propositions to be defended in the thesis:

1. Biodynamic preparations have positive influence on soil enzymatic activity.
2. Biodynamic preparations positively influence the development of pumpkin plants and physiological effects.
3. Biodynamic preparations promote the accumulation of biologically active compounds in the fruits of great pumpkin.

Originality of the research work. For the first time was identified and assessed the effects of biodynamic preparations in the great pumpkin cultivation technology on development of pumpkin plants and their fruit quality (on the content of biologically active compounds).

Practical value of the research work. Our experimental findings proved that with the inclusion of biodynamic preparations in the cultivation technology it is possible to improve soil composition, to increase activity of soil enzymes, to enhance the nutritive value of pumpkin fruits. Moreover, they provide preconditions for the development of novel cultivation technologies in Lithuania as well as for innovative outlook on harmonious interaction between man and nature.

Approval of the thesis work. The research results were presented and discussed in the national and international conferences: *„Optimization of ornamental and garden plant assortment, technologies and environment“* (Lithuania, Mastaičiai, 2012), *„Young scientists for agricultural progress”* (Lithuania, Vilnius, 2012), *„Human and nature safety”* (Lithuania, Akademija, 2012), *„Innovative and healthy food for consumers”* (Lithuania, Kaunas, 2012), „*Ecology and health*“ (Kaliningrad, 2012), *„4th International Course Advanced Food Analysis“* (Netherlands, Wageningen, 2013), *„19th Baltic agronomy forum“* (Lithuania, Akademija, 2013), *„Youth seeks progress”* (Akademija, 2013), *„3rd International Horticulture Conference for Post-graduate Students“* (Czech Republic, Lednice, 2013), *„Rural Development 2013: Innovations and Sustainability“* (Lithuania, Akademija, 2013), 6th *„Quality and Safety in Food Production Chain“* (Poland, Wrocław, 2014), *„3rd International ISEKI_Food Conference“* (Greece, Athens, 2014), *„Horticulture in shaping life quality“* (Poland, Lublin, 2015).

Volume and structure of the work. The doctoral thesis consists of: introduction; literature analysis; research object, conditions and methods; research results and discussion; conclusions; list of publications, and list of references (241 references). The thesis includes 15 tables, 21 figures. The thesis contains 83 pages.

RESEARCH OBJECT, CONDITIONS AND METHODS

Research object – plants of varieties 'Justynka', 'Karowita', 'Amazonka' of the great pumpkin (*Cucurbita maxima* D.) in their cultivation technology using the biodynamic preparations.

The experiment was conducted in 2012–2014.

A field experiment was set up on an organically-managed plot in Kaunas district, Ringaudai village. The study site is situated in the Middle Lithuania Lowland. The relief is little undulating plain. **The soil of the experimental site is** *Calc(ar)i-Endohypogleyic Luvisol* (Buivydaitė, Vaičys, 2001). The soil texture is light loam, humus content 2.4 % (high), pH ~ 7 (somewhat neutral); P_2O_5 – 139–173 mg kg^{-1} (high); K_2O – 173–209 mg kg^{-1} (high).

Experimental design:

Four treatments were chosen to estimate the effects of the biodynamic preparations. A two-factor field experiment was established according to the following design:

Factor A. Varieties of the great pumpkin:

1. 'Justynka'
2. 'Karowita'
3. 'Amazonka'

Factor B. Use of biodynamic (BD) preparations:

1. BD preparations were not applied – control;
2. The soil was sprayed with the BD preparation *500* (P 500);
3. The plants were sprayed with the BD preparation *501* (P 501);
4. The soil was sprayed with the BD preparation *500* (P 500) and the plants were sprayed with the BD preparation *501* (P 501).

The biodynamic preparations 500 (fermented manure) and 501 (ground silica SiO_2) were obtained from the Demeter certified farm in Germany (CvW KG, Internationale Biodynamische Präparatezentrale) (Fig. 1).

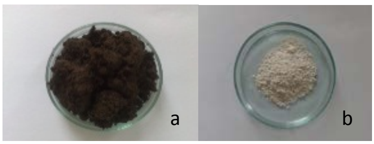

Fig. 1. Biodynamic preparations *500* (a) and *501* (b)

The P 500 had a very high phosphorus content and high urease enzyme activity (Table 1).

Table 1. Composition of the biodynamic preparation 500, in 2012–2014

pH_{KCl}	P_2O_5 (g·kg^{-1})	K_2O (mg·kg^{-1})	N (%)	Urease activity (mg NH_3 1 g soil 24 h^{-1})	Saccharase activity (mg glucose 1 g soil 48 h^{-1})
6.96	1.96	259.20	2.10	1.56	32.7

The soil was sprayed twice with the P 500 at 1 % concentration of the solution two weeks before planting of the pumpkin seedlings and after harvesting. P 501 – ground silica (SiO_2). The system of this transparent, colourless crystal is hexagonal and light fracture is shell-like. The P 501 was sprayed in the morning when the ray energy utilization efficiency in photochemical reactions is higher. The planting was done in the third ten-day period of May in four replications. Pumpkin leaves were sprayed twice with the solution of 0.5 % concentration of the P 501 (ground silica) at the beginning of flowering (BBCH 605 – 5th flower open on main stem) and at the beginning of fruit formation (BBCH 702 – 2nd fruit on main stem has reached typical size and fruit). The BBCH-identification keys were established by methodology of Feller et al. (1995) for pumpkin plants. The crop was harvested within the first ten-day period of September. The treatments in the replicated plots were arranged in a systematic order. The total area of a plot was 12 m^2, the width of the protection strip – 0.5 m, the harvested plot area – 6 m^2. Six plants were planted per each treatment's harvested plot area.

Laboratory analyses. **Soil chemical composition** was determined prior to the trial establishment. To determine the effects of the P 500, soil samples (up to 20 cm depth) were taken 4 times – 7 days, 14 days, 65 days and 130 days (at the end of vegetation) after application as soil spray.

Quality indicators were estimated using the standard methods:

Soil reaction (pH) was measured potentiometrically with a pH-meter in 1 N KCl extract (ISO 10390:2005).

Total nitrogen concentration (%) was determined by the Kjeldahl method, the contents of mobile phosphorus (P_2O_5) (mg·kg^{-1}) and mobile potassium (K_2O) (mg·kg^{-1}) were measured by the CAL method.

Activity of urease and saccharase enzymes in the soil was estimated three times – 7 days, 14 days, and 130 days after soil spray. Urease activity was estimated according to Hofmann and Schmidt (1953) method and that of saccharase according to Hofmann and Seegerer (1950) methods.

Concentrations of nitrogen (nitrate + nitrite) and ammonia nitrogen (mg·kg^{-1}) were determined by a flow injection analysis (FIA) spectrometric method using an inductively coupled mass spectrometer (ICP-MS, Thermo Finnigan MAT, Germany).

Mineral nitrogen concentration (mg·kg^{-1}) – a sum of nitrate nitrogen, nitrite nitrogen and ammonia nitrogen.

Net photosynthetic productivity (Fpr) was calculated according to the formula:

$Fpr = 2 (M_2 - M_1) / (L_1 + L_2) T$ (1), where:

$(M_2 - M_1)$ – dry mass increment during a given time;

L_1 and L_2 – leaf area at the beginning and end of the period;

T – time duration in days (Bluzman et al., 1991).

A composite fruit sample was formed: treatment x 4 fruit x 4 replications – 16 fruit – from the selected 5 fruit a test sample was formed. For the analyses of pumpkin flesh chemical composition, a 1000 g sample was formed from the composite sample of each treatment by selectively cutting pieces of flesh from several places (LST ISO 2859-10:2007). A size of a peel sample was ~1000 g and that of seed 100 g. Chemical analyses of pumpkin fruit peel, flesh and seed were performed in four replications.

Standard methods were used **to establish the below listed parameters in the dry matter (d. m.) (**exept dry matter (%) – by drying the samples at 105°C to a constant weight (LST ISO 751:2000) **of the great pumpkin peel and flesh and in the fresh mass (f. m.) of fruit flesh**:

- **macroelement (K, P, Ca, Mg) content** (% d. m.) – by atomic absorption spectrometry method;
- **carotenoid content** ($mg·100g^{-1}$ f. m.) – by a spectrophotometer Spectro UV-VIS dualbeam UVS-2800 (Labomed Inc., USA);
- **lutein+zeaxanthin, lycopene, β-carotene** ($mg·100g^{-1}$ f. m.) – by high performance liquid chromatography (HPLC).

***The below listed parameters were determined at the Institute of Chemistry and Biology of Immanuel Kant Baltic Federal University* using a spectrophotometer SF-2000 (manufacturer ZAO "OKB SPECTRUM", Russia) according to Gupta and Verma (2011) methodology:**

- **leuco-anthocyanin content** ($mg·100g^{-1}$);
- **catechin content** ($mg·100g^{-1}$);
- **total phenolics content** ($mg·g^{-1}$).

The below listed parameters were measured in **the fresh mass of the great pumpkin seeds** using a spectrophotometer SF-2000 according to Gupta and Verma (2011) methodology at *the Institute of Chemistry and Biology of Immanuel Kant Baltic Federal University*:

- **anthocyanin content** ($mg·100g^{-1}$);
- **leuco-anthocyanin content** ($mg·100g^{-1}$);
- **carotenoid content** ($mg·g^{-1}$).

The statistical analysis of the research data. The research data were statistically processed by the analysis of variance (ANOVA) using the software STATISTIKA (*STATISTICA 7*). Statistical significance of the differences between the means was estimated by Fisher's LSD test ($p<0.05$). Correlation analysis was performed to determine the strength and nature of the relationship between the variables.

RESEARCH DATA ANALYSIS AND DISCUSSION

Effects of the biodynamic preparation 500 on the soil properties

Soil composition and plant yield depend largely on the agricultural production system employed. Organic fertilizers and crop rotation applied in organic farms determine better soil quality indicators. In biodynamic farms, soil viability is maintained, special preparations are used, conditions are created for the production of products characterised by high quality and nutrition value.

Our research indicated that phosphorus and potassium contents were significantly higher during the entire vegetation period in the treatment where the soil had been sprayed with the P 500 (Table 2). Seven days after the spray application, P_2O_5 content was by 13.95 % and that of K_2O by 5.88 % higher compared with the unsprayed control treatment. Significantly highest contents of all nitrogen forms in the soil were established 14 days after the spray application (nitrogen – by 12.79 %, ammonia nitrogen – by 18.46 %, mineral nitrogen – by 13.64 % higher). From fourteen days after the application until the end of vegetation the contents of nutrients were decreasing in the soil (the larger part of them was accumulated by the pumpkin plants); however, they remained significantly higher in the spray-applied treatment. In the P 500 soil spray treatment, the soil pH was significantly decreasing until the end of pumpkin vegetation season – 7 days after treatment it decreased to 6.68, after 14 days – to 6.64, and after 130 days – to 6.52. The variation of the soil reaction had influence on phosphorus uptake by pumpkin plants. The soil pH tended to decrease. Therefore it can be concluded that significant changes occurred within a 14-day period after the soil spray with the P 500. It is maintained that soil bacteria identify and respond to very small amounts of signalling molecules in their environment. It may be presumed that the fermented manure preparation influenced the activity and proliferation of soil bacteria by promoting nutrient accumulation from soil.

Table 2. The effect of spray with preparation 500 on soil agrochemical composition, in 2012–2014

Indicator	*Spray treatments (factor B)*						
	Not sprayed	Sprayed with P 500		Not sprayed	Sprayed with P 500	Not sprayed	Sprayed with P 500
	Terms after spray (factor A)						
	before spray	*7 days*	*14 days*	*65 days*		*130 days*	
P_2O_5 ($mg \cdot kg^{-1}$)	350.12c	398.97a*	365.04b	190.20f	252.95d*	180.44g	243.17e*
K_2O ($mg \cdot kg^{-1}$)	285.60b	302.40a*	271.20b	150.32e	214.80c*	127.20f	180.00d*
Nitrogen (nitrate+ nitrite) ($mg \cdot kg^{-1}$)	25.95b	24.85b	29.27a*	3.90d	5.99c*	3.21d	5.41c*
Ammonia nitrogen ($mg \cdot kg^{-1}$)	4.55c	4.96c*	5.39b*	3.45d	9.38a*	2.46e	2.56e*
Mineral nitrogen ($mg \cdot kg^{-1}$)	30.50b	29.81b	34.66a*	7.35d	15.37c*	5.67e	7.97d*
pH	6.81b	6.68c*	6.64c*	6.90a	6.82b*	6.66c	6.52*

Note: factor A – spray term: before spray, 7, 14, 65 and 130 days after spray; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500. Differences between the means of treatments of factor A marked by a different letter (a, b, c...g) and differences between the means of treatments of factor B marked by an asterisk are significant, $p \le 0.05$.

Changes in the enzyme activity in the soil occur more rapidly than those of other indicators. Our research evidenced that the P 500 significantly increased soil enzymatic activity (Table 3).

Table 3. The effect of spray with preparation 500 on enzyme activity, in 2012–2014

Enzym	*Spray treatments (factor B)*				
	Not sprayed	Sprayed with P 500		Not sprayed	Sprayed with P 500
	Terms after spray (factor A)				
	before spray	*7 days*	*14 days*	*130 days*	
Urease activity (mg NH_3 1 g soil 24 h^{-1})	0.45c	0.62a*	0.52b*	0.28d	0.54b*
Sacharasse activity (mg glucose 1 g soil 48 h^{-1})	33.79c	35.64a*	35.64a*	33.22d	35.00b*

Note: factor A – spray term: before spray, 7, 14, 65 and 130 days after spray; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500. Differences between the means of treatments of factor A marked by a different letter (a, b, c, d) and differences between the means of treatments of factor B marked by an asterisk are significant, $p \le 0.05$.

Significantly higher urease (by 37.78 %) and saccharase (by 5.33 %) activity was identified 7 days after application, while a slight decrease in urease activity was recorded 14 days after application. Urease plays an important role in the soil nitrogen cycle and characterises it. This can explain greater changes in this enzyme activity over the same period of time compared with saccharase. At the end of vegetation of pumpkin plants urease activity was significantly by 1.93 times higher and that of saccharase by 1.05 times higher compared with the unsprayed treatment. It can be assumed that active proteolytic activity of the P 500 is associated with its specific composition.

Effects of the biodynamic preparations 500 and 501 on the development of the great pumpkin plants

Net photosynthetic productivity. Our research showed that genetic characteristics of a variety determined the photosynthetic productivity of the great pumpkin. The dry mass increase for the variety 'Justynka' over the same period was 1.24 times higher than that of 'Karowita' and 2.81 times higher than that of 'Amazonka' (Fig. 2). The use of both biodynamic preparations (500+501) had a positive effect on the photosynthetic productivity of the great pumpkin. The productivity of the variety 'Justynka' significantly increased by 11.53 % that of 'Karowita' by 8.60 %, and that of 'Amazonka by 14.57 % (Fig. 2). The biodynamic preparation 501 is ground silica (SiO_2) powder. We believe that the specific ray fracture might be associated with a larger amount of photosynthetically active radiation in the upper canopy of the pumpkin. Part of the radiation diffusely spreads in the leaf canopy mass. Apparently such light is additionally utilized as a source of light for the process of photosynthesis.

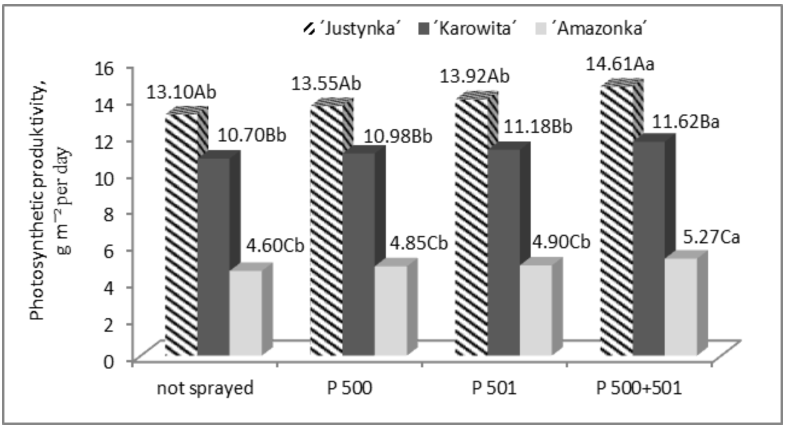

Fig. 2. The effect of preparations 500 and 501 on net photosynthetic productivity of the great pumpkin plants, in 2012–2014

Note: factor A – varieties: Justynka, Karowita, Amazonka; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500, P 501 – sprayed with P 501, P 500+501 – sprayed with P 500 and P 501. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) and differences between the means of treatments of factor B marked by a different lowercase letter (a,b) are significant, $p \leq 0.05$.

The effect of P 500 and 501 on the fruit quality of the great pumpkin

Our findings suggest that dry matter content in the peel of different pumpkin varieties varied from 14.79 to 21.01 % (Fig. 3). Significantly highest dry matter content was established in the peel of 'Justynka', i.e. it was by 5.3 percentage points higher than that in 'Karowita' peel and by 1.54 percentage points higher than that in 'Amazonka' peel. Pumpkin flesh accumulated markedly lower contents of dry matter (7.55–14.29 %). Of all varieties 'Justynka' had the highest dry matter content ranging from 12.44 to 14.29 %. 'Karowita' and 'Amazonka' had similar flesh dry matter content. The spray with the preparations exerted a significant effect on the accumulation of dry matter. In the treatments sprayed with both preparations the dry matter content in the peel of 'Justynka' increased by 2.34 %, in the peel of 'Karowita' it increased by 2.60 %, and in the peel of 'Amazonka' – by 4.65 %. The flesh of 'Justynka' accumulated 13.26 % higher dry matter content when sprayed with P 501 and by 14.87 % higher content when sprayed with both preparations. The flesh of 'Karowita' contained by 16.63 % and that of 'Amazonka' by 25.43 % more dry matter under the effect of both preparations – P 500 and 501.

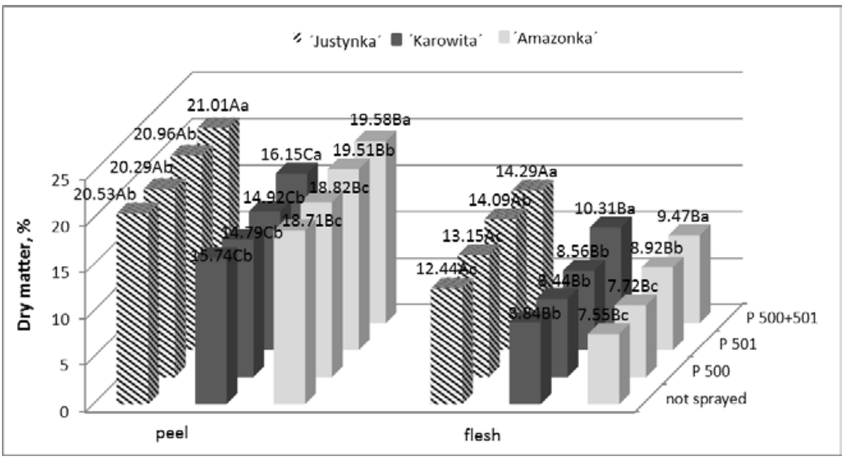

Fig. 3. The effect of preparations 500 and 501 on dry matter content in the peel and flesh of the great pumpkin fruits, in 2012–2014

Note: factor A – varieties: Justynka, Karowita, Amazonka; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500, P 501 – sprayed with P 501, P 500+501 – sprayed with P 500 and 501. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) and differences between the means of treatments of factor B marked by a different lowercase letter (a,b,c) are significant, p≤0.05.

Pumpkin plants cannot complete their life cycle without an optimal content of macroelements. Our research findings indicated higher contents of macroelements to be present in the pumpkin peel (Table 4).

'Amazonka' peel was found to have the highest phosphorus content (1.46 % d. m.) and magnesium (0.54 % d. m.). The highest content of magnesium (0.17 % d. m.) in 'Justynka' flesh was detected in the not sprayed treatment. The content of potassium, phosphorus, calcium and magnesium significantly increased in the peel and flesh of all pumpkin varieties tested in the treatments sprayed with P 501 or with both preparations. P 501 spray-application on plants had a greater positive effect on macroelement content. The content of potassium in the peel increased by on average 15.24 %, in the flesh by 17.54 %; the content of phosphorus in peel increased by 14.54 %, in flesh by 23.34 %. Using the P 501, the peel of the great pumpkin accumulated by on average 1.69 times more calcium and flesh by 1.33 times. Magnesium contents were by respectively 1.47 and 1.90 times higher. The research literature indicates that silicon is involved in binding inorganic phosphate to ATP, ADP and sugar phosphates in sugar cane. We established a strong positive correlation between phosphorus content and spray application of pumpkin plants with the P 501 ($r = 0.760$, $p < 0,01$).

Table 4. The effect of preparations 500 and 501 on the content of macroelements in the peel and flesh of the great pumpkin fruits, in 2012–2014

Spray treatments *(factor B)*	K		P		Ca		Mg	
	% d. m.							
	p*	f*	p	f	p	f	p	f
´Justynka´ (factor A)								
Not sprayed	0.22Aa	0.19Aa	0.94Bb	0.82Aa	0.25Ab	0.16Aa	0.30Bb	0.17Aa
P 500	0.23Aa	0.20Aa	1.02Bab	0.81Aa	0.26Ab	0.16Aa	0.28Bb	0.14Aa
P 501	0.25Aa	0.22Aa	1.24Aa	0.88Aa	0.47Aa	0.23Aa	0.51Aa	0.25Aa
P500+501	0.25Aa	0.22Aa	1.16Aab	0.86Aa	0.36Aa	0.21Aa	0.51Aa	0.21Aa
´Karowita´								
Not sprayed	0.23Aa	0.19Aa	1.44Aa	0.87Aa	0.27Abc	0.18Aa	0.33Bb	0.14Aab
P 500	0.24Aa	0.20Aa	1.42Aa	0.86Aa	0.33Ab	0.17Aa	0.32Bb	0.13Ab
P 501	0.26Aa	0.23Aa	1.52Aa	1.02Aa	0.46Aa	0.23Aa	0.54Aa	0.33Aa
P500+501	0.25Aa	0.23Aa	1.50Aa	0.99Aa	0.41Aab	0.21Aa	0.48Aab	0.25Aa
´Amazonka´								
Not sprayed	0.21Aa	0.19Aa	1.46Aa	0.88Ab	0.26Ab	0.19Aa	0.54Aa	0.14Ab
P 500	0.22Aa	0.20Aa	1.43Aa	0.86Ab	0.28Aa	0.18Aa	0.52Aa	0.14Ab
P 501	0.25Aa	0.22Aa	1.55Aa	1.28Aa	0.39Aa	0.24Aa	0.58Aa	0.26Aa
P500+501	0.24Aa	0.22Aa	1.52Aa	1.02Ab	0.30Aa	0.22Aa	0.56Aa	0.22Aab

Note:*p – peel,*f – flesh; P 500 soil spray, P 501– plant spray. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B) and differences between the means of treatments of factor B marked by a different lowercase letter (a,b,c) are significant, $p \leq 0.05$.

Our findings suggest that **the total carotenoid content** in the peel of the great pumpkin fruit was by on average 1.51 times higher than in the flesh (Fig. 4). In the peel the content of these antioxidants amounted to from 21.08 to 33.81 $mg \cdot 100g^{-1}$ f. m., in the flesh – from 14.80 to 20.50 $mg \cdot 100g^{-1}$ f. m. The fruit of 'Karowita' accumulated the highest content of carotenoids.

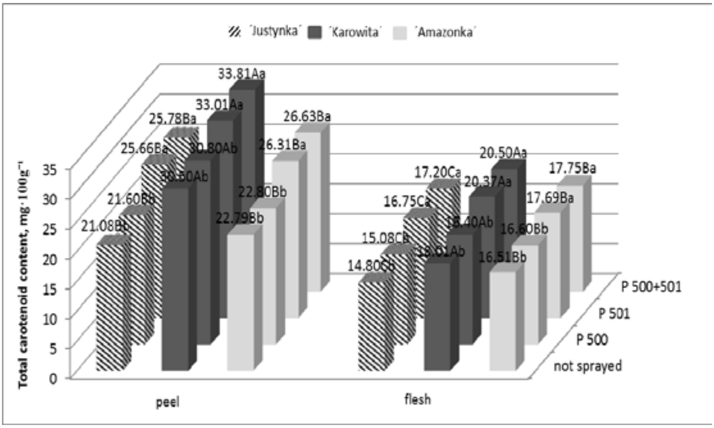

Fig. 4. The effect of preparations 500 and 501 on the total carotenoid content in the peel and flesh of the great pumpkin fruits, in 2012–2014

Note: factor A – varieties: Justynka, Karowita, Amazonka; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500, P 501 – sprayed with P 501, P 500+501 – sprayed with P 500 and 501. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) and differences between the means of treatments of factor B marked by a different lowercase letter (a,b) are significant, $p \leq 0.05$.

The total carotenoid content in all pumpkin varieties tested was positively influenced by the use of P 501 and both preparations. In the P 501 treatment, carotenoid content in the peel increased by on average 15.14 % and in flesh by 11.14 %. In the treatment where both preparations had been used, carotenoid content increased by 16.67 % in the peel and by 12.52 % in the flesh.

The use of P 500 and 501 together had the greatest positive effect on lutein and zeaxanthin, lycopene and β-carotene content in pumpkin fruit flesh (Fig. 5 a, b, c). The flesh of 'Karowita' fruit accumulated the highest concentrations of lutein and zeaxanthin (12.31 mg·100g-1 f. m.). The flesh of 'Justynka' and 'Amazonka' contained by one average 1.58 times less of these carotenoids. In the treatments sprayed with both preparations, their content in 'Justynka' fruit increased by 2.01, in 'Karowita' fruit by 1.15, in 'Amazonka' fruit by 2.09 times. The literature sources indicate that the use of 500 and 501 can significantly increase lutein and zeaxanthin content in cultivated plants. Our findings indicate that lycopene concentrations differed insignificantly between the varieties and spray treatments (Fig. 5a).

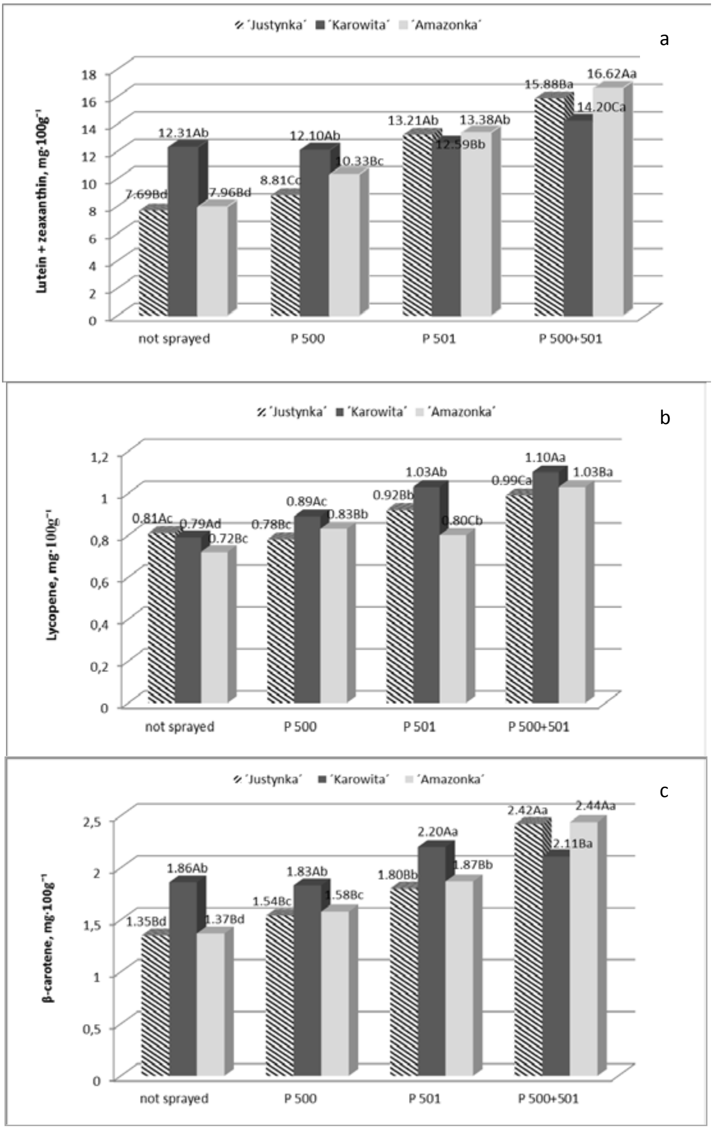

Fig. 5 (a, b, c) The effect of preparations 500 and 501 on antioxidant content in the flesh of the great pumpkin fruits, in 2012–2014

Note: factor A – varieties: Justynka, Karowita, Amazonka; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500, P 501 – sprayed with P 501, P 500+501 – sprayed with P 500 and 501. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) and differences between the means of treatments of factor B marked by a different lowercase letter (a, b, c, d) are significant, $p \leq 0.05$.

The flesh of 'Justynka' and 'Karowita' contained 0.81 and 0.79 mg·100g^{-1} f. m. of lycopene, respectively. 'Amazonka' tended to accumulate less lycopene – 0.72 mg·100g^{-1} f. m. In the treatments applied with both preparations lycopene content in the flesh of 'Justynka' significantly increased by 22.22 %, in 'Karowita' by 39.24 %, and 'Amazonka' by 43.06 %. The highest content of β-carotene (1.86 mg·100g^{-1}) was identified in the flesh of 'Karowita' (Fig. 5c). In the treatment applied with both preparations 'Justynka' flesh contained 1.79 times more of this carotenoid, 'Amazonka' – 1.78 times more than in the control treatment. 'Karowita' fruit accumulated the highest β-carotene content (2.20 mg·100g^{-1}) in the treatment sprayed with P 501. The increase in carotenoid content can be associated with the improvement of the nutrient medium and with the changes that had occurred in the light absorption process. Literature suggests that apart from direct effect on photosynthetic intensity, light is important for nitrate reductase. It can be presumed that the small changes in light wave length, caused by the P 501, affected gene expression of nitrate reductase, which resulted in the formation of more complex compounds – amino acids.

In the current study, the content of leuco-anthocyanins in the pumpkin peel was by on average 2.03 times higher than that in the flesh (Table 5). The peel and flesh of 'Amazonka' accumulated significantly highest content of these antioxidants (148.31 and 91.57 mg·100g^{-1} d. m. respectively).

Table 5. The effect of preparations 500 and 501 on antioxidant content in the peel and flesh of the great pumpkin fruits, in 2012–2014

Spray treatments *(factor B)*	Leuco-anthocyanins		Catechins		Total content of phenols	
	mg·100g^{-1} d. m.				mg·g^{-1} d. m.	
	peel	flesh	peel	flesh	peel	flesh
´Justynka´ (factor A)						
Not sprayed	60.02Cb	41.40Bb	181.72Aab	165.31Ab	4.16Cab	2.49Bab
P 500*	55.31Cb	42.08Bab	172.51Ab	157.61Abc	3.06Bb	2.10Bb
P 501	76.89Ba	48.75Ba	194.99Aa	177.83Aa	5.72Ba	3.35Ba
P 500+501	73.20Ba	46.74Bab	192.13Aa	175.51Aab	5.16Cab	3.16Ba
´Karowita´						
Npt sprayed	122.14Bc	40.30Bb	153.84Bb	172.61Ab	7.27Ab	5.25Ab
P 500	110.32Bc	41.52Bb	130.23Bc	164.11Ab	4.05Ac	4.77Ab
P 501	153.77Aa	49.51Ba	174.04Ba	188.81Aa	8.76Aa	6.20Aa
P 500+501	140.64Ab	46.71Bab	160.12Bb	185.67Aa	7.96Aab	6.60Aa
´Amazonka´						
Not sprayed	148.31Ab	91.57Ab	173.72Ab	174.01Ab	5.09Bab	5.91Ab
P 500	135.93Ac	80.12Ac	167.63Abc	157.12Ac	4.73Ab	4.96Ac
P 501	160.49Aa	100.54Aa	199.75Aa	185.26Aa	6.47Ba	6.72Aa
P 500+501	152.36Aab	98.19Aab	185.59Ab	182.40Aab	6.27Ba	6.10Aab

*– P 500 soil spray, P 501– plant spray. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) and differences between the means of treatments of factor B marked by a different lowercase letter (a, b, c) are significant, $p \le 0.05$.

Similar contents of catechins were detected in the peel and flesh of all the pumpkin varieties tested. The highest phenolics content was identified in the peel of 'Karowita' and flesh of 'Amazonka'. In the treatments sprayed with P 501 or both preparations, leuco-anthocyanin and total phenolics content significantly increased in the peel. Significantly higher catechin content in pumpkin peel was established in the P 501-sprayed treatment.

The use of P 501 had the greatest positive effect on the concentration of the above-mentioned antioxidants in the pumpkin peal and flesh: leuco-anthocyanin content in the peel increased by on average 20.74 %, catchetin content by 11.80 %, and total phenolics by 28.37 %. In the flesh, the increase was 16.80 %, 7.81 % and 22.12 %, respectively. We suppose that the application of biodynamic preparations alleviates the stress in plants, which results in higher concentrations of antioxidants in them. It is likely that pumpkin plant spray-application with P 501 had influence on the speed of photochemical reactions and CO_2 assimilation rate. With more intensive photochemical reactions and faster CO_2 assimilation, more assimilates in plants are provided for metabolism reactions. The antioxidant compounds (phenols) investigated in our study are attributed to the products of the secondary metabolism of plants.

Our research findings suggest that the highest dry matter content was accumulated by the seed of 'Justynka' (93.20 %) and the lowest by the seed of 'Karowita' (84.70 %) (Fig. 6). The use of the preparations did not have significant effect on dry matter content in pumpkin seed.

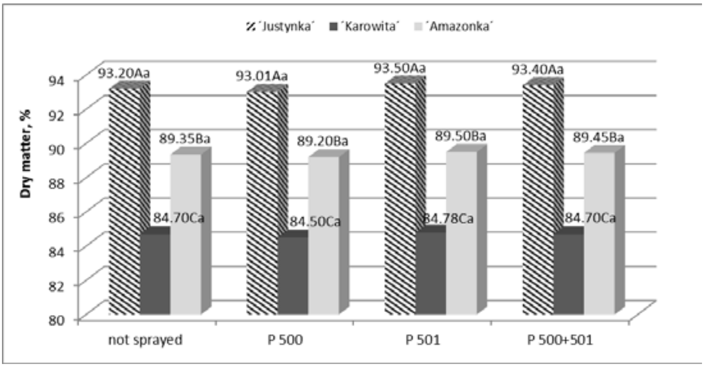

Fig. 6. The effect of preparations 500 and 501 on dry matter content in the seeds of the great pumpkin, in 2012–2014

Note: factor A – varieties: Justynka, Karowita, Amazonka; factor B – spray treatments: not sprayed, P 500 – sprayed with P 500, P 501 – sprayed with P 501, P 500+501 – sprayed with P 500 and 501. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) are significant, $p \leq 0.05$ and treatments of factor B marked by a lowercase letter a are not significant, $p \leq 0.05$.

In our experiment, we established similar and relatively low concentrations of **anthocyanins** in the seed of all varieties tested (Table 6). Our research findings indicate that the seeds of the great pumpkin are rich in carotenoids. 'Amazonka' seeds accumulated significantly highest contents of **carotenoids and leuco-anthocyanins** (6.50 and 32.20 $mg \cdot 100g^{-1}$ f. m. respectively). Carotenoid content in 'Amazonka' seed was 1.35 times higher than that in 'Karowita' seed and as many as 3.16 times higher than in 'Justynka' seed. The application of P 501 had the greatest positive effect on the leuco-anthocyanin and total carotenoid content in seed. The content of leuco-anthocyanins increased by on average 1.47 times and that of carotenoids by 1.64 times. It can be presumed that the increase in the antioxidant compounds in the great pumpkin seed is associated with better light absorption when the plants had been sprayed with the P 501.

Table 6. The effect of preparations 500 and 501 on antioxidant content in the seeds of the great pumpkin, in 2012–2014

Spray Treatments *(factor B)*	Anthocyanins $\mu g \cdot 100g^{-1}$ f. m.	Leuco-anthocyanins $mg \cdot 100g^{-1}$ f. m.	Total carotenoid content $mg \cdot 100g^{-1}$ f. m.
´Justynka´ (factor A)			
Not sprayed	0.09Aa	4.50Bb	10.20Cb
P 500*	0.06Aa	4.20Bb	18.70Ca
P 501	0.10Aa	5.70Ba	23.70Ca
P 500+501	0.09Aa	4.80Bb	21.50Ca
´Karowita´			
Not sprayed	0.10Aa	2.40Cc	23.90Bb
P 500	0.06Aa	4.80Ba	24.50Bb
P 501	0.11Aa	4.90Ca	32.20Ba
P 500+501	0.10Aa	4.20Bb	29.60Bab
´Amazonka´			
Not sprayed	0.10Aa	6.50Ab	32.20Ab
P 500	0.07Aa	6.30Ab	35.00Aab
P 501	0.11Aa	7.10Aa	40.70Aa
P 500+501	0.10Aa	6.70Aab	38.00Aab

*– P 500 soil spray, P 501– plant spray. Differences between the means of treatments of factor A marked by a different uppercase letter (A, B, C) and differences between the means of treatments of factor B marked by a different lowercase letter (a, b, c) are significant, $p \leq 0.05$.

CONCLUSIONS

1. In the soil sprayed with P 500, the contents of phosphorus, potassium, nitrogen and the activity of soil enzymes – urease and saccharase – during the entire vegetation period of the great pumpkin were significantly higher than those in the untreated soil. (TASK 1)
2. The use of the both preparations (500 and 501) had the greatest positive effect on the great pumpkin roots and leaf biometric parameters, chlorophyll index value and net photosynthetic productivity. (TASK 2)
3. The peel and flesh of 'Justynka' variety were found to contain significantly highest concentrations of dry matter and water soluble carbohydrates. The use of P 500 and P 501 had a significant positive influence on the accumulation of dry matter, water soluble carbohydrates, crude fibre, the use of P 501 – on the accumulation of crude ash in the peel and flesh of pumpkin fruit tested. (TASK 3)
4. The results of the sensory analysis indicated that 'Justynka' flesh was characterised by the best appearance, texture, taste and smell. The application of P 501 had a positive effect on the sensory characteristics of flesh of all pumpkin varieties tested. (TASK 3)
5. The flesh of 'Amazonka' contained the highest concentrations of vitamin C, leuco-anthocyanins and total phenols. The peel and flesh of all varieties tested had a similar content of catechins. The spray-application of the P 501 had the greatest positive effect on the concentrations of vitamin C, leuco-anthocyanins, catechins and total phenols in the peel and flesh of the great pumpkin fruit. (TASK 4)
6. The fruit flesh of the 'Karowita' variety were characterised by the highest contents of carotenoids, lutein+zeaxanthin and β-carotene. The use of both preparations had a positive effect on the total carotenoid, lutein+zeaxanthyn, lycopene and β-carotene content in flesh for all great pumpkin varieties tested. The use of P 501 had a positive effect on accumulation for these compounds in seeds. (TASK 4)

REZIUMĖ

Tyrimų hipotezė – didžiųjų moliūgų agrotechnikoje naudojami biodinaminiai preparatai aktyvina fiziologinius procesus, metabolizmo reakcijas, skatinančias biologiškai aktyvių junginių sintezę ir kaupimąsi augaluose bei vaisiuose.

Tyrimų tikslas – ištirti ir įvertinti biodinaminių preparatų poveikį dirvos agrocheminei sudėčiai, didžiųjų moliūgų vaisių kokybei.

Tyrimų uždaviniai:

1. Ištirti ir įvertinti biodinaminių preparatų poveikį dirvos agrocheminėms savybėms ir fermentų aktyvumui.
2. Nustatyti biodinaminių preparatų poveikį moliūgų augalų vystymuisi.
3. Palyginti įvairių veislių didžiųjų moliūgų vaisių morfologinių dalių – žievės, minkštimo ir sėklų – kokybę.
4. Nustatyti biodinaminių preparatų poveikį biologiškai aktyvių junginių kiekiui didžiųjų moliūgų vaisiuose.

Disertacinio darbo ginamieji teiginiai:

1. Biodinaminiai preparatai turi teigiamos įtakos dirvos fermentų aktyvumui.
2. Biodinaminiai preparatai teigiamai veikia moliūgų augalų vystymąsi bei fiziologinius efektus.
3. Biodinaminiai preparatai skatina biologiškai aktyvių medžiagų kaupimąsi didžiųjų moliūgų vaisiuose.

Mokslinio darbo naujumas. Pirmą kartą nustatytas ir įvertintas biodinaminių preparatų poveikis moliūgų auginimo agrotechnikoje, moliūgų augalų vystymuisi ir jų vaisių kokybei (biologiškai aktyvių medžiagų kiekiui).

Praktinė darbo vertė. Mūsų tyrimų rezultatais įrodyta, kad panaudojus biodinaminius preparatus pagerėja dirvos sudėtis, joje padidėja fermentų aktyvumas, pagerėja moliūgų vaisių maistinė vertė. Sudaromos prielaidos taikyti pažangesnes auginimo technologijas Lietuvoje, skatinti visuomenės inovatyvų požiūrį į darnų žmogaus ir gamtos santykį.

IŠVADOS

1. Per visą didžiųjų moliūgų vegetacijos laikotarpį dirvoje purkštoje preparatu 500 fosforo, kalio ir azoto kiekiai bei ureazės ir sacharazės fermentų aktyvumas buvo esmingai didesni lyginant su nepurkšta dirva (1 UŽDAVINYS).
2. Moliūgų vegetacijos metu šaknų ir lapų biometrinių rodiklių, chlorofilo indekso vertės dydžiams bei grynajam fotosintezės produktyvumui didžiausią esminę įtaką turėjo abiejų preparatų (500 ir 501) taikymas (2 UŽDAVINYS).
3. Esmingai didžiausi sausųjų medžiagų, vandenyje tirpių angliavandenių kiekiai nustatyti 'Justynka' veislės moliūgų vaisių žievėje ir minkštime. Tirtų moliūgų vaisių žievėje ir minkštime sausųjų medžiagų, vandenyje tirpių angliavandenių, žalios ląstelienos kaupimuisi esminę įtaką turėjo abiejų preparatų (500 ir 501), žalių pelenų kiekiui – preparato 501 taikymas agrotechnikoje (3 UŽDAVINYS).
4. Pagal juslinės analizės rezultatus, 'Justynka' veislės minkštimo buvo geriausia išvaizda, tekstūra, skonis ir kvapas. Visų tirtų veislių moliūgų minkštimo juslinėms savybėms teigiamos įtakos turėjo augalų purškimas preparatu 501 (3 UŽDAVINYS).
5. Didžiausias vitamino C, leukoantocianų ir bendras fenolių kiekis nustatytas 'Amazonka' veislės moliūgų minkštime. Tirtų moliūgų vaisių žievėje ir minkštime katechinų kiekiai buvo panašūs. Antioksidacinių junginių (vitamino C, leukoantocianų, katechinų ir bendram fenolių kiekiui) kaupimuisi didžiųjų moliūgų žievėje ir minkštime didžiausios esminės įtakos turėjo augalų purškimas preparatu 501 (4 UŽDAVINYS).
6. 'Karowita' vaisių minkštimas pasižymėjo esmingai didžiausiu bendru karotenoidų, liuteino+zeaksantino ir β-karoteno kiekiu. Šių junginių kaupimuisi moliūgų minkštime esminę įtaką turėjo abiejų preparatų (500 ir 501), sėklose – preparato 501 taikymas auginimo agrotechnikoje (4 UŽDAVINYS).

TRUMPOS ŽINIOS APIE DISERTANTĘ

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Edita Juknevičienė was born on January 2, 1987 in Jurbarkas. In 2005 she finished Vytautas Didysis secondary school and entered Lithuanian University of Agriculture's Faculty of Forestry and Ecology. In 2009 she completed her studies with the B.A. qualifications in Ecology and Environmental Science. In 2009–2011 she continued her studies at the same university and graduated with the M.A. degree in Ecology and Environmental Science. Since 2011 she has been doing her PhD studies at Aleksandras Stulginskis University's Institute of Agricultural and Food Science.

BIODINAMINIŲ PREPARATŲ ĮTAKA DIRVOS SAVYBĖMS, DIDŽIŲJŲ MOLIŪGŲ VAISIŲ DERLIUI IR KOKYBEI

Edita Juknevičienė

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