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SPIS TREŚCI

VARIA

Articles published in this issue were presented during Scientific Conference *Horticulture of Tomorrow – Challenges and Risks*, that took place on 10–11 September 2009 in Krakow. The conference was organized by the Polish Society for Horticultural Science and the Faculty of Horticulture of Agricultural University in Krakow and co-financed by the Ministry of Science and High Education. All articles have been reviewed.

220 participants represented Faculties of Horticulture of three Polish universities: Wroclaw University of Environmental and Life Sciences, Poznan University of Life Sciences and University of Life Sciences in Lublin, and also from Warsaw University of Life Sciences – SGGW, University of Technology and Life Sciences in Bydgoszcz, West Pomeranian University of Technology in Szczecin, University of Warmia and Mazury in Olsztyn, University of Podlasie, University of Rzeszow, Public Technical Higher School in Sandomierz, School of Engineering and Economic in Ropczyce, Agricultural University of Plovdiv (Bulgaria) and Agricultural University in Krakow. Also scientists from some institutes such as Research Institute of Vegetable Crops, Research Institute of Pomology and Floriculture in Skierniewice, Institute of Natural Fibres and Medicinal Plants and Institute of Biopolymers and Chemical Fibres in Lodz took part in the conference.

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Uczestnicy w liczbie 220 reprezentowali 3 Wydziały Ogrodnicze Uniwersytetów Przyrodniczych we Wrocławiu, Lublinie i Poznaniu oraz SGGW w Warszawie, Uniwersytet Technologiczno-Przyrodniczy w Bydgoszczy, Zachodniopomorski Uniwersytet Technologiczny w Szczecinie, Uniwersytet Warmińsko-Mazurski w Olsztynie, Akademię Podlaską w Siedlcach, Uniwersytet Rzeszowski, Państwową Wyższą Szkołę Zawodową w Sandomierzu, Wyższą Szkołę Inżynieryjno-Ekonomiczną w Ropczycach, Uniwersytet Rolniczy w Płowdiw (Bułgaria) oraz Uniwersytet Rolniczy w Krakowie. W Konferencji wzięli udział także pracownicy naukowi instytutów branżowych IW i ISK w Skierniewicach, Instytutu Włókien Naturalnych i Roślin Zielarskich w Pozna-

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CHARACTERISATION OF CARROTS OF VARIOUS ROOT COLOUR

OCENA ODMIAN MARCHWI O RÓŻNEJ BARWIE KORZENI

Abstract: Carrot (*Daucus carota* L.) is one of the main vegetables grown world-wide with orange roots occurring in Europe and purple, red, yellow and white in other world regions. Eight accessions were characterized with regard to their morphological traits, root yield and root chemical composition.

The most significant differences were observed in root colour, and colour homogeneity in flesh and core. The accessions differed in the proportion of marketable yield in total yield due to their different susceptibility to diseases, and the tendency to development of forked roots. Some populations produced bolters in the first year of cultivation, which additionally limited the number of marketable roots. Great variation was observed with regard to alpha- and beta-carotene as well as lutein content. Purple roots contained more lutein, and yellow roots had higher proportion of lutein to beta-carotene than roots of orange cultivars. Purple roots of carrot were particularly rich in phenolic compounds including anthocyanins, which corresponded with high antioxidant activity of root tissue extracts. The obtained results show valuable traits of coloured carrots, which are novel to European market, but also indicate on the need for the improvement of several characteristics of this crop before commercialization.

Keywords: anthocyanin, antioxidant activity, carotene, *Daucus carota* L., morphology, root yield

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Production of carrot (*Daucus carota* L. ssp. *sativus* Hoffm. Arc.) has tripled during recent 30 years and reached about 24 million tons ($24 \cdot 10^{12}$ g), with Europe covering about one quarter of world production [1]. Modern cultivars must now conform not only to high yield but also to advanced standard of root quality and nutritional value. The later is particularly important as carrots consumed raw or processed all-year-round is an important source of bioactive compounds, especially beta-carotene, a vitamin A precursor [2]. Roots of European cultivars are orange in colour, but this type of carrot emerged relatively recently around the 17th century. First edible carrots, probably developed in Afghanistan around 10th century, had yellow and purple roots, and they are still grown in Asia nowadays. Also white carrots are known in various world regions [3]. Root colour results from pigment composition, mainly carotenoids ie, orange beta-carotene and alpha-carotene, and yellow lutein, purple colour is determined by anthocyanins [4–6]. All these compounds are essential phytonutraceuticals of antioxidant activity and are important in prevention of cancer, cardiovascular diseases and age related dysfunctions [7, 8]. The composition and content of these compounds in carrot root is genetically determined by several main and accessory genes. Additionally, environmental factors influence their level [3, 9]. Few reports describing non-orange carrots are available till now indicating that yellow and purple carrots can be richer in bioactive compounds than orange roots [5, 10]. Utilization of these carrot types for production in temperate climate of Central Europe may be, however, not possible without further adaptation by breeding. They evolved in warmer climate or at the regions with longer day. Modified light conditions strongly affect plant reproductive biology, and as a result, Asian carrots grown in European conditions tend to flower in the first vegetation year, which disqualifies such plants for root production. Therefore, it is important to evaluate carrots of various root colours both for their nutritional value and suitability for commercial production.

Material and methods

Characterization of eight carrot (*Daucus carota* L.) accessions comprising commercial cultivars, and a breeding line was carried out in a field trial set up in an experimental station near Krakow (Poland). Seeds were sown (80 seeds $\cdot$ m^{-1}) in flat beds with two rows, each of 3 m long. Plots were arranged in a randomized block design with four replications. During vegetation the accessions were evaluated and characterized according to the list of IPGRI descriptors [11]. After harvest at 128 vegetation day, the same guideline was used for root description complementing yield assessment. Each harvested root was also classified into a marketable, small, forked, split or diseased fraction. Analyses of phenolic compounds and antioxidant activity were performed using 15 healthy, and untouched marketable roots from each field replication. Washed and homogenized root tissue samples were frozen and kept for analyses. Radical scavenging activity (RSA) was assessed by the reaction of 80 % MeOH root extract with DPPH (1,1-diphenyl-2-picrylhydrazyl) and measuring the absorbance after 30 min incubation [12]. The amount of total phenolics and anthocyanins was determined by measuring UV/VIS spectrum according to the method

described by Fukumoto [13]. For carotenoid analysis, 10 roots were cut into cubes, lyophilized and milled. Acetonitrile/*n*-butanol extracts obtained using automated sample extractor were applied to Develosil RP-aqueous C30 (150 × 3 mm; 3 μm) column and analysed by HPLC-DAD (*diode array detector*). Alpha-carotene and lutein were monitored at λ = 488 nm and beta-carotene at λ = 455 nm. The data were subjected to one-way analysis of variance and the means were separated using a multiple range Duncan test at $p = 0.05$.

Results and discussion

Carrot cultivars of orange roots are common vegetable crop grown in Europe. We have compared two orange carrots, an old 'Nantes Fancy' and new developed F1 hybrid 'Nerac' with accessions of white, yellow and purple roots originating from various world regions. The most distinguished characters of these accessions are provided in Table 1. White, yellow and orange carrots were mostly homogeneous in colour both at skin along the root length and on a transverse section. Particularly, white and yellow roots had no colour difference between core and flesh, which is often observed in orange cultivars and considered as an undesirable trait. A great variation in colour was observed for 'Syrian Purple', which had purple flesh, but the core was not completely coloured and had white or yellow areas of various size. White carrots had very smooth skin surface although many lateral roots developed on storage roots of 'Blanche 1/2 longue des vosges'. For this trait, orange and yellow accessions were much more advanced as they showed very low number of lateral roots. Distinct morphological features of canopy, not observed in European carrots, were characteristic for 'Syrian Purple'. The leaves had unusual grey-green colour and were densely covered with long hairs on both sides of the lamina, similarly as the petioles. Another purple cultivar 'Anthonina' had strong anthocyanin pigmentation along the petioles.

A great variation was observed in root yield. Most accessions yielded in the range determined by two common orange cultivars (Table 1) ie 42–75 kg · 10 m^{-1}. Yield of yellow and white roots was superior to purple ones. Except modern orange hybrid 'Nerac' and old white 'Blanche 1/2 longue des vosges' a marketable yield was reduced by over 15 %. This was caused by a great share of roots classified as unmarketable, mainly forked. In particular, roots of 'Syrian Purple' were highly affected by bacterial diseases causing rotting in field conditions while yellow 'Gelbe Rhenische' developed large roots, which easily split probably due to prolonged vegetation period. Additionally, emergence of shoots with inflorescences limited the number of plants suitable for root production. A remarkable number of bolters in the first year of cultivation was observed in 'Syrian Purple' (over 3 %), which is not adopted to long day conditions while other accessions flowered with a frequency below 1 %. None of the orange cultivars developed shoots with inflorescences.

The biochemical parameters varied depending on the accession evaluated (Table 2).

Orange cultivars contained 26–38 mg phenolic compounds in 100 g root tissue and very low amounts of anthocyanins (below 1 mg · 100 g^{-1} f.m.). Similar level was also found in white and yellow cultivars, which did not differ significantly to each other with

Table 1

Root yield and morphological characters of evaluated carrot accessions

Accession Origin/propagator	Type	Skin colour	Flesh colour	Core colour	Root surface	Lateral roots	Yield [kg · 10 m^{-2}]	Yield structure [%]			
								Marketable roots	Split roots	Diseased roots	Bolters
Blanche 1/2 longue des vosges INH / JKI	old French cultivar	white	white	white	smooth	medium	68.74 ± 2.3 cd	92.5	0.5	0	0.14
Kuettiger HRIGRU / JKI	old Swiss local cultivar	white	white	white	smooth/light dimpled	medium	55.83 ± 3.7 bc	85.8	1.0	1.7	0.2
Gelbe Rheinische HRIGRU / JKI	old German cultivar	yellow	yellow	yellow	light dimpled	low	64.43 ± 3.5 cd	82.1	2.4	2.1	0.0
Line 710015 Seminis	modern breeding line	yellow	yellow	yellow	smooth/light dimpled	low	119.34 ± 8.7 e	83.6	1.3	0.2	0.2
Anthonina Seminis	modern cultivar	purple	purple	purple/yellow purple/white purple	medium/strong ringed	medium	58 ± 2.3 c	87.2	0.5	0.4	0.8
Syrian Purple HRIGRU / JKI	Syrian landrace	purple	purple	white/yellow purple/white purple	strong ridged	medium	39.8 ± 2.6 a	82.1	0.0	8.4	3.1
Nantes Fancy NGB / JKI	old cultivar	orange	orange	orange	light/medium dimpled	low	42.34 ± 3.2 ab	83.3	0.0	1.1	0.0
Nerac Bejo Zaden	modern F1 hybrid	orange	orange	orange	light dimpled	low	75.1 ± 3.5 d	94.1	0.8	0.0	0.0

Explanation: HRIGRU – Horticulture Research International Genetic Resources Unit, Warwick, UK; INH Institut National d'Horticulture, Angers, FR; JKI – Julius Kuehn Institute, Quedlinburg, DE; NGB – Nordgen Plants, Alnarp, SE; Means followed by the same letter in a column do not differ significantly at p = 0.05.

regard to those components. In contrast, both purple carrots possessed elevated amounts of anthocyanins additionally accompanied by other phenolics, which in total reached the level of 130 and 290 mg · 100 g^{-1} f.m. in 'Syrian Purple' and 'Anthonina', respectively. The presence of phenolics and anthocyanins highly correlated with the root extract ability to neutralize free radicals (0.99 and 0.97, respectively), so both purple cultivars showed very high antioxidant activity. On the other hand, roots of other colours did not show any antioxidant activity or exhibited a tendency for a prooxidant activity like the line 710015. The presence of phenolic compounds in plant tissue may increase nutritional value of fruits and vegetables because of their antioxidant properties [14]. Particularly flavonoids, like anthocyanins, are considered as valuable in protection against reactive oxygen species [15].

Table 2

Chemical composition and radical scavenging activity (RSA) of carrot root tissue [mg · 100 g^{-1} fresh mass]

Accession	Lutein	Alpha--carotene	Beta--carotene	Total phenolics	Antho-cyanins	RSA [%]
Blanche 1/2 longue des vosges	0.02 ± 0.01* a	0.1 ± 0.01 a	1.5 ± 0.1 a	31.0 ± 0.8 a	0.4 ± 0.1a	–0.5 ± 0.3 b
Kuettiger	0.04 ± 0.02 a	0.1 ± 0.02 a	2.1 ± 0.1 a	28.2 ± 1.4 a	0.1 ± 0.03 a	–2.1 ± 0.2 ab
Gelbe Rheinische	0.9 ± 0.03 b	0.2 ± 0.10 ab	6.2 ± 1.6 ab	27.7 ± 0.8 a	0.4 ± 0.02 a	0.6 ± 0.2 b
Line 710015	0.7 ± 0.06 b	0.1 ± 0.02 a	4.3 ± 0.5 ab	22.2 ± 1.0 a	0.1 ± 0.1a	–7.0 ± 0.4 a
Anthonina	2.5 ± 0.27 d	1.6 ± 0.40 c	23.6 ± 6.2 cd	290.2 ± 18.3 c	65.6 ± 7.4 c	61.5 ± 2.7 d
Syrian Purple	1.7 ± 0.25 c	0.1 ± 0.02 bc	16.2 ± 2.9 bc	129.6 ± 7.0 b	14.2 ± 1.2 b	24.3 ± 3.0 c
Nantes Fancy	1.0 ± 0.05 b	3.5 ± 0.30 e	48.7 ± 6.9 e	38.1 ± 2.8 a	0.4 ± 0.04a	–2.8 ± 0.6 ab
Nerac	0.8 ± 0.04 b	2.4 ± 0.10 d	30.0 ± 1.3 d	25.6 ± 1.2 a	0.3 ± 0.1a	–1.8 ± 0.1 ab

* Mean values with standard errors; Means followed by the same letter in a column do not differ significantly at $p = 0.05$.

Carotenoids were detected in all roots (Table 2). Orange ones contained these compounds in the amounts of 32–52 mg · 100 g^{-1} f.m. with xanthophylls (alpha--carotene and lutein) contributed about 10 % of beta-carotene level, and with lutein to alpha-carotene ratio of 1 to 3. Purple and yellow roots had two- and six-fold lower amounts of carotenoids than orange roots, respectively. However they contained two-fold higher proportion of both xanthophylls to beta-carotene. Particularly rich in lutein were purple carrots possessing 1.7 and 2.5 mg · 100 g^{-1} f.m. Thus in contrast to common edible carrots considered rather as a poor source of lutein, purple roots may provide a substantial portion of a recommended allowance, which is proposed to be 6 mg per day [16]. White roots were almost free of carotenoids as reported previously [17]. Although carotenoids are known as strong antioxidants, there was no correlation found between the content of these compounds and RSA. This discrepancy results from the analytical method used. The DPPH assay is convenient for the assessment of antioxidant activity of phenolics but not carotenoids, which spectra interfere with DPPH [18].

Vegetable consumption is recommended to limit numerous civilisation diseases initiated by reactive oxygen species (ROS), which are generated either during the normal cell function or result from environmental pollution [19]. Carrots possess carotenoids and phenolic compounds functioning as ROS antagonists. Our study shows that purple coloured roots are particularly rich in anthocyanins and other phenolics as well as in lutein, the macular pigment of human retina, which deficiency leads to age-related macular degeneration [20]. Also yellow coloured roots may contain a high proportion of lutein. These properties makes coloured carrots valuable source of health promoting compounds superior to orange cultivars. They may also become attractive to consumers due to their root colour, which is unusual at European market. Purple roots additionally may become a source of monoacylated anthocyanins, which possess increased stability at food pH, during heating and storage, and are advantageous for food colouring in comparison with anthocyanins produced from red grape or berries [21]. However utilization of such types for commercial production requires the development of advanced, highly uniform populations with high marketable yield. Our field trials indicate that the accessions grown in other world regions are not suitable for direct implementation into commercial production and require improvement through breeding programs. Among the most crucial traits to be enhanced are resistance to diseases and root homogeneity as well as reducing the number of forked roots and bolting tendency.

Conclusions

Carrot accessions of various root colour and origin were grown in temperate climate of Poland and compared with regard to their morphological traits, yield and chemical composition. The results indicate that accessions with other than orange root colour may: 1) possess valuable compounds ie, yellow and purple roots contain high proportion of lutein in total carotenoids, and purple roots have high content of antioxidants, including anthocyanins, 2) exhibit undesirable characters like tendency for bolting, high share of forked roots and susceptibility to diseases, which make them difficult for direct commercialization.

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CHARAKTERYSTYKA ODMIAN MARCHWI O RÓŻNEJ BARWIE KORZENI

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Abstrakt: Marchew jadalna (*Daucus carota* L.) jest jednym z głównych warzyw uprawianych na świecie. W Europie znana jest głównie marchew o korzeniach pomarańczowych, natomiast w innych rejonach świata również o korzeniach fioletowych, czerwonych, żółtych i białych. W niniejszej pracy scharakteryzowano osiem odmian i linii pod względem różnych cech morfologicznych, plonu oraz składu chemicznego korzeni.

Największe zróżnicowanie zaobserwowano ze względu na barwę oraz stopień ujednolicenia barwy miąższu i rdzenia. Badane obiekty różniły się udziałem korzeni handlowych w plonie całkowitym, co wynikało z ich zróżnicowanej podatności na choroby oraz tendencji do wytwarzania korzeni rozwidlonych. Niektóre populacje wytwarzały kwiatostany w pierwszym roku uprawy, co dodatkowo obniżało plon korzeni handlowych. Zaobserwowano duże zróżnicowanie pod względem zawartości alfa- i beta-karotenu oraz luteiny. Marchew o korzeniach fioletowych zawierała więcej luteiny natomiast żółte miały więcej luteiny w stosunku do beta-karotenu w porównaniu z marchwią pomarańczową. Marchew o korzeniach fioletowych zawierał więcej związków fenolowych, w tym antocyjany, co korespondowało ze zwiększoną aktywnością antyoksydacyjną tkanki korzeniowej. Uzyskane wyniki potwierdzają korzystne cechy marchwi kolorowych, będących nowością na rynkach europejskich, jednocześnie wskazując na potrzebę poprawy niektórych cech przed wprowadzeniem ich do uprawy na skalę komercyjną.

Słowa kluczowe: aktywność antyoksydacyjna, antocyjany, *Daucus*, karoten, morfologia, plon korzeni

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EFFECT OF THE AGE OF PLANTATION AND HARVEST TERM ON CHEMICAL COMPOSITION AND ANTIOXIDANT AVCTIVITY OF STINGING NETTLE (*Urtica dioica* L.)

WPŁYW WIEKU PLANATCJI I TERMINU ZBIORU NA SKŁAD CHEMICZNY I AKTYWNOŚĆ ANTYOKSYDACYJNĄ POKRZYWY ZWYCZAJNEJ (*Urtica dioica* L.)

Abstract: In 2004–2006 field experiment aimed at the assessment of the effect of plantation age and harvest term on chemical composition and antioxidant activity of stinging nettle.

The research showed that the highest content of pigments (chlorophyll *a*+*b* and carotenoids) and calcium were characterized plants grown on 1-year-old plantation, whereas the highest amount of magnesium and potassium contained the oldest plants. The most considerable antioxidant activity featured stinging nettle harvested in May, while the following harvests brought about the decrease in this property. Antioxidant activity depends on polyphenols content.

Keywords: stinging nettle, antioxidant activity, chemical composition

Stinging nettle constitutes a valuable source of numerous biologically active substances: vitamins (A, B, C, K), macro- and microelements (P, Mg, Ca, K, Fe, Se), tannins, polyphenols, silicic acid and volatile oil [1, 2]. The mentioned properties provide different ways of its utilization – as medicinal or fibrous plant, leafy vegetable used in soups and omelets, as well as fodder for animals [3, 4].

Medicinal properties of stinging nettle have been known and applied for a long time, among others, in prophylaxis and treatment of lung diseases, rheumatism or cirrhosis of liver [5]. It is also used as mild duretic, antimycoric and bactericide agent. Main application of stinging nettle, however, is connected with obtaining chlorophyll, a raw

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material in pharmaceutical and food industry, as well as cosmetics production [6]. A number of substances stinging nettle contains characterize antiradical and antioxidant activity. The latter one prevents uncontrolled oxidation reaction, inhibit oxidation processes which take place in the cells and normalize redox potential, thus protecting human organism from such civilization diseases as artheroma, hypertension or neoplasms [7, 8]. The presence of chemical components in plants is not permanent and it depends on such factors as environmental conditions, phase of plant growth, plant form and organ, harvest term, age of plantation, as well as conditions of raw material storage [9].

The purpose of the investigation conducted in the years 2004–2006 was the assessment of the effect of plantation age and harvest term on yielding, chemical composition and antioxidant activity of stinging nettle.

Material and methods

Field experiment was carried out in Horticultural Research Station in Piastow, on sandy clay soil of pH = 7.8, containing 1.8 % humus, 138 mg P, 96 mg Mg, 220 mg K and 1538 mg Ca in 1 dm^3. Stinging nettle seedlings were produced in multicells filled with the mixture of peat substrate and loamy soil. In the last week of March 3–4 seeds were sown into each cell of 76.5 cm^3 volume. After germination seedlings were thinned, leaving one best developed plant in each pot. 7-week-old ready seedlings were planted on the field in the second decade of May, in spacing 50 × 25 cm. In the subsequent years experimental plots were fertilized with nitrogen in the amount of 200 kg N · ha^{-1}, half of which was applied before planting, while in further years of cultivation fertilization took place before the beginning of plant growing period and the remaining part – after the first harvest of herbaceous plant. The experiment was established according to randomized pattern of split-plot method in three replications and the area of one plot for harvesting equaled 1 m^2. Herb harvest took place at the beginning of blossom stage, twice in the first year (in the middle of July and September) and three times in the subsequent years (in the half of May, July and September) using electric knife-mower for hedges. Samples of leaves were collected from every harvest. There was estimated level of dry matter, chlorophyll, caroteniods, polyphenols, nitrates, total N, macroelements and proteins in nettle leaves as well as antioxidant activity of raw products. Fresh leaves were blended with BOSCH blender and extracted with 100 cm^3 of methanol (80 %). The content of total phenolic compounds was assayed using Folin-Ciocalteu method [10], chlorophyll *a*+*b* and total carotenoids were estimated using spectrophotometer, due to the method by Ruminska et al [11].

Dry matter was estimated by drying to constant mass at 105 ^oC. There was also assayed nitrates content (using potentiometry method), total N by Kjeldahl method and protein content was calculated with the use of 6.25 coefficient, as well as macro-elements P, K, Mg and Ca following standard method [12]. W 2005 and 2006 antioxidant activity was assessed by DPPH [13], ABTS [14] and FRAP [15] tests in leaves samples collected from all treatments.

Results and discussion

The content of dry matter in stinging nettle leaves was to a low degree dependent on plantation age and it ranged average from 27.93 to 28.15 % (Table 1).

Table 1

The effect of plant age and harvest term on chemical composition of stinging nettle

Age of plant and harvest term		Dry matter [%]	Chlorophyll *a*+*b*	Carotenoids	Polyphenols
			[mg · g^{-1} d.m.]		
1-year-old	July	30.20	9.57	1.37	13.20
	September	26.09	12.86	1.61	8.13
Mean		28.15	11.22	1.49	10.67
2-year-old	May	28.79	8.39	1.32	20.76
	July	29.30	8.63	1.07	15.92
	September	25.71	8.57	1.00	8.96
Mean		27.93	8.23	1.13	15.21
3-year-old	May	32.40	7.38	1.12	19.06
	July	26.69	10.80	1.61	15.19
	September	25.03	12.90	1.47	9.11
Mean		28.13	10.36	1.40	14.45
Mean	May	30.59	7.88	1.22	19.91
	July	28.82	9.67	1.35	14.77
	September	25.61	11.44	1.36	8.73
Mean		28.34	9.66	1.31	14.47
LSD α = 0.05 for:					
age of plant		ns	0.89	0.36	1.56
term of harvest		ns	1.12	0.12	1.15

Higher diversity of this parameter was observed in I particular cuts. The leaves of stinging nettle harvested in September contained the lowest amount of dry matter, while the highest quantity of dry matter was obtained from May harvest. Chlorophyll *a*+*b* and carotenoids content was related to both plantation age and herb harvest term. Higher content of pigments was recorded in younger plants (one-year-old). In the second and third year of cultivation their level was relatively even. Higher content of carotenoids was reported in the first and third year of cultivation, while in two-year-old plants their content was considerably lower. Concentration of chlorophyll *a*+*b* increased in leaves in subsequent cuts, similar results was observed in carotenoids content of 1- and 3-year-old plantation whereas in 2-year-old nettle highest level of carotenoids was noticed in first cut. Weglarz and Karaczun [16] did not observe any correlation between herb harvest term and the content of carotenoids and chlorophyll in stinging nettle leaves. In the research presented by these authors, however the content of these pigments did decrease according to the age of plants (older plants from 3-year-old and 4-year-old plantations).

Polyphenols content in stinging nettle leaves from 1-, 2- and 3-year-old plantation was similar and it ranged from 14.45 to 15.19 mg · g^{-1} d.m. Regardless the age of plantation, polyphenols content decreased in the subsequent cuts and plants harvested in

May contained average 20.84 mg · g^{-1} d.m. of polyhenols, while those of September harvest – 8.73 mg · g^{-1} d.m. Węglarz and Karaczun [16] examined correlation between plantation age and harvest term regarding the content of flavonoids. They reported no effect of plantation age on flavonoids content, as well as diminishing content of flavonoids in raw material coming from the subsequent cuts.

The level of total nitrogen and total protein in stinging nettle leaves gradually increased in the subsequent years, according to plant age (Table 2). On average, their highest value was recorded in 3-year-old plants and it ranged from 3.86 to 4.11 % d.m. for total N and from 24.12 to 25.68 % for total protein. Stinging nettle is a plant of slight tendency to nitrates accumulation since their average content in leaves, regardless plantation age and harvest term, did not exceed average 400 mg.

Table 2

The effect of plant age and harvest term on total nitrogen, protein and nitrates content of stinging nettle

Plant age at harvest	Nitrogen – total			Protein			Nitrates		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
	[% d.m.]						[mg · kg^{-1} f.m.]		
1-year-old		3.10	3.48		19.37	21.75		158	288
2-year-old	2.77	2.88	3.43	17.31	18.00	21.43	133	121	125
3-year-old	3.86	3.92	4.11	24.12	24.50	25.68	175	375	123
Mean	3.32	3.30	3.67	20.72	21.25	23.56	154	218	178.66
LSD α = 0.05 for:									
age of plant		0.22			1.16			12	
term of cut		0.11			1.07			22	

Our own investigation proved that stinging nettle is a valuable source of macroelements. In the conditions of cultivation on the soil rich in P, K, Ca and Mg their content in dry matter of raw material was as 0.4 % P, 1.77 % K, 3.48 % Ca and 0.34 % Mg.

The content of phosphorus in 1-, 2- and 3-year-old stinging nettle leaves was similar, while the amount of magnesium and potassium increased and Ca content decreased, as plants were getting older. There was not observed any apparently directed effect of herb harvest term on P and K content in stinging nettle leaves and Mg amount in the leaves originating from the subsequent cuts increased, while the one referring to Ca decreased. Weglarz and Karaczun [16] also recorded the decrease in Ca content in the leaves coming from older, 3- and 4-year-old plantation. Our own investigation proved Ca value in the leaves of stinging nettle, cultivated on the soil of pH = 7.8, was relatively high and amounted 4.96–5.79 % in 1-year-old plants, 3.51–3.88 % in 2-year-old and 1.34–2.33 % in 3-year-old plants (Table 3). In the experiment conducted by Weglarz and Karaczun [16] this value gradually decreased according to plantation age from 2.06 % Ca in 1-year-old plants to 1.87 % Ca in 4-year-olds, while in the work by Szewczuk and Mazur [17], who cultivated stinging nettle on acid soil of pH = 5.6, it ranged average merely 0.5.

Table 3

The effect plant age and harvest term on macroelements content in leaves of stinging nettle

Age of plant	P			K			Ca			Mg		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
	[% d.m.]											
1-year-old		0.44	0.41		1.39	1.58		5.79	4.96		0.28	0.31
2-year-old	0.43	0.34	0.31	1.89	1.85	1.00	3.88	3.75	3.51	0.22	0.28	0.29
3-year-old	0.37	0.33	0.44	2.02	2.33	2.45	2.33	2.16	1.34	0.39	0.36	0.59
Mean	0.40	0.37	0.39	1.96	1.86	1.68	3.11	3.90	3.27	0.31	0.31	0.40

Antioxidant activity measured by FRAP and DPPH test reached the highest values in stinging nettle harvested in the earliest period, in May and it decreased with the subsequent cuts. In 1-year-old plants this parameter achieved the highest value at the first cut performed in the half of June. Yet no effect was recorded, as far as the age of plantation was taken into account, on antioxidant activity of raw material analyzed. Jamroz et al [7], determining antioxidant properties of hop cultivars, proved strict positive correlation between polyphenols content and antioxidative activity. Similarly, in authors own investigation it was possible to confirm high antioxidant activity of stinging nettle at considerably elevated level of phenolic compounds. These results were also in agreement with the data obtained by Katsube et al [18] in research involving berry fruits. Antioxidant activity, measured according to FRAP – quick and easy to use test, ranged from 45.9 to 130 $\mu M \cdot 100\ g^{-1}$ (Table 4).

Table 4

Effect of plant age and harvesting term on antioxidant activity of stinging nettle

Plant age and harvest term		DPPH	FRAP	ABTS
		[μM Trolox $\cdot$ g^{-1} d.m.]		
1-year-old	July	0.6	84.6	17.3
	September	0.4	45.9	34.0
Mean		0.5	65.3	25.6
2-year-old	May	1.2	129.6	23.7
	July	0.8	102.1	20.8
	September	0.5	50.0	37.9
Mean		0.8	93.9	27.5
3-year-old	May	1.2	126.5	22.5
	July	0.6	93.9	20.6
	September	0.4	51.9	38.6
Mean		0.7	90.8	27.2
Mean	May	1.2	128.0	23.1
	July	0.7	93.5	19.6
	September	0.4	49.3	36.8
Mean		0.8	90.3	26.5

Assuming classification introduced by Wojdylo et al [18] for the assessment of 32 herbaceous plants, the values obtained place this species in the group of plants featuring good and high antioxidant activity. The research by Gulcin et al [5], conducted *in vitro*, also proved that water extract of stinging nettle is a significant source of antioxidants and that this raw material can be utilized by pharmaceutical industry as diet supplement. According to the authors quoted above main source of antioxidants are polyphenols [5].

Conclusions

1. Chlorophyll content in stinging nettle leaves decreased with age of plantation, while polyphenols content showed constant level in the course of the whole cultivation period.

2. Higher amounts of polyphenols were accumulated in stinging nettle leaves harvested in May and July, while chlorophyll and carotenoids content reached the highest values in raw material harvested from September cut.

3. There was not found any directed dependence between harvest term and the content of phosphorus and potassium in stinging nettle leaves, while the amount of magnesium gradually decreased in raw material from the subsequent cuts.

4. The content of total nitrogen and total protein in this herb leaves increased according to both plantation age and in the subsequent cuts.

5. The highest antioxidant activity featured stinging nettle harvested in the earliest term, in May and June, while the lowest one was recorded in raw material from September harvest.

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WPŁYW WIEKU PLANATCJI I TERMINU ZBIORU NA SKŁAD CHEMICZNY I AKTYWNOŚĆ ANTYOKSYDACYJNĄ POKRZYWY ZWYCZAJNEJ (*Urtica dioica* L.)

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Abstrakt: W badaniach polowych przeprowadzonych w latach 2004–2006 oceniano wpływ wieku plantacji i terminu zbioru ziela na skład chemiczny i aktywność antyoksydacyjną pokrzywy zwyczajnej.

Największą zawartość barwników (chlorofilu *a*+*b* i karotenoidów) oraz wapnia stwierdzono w roślinach jednorocznych, natomiast najwięcej magnezu i potasu zawierały rośliny najstarsze. Największą aktywność antyoksydacyjną wykazywał surowiec pokrzywy zbieranej w maju, natomiast zmniejszała się ona w zielu z następnych pokosów. Stwierdzono, że aktywność antyoksydacyjna surowca zależy od zawartości w nim polifenoli.

Słowa kluczowe: pokrzywa zwyczajna, aktywność antyoksydacyjna, skład chemiczny

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INFLUENCE OF SOILLESS CULTURES ON SOIL ENVIRONMENT

WPŁYW UPRAW BEZGLEBOWYCH NA ŚRODOWISKO GLEBOWE

Abstract: Soilless culture is used for the growing of vegetables and ornamental plants. In this technology plants in inert medium are frequently cultivated. However, in order to stabilize the concentration and the pH value of the solution in the root zone and in order to adjust the substrate moisture, the volume of nutrient solution must be higher than the nutritional requirements of plants. In result, there are significant leakages of nutrient solution to the soil. The aim of the presented research was the investigation of chemical properties of soils in the greenhouses where soilless culture technology is used for ornamental plant cultivation.

In comparison with control soils, the 1:2 soil water extract from greenhouse soils showed a higher electrical conductivity. In result of nutrient solution leakages, in the soil increased the concentration of almost all nutrients and particularly of potassium, nitrates, magnesium, while the content of phosphorus, sulphates and of microelements decreased in a lesser degree. The highest threat results from the easy translocation of NO_3-N. The degradation rate of soil environment depended primarily on the length of greenhouse utilization.

Keywords: greenhouse, soil, degradation, soilless culture, leakage

Soilless culture represents a technology for plant growing in nutrient solutions that supply all nutrient elements needed for optimum plant growth with or without the use of an inert medium or organic growing medium to provide mechanical support. Hydroponics or soilless culture offers a means of control over soil-borne diseases and pests. Thanks to that fact, one can avoid the costly and time-consuming soil replacement or sterilization. Soilless culture in commercial ornamental plant production is used for the growing of roses, gerbera, carnations and even asparagus. However, in order to stabilize the concentration and the pH value of the solution in the root zone and in order to adjust the substrate moisture, the volume of nutrient solution must be higher than the nutritional requirements of the plants. In effect, there are significant leakages of the nutrient solution to the soil. Currently, for soilless culture, 30–50 % of overflow is recommended [1].

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The aim of the present paper was to investigate the chemical properties of soils in greenhouses where soilless culture technology is utilized for ornamental plants cultivation.

Material and methods

Studies were carried out in September 2008 in three horticultural farms localized in different regions of Wielkopolska. The first one specialize in the production of carnations in rockwool (8 years), in the second farm roses are cultivated in rockwool (10 years) and in the third one, gerberas are grown in perlite (12 years). In those objects, open fertigation systems are applied, ie the drainage water is leaking from the slabs directly to the soil. Overflow is not collected and nutrient recirculation is not applied. In each of the objects, one greenhouse was selected, where soil samples were taken every 20 cm to the depth of one meter. Samples were taken in three randomly selected places. Drilling was carried out under the rockwool slabs (rose and carnation), or under the containers with perlite (gerbera), where the plants were grown. For the sake of comparison, soil samples (0–100 cm) were taken as well from sporadically fertilized lawns growing near the greenhouses. The available forms of macroelements in the soil were extracted with 0.03 mol · dm^{-3} CH_3COOH [2]. For the extraction of micro-elements, the modified Lindsey solution was used [3]. The following elements were determined: NH_4-N and NO_3-N (by distillation method in modification of Starck), P, SO_4-S (by colorimetric analysis), K, Ca, Na (by spectrophotometric analysis), Mg, Fe, Cu, Zn, Mn (by atomic absorption spectrophotometry). Electrical conductivity and pH value were measured as well. Soil texture was determined by sedimentation method. Results of the determinations are given according to FAO classification [4].

Results and discussion

Drainage waters from typical agricultural areas are the beginning of the migration tracks of nutrients. Similar process takes place with the leakages from soilless cultures. They transfer mineral components first to the upper layer and then to the lower-layer of soil. Subsequently, the nutrients penetrate the surface waters or the groundwaters. The threat is significant, because of the high fertilizer doses used for vegetable and ornamental plants fertigation grown in greenhouses. For example, from 1 ha of roses cultivated in perlite or lapillus (volcanic rock) and with the average overflow about 40 %, the effluent of 2000 m^3 contained 700 kg of nitrogen [5]. During one growing season of gerbera in rockwool, together with the drainage waters, the monthly overflow to the soil was: 10–39 kg N, 13–52 kg K, 3–13 kg Mg and 3–12.5 kg Na · ha^{-1} [6].

During plant cultivation, the chemical composition of the leakages depending on the chemical composition of the nutrient supplied to plants, the plant age, course of climatic conditions (especially of temperature), time of the day and fertigation frequency. In consequence, there follows an uncontrolled leakage of solutions to the soil, and the concentration of the majority of components in the soil increases. These changes depended only in a small degree on the soil texture (Tables 1–3), but in a greater extent,

Table 1

Influence of nutrient solution leaching during 8 years of carnation cultivation in rockwool on chemical properties of greenhouse soil in comparison to garden soil

Layer [cm]	Soil text.*	NH_4–N	NO_3–N	P	K	Ca	Mg	Na	SO_4–S	Fe	Mn	Zn	Cu	pH	EC [mS · cm^{-1}]
		[mg · dm^{-3}]													
Greenhouse															
0–20	SL	7	74	338	657	2005	422	39	37	109	58	31	17	7.0	0.57
20–40	SL	14	77	299	719	1993	376	38	76	89	15	20	9	7.0	0.56
40–60	L	18	53	295	772	1250	315	39	78	83	16	10	6	7.0	0.48
60–80	L	18	49	57	778	5519	373	30	50	31	6	3	2	7.2	0.40
80–100	L	18	39	63	655	5850	350	27	36	19	4	2	2	7.7	0.50
Lawn															
0–20	SL	28	28	47	59	2497	183	44	123	50	2	2	1	7.6	0.50
20–40	SL	< 1	< 1	20	17	2220	170	30	94	64	2	2	1	7.9	0.44
40–60	L	< 1	< 1	8	5	2387	160	24	86	87	2	1	1	8.0	0.36
60–80	L	< 1	< 1	7	5	2863	187	23	82	64	2	1	1	8.0	0.30
80–100	L	< 1	< 1	9	5	6221	176	24	75	25	2	1	1	8.1	0.26

* Soil texture according to FAO [4]: S – sand, LS – Loamy sand, SL – Sandy loam, L – loam.

Table 2

Influence of nutrient solution leaching during 10 years of roses cultivation in rockwool on chemical properties of greenhouse soil in comparison with garden soil

Layer [cm]	Soil text.*	NH_4–N	NO_3–N	P	K	Ca	Mg	Na	SO_4–S	Fe	Mn	Zn	Cu	pH	EC [mS · cm^{-1}]
		[mg · dm^{-3}]													
Greenhouse															
0–20	LS	7	399	58	1098	461	163	110	95	160	7	8	1	6.2	0.99
20–40	LS	7	245	45	711	355	123	70	103	192	8	5	1	6.3	0.68
40–60	LS	21	91	23	536	254	80	48	30	197	8	4	1	6.3	0.48
60–80	LS	7	42	7	263	223	40	36	34	180	5	1	< 1	6.4	0.20
80–100	SL	7	35	12	205	281	52	41	9	180	3	< 1	< 1	6.5	0.25
Lawn															
0–20	LS	28	14	18	24	452	72	19	2	138	5	78	3	6.6	0.09
20–40	LS	11	< 1	13	19	542	28	22	2	155	7	49	2	6.3	0.03
40–60	LS	7	< 1	10	16	363	13	16	1	181	16	21	3	6.3	0.02
60–80	S	7	< 1	7	18	324	13	15	1	177	12	18	2	6.4	0.02
80–100	S	4	< 1	4	20	263	13	15	1	180	11	13	1	6.4	0.02

* Abbreviation: see Table 1.

Table 3

Influence of nutrient solution leaching during 12 years of gerbera cultivation in perlite on chemical properties of greenhouse soil in comparison with garden soil

Layer [cm]	Soil text.*	NH_4–N	NO_3–N	P	K	Ca	Mg	Na	SO_4–S	Fe	Mn	Zn	Cu	pH	EC [mS · cm^{-1}]
		[mg · dm^{-3}]													
Greenhouse															
0–20	SL	11	77	295	524	1937	379	63	42	119	13	32	18	6.4	0.64
20–40	SL	18	56	333	341	956	227	41	98	123	27	23	17	6.4	0.56
40–60	S	11	28	327	271	761	164	34	221	123	45	18	14	6.2	0.71
60–80	S	14	25	295	297	800	166	34	326	120	32	16	12	6.3	0.68
80–100	S	7	10	352	360	937	255	42	246	120	36	20	16	6.0	1.02
Lawn															
0–20	SL	7	7	139	30	638	59	24	4	84	2	7	2	6.8	0.07
20–40	SL	14	< 1	86	27	614	75	30	15	87	6	10	2	6.7	0.07
40–60	S	7	< 1	55	26	471	55	30	7	99	6	11	2	6.7	0.07
60–80	S	7	< 1	28	16	276	24	23	4	93	4	7	1	6.8	0.05
80–100	S	7	< 1	14	10	270	25	12	4	90	4	6	1	6.2	0.04

* Abbreviation: see Table 1.

they depended on the species of the grown plants and on the length of the greenhouse exploitation (8–12 years).

In comparison with the samples from control soils, the water extract from greenhouse soils showed a higher electrical conductivity, but a slightly lower pH value. The highest increment of EC in relation to the control soil was shown by the greenhouse with roses (to the depth of 60 cm), while the lowest increment of EC value was found in the greenhouse with carnation (Tables 1–3). Probably it is an effect of nutrient solutions concentration.

Electrical conductivity of the basic nutrient solution used for plants fertigation cultivated in soilless cultures were 2.2 for rose, 1.9 for gerbera and 1.7 mS · cm^{-1} for carnation. In the soils from greenhouses with roses, because of the scale of concentration in the soil, the elements can be arranged in the following order: K > NO_3-N > Mg > SO_4-S > Na > P > Fe. At the same time, there was found a decrease in the content of Mn, Zn and Cu. In the soil from greenhouses with carnations, the elements, regarding their concentration increase formed the following sequence: K > P > Mg > NO_3-N > Fe > Mn > Zn > NH_4-N > Cu. In the soil samples from greenhouses with gerbera, the order of elements was the following one: K > Ca ≈ P > Mg > SO_4-S > N-NO_3 > Fe > Na > Mn > Zn > Cu. A particularly high concentration increase was usually found in case of potassium (up to 1098 mg · dm^{-3}), magnesium (up to 422 mg · dm^{-3}) and in the nitrate(V) form of nitrogen (up to 399 mg · dm^{-3}). High concentration of Ca in soil from the greenhouse with carnations should be explained by the naturally high content of this element in loams.

An increase of the component content in the soil is connected with the exchange--adsorption of cations and chemisorption of cations and anions. Because of a negative charge of the sorption complex of soils, anions are not exchange-adsorbed. They migrate most easily into the depth of soil. In literature, attention is called to the rather significant speed of nitrates translocation in the soil – the presence of NO_3^- ions at the depth of 90 cm was found already several weeks after the application of mineral fertilizers in nurseries and in greenhouses [7, 8]. Utilization of the total applied nitrogen fertilizer by plants is not possible, even in field conditions where smaller doses of fertilizers are applied than in greenhouses. An example of a possible pollution effect caused by nitrogen application was quoted in a fertilization experiment with avocado grown in a field [9]. Four levels of N were applied over four years. Increasing the N applied from 80 kg · ha^{-1} to 640 kg · ha^{-1} increased the NO_3-N concentration in soil from 4.2 to 427.2 mg · kg^{-1} in the 0–30 cm layer and from 0.5 to 232.0 mg · kg^{-1} in the 60–90 cm layer.

Effect of long-term fertilization on the content of macro- and microelements in the profiles of greenhouse soils was evaluated already 14 years ago. However, the research referred to the conventional methods of plant fertilization grown in greenhouse soils [10, 11]. Traditional fertilization (organic plus mineral fertilizers) caused a significant increase of elements not only in the arable layer, but also in the deeper soil layers. The range of changes in the chemical properties of soils depended more on the time length of the greenhouse exploitation (20–40 years) and on the accepted production program than on the soil texture. Results of the above-mentioned studies [10, 11] were similar to

those received in our actual studies shown in Tables 1–3. Differences refer to concentrations which in case of potassium were higher in soil samples taken from greenhouses with soilless culture. On the other hand, the concentrations of SO_4-S, Fe, Mn and Zn in soils with the conventional fertilization were significantly higher than in the greenhouses with soilless culture and fertigation. It indicates a quicker rate of soil degradation in greenhouse where the soilless cultures with open fertigation system are used.

The danger following from excessive doses of the applied fertilizers in greenhouse was noticed in Spain [12], Netherlands [13] and in Italy [14]. It was found that during plant cultivation in greenhouse soil, 83 % of NO_3-N originating from mineral fertilizers was translocated to the depth of 60–100 cm, while 77 % of NH_4-N was accumulated in the upper soil layer (0–60 cm). On the other hand, 90 % of N originating from organic fertilizers was found in the 0–10 cm layer [15]. The ion of NO_3^- can migrate both with water percolation and with the groundwater ascension. Therefore, the place of nitrate accumulation depends on the intensity of these two opposite water movements [16].

Conclusions

Leakages of nutrient in soilless cultures cause the accumulation of significant amounts of elements in soil. At the same time, the not absorbed ions migrate to groundwaters. The greatest threat results from the easy translocation of NO_3-N. Degradation rate of soil environment depends primarily on the length of greenhouse exploitation. A complete elimination of losses from soilless culture is unrealizable. A smaller application of nutrients and in consequence smaller losses to the soil can be obtained by the introduction of the closed system. However, because of increase of ions concentration in root environment, unending recirculation of the whole volume of nutrient solution is impossible. A significant part must be mixed with pure water. The rest constitutes an unusable post-production waste.

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WPŁYW UPRAW BEZGLEBOWYCH NA ŚRODOWISKO GLEBOWE

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Abstrakt: Uprawy bezglebowe wykorzystuje się do uprawy warzyw i roślin ozdobnych. W tego typu technologiach w celu ustabilizowania stężenia i pH pożywki oraz wilgotności strefy korzeniowej, ilość stosowanych roztworów musi być większa od rzeczywistych wymagań rośliny. Efektem tego są znaczne wycieki pożywki do gleby. Celem badań była ocena właściwości chemicznych gleb w szklarniach, w których są prowadzone uprawy bezglebowe roślin ozdobnych.

W porównaniu z próbkami gleb kontrolnych (trawnik), wyciąg wodny z gleb szklarniowych miał większą konduktywność elektryczną. Na skutek wycieków pożywek w glebie wzrastała koncentracja niemal wszystkich składników, a w szczególności potasu, azotu azotanowego i magnezu, w mniejszym stopniu fosforu, siarczanów i mikroelementów. Największe zagrożenie dla środowiska wynikało z łatwego przemieszczania się $N\text{-}NO_3$. Tempo degradacji środowiska glebowego zależało przed wszystkim od długości okresu eksploatacji szklarni.

Słowa kluczowe: szklarnia, gleba, degradacja, kultury bezglebowe, wyciek

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POSSIBILITIES FOR USE OF SEED EXTRACTS FROM SELECTED *APIACEAEUS* PLANTS IN PLANT PROTECTION AGAINST DISEASES

MOŻLIWOŚCI WYKORZYSTANIA EKSTRAKTÓW Z NASION WYBRANYCH ROŚLIN Z RODZINY *APIACEAE* W OCHRONIE ROŚLIN PRZED CHOROBAMI

Abstract: Fungistatic activity of seed extracts from *Heracleum Sosnowskyi*, *Levisticum officinale* and *Aegopodium podagraria* was investigated.

The extract from *H. Sosnowskyi* at concentrations 0.5–1.5 $cm^3 \cdot 100\ cm^{-3}$ highly inhibited the growth of fungi colonies of *Fusarium culmorum* and *Botrytis cinerea* while a seed extract from *A. podagraria* exhibited no fungistatic activity. The spraying of rose and bean leaves with extracts from *H. Sosnowskyi* and *A. podagraria* substantially reduced necroses caused by *Botrytis cinerea.* Under field conditions the extracts from *H. Sosnowskyi* and *A. podagraria* effectively decreased the infestation of azalea leaves with *Microsphaera penicillata.*

Keywords: *Apiaceaeus* plants, seed extracts, fungistatic activity

Many plant species contain in its tissues some substances of bacteriostatic and fungistatic activity [1]. Due to such properties these plants can be potentially the source of natural fungicides.

Special attention should be paid to umbellifiers (*Apiaceae*). It was found that methanol-water extracts from seeds of dill, parsnip and parsley highly inhibited the growth of colonies of such phytopathological fungi as *Fusarium solani*, *Rhizoctonia solani* and *Botrytis cinerea* [2] and parsley extract also strongly reduced the germination

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of urediospores of *Puccinia tritici* [3]. Similar results was obtained for seeds of several other umbellifiers [4].

The aim of this research is to determine the suitability of seed extracts from *Heracleum Sosnowskyi*, *Levisticum officinale* and *Aegopodium podagraria* prepared by using ethyl acetate in plant protection against phytopathological fungi.

Materials and methods

Dried and ground seeds of plants under investigation were extracted with ethyl acetate in Soxhlet apparatus. The composition of obtained extracts was determined by employing gas chromatography combined with mass analysis. The GC-MS results were interpreted basing on chromatograms and spectrograms as well as data from databases available by Internet (SDBS and NIST).

An effect of extracts on linear growth of the fungi *Fusarium culmorum* (W.G. Sm.) Sacc. and *Botrytis cinerea* Pers. ex. Fries was assessed under laboratory conditions. The crude extracts or extracts appropriately diluted with ethyl acetate were added to potato-dextrose agar (PDA Fluka) to obtain concentrations 1.5, 1.0 and 0.5 % (v/v). Such prepared substrate was poured into 6 cm Petri dishes and inoculated after 24 hours with 5 mm mycelial disk of appropriate test fungi species cut for a 14-day culture. In control combination the agar contained 1.5 % of ethyl acetate. To determine an effect of solvent on the growth of test fungi, the experiments included also a combination with agar without its addition. The culture was carried out at 20 (±2) ^{o}C. The colony diameter was measured at four days after inoculation. Fungistatic activity was determined from Abbott's formula [5] by comparing the fungi growth on the substrates containing extracts with the controls. The experiments were carried out in five replications. A replication was considered to be a Petri dish with one inoculum disk. The above experiments were supplemented with an assessment of activity of furanocoumarin compounds being important secondary metabolites of selected umbellifiers considered by some authors [6] to be strongly fungistatic. In these experiments the test fungi were grown on agars containing 50 mg $\cdot$ dm^{-3} of xantotoxin and the mixture of angelicin and pimpinelin.

The efficiency of extracts in reducing plant infestation with the fungus *Botrytis cinerea* was derived from laboratory experiments. Healthy and superficially disinfected rose and bean leaves were injured with silicon carbide, sprayed with water solutions of extracts at concentrations of 1.5, 1.0 and 0.5 %, and after drying, infected with the pathogen mycelium-grown agar disk and finally incubated inside a wet chamber. The diameter of necrosis developing around inoculum was measured after three days.

The results of laboratory experiments were analyzed statistically by using variance analysis designed for two-factor experiments (extract type, concentration) in independent array. The significance of differences was estimated based on the multiple Duncan's test (α = 0.05).

The extract efficiency in protection of azalea (*Rhododendron glomeatum*) 'Glowing Embels' cv. against powdery mildew *Microsphaera penicullata* (Wallr. ex Link) Lev. was estimated under field conditions. The experiment was carried out at plant nursery in

Wygielzow (Malopolska province, southern Poland). Once the first disease symptoms have occurred, azalea shrubs were sprayed trice, every 7 days, with water solutions of extracts under investigation at concentration 6 % with addition of the adjuvant Superam 10 AL. The health state of the plants was assessed by the extent of leaf surface covering with disease symptoms on the following scale: 0 – no symptoms; 1 – up to 10 %; 2 – 11–25 %; 3 – 26–50 %; 4 – 51–75 %, 5 – above 75 %. After each treatment its efficiency was calculated. The experiment was carried out as complete random in three replications, each of 5 azalea shrubs.

The results were analyzed statistically based on variance analysis. The significance of differences between combinations was evaluated based on Student's t-test ($\alpha = 0.05$).

Results and discussion

The *in vitro* extracts under investigation differed in fungistatic activity. It was depending on the plant species used for extract preparation, its concentration in agar and test fungus species.

The seed extract from *H. Sosnowskyi* was of particular fungistatic activity. For *F. culmorum* the growth inhibition on agars containing this extract was from 86 to 67 %, while in the case of *B. cinerea* these figures were 40–28 % (Table 1). This confirms the results obtained in previous studies [4]. The data indicating fungistatic activity of extracts from *Heracleum* species are reported also by other authors [7, 8].

Table 1

Fungistatic activity of tested extracts

Extract and concentration in the medium [%]	Growth inhibition [%]	
	Fusarium culmorum	*Botrytis cinerea*
Heracleum Sosnowskyi		
1.5	86.6 g*	40.8 d
1.0	72.7 f	32.6 c
0.5	66.2 e	28.6 c
Levisticum officinale		
1.5	43.3 d	15.6 b
1.0	28.2 c	2.0 a
0.5	23.7 b	+1.3 a
Aegopodium podagraria		
1.5	0.9 a	0.9 a
1.0	+1.1 a	+0.3 a
0.5	+1.7 a	+1.5 a

* Means in columns marked by the same letter do not differ significantly according to Duncan ($\alpha = 0.05$).

Chemical analysis revealed that aliphatic alcohols (octanol), aliphatic esters (octyl acetate), coumarin and its derivatives as well as furanocoumarins (angelicin, bergapten,

xantoxin, pimpinelin) are the primary constituents of seed extracts from *H. Sosnowskyi*. It follows from the literature that the latter compounds inhibits development of many fungi [9] but the studies presented above did not confirm their activity to *F. culmorum* and *B. cinerea.* There were no significant differences between the growth of these pathogens on agars containing xantotoxin and the mixture of angelicin and pimpinelin and that of the controls.

The extract from *L. officinale* exhibited significantly weaker activity to test fungi. This was evident particularly for the fungus *B. cinerea*. For this pathogen the inhibition of development was clearly visible in combination with 1.5 % extract (Table 1). Ligustilid of fungistatic activity is an important constituent of this extract [10]. The extract contained terpens (β-felandren, α-pinen, α-terpinol), aliphatic acids (for example 9-octadecenoic, lauric, oleic), coumarin derivatives (6-hydroxy-7-metoxycoumarin), aliphatic esters (citronellol octanate), aliphatic (tetradecane, hexadecane, nonacosane).

The seed extract from *A. podagraria* had practically no effect on the growth of test fungi (Table 1).

The spraying of bean and rose leaves with water solutions of seed extracts from the plants under examination reduced its infestation with *Botrytis cinerea.* The treatment effectiveness depended on extract type and its concentration in working liquid. The distinct protective action was observed for combinations with 1.5 % and 1 % extracts from *H. Sosnowskyi* and *L. officinale*. The extract from *A. podagraria*nie no effected on health state of leaves (Table 2).

Table 2

An effect of extracts on infestation of rose and bean leaves by *Botrytis cinerea*

Extract and concentration in the medium[%]	Necrosis diameter [mm]	
	been	rose
Control	15.5 c*	16.5 de
Heracleum Sosnowskyi		
1.5	3.5 a	8.0 a
1.0	10.0 b	12.7 bc
0.5	13.0 bc	15.7 cd
Levisticum officinale		
1.5	6.2 a	10.0 ab
1.0	11.7 b	15.7 cd
0.5	13.2 bc	15.5 cd
Aegopodium podagraria		
1.5	14.2 c	16.0 de
1.0	14.0 c	19.5 e
0.5	14.5 c	17.2 de

* Means in columns marked by the same letter do not differ significantly according to Duncan ($\alpha = 0.05$).

The spraying of azalea shrubs with solutions of examined extracts significantly inhibited infestation with *Microsphaera penicullata* but the largest differences were

recorded only after third treatment. This resulted from constraint of disease symptoms on young leaves of the treated plants. Also in this case extracts from *Heracleum Sosnowskyi* and *Levisticum officinale* showed high effectiveness (Table 3). The efficiency of methanol extracts from seeds of these plants was found in previous studies [4].

Table 3

Influence of extracts on the infestation of azalea by *Microsphaera penicillata*

Extract	Treatment					
	I		II		III	
	Adi*	Ef**	Adi	Ef	Adi	Ef
Control	5.0 b***	—	5.0 c	—	5.0 d	—
Heracleum Sosnowskyi	4.4 ab	12	3.6 a	28	2.6 a	48
Levisticum officinale	4.6 ab	8	4.0 ab	20	3.4 b	32
Aegopodium podagraria	4.8 ab	4	4.6 bc	8	4.2 c	16

* Average degree of infestation; ** Treatment effectiveness [%]; *** Means marked by the same letter do not differ significantly.

Conclusions

1. Seed extracts in ethyl acetate from *Heracleum Sosnowskyi* and *Levisticum officinale* are of high fungistatic activity on the fungi *F. culmorum* and *Botrytis cinerea*.
2. The activity of above-mentioned extracts is not related to the presence of furanocoumarin derivatives.
3. Seed extracts from *Heracleum Sosnowskyi* and *Levisticum officinale* can be suitable in plant protection against powdery mildews.

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MOŻLIWOŚCI WYKORZYSTANIA EKSTRAKTÓW Z NASION WYBRANYCH ROŚLIN Z RODZINY *APIACEAE* W OCHRONIE ROŚLIN PRZED CHOROBAMI

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Abstrakt: Badano aktywność fungistatyczną ekstraktów z nasion roślin selerowatych: *Heracleum Sosnowskyi*, *Levisticum officinale* i *Aegopodium podagraria.*

Wyciąg z *H. Sosnowskyi* w stężeniach 0,5–1,5 $cm^3 \cdot 100\ cm^{-3}$ silnie hamował wzrost kolonii grzybów *Fusarium culmorum* i *Botrytis cinerea*, natomiast wyciąg z nasion *A. podagraria* nie wykazywał aktywności fungistatycznej. Opryskanie liści róży i fasoli wyciągami *H. Sosnowskyi* i *A. podagraria* znacznie ograniczało powstawanie nekroz powodowanych przez *Botrytis cinerea*. W warunkach polowych wyciągi *H. Sosnowskyi* i *A. podagraria* skutecznie zmniejszały porażenie azalii wielkokwiatowej przez *Microsphaera penicullata.*

Słowa kluczowe: rośliny selerowate, ekstrakty z nasion, aktywność fungistatyczna

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EVALUATION OF PRODUCTIVE VALUE OF SEVERAL WHITE CABBAGE CULTIVARS RESISTANT TO CLUBROOT (*Plasmodiophora brassicae* WOR.)

OCENA WARTOŚCI UŻYTKOWEJ KILKU ODMIAN KAPUSTY GŁOWIASTEJ BIAŁEJ ODPORNYCH NA KIŁĘ (*Plasmodiophora brassicae* WOR.)

Abstract: In a 3-year experiment three late cultivars of clubroot-resistant white cabbage: 'Kilafur F_1', 'Kilaton F_1' and 'Kilaxy F_1' were compared with 3 standard cultivars: 'Bloktor F_1', 'Kingston F_1' and 'Novator F_1'. The experiment was located on a stand where in the previous year also cabbage was cultivated. During vegetation the degree of plants affected by clubroot was determined. Yield as well as commercial and nutritional quality of cabbage heads were evaluated.

In all three years of the experiment it was demonstrated that clubroot-resistant cultivars were not at all affected by *Plasmodiophora brassicae* pathogen while other cultivars were affected to a varied degree. High commercial yield of clubroot-resistant cultivars and a very good head quality were obtained. In the same conditions, non-resistant cultivars demonstrated a significantly lower yield level.

Keywords: white cabbage, clubroot, resistant cultivars

White cabbage is a basic vegetable species grown in Poland [1]. High concentration of cultivation in certain regions results in a considerable increase of the occurrence of clubroot, the most arduous and difficult to control pathogen for this vegetable group. Among preventive measures against clubroot agrotechnical methods, such as crop rotation or calcium fertilization dominate [2]. Apart from these, fungicides are also used to pickle seedlings or disinfect soil. These methods are not always effective [3, 4]. Thus, since long, scientists have concentrated on searching for such genetic resistance which

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would provide full protection against the infection caused by *Plasmodiophora brassicae* fungus. Extensive breeding researches culminated in a spectacular success, namely creating, by Syngenta Seeds Corporation, of several cultivars resistant to most usual on the European continent clubroot races [5]. The introduction of such cultivars to the production of cabbage is the most rational and effective method of protection against clubroot. At the same time, such cultivars should possess important use features, such as good productivity and, in particular, high commercial and nutritional values of the heads [6–8].

In the present experiment the actual susceptibility to clubroot of several such cultivars of white head cabbage was checked in field conditions. At the same time the attempt was made to assess their yielding and quality as compared with other popular cultivars.

Materials and methods

Field experiment was carried out in the years 2005–2007 on the site where cabbage was grown in a previous year. Three late cultivars of clubroot-resistant white cabbage were compared, ie 'Kilafur F_1', 'Kilaton F_1' and 'Kilaxy F_1' with three standard cultivars having a comparable vegetation period and growth pattern: 'Bloktor F_1', 'Kingston F_1' and 'Novator F_1'.

Seeds were sown to multipots (palettes of 96 cells each) filled with peat substrate in the first half of April (11, 6 and 12, respectively in consecutive years) and cabbage seedlings were planted out to the field in the first half of May (12, 8 and 9, respectively in consecutive years). The experiment was established in randomized blocks with four replications. Plants were planted in 67.5 × 40 cm distance into plots of the area of 10.8 m^2 (40 plants). Measurements were carried out on 32 plants of each plot, excluding side rows.

During the growth period plants were weeded and they were fertilized with nitrogen. When necessary plants were watered and protected against diseases and insect pests.

During vegetation, the percentage of plants affected with clubroot was assessed using a 6-point scale, ie: 0 – healthy plant; 1 – first symptoms of disease, wilting; 2 – more noticeable symptoms, wilting and yellowing of leaves; 3 – clearly noticeable symptoms, delayed growth, wilted or yellowed leaves; 4 – strong symptoms, inhibited growth, yellowed or lacking leaves; 5 – plant completely diseased, dried or lacking.

Heads were harvested in mid-October (between 8 and 18 in individual years). At harvesting yield was assessed by determining the mass and number of cabbage heads as per quality grades (I, II and unmarketable). Commercial quality (mean weight, yield structure) and consumption quality (dry matter, sugars, L-ascorbic acid, chlorophylls and carotenoids content) of cabbage heads were an important element in assessment process. Dry matter was determined using hair-dryer method at 95 ^{o}C, sugars after inversion with Luff-Schoorl method, L-ascorbic acid with Tillmans method, and chlorophylls and carotenoids with Lichtenthaler method.

The obtained results were compared as averages from three years of the experiment and subjected to statistic analysis with variation method at $p = 0.05$.

Results and discussion

The most important result obtained in this study was the finding that in all the years of the experiment cultivars 'Kilafur F_1', 'Kilaton F_1' and 'Kilaxy F_1' were not, even to the smallest degree, affected by clubroot. Not a single plant of clubroot-resistant cultivars was infected by *Plasmodiophora brassicae* pathogen, whereas the remaining cultivars were affected to a varied degree.

Most attacked plants (Fig. 1) were found for 'Kingston F_1' cultivar (86.0 %) with affection degree varying from first symptoms (8.5 %) to total disease (25.0 % of the whole population). 'Bloktor F_1' cultivar was affected to the smallest extent, however also in this case as many as 55.4 % of plants were diseased. Complete lack of affection of clubroot-resistant cultivars in three consecutive years of the experiment at varied weather conditions and in the direct vicinity of standard cultivars, which showed strong clubroot symptoms, confirms the success of resistant cultivar breeding.

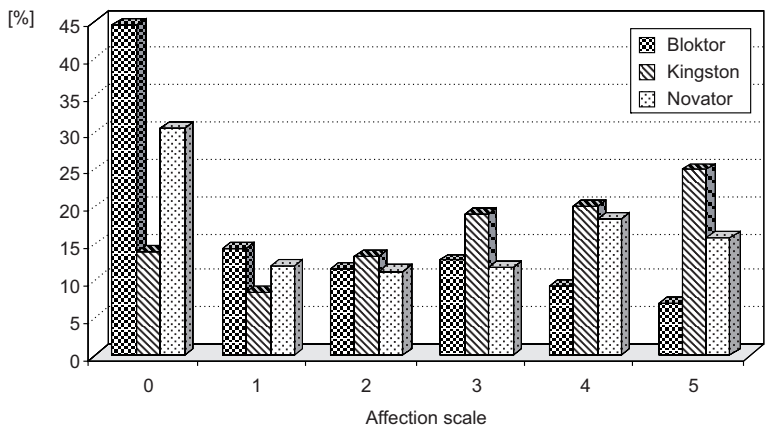

Fig. 1. Percentage of non-resistant cultivar plants affected by clubroot; 0–5: affection scale, for description see Materials and methods

In effect, clubroot-resistant cultivars generated almost twice as big commercial yield, determined in mass units, than non-resistant ones (Table 1).

The highest yields were obtained from 'Kilafur F_1' cultivar (116.8 Mg · ha^{-1}) and slightly lower from 'Kilaxy F_1' and 'Kilaton F_1' cultivars (90.9–99.3 Mg · ha^{-1}). Such a high yield is the best proof of a high production potential of these cultivars and is comparable with the productivity of best world cultivars, grown on clubroot-free sites [9]. In the same conditions, from non-resistant cultivars significantly lower yields were obtained (51.3–65.1 Mg · ha^{-1}) with no significant statistical differences observed between individual cultivars. Such a significant reduction in yield regarding sensitive cultivars shows how economically important and serious disease leading to considerable loss is clubroot. However, it is worth mentioning that a group of such cultivars, despite evident infection, was not totally destroyed, the fact which may prove lower genetic susceptibility of such cultivars to clubroot pathogen. Studies conducted by some authors

demonstrate variations in sensitivity of cultivars or production lines of white head cabbage [2, 10].

Table 1

Marketable yield and mean weight of heads

Cultivars	Marketable yield				Mean weight of heads [kg]	
	[Mg · ha^{-1}]		[number · ha^{-1}]		for cultivar	mean for group
	for cultivar	mean for group	for cultivar	mean for group		
Resistant:						
Kilafur F_1	116.8 c		32 790 d		3.56 ab	
Kilaton F_1	99.3 b	102.3	24 305 bc	28 517	4.09 b	3.61
Kilaxy F_1	90.9 b		28 455 cd		3.19 a	
Non-resistant:						
Bloktor F_1	65.1 a		21 025 b		3.10 a	
Kingston F_1	51.3 a	57.7	14 180 a	16 943	3.62 ab	3.45
Novator F_1	56.8 a		15 625 a		3.64 ab	

a, b – means in the columns marked with different letters differ significantly at p = 0.05; 1 Mg = 1 ton.

Mean head weight of clubroot-resistant and non-resistant cultivars was comparable (3.61 and 3.45 kg, respectively for each group). The highest one was characteristic for 'Kilaton F_1' (4.09 kg) cultivar only. On the other hand, the analysis of total yield structure (Fig. 2) demonstrates a high share of 1st grade heads for 'Kilafur F_1' (87.8 %) and 'Novator F_1' (90.8 %) cultivars and relatively low one for 2nd grade and unmarketable cabbage heads. In the case of other cultivars the number of highest quality heads collected was slightly lower and the number of lower quality ones relatively higher. The lack of significant differences regarding this aspect may seem surprising but it is a direct consequence of including these heads of sensitive cabbage cultivars which

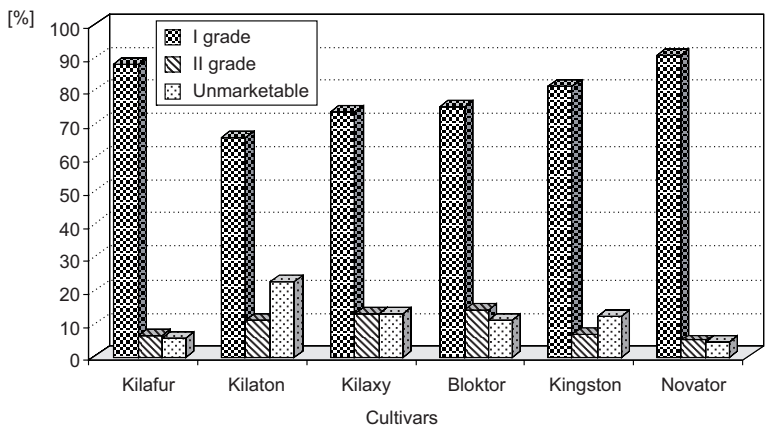

Fig. 2. Structure of total cabbage yield

had not been destroyed by clubroot in the above classification. The presented data are a proof of full commercial values of new cultivars which are comparable with the commonly cultivated ones [7, 9].

As it can be seen from the data presented in Table 2, nutritional value of the compared cultivars was pretty similar, although, in certain cases, statistically differentiated.

Table 2

Content of some compounds in cabbage heads

Cultivars	Dry matter	Total sugars	L-ascorbic acid [mg · 100 g^{-1} f.m.]	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
	[g · 100 g^{-1} f.m.]			[mg · g^{-1} f.m.]		
Resistant:						
Kilafur F_1	8.87 d	4.47 a	24.92 a	0.015 c	0.008 a	0.006 a
Kilaton F_1	8.29 a	4.77 b	24.66 a	0.011 ab	0.007 a	0.006 a
Kilaxy F_1	8.73 cd	4.79 b	25.39 a	0.012 b	0.006 a	0.006 a
Non-resistant:						
Bloktor F_1	8.57 bc	4.47 a	26.43 a	0.012 b	0.008 a	0.007 a
Kingston F_1	8.63 bc	4.52 a	65.44 b	0.011 ab	0.008 a	0.006 a
Novator F_1	8.45 ab	4.58 a	23.33 a	0.009 a	0.006 a	0.005 a

a, b – means in the columns marked with different letters differ significantly at p = 0.05.

From all cultivars, 'Kingston F_1' distinguished with a very high L-ascorbic acid content (65.44 mg in 100 g of fresh matter), whereas for all other cultivars the value ranged from 23.33 to 26.43 mg. Dry matter content for all cultivars ranged from 8.29–8.87, and total sugars content from 4.47–4.79 g · 100 g^{-1} f.m. Chlorophyll *a* level ranged from 0.009 to 0.015, chlorophyll *b* from 0.006 to 0.008, and caretonoid level from 0.005 to 0.007 mg in 1 g of fresh mass. These values, except for the above-mentioned case of vitamin C fall into limits of standard element content determined for white head cabbage [8, 11, 12].

Summarizing, clubroot-resistant cultivars under investigation remained unaffected by the disease in the conditions of the experiment and gave good quality, high yield, proving suitable for commercial production.

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OCENA WARTOŚCI UŻYTKOWEJ KILKU ODMIAN KAPUSTY GŁOWIASTEJ BIAŁEJ ODPORNYCH NA KIŁĘ (*Plasmodiophora brassicae* WOR.)

Katedra Warzywnictwa z Ekonomiką Ogrodnictwa
Katedra Ochrony Roślin
Uniwersytet Przyrodniczy im. Hugona Hołłątaja w Krakowie

Abstrakt: W trzyletnich badaniach porównywano 3 późne odmiany kapusty głowiastej białej odporne na kiłę: 'Kilafur F_1', 'Kilaton F_1' i 'Kilaxy F_1' oraz 3 standardowe: 'Bloktor F_1', 'Kingston F_1' i 'Novator F_1'. Doświadczenie zlokalizowano na stanowisku, na którym w poprzednim roku była również uprawiana kapusta. W czasie wegetacji szacowano stopień porażenia roślin przez kiłę. Oceniano plonowanie oraz jakość handlową i odżywczą główek kapusty.

We wszystkich latach prowadzenia badań wykazano brak jakiegokolwiek porażenia odmian odpornych przez patogena *Plasmodiophora brassicae*, podczas gdy pozostałe uległy w różnym stopniu infekcji. Uzyskano duży plon handlowy odmian odpornych oraz bardzo dobrą jakość ich główek. W tych samych warunkach uzyskano znacząco niższe plony odmian nieodpornych.

Słowa kluczowe: kapusta głowiasta biała, kiła, odmiany odporne

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Agnieszka DOBROWOLSKA[1]

INFLUENCE OF THE MEDIUM WITH ADDITION OF COCOA HUSK ON THE PRODUCTION OF SEEDLINGS OF SELECTED SPECIES AND CULTIVARS OF BEDDING PLANTS

WPŁYW PODŁOŻA Z DODATKIEM ŁUSKI KAKAOWEJ NA PRODUKCJĘ ROZSADY WYBRANYCH GATUNKÓW I ODMIAN ROŚLIN KWIETNIKOWYCH

Abstract: The experiment was conducted in the years 2007–2008. Its purpose was to compare the germination, further growth and the quality of seedlings of annual plants grown on a traditional medium (peat) and on a medium with the addition of cocoa husks. The test plants were: *Tagetes erecta*, 'Klaun' cv., *Tagetes patula nana*, 'Bolero' cv., *Impatiens walleriana*, 'Candy White' and 'Candy Red' cvs. Seeds of all the taxons were sown onto two media: 1. deacidified high peat with the addition of Azofoska fertilizer in the dose of 2 g · dm^{-3} (control medium); 2. peat + cocoa husks (1:1 v/v). Cocoa husk constituted a waste product from a confectionery plant, prior to use it had been composted for a period of approx. 12 months.

Chemical analysis of the cocoa husks has shown that this component is rich in nutrients. After the 12-month period of composting the structure of the husks changed, the pH value of the husks dropped, their electrical conductivity (EC) lowered, and the amount of plant-available nitrogen increased. It was found that the 1:1 v/v cocoa husk medium, used for sowing shortened the time of plant emergence and also shortened the production period of marigold and impatiens seedlings by 6 to 14 days. Cocoa husks favourably influenced the development of the root system and accelerated the blossoming time of the tested cultivars.

Keywords: flower bed plants, medium, cocoa husk, *Tagetes, Impatiens*

Bedding plants constitute an important part of Polish ornamental horticulture. By devising a production technology of these plants the improvement of growing conditions plays an important part, on the other hand, however, a lot of emphasis is put on environmental protection. Peat is the basic medium for protected cultivation of ornamental plants. However, its resources are constantly decreasing. In order to reduce peat extraction, only to a small extent, research is being conducted to explore the

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possibility of using other substances, including waste products, which might partially replace it [1–3].

In recent years new medium components have been more and more widely used in horticultural cultivations, especially organic ones [4, 5]. They are frequently treated as organic fertilizers, as they constitute a valuable source of macro- and micronutrients for cultivated plants, improve soil structure and play an important role in organic matter balance [6]. On the other hand, their composting and application in cultivation allows efficient utilization of wastes, especially in urban areas or in the vicinity of processing plants where such by-products are produced. Cocoa husks, constituting a waste product of chocolate production, are an example of such materials. Owing to the high dry mass content they are used to stabilize other waste materials, especially liquid ones, as a structure-forming component [2].

The studies conducted were aimed at determining the cocoa husk value as a component of the medium and defining its fertilizing value. The applicability of cocoa husks in the production of *Tagetes erecta*, *Tagetes patula nana* and *Impatiens walleriana* was also verified.

Material and methods

The experiment was conducted at the Department of Ornamental Plants, University of Agriculture in Szczecin within the Vegetation Hall in the years 2007–2008. Cocoa husk used for the study was a waste product from a confectionery plant. Prior to use, it had been composted for a period of 12 months with the addition of 1.5 kg of urea per 1 m^3 of husks. Before the commencement of the experiments the chemical and physical properties of cocoa husks were analyzed. They allowed to determine the content of individual nutrients, the reaction of the husks, their salinity and moisture capacity.

The test plants were: *Tagetes erecta*, 'Klaun' cv., *Tagetes patula nana*, 'Bolero' cv., *Impatiens walleriana*, 'Candy White' and 'Candy Red' cvs. The seeds of all the taxons were sown after the March 20th into seedling punnets. As the control medium sphagnum peat with the addition of Azofoska fertilizer in the dose of 1 g · dm^{-3} was used. The other medium consisted of a mixture of peat and cocoa husks in a 1:1 volume ratio with no fertilizer added. The pH value of both media was increased to 6.0 by means of chalk and dolomite. Young marigold plants were planted in pots 4–6 weeks after sowing, impatiens plants – after 8 weeks after sowings. These differences resulted from the growth rate of plants on the individual media. Pots with ∅ 12 cm were used for planting, also two media were used – peat and a mixture of peat and cocoa husks (1:1 v/v). Osmocote Exact Hi-Start fertilizer at a dose of 5 g · dm^{-3} was added to the peat medium. The plants grew in patches in an unheated high tunnel until the end of the experiment.

In the experiment the seed ability to germinate and the rate of germination were evaluated and the length of the aboveground parts of the seedlings as well as the size and quality of the root system were measured. In the second part of the experiment, after planting the seedlings in pots, the time needed to obtain ready-to-sell blossoming seedlings was evaluated, the size of the plants was measured and the number of buds

and flowers and their general ornamental value were assessed. The results of the measurements of the plant biometric features were analyzed statistically by means of year synthesis. Differences between means were verified statistically by the means of Tukey's test at the significance level $\alpha = 0.05$.

Results and discussion

Cocoa husks are characterized by a high dry mass content, a high content of total nitrogen and the C:N ratio is lower compared with other structure-forming materials such as straw or shredded paper [7]. The chemical analysis showed that cocoa husks, after the period of composting, had a high NO_3-N content, on the other hand, the content of P and K decreased. Similar dependencies were found by Lis-Krzyscin [8], although in her studies the medium with the addition of cocoa husks had been composted only for six months. According to the literature, the decrease in potassium content is beneficial as too high its amount may block the absorption of other elements and the plants wither easily [9]. It was found that the composting process of cocoa husk improved its physical properties, increasing its porosity and moisture capacity. Composted cocoa husks also had a lower electrical conductivity and the medium was characterized by a low pH (Table 1). As cocoa husks decompose quickly under the influence of water and fertilizers, they can be primarily used as a component of mixtures in protected cultivations of ornamental plants, eg in seedling production which does not last longer than 12 weeks [10].

Table 1

Physical and chemical properties of fresh cocoa husks and after composting

Cocoa husks	Physical properties				The contents of macroelements [mg · dm^{-3} of medium]					
	Bulk weight [g · dm^{-3}]	pH in H_2O	EC [mS · cm^{-1}]	Full water capacity [% v/v]	NO_3-N	P	K	Ca	Mg	Cl
Fresh cocoa husk	250	6.8	3.102	—	81	377	2154	137	380	168
Composted cocoa husk	380	4.3	1.156	84.6	161	126	932	443	203	43

After the analysis of the content of the most important macronutrients in cocoa husks and commonly used fertilizers it can be concluded that cocoa husks have a high fertilizing value. The dose of 142.5 g per pot used in the experiment introduced large amounts of NO_3-N into plants (60 mg per plant), and a similar amount of potassium as Osmocote Exact Hi-Start fertilizer (Table 2). Although no fertilizer had been added to the medium containing the composted cocoa husks the plants developed properly and grew even faster than the plants grown on the peat medium.

Table 2

Comparison of fertilizer value of cocoa husk and common fertilizers: Azofoska and Osmocote Exact Hi-Start

Fertilizer	Dose [g per plant]	Amount of N	Amount of P	Amount of K
		[mg per plant]		
Cocoa husk	142.5	60.4	47.2	349.5
Azofoska	3.75	510	240	600
Osmocote Exact Hi-Start	3.75	637	375	375

On the basis of the measurements and observations of the plants grown on both media it was found that the seeds of almost all cultivars germinated 1–2 days more quickly and the germination was more balanced when the medium with cocoa husks was used. Only in *Impatiens Walleriana*, 'Candy Red' cv., a larger percentage of germination was observed on the peat medium than on the medium with cocoa husks (Table 3).

Table 3

Influence of cocoa husk on quality of marigolds and impatiens seedlings – synthesis of 2 year experiments

Species and cultivar	Medium	Seedlings traits				
		Height of seedling [cm]	Number of leaves	Number of roots	Number of roots longer than 3 cm	Germination [%]
Tagetes erecta 'Klaun'	peat	11.5 a**	5.97 a	11.83 a	3.75 a	76
	*peat + cocoa husk (1:1 v/v)	*12.1 a	*5.45 a	*12.26 a	*3.22 a	94
Tagetes patula nana 'Bolero'	peat	6.08 a	4.9 b	12.71 b	3.92 b	59
	*peat + cocoa husk (1:1 v/v)	*6.51 a	*6.4 a	*14.82 a	*4.59 a	76
Impatiens walleriana 'Candy Red'	peat	3.23 b	3.51 b	10.02 a	4.75 a	82
	*peat + cocoa husk (1:1 v/v)	8.23 a	4.66 a	7.42 b	3.53 b	75
Impatiens walleriana 'Candy White'	peat	3.26 b	3.36 b	6.92 a	4.07 a	73
	*peat + cocoa husk (1:1 v/v)	7.41 a	4.64 a	7.57 a	3.17 b	90

* The measurements were performed 2 weeks earlier due to the large size and the necessity to replant the seedling growing on the cocoa husk medium; ** Averages followed by the same letter do not differ significantly at $\alpha = 0.05$.

Marigold cultivars growing on the cocoa husk medium needed to be pinched out 14 days earlier than the same cultivar growing on the peat medium (Table 3). Marigolds of the 'Bolero' cultivar grown on the cocoa husk medium had more leaves and roots,

including roots longer than 3 cm, although the measurements were performed 2 weeks earlier due to the large size and the necessity to replant the seedling growing on this substrate.

The addition of cocoa husks also influenced the growth rate at the further stage of cultivation (Table 4).

Table 4

Influence of cocoa husk on vegetative and generative traits of marigolds and impatiens – synthesis of 2 year experiments

Species and cultivar	Medium	Seedlings traits			
		Height of plants	Diameter of plants	Number of buds	Number of flowers
		[cm]			
Tagetes erecta 'Klaun'	peat	55.2 a**	43.3 a	3.33 b	2.00 a
	*peat + cocoa husk (1:1 v/v)	*43.8 b	*30.2 b	*5.00 a	*1.00 a
Tagetes patula nana 'Bolero'	peat	17.6 a	25.7 a	7.67 b	3.00 a
	*peat + cocoa husk (1:1 v/v)	*17.7 a	*18.8 b	*9.75 a	*0.75 b
Impatiens walleriana 'Candy Red'	peat	9.0 b	23.0 a	17.25 a	3.23 b
	peat + cocoa husk (1:1 v/v)	15.3 a	23.3 a	19.00 a	10.50 a
Impatiens walleriana 'Candy White'	peat	9.5 b	27.5 a	24.75 a	2.00 b
	peat + cocoa husk (1:1 v/v)	13.8 a	22.5 a	21.00 a	15.65 a

Explanation, see Table 3.

Both marigold and impatiens plants grown on the cocoa husk medium entered the generative growth stage earlier and were ready for sale. *Tagetes erecta*, 'Klaun' cv., grown on the cocoa husk medium was more compact, lower and less expansive than those grown on the peat medium, however, it had more flower buds. Both cultivars of *Impatiens walleriana* were taller when grown on the cocoa husk medium, they also blossomed more abundantly. They started to blossom on average 6 days ('Candy White' cv.) and 9 days ('Candy Red' cv.) earlier than plants grown on the peat medium. Also in the studies by Lis-Krzyscin [8] it was found that compost with the addition of cocoa husks had a beneficial effect upon the development of plants and the abundance of blossom of bedding geranium, 'Susan Improved' cv. In the experiments conducted by Ochmian et al [11] the influence of cocoa husk addition on the yield and quality of fruits of highbush blueberry, 'Sierra' cv., was examined. It was found that plants cultivated in sphagnum peat and cocoa husk were characterized by lower yield, smaller fruits, but higher content of phenolics and anthocyanins in fruits.

Conclusion

1. Composted cocoa husk constitutes a valuable addition to the medium, it also has a high fertilizing value.

2. The addition of composted cocoa husks to the peat medium (1:1 v/v) with no other fertilizers favourably influences the growth and development of .

3. By growing plants in a mixture of peat and cocoa husks (1:1 v/v) ready for sale seedlings of *Tagetes erecta*, 'Klaun' cv., can be obtained by approx. 14 days earlier than in the case of a peat medium.

4. *Impatiens walleriana* from the Candy group develop better, grow more strongly and blossom 6–9 days earlier when they are grown on a mixture of peat and cocoa husks (1:1 v/v).

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WPŁYW PODŁOŻA Z DODATKIEM ŁUSKI KAKAOWEJ NA PRODUKCJĘ ROZSADY WYBRANYCH GATUNKÓW I ODMIAN ROŚLIN KWIETNIKOWYCH

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Abstrakt: Celem doświadczenia przeprowadzonego w latach 2007–2008 było porównanie kiełkowania i dalszego wzrostu oraz jakości rozsady roślin jednorocznych uprawianych w podłożu tradycyjnym (torf) oraz w podłożu z dodatkiem łuski kakaowej. Roślinami testowymi były: *Tagetes erecta* odm. 'Klaun', *Tagetes patula nana* odm. 'Bolero', *Impatiens walleriana* odm. 'Candy White' i 'Candy Red'. Nasiona wszystkich taksonów wysiano do dwóch podłoży: 1. odkwaszony torf wysoki z dodatkiem nawozu Azofoska w dawce 2 g · dm^{-3} (podłoże kontrolne); 2. torf + łuska kakaowa (1:1 v/v). Łuska kakaowa stanowiła odpad pochodzący z zakładu cukierniczego, przed użyciem do doświadczeń kompostowano ją przez okres około 12 miesięcy.

Analiza chemiczna łuski kakaowej wykazała, że komponent ten jest zasobny w składniki pokarmowe. Po rocznym okresie kompostowania zmieniła się struktura łuski, obniżyło się pH łuski, obniżyła się także konduktywność (przewodność właściwa EC), wzrosła natomiast ilość dostępnego dla roślin azotu. Stwierdzono, że podłoże z dodatkiem łuski kakaowej (1:1 v/v), zastosowane do wysiewu przyspieszyło wschody, a także skróciło okres produkcji rozsady aksamitki i niecierpka od 6 do 14 dni. Łuska kakaowa korzystnie wpłynęła na rozbudowę systemu korzeniowego i przyspieszyła kwitnienie roślin badanych odmian.

Słowa kluczowe: rośliny kwietnikowe, podłoże, łuska kakaowa, *Tagetes, Impatiens*

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Iwona DOMAGAŁA-ŚWIĄTKIEWICZ[1] and Jan BŁASZCZYK[2]

EFFECT OF AUTUMN FOLIAR UREA SPRAY ON NUTRIENTS STATUS OF 'ELISE' APPLE TREES

WPŁYW JESIENNEGO DOLISTNEGO NAWOŻENIA MOCZNIKIEM NA STAN ODŻYWIENIA MINERALNEGO JABŁONI ODMIANY 'ELISE'

Abstract: The present project was undertaken to study the influence of autumn foliar urea application on leaf and twigs macroelements content and yield of 5 year old 'Elise' apple. The study was carried out in years 2004–2007 in the experimental orchard at Garlica Murowana near Krakow. Trees were sprayed with 2 % and 4 % of urea at post-harvest in October. The yield of fruits and the contents of N, P, K, Mg and Ca were examined in plant samples collected.

The N status of apple trees was optimal or high independent on treatments. Autumn foliar urea sprays increased N content in twigs, but only in 2007 (after dry and warm 2006) affected fruit yield. There is some evidence that autumn application of urea would be most useful for trees with low nitrogen status. The results indicate that use of foliar urea at the high solution concentration (4 % m/v) is more efficient way to N supply than 2 %.

Keywords: 'Elise' apple, urea sprays, nutritional status

Foliar application of mineral nutrients by means of sprays offers a method of supplying nutrients to plants more rapidly than methods involving root application [1, 2]. Foliar nitrogen supply in orchard is gaining importance as a technique to integrate soil N applications or as an alternative to them [3, 4].

In fruit trees, spring growth, including flowering, depends on translocation of nitrogen stored in perennial organs [5]. Nitrogen is stored during the winter, predominantly as protein in the bark of twigs and trunk or roots, and translocated in the spring when the buds break [5, 6]. Translocation of N in spring provides nitrogen for leaf growth before rapid root uptake occurs and so is unaffected by the N supply to the soil [6–9]. Nitrogen utilized during bloom and the period of rapid shoot elongation

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following bloom is dependent upon the redistribution of stored nitrogen from previous year application [5].

Urea is considered the most suitable form of N to supply as a foliar spray because its non-polarity, and rapid absorption, low phytotoxicity and high solubility [1, 10]. Foliar supplied urea is absorbed by leaves and rapidly converted to amino acid [11]. It indicates that the metabolism of root-absorbed and leaf absorbed N do not differ [5]. Most studies were focused on plant N status and growth in response to urea sprays [2, 12–14]. Although urea may be used at any time during the growing season, autumn application may be most effective for deciduous trees because high urea concentration can be used with minimal concern about phytotoxicity [15]. The autumn foliar application of urea can increase N reserves and improve flowering, fruit set and growth in the following season. Nitrogen uptake through leaf surfaces may permit the reduction of soil N application in orchard, thus decreasing the potential nitrate leaching to the groundwater [16].

As a side effect of foliar application of urea, microflora populations on the leaf surface might be changed and spore germination and colony growth of pathogens thereby reduced [17].

The aim of the present research was to assess the effect of 2 % and 4 % urea solutions on the leaves and twigs macronutrients concentration, and yield of 'Elise' apples.

Materials and methods

The study was carried out in years 2004–2007 in the experimental orchard at Garlica Murowana near Krakow. Trees of 'Elise' cultivar, grafted on M.9 rootstock were planted in 2000 at 4 × 1.6 m spacing. The soil was kept in the herbicide strips in tree rows with grass between them.

Every year of the experiment in spring nitrogen soil fertilization was applied at the rate of 50 kg · ha^{-1} using ENTEC 26 (26 % N including 18.6 % NH_4-N with the addition of nitrification inhibitor). Nitrogen fertilizer was spread around each tree, in the area of herbicide strip.

The experiment was carried out in a complete randomized blocks in four replications (6 trees in one replication) and comprised:

1. Control (no foliar spraying, no soil N applied),
2. Control + soil N applied,
3. Trees sprayed with 2 % urea (one time),
4. Trees sprayed with 4 % urea (one time).

Solutions were used with an addition of the surfactant Aptolan 80EC (76 % of paraffin oil) in the amount of 1000 dm^3 · ha^{-1}. The sprayings were conducted after fruits harvest in the first decade of October.

Soil analysis

Soil samples were taken from the layers of 0–20 cm and 20–40 cm, each separately from herbicide stripes and grass strips, at the time of taking leaf assays. Nutrient

contents in soil were estimated according to the Egner-Riehm (P and K) and Schachtschabel (Mg) methods as well as by universal methods (Ca) [18]. The pH value in water suspension (pH_{H_2O}) and in 1 mol · dm^{-3} KCl (pH_{KCl}) in 1 : 2 the soil : water (or soil : solution) ratio were measured. In soil samples granulometric composition by the aerometric method of Proszynski [19] and the organic carbon content by Tiurin's method were determined [18].

Leaf analysis

To estimate the nutritional status of the trees the leaves samples were collected every year at the end of July or the beginning of August. Samples of 10 mid-shoot leaves from current season's extension growth on shoots of representative vigor, in the periphery area around the each tree were collected. Plant material was dried at 70 ^{o}C and digested in the mixture of HNO_3, $HClO_4$ and H_2SO_4 (6 : 2 : 0.8 ratio). The contents of K, Mg and Ca were measured by atomic absorption spectrophotometer using the flame method; P was determined by spectrophotometric method with ammonium molybdate. Total nitrogen content in leaves was determined by Kjeldahl's methods [19].

Twigs analysis

At the beginning of December after leaves abscising 5 twigs (10 cm a length) of each tree were taken from the outer part of the tree, at the height of 1 m. The bark with a cambium layer and the wood of twigs were analyzed separately. Nitrogen, phosphorus, potassium, magnesium and calcium contents were determined after dried, milled and mineralization in acid mixture as previously described.

Every year in the first decade of September the yield of fruits was also estimated.

Statistical analysis

Data were subjected to two or tree-factor analysis of variance (ANOVA). Differences between means were assessed by Fisher's test. All statistical analyses were performed with Statistica 8.0 software.

Results and discussion

Soil analysis

The experimental orchard was established on heavy soil (> 35 % of fraction < 0.02 mm) of silt loam with a low pH_{KCl} 4.05–4.19. The phosphorus and magnesium content was high (> 4 mg P · 100 g^{-1} and 5–6 mg Mg · 100 g^{-1}, respectively) both in the herbicide and grass stripe samples of 0–20 cm and 20–40 cm layers. The potassium content was high and ranged between 16.3 (in 20–40 cm layer) to 22.4 mg K · 100 g^{-1} (in the humus layer). The potassium to magnesium ratio (K:Mg) in the soil was proper, lower than 3.5 (Table 1).

Table 1

Contents of P, K, Mg and Ca in the orchard soil

Factor		pH in KCl	P	K	Mg	Ca	K : Mg
			[mg 100 · g^{-1}]				
Place of sampling	Herbicide strip	4.19	5.0	19.1	5.9	38.8	3.2
	Grass strip	4.05	4.8	19.7	6.4	27.4	3.1
Soil layer [cm]	0–20	4.17	5.6	22.4	6.1	32.6	3.7
	20–40	4.06	4.3	16.3	6.2	33.6	2.6

Leaves analyses

Environmental factors affected nutrient uptake by plant in years of research. Any year of the experiment magnesium and calcium content in the leaves ranged in the optimum values for the apple trees [20] (Table 2).

Table 2

Nutrient contents in leaves of apple 'Elise' cv.

Factor		N	K	Mg	Ca	P
		[% d.m.]				
Year	2005	2.53	1.72	0.25	1.32	0.17
	2006	2.36	1.21	0.26	1.34	0.08
	2007	2.44	1.21	0.24	1.52	0.14
Treatment	Control	2.46	1.33	0.26	1.41	0.13
	Control + N	2.39	1.42	0.25	1.37	0.13
	2 % urea	2.48	1.43	0.23	1.40	0.13
	4 % urea	2.45	1.34	0.26	1.40	0.13
$LSD_{0.05}$ for	Year	0.054	0.170	ns	0.075	0.015
	Treatment	0.063	ns	0.023	ns	ns

ns – not significant.

The phosphorus level reached the optimum range in 2005 and 2007, however, it was low in 2006. In 2006 the nitrogen level in leaves was in the optimum, while in 2005 and 2007 in the high range (more than 2.4 % N in d.m.). In 2005, high K content in the apple leaves was detected (> 1.5 % K in dm). In the present study spring soil N application (Control + N) resulted in a little lower than average leaf N. This result did not confirm conclusions of Khemira et al [21], who reported that spring-applied broadcast fertilizer in apple was found in aboveground tissues rather than roots, but that nitrogen from preharvest broadcast application was translocated preferentially to the roots.

Autumn foliar urea application did not affect P, K and Ca content in apple leaves. The lower content of Mg was measured in leaves treated 2 % urea solution than in the control trees and foliar applied of 4 % urea solution.

Twigs analyses

Oland [22] reported that foliar application of 4 % urea solution to apple trees in autumn increased the amount of nitrogen translocation from leaves during senescence, which resulted in a 31 % increase in N content of reproductive spurs by leaves fall and increased fruit yield in the subsequent year. In present studies foliar 4 % urea solution application significantly increased N content in bark of the one-year old apple twigs analyzed in the beginning of December in 2005 and 2006 (Table 3).

Table 3

Nitrogen content in the bark and wood of 'Elise' apple twigs

Treatment	Part of branch	N content [%]			
		2005	2006	2007	Mean
Control	Bark	1.41 a	1.43 ab	1.33 a	1.39 a
Control + N		1.48 a	1.35 a	1.29 a	1.37 a
2 % urea		1.48 a	1.50 bc	1.41 a	1.46 b
4 % urea		1.61 b	1.58 c	1.40 a	1.53 c
Control	Wood	0.84 a	0.69 ab	0.86 a	0.80 a
Control + N		0.84 a	0.58 a	1.04 b	0.82 ab
2 % urea		0.91 a	0.66 a	1.03 b	0.87 bc
4 % urea		1.06 b	0.80 b	0.90 a	0.92 c

In 2007 this trend was also observed, but was not statistically significant. In the wood samples only in 2005 application of 4 % urea statistical significantly augmented N content. The nitrogen content in bark was above 60 % higher than in wood tissues. An average (in 3 years of experiment) the amount of nitrogen in bark ranged between 1.37 % (control + N) to 1.53 % d.m. (4 % urea solution), while wood contained 0.80 % N (control) to 0.92 % d.m. (4 % urea solution). Khemira et al [23] reported that in mature spur and standard apple trees, very little N derived from autumn foliar applications was found in any tissues the following season. Authors showed that for trees with sufficient N at senescence, foliar applied urea may only replace leaves nitrogen that would normally be withdrawn, rather than augmenting it.

The phosphorus content in bark and wood of shoots was similar and independent on treatments in case of wood. Mean values of P in bark were 0.15–0.18 % d.m. (Table 4). The lowest P content was measured in bark of shoots collected from control treatment without N fertilization. In wood tissues the phosphorus content ranged from 0.16 % to 0.17 % d.m. The potassium content in bark as well as in wood did not affect by treatments. In wood measured above 40–46 % of K detected in bark (Table 4). The amount of Mg and Ca in wood tissues was much lower than in bark of apple twigs and amounted above 30 % and 23 % of bark content, respectively. The magnesium level in twigs wood tissues was not very differentiated following treatments, however statistical significant. The Mg content in bark and wood was higher in the control trees with soil applied N. These results are difficult to conclusive interpretation. The twigs collected

from trees without N fertilization (control) or sprayed with 2 % urea solution had significantly higher calcium content.

Table 4

Nutrient content in the bark and wood of 'Elise' apple twigs (mean of 3 years)

Treatment	Part of branch	P	K	Mg	Ca
		[% d.m.]			
Control	Bark	0.15 a	0.61 a	0.15 a	1.92 ab
Control + N		0.18 b	0.76 a	0.17 b	1.74 a
2 % urea		0.17 b	0.67 a	0.15 a	2.01 b
4 % urea		0.17 b	0.62 a	0.15 a	1.73 a
Control	Wood	0.16 a	0.28 a	0.04 ab	0.45 a
Control + N		0.17 a	0.30 a	0.05 c	0.42 a
2 % urea		0.16 a	0.28 a	0.04 a	0.44 a
4 % urea		0.16 a	0.29 a	0.05 bc	0.42 a

Yield

In 2005 and 2006 yield of trees was similar and ranged between 12.3 to 13.1 kg per tree, while in 2007 over twice higher yield was obtained (Table 5).

Table 5

Yield [kg per tree] of 'Elise' apple in 2005–2007

Combination	Yield [kg per tree]			
	2005	2006	2007	Sum of 2005–2007
Control	14.3	11.3	29.5	55.1
Control + N	11.1	13.9	27.1	69.5
2 % urea	13.3	13.1	30.6	57.0
4 % urea	14.0	11.1	34.4	59.5
Mean for year	13.1	12.3	30.4	
$LSD_{0.05}$ for Year		2.05		
$LSD_{0.05}$ for Treatment	ns	ns	ns	

Severe spring frost affected the fruit production for 2005 and 2006, whereas growing condition in 2007 was ideal with adequate rainfall during the summer and no significant frost/freeze conditions in the spring during bloom. Any year were not significant differences between treatments although, average yield from trees treated with 2 % and 4 % urea solutions was slightly higher (Fig. 1).

The growing season of 2006 was the warmest and driest. This year extremely dry month were recorded July and October. There were not favorable conditions to N uptake by roots and storing nitrogen in the woody tissues of the trees as proteins or

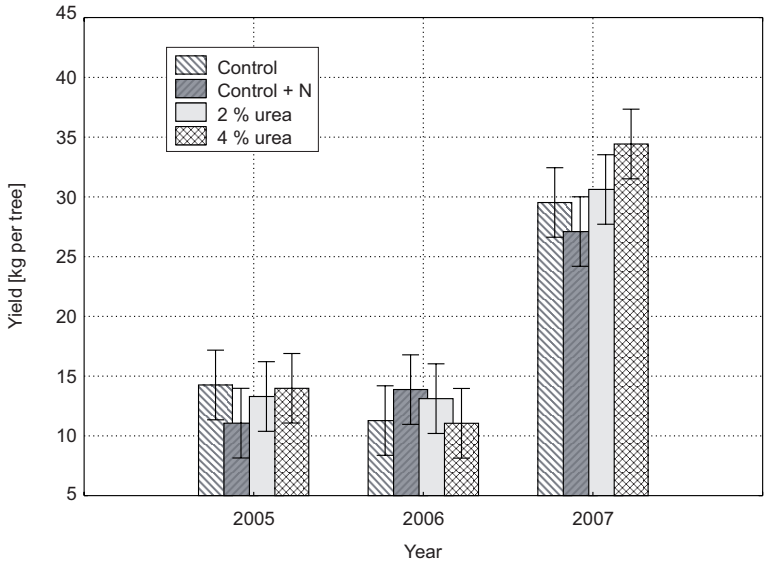

Fig. 1. Yield [kg per tree] of 'Elise' apple in 2005–2007

amino acids for the growth the following spring. Autumn urea-N applied could improve nitrogen sink for next year and affected yield in 2007. Fallahi et al [24] reported that a post-harvest urea application at the beginning of leaf senescence appears to be most efficient in providing N to the developing flower buds. Neilsen et al [9] showed that translocated nitrogen contributed 50 % of N in shoot leaves, 90 % of N in the spur leaves that subtend the fruit and 60 % of the nitrogen in the fruit.

Conclusion

Conditions which potentially limit the availability of nitrogen in the soil or their utilization (ie light textured soils, late fruit harvests, environmental conditions) represent situations in which foliar N application offers an important alternative means of supply for maintain nutrient N status. Post-harvest foliar N applications are typically made in summer/autumn to augment N reserves in the branches available to support the spring vegetative and reproductive growth occurring before the roots are capable of significant uptake of soil nitrogen. The results discussed here demonstrate the benefits of N post-harvest urea application in the average well-maintained apple orchard. In present study the nitrogen N status of apple trees was optimal or high N independent on treatments. Autumn foliar 4 % urea sprays slightly increased N content in twigs, but only in 2007 (after dry and warm 2006) affected fruit yield. There is some evidence that autumn application of urea would be most useful in trees with low nitrogen status. The results reported indicate that the use of foliar urea at the high concentration (4 % m/v) is more efficient way to supply N than 2 % solution.

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WPŁYW JESIENNEGO DOLISTNEGO NAWOŻENIA MOCZNIKIEM NA STAN ODŻYWIENIA MINERALNEGO JABŁONI ODMIANY ‘ELISE’

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Abstrakt: Badano wpływ jesiennego dolistnego nawożenia mocznikiem na zawartość makroskładników w liściach i pędach 5-letnich jabłoni odmiany ‘Elise’. Doświadczenie prowadzono w latach 2004–2007 w Garlicy Murowanej koło Krakowa. Zabiegi dolistnego nawożenia 2 % i 4 % roztworem mocznikiem wykonywano po zbiorze owoców w pierwszej dekadzie października. Określano plon owoców oraz zawartość azotu, fosforu, potasu, magnezu i wapnia w liściach i pędach jabłoni.

Przeprowadzone analizy materiału roślinnego wykazały, że we wszystkich latach prowadzenia badań stan odżywienia jabłoni azotem był optymalny lub duży, niezależnie od zastosowanego nawożenia. Jesienne pozakorzeniowe stosowanie mocznika zwiększało zawartość N w pędach jabłoni, ale tylko w 2007 r. (po suchym i gorącym roku 2006) zwiększyło plony owoców. Lepsze wyniki uzyskano, stosując większe stężenia mocznika (roztwór 4 %).

Słowa kluczowe: jabłka odmiany ‘Elise’, dolistne nawożenie mocznikiem, odżywienie mineralne

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INFLUENCE OF ECOLOGICAL FRIENDLY MEDIUMS ON CHEMICAL COMPOSITION OF GREENHOUSE-GROWN EGGPLANTS

WPŁYW EKOLOGICZNYCH PODŁOŻY NA SKŁAD CHEMICZNY OBERŻYNY W UPRAWIE SZKLARNIOWEJ

Abstract: The objective of the study was to examine the influence of ecological friendly growing mediums – coconut fiber and wood fiber – on chemical composition of eggplant (*Solanum melongena* L.), grown in the greenhouse, in comparison with standard rockwool medium. Cultivars used in the study were: 'Scorpio', 'Oscar', 'Tango' and DRA 2086. Fruits were harvested in June, at marketable maturity. There were determined in the fruits: soluble solids, dry matter, vitamin C, nitrates(V) and total phenolic compounds. Separation of phenolic acids was performed with HPLC. Antioxidant activity was determined with DPPH method. Nutrients content (N, P, K, Ca) in plants leaves was also determined. Results showed that growing mediums influenced some quality traits of the fruits, but genotype showed stronger influence on their chemical composition. Therefore, the two ecological friendly mediums are suitable for eggplant cultivation and have similar effect on chemical composition of plants, as standard rockwool medium.

Keywords: aubergine, ecology, growing mediums, greenhouse, phenols, nitrates, vitamin C, antioxidants

In temperate climate, eggplant (aubergine) can be grown successfully only in greenhouses or foil-made tunnels [1]. Eggplant fruits contain about 7 % of dry matter, 1 % of proteins, 4 % of carbohydrates, and also vitamins B_1, B_2, B_6 and C [2]. The fruits have relatively high amount of phenolics. There are reports on positive influence of phenolics on human health [3, 4]. Polyphenolic acids are components of plant lignins and tannins, but also occure in a free form [5]. During eggplant fruits development reducing total sugars, ascorbic acid, proteins and total phenolics contents increase [2, 6], and during fruits storage sugars content decreases [7]. Antioxidants in foods inhibit or delay the oxidation of other molecules and protect cells against the damaging effects of reactive oxygen species. Antioxidant activity of plant products has been reported in

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literature [3, 4, 8]. Main antioxidants found in plants are phenolic compounds (tocopherols, flavonoids and phenolic acids), carotenoids and ascorbic acid. Soilless plant cultures have been popular in vegetables production during the last decades because of their important advantages [9]. Up to now, in greenhouse cultivation rockwool slabs are most commonly used as a growing medium. This medium has inert characteristics, which is a valuable trait in cultivation. Troubles with rockwool utilization after completing growing cycle stimulate researches to introduce more environmentally friendly mediums. Since peat use in horticulture is negatively rated from ecological reason, other natural organic materials are looking for. Plant fiber has good physical and chemical characteristics for plant growing. Wood fiber and coconut fiber are especially perspective as ecological mediums [10]. Moreover, coconut fiber has a larger oxygen capacity than rockwool and good water holding ability, which makes it particularly suitable for hydroponic systems with intermittent watering cycles.

The aim of this work was to compare chemical composition of eggplants of four cultivars in relation to growing medium used in greenhouse cultivation. Two ecological mediums – coconut fiber and wood fiber were used, and compared with standard rockwool medium.

Material and methods

Eggplant hybrid cultivars used in the experiment were: 'Scorpio', 'Oscar', 'Tango' and DRA 2086 (De Ruiter Seeds). Fruits of 'Scorpio', 'Oscar' and DRA 2086 are of violet-black skin, and fruits of 'Tango' are of plain creamy-white skin. Growing mediums applied were: slabs made of coconut fiber (Ceres Intern.), slabs made of wood fiber (Steico SA) and standard slabs made of rockwool (Grodan BV). Slabs dimensions were 100 × 15 × 7.5 cm (length × width × height). The experiment was established in a random design, in three replicates, with 8 plants in each. Eggplants were planted in the middle of April. Drip irrigation system was used, and nutrients concentration in the solution, EC and pH were controlled and kept at uniform levels for all objects. The concentrations of nutrients in water were as follows: 140 mg NO_3-N, 70 mg P, 360 mg K, 60 mg Mg, 200 mg Ca, 2 mg Fe, 0.6 mg Mn, 0.3 mg B, 0.15 mg Cu, 0.3 mg Zn and 0.05 mg Mo in 1 dm^3. During fruits development (since June) temperature in the greenhouse ranged from 20–25 ^oC during the day to about 18–20 ^oC during the night. Fruits for the evaluation were harvested in June, at the peak of plants fruiting, at marketable maturity.

Nitrates (NO_3) in the fruits were determined with spectrophotometrical method, with Fiastar (Tecator, Sweden), at wavelength of λ = 440 nm. Vitamin C was determined with Tillmans' method. Soluble solids (^oBx) in raw and roasted fruits were determined with digital refractometer. Dry matter was determined by drying samples at 105 ^oC, until stable weight. Antioxidant activity was determined spectrophotometrically [4], as the percent of DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition in methanol extracts. Fresh fruit samples of 5 g were ground and extracted in methanol. Light inhibition was measured after 10 min of reaction, with the wavelength of λ = 517 nm. Total phenolic compounds were determined with Folin-Ciocalteu method [8]. The absorbance was read at λ = 725 nm, with UV Shimadzu spectrophotometer.

Free phenolic acids in the fruits were determined with HPLC. For this purpose, ground raw material was extracted with methanol in Büchi B-811. After evaporation of solvent, the residue was dissolved in methanol, filtered and subjected to HPLC. Shimadzu chromatograph with SPD-M10A VPDAD detector, equipped with Luna 5 μm C18(2) 250 mm × 4.6 mm column was used. The gradient of 10 % ACN(A) and 55 % ACN(B) was used. Injection volume was 1.0 $cm^3 \cdot min^{-1}$, oven temperature 36 ^oC, time of analysis 40 min.

Plants leaves were analyzed after growing cycle for macronutrients (N, P, K, Ca) content, according to Nowosielski [11].

Data obtained were subjected to the ANOVA with 'Statgraphics Plus 4.1' software. Tukey's HSD test was used to separate homogenous groups at $p = 0.05$.

Results and discussion

Nutrients availability for plants in greenhouse cultivation, related to soil conditions, is one of the most important factors influencing chemical composition of vegetables [12]. There is shortage of data concerning the influence of ecological friendly mediums on quality of vegetables grown under protection. Nitrates content in vegetables is of major concern due to negative influence on human health. Acceptable intake of nitrates was established by FAO/WHO Expert Committee on the level of 3.7 $mg \cdot kg^{-1}$ of body per day in 2002 [13]. In the study, nitrates(V) content in eggplant fruits was generally on a high level (about 400–500 $mg \cdot kg^{-1}$ f.m.), but on average the content was the lowest for plants grown in coconut fiber (Table 1). The lowest nitrates content was found in DRA 2086 fruits.

Vitamin C content in fruits was rather low (about 14–15 $mg \cdot 100\ g^{-1}$ f.m.), but typical to eggplant [2]. Fruits of plants grown in wood fiber showed the lowest vitamin C content, but the differences were only marginal. The highest dry matter content was characteristic to fruits from plants grown in coconut fiber, and the lowest for ones grown in wood fiber. The lowest dry matter content showed fruits of 'Tango' cv. The influence of mediums on soluble solids content was insignificant in the case of raw fruits and significant in the case of roasted fruits. Roasted fruits of plants grown in wood fiber showed the lowest soluble solids content. 'Oscar' cv. had fruits of the highest soluble solids content in the raw fruits from the examined cultivars.

Total phenolics content in the fruits was related to growing mediums and was the highest in the case of coconut fiber, where it reached level of 14 $mg \cdot g^{-1}$ d.m. in the case of fruits of 'Oscar'. On average, the highest content of phenolics showed fruits of 'Oscar' cv. Phenolic acids content in eggplant fruits can influence their sensory characteristics, and fruits high in these compounds are rated as more bitter [6]. Phenolic acids in the fruits were: chlorogenic acid, 3,4-dihydroxycinnamic acid and rosmarinic acid (Table 2). Chlorogenic acid was found in the highest amount. Sum of polyphenolic acids determined was much lower than total phenolics amount, and was related to growing mediums and cultivars. The influence of growing medium was evident in the case of chlorogenic acid, which was found in the lowest concentration in fruits of plants grown in coconut fiber. Chlorogenic acid concentration was the highest in fruits of 'Tango' cv.

Table 1

Chemical related traits of eggplant fruits as affected by growing mediums and cultivars

Cultivar	Growing medium	Nitrates [mg $NO_3 \cdot kg^{-1}$ f.m.]	Vitamin C [mg · 100 g^{-1} f.m.]	Total phenolics [mg · g^{-1} d.m.]	Dry matter [%]	Soluble solids	
						Raw fruits	Roasted fruits
						[°Bx]	
Scorpio	Coconut fiber	461	15.1	11.2	6.15	4.7	4.1
	Wood fiber	549	13.6	9.3	5.79	5.3	3.4
	Rockwool	530	14.9	7.5	6.13	4.3	3.4
Oscar	Coconut fiber	373	15.3	13.9	6.69	5.6	4.2
	Wood fiber	559	13.3	11.4	6.00	5.2	3.8
	Rockwool	504	15.3	11.8	6.61	6.2	4.7
Tango	Coconut fiber	447	15.2	9.6	6.07	5.2	3.4
	Wood fiber	449	13.7	9.5	5.33	4.7	2.8
	Rockwool	513	14.8	9.5	5.37	5.5	3.3
DRA 2086	Coconut fiber	433	15.0	11.1	6.63	5.5	4.3
	Wood fiber	469	13.3	8.1	6.30	5.3	3.7
	Rockwool	427	15.5	8.1	6.49	5.2	3.9
Means for mediums	Coconut fiber	429 a	15.2 b	11.5 b	6.39 c	5.3 a	4.0 b
	Wood fiber	507 b	13.5 a	9.6 a	5.86 a	5.1 a	3.4 a
	Rockwool	494 b	15.2 b	9.2 a	6.15 b	5.3 a	3.8 b
Means for cultivars	Scorpio	513 b	14.5 a	9.3 a	6.02 b	4.8 a	3.6 b
	Oscar	479 ab	14.6 a	12.4 b	6.43 c	5.7 c	4.2 c
	Tango	470 ab	14.5 a	9.5 a	5.59 a	5.1 b	3.2 a
	DRA2086	443 a	14.6 a	9.1 a	6.47 c	5.3 b	4.0 c

Note: means which do not differ according to Tukey's HSD test at p = 0.05 are marked with the same letters.

Table 2

Free phenolic acids in raw eggplant fruits as affected by growing mediums and cultivars [mg · 100 g^{-1} d.m.]

Cultivar	Growing medium	Chlorogenic acid	Dihydroxycinnamic acid	Rosmarinic acid	Total free phenolic acids
Scorpio	Coconut fiber	179.4	1.0	1.6	182.0
	Wood fiber	160.4	0.6	1.2	162.2
	Rockwool	205.0	1.5	1.1	207.5
Oscar	Coconut fiber	180.7	0.7	0.7	182.1
	Wood fiber	170.8	0.7	1.5	173.0
	Rockwool	194.6	1.2	0.7	196.5
Tango	Coconut fiber	197.9	1.5	1.7	201.2
	Wood fiber	378.8	1.8	1.2	381.8
	Rockwool	345.8	1.9	1.6	349.4
DRW 2086	Coconut fiber	161.8	0.6	1.5	163.8
	Wood fiber	221.4	1.0	0.6	223.0
	Rockwool	154.4	0.5	1.0	155.9
Means for mediums	Coconut fiber	180.0 a	1.0 a	1.4 a	182.3 a
	Wood fiber	232.9 b	1.0 a	1.1 a	235.0 b
	Rockwool	225.0 b	1.3 a	1.1 a	227.3 b
Means for cultivars	Scorpio	181.6 a	1.0 a	1.3 a	183.9 a
	Oscar	182.0 a	0.9 a	1.0 a	183.9 a
	Tango	307.5 b	1.7 b	1.5 a	310.8 b
	DRA2086	179.2 a	0.7 b	1.0 a	180.9 a

Note: see Table 1.

The DPPH method of antioxidant activity (AA) determination is based on free radicals scavenging by plant extracts and is used in direct comparison of plant raw materials in this respect [14]. AA of eggplant fruits was generally rather low and did not relate to growing mediums, but to genotype only (Table 3). AA was the highest for fruits of 'Scorpio' cv., but was only a little higher than that for other cultivars. Phenolic compounds are believed to be very active free radicals scavengers [3]. However, it seems that AA of the fruits used in the study did not related to phenolics content.

Table 3

Antioxidant activity of raw eggplant fruits in relation to growing mediums and cultivars [% DPPH]

Cultivar	Growing medium			Means for cultivars
	cocos fiber	wood fiber	rockwool	
Scorpio	9.72	7.93	7.16	8.27 b
Oscar	7.09	6.27	5.82	6.39 a
Tango	4.64	5.66	5.52	5.27 a
DRA2086	4.64	7.29	5.79	5.91 a
Means for mediums	6.52 a	6.79 a	6.07 a	

Note: see Table 1.

Amount of nutrients accumulated in eggplant leaves during growing cycle was similar for plants cultivated in the three mediums (Table 4). Small differences were found in the case of nitrogen and phosphorus content, which were lower in plants grown in rockwool slabs than in plants grown in the two ecological-friendly mediums.

Table 4

Nutrients content in eggplant leaves after growing cycle, in relation to growing mediums [mg · g^{-1} d.m.]

Nutrient	Growing medium		
	coconut fiber	wood fiber	rockwool
NO_3-N	1.00 b	1.10 b	0.80 a
P	0.55 b	0.62 b	0.38 a
K	3.50 a	3.50 a	3.51 a
Ca	0.47 a	0.45 a	0.49 a

Note: see Table 1.

Conclusion

Kind of growing medium used in greenhouse influenced some quality attributes of eggplant fruits, including their chemical composition, but the differences between the fruits related to growing mediums were small, compared with the differences related to cultivars. Basic nutrients contents in plants leaves, related to growing mediums, were similar, except nitrogen and phosphorus. Generally, all three growing mediums proved their suitability in eggplant cultivation. Therefore, it can be concluded that both ecological-friendly mediums, ie coconut fiber and wood fiber, could be used in horticultural practice as the replacement of standard rockwool medium for growing eggplant in greenhouses.

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WPŁYW EKOLOGICZNYCH PODŁOŻY NA SKŁAD CHEMICZNY OBERŻYNY W UPRAWIE SZKLARNIOWEJ

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Abstrakt: Celem pracy było zbadanie wpływu dwóch ekologicznych podłoży – włókna kokosowego i włókna drzewnego na skład chemiczny oberżyny (*Solanum melongena* L.) w uprawie szklarniowej, w porównaniu z konwencjonalnym podłożem z wełny mineralnej. W badaniach użyto odmian 'Scorpio', 'Oscar', 'Tango' i DRA 2086. Owoce zbierano w czerwcu, w fazie dojrzałości handlowej. W owocach oznaczano zawartość ekstraktu, suchą masę, witaminę C, azotany(V) oraz związki fenolowe ogółem. Rozdział kwasów polifenolowych przeprowadzono metodą HPLC. Aktywność antyoksydacyjną określono metodą DPPH. Oznaczono zawartość składników pokarmowych (N, P, K, Ca) w liściach pod koniec wegetacji.

Rodzaj podłoża miał wpływ na niektóre cechy jakościowe owoców, ale genotyp wykazywał większy wpływ na jakość oberżyny. Badane ekologiczne podłoża nadają się do uprawy oberżyny, wpływając na jakość owoców w sposób zbliżony do podłoża z wełny mineralnej.

Słowa kluczowe: oberżyna, ekologia, podłoża, szklarnia, związki fenolowe, azotany, witamina C, antyoksydanty

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PHYTOREMEDIATION OF ZINC, LEAD AND CADMIUM RICH POST-FLOTATION TAILINGS USING TREE CLONES

FITOREMEDIACJA ODPADÓW POFLOTACYJNYCH O DUŻEJ ZAWARTOŚCI CYNKU, OŁOWIU I KADMU Z WYKORZYSTANIEM KLONÓW ROŚLIN DRZEWIASTYCH

Abstract: It was tested the usefulness of *Betula pendula*, *Prunus cerasus* L.'Tabel® Edabriz', *Prunus domestica* 'Dabrowicka purple plum' and *Taxus baccata* clones in removal of metallic elements from post flotation tailings contaminated with cadmium, lead and zinc.

Obtained results indicated a certain potential in that respect of plants belonging to the genus *Prunus*, therefore it is recommended to monitor carefully the orchard farms located within impact of metalliferous dusts containing heavy metals. Examined clone of *B. pendula* expressed the ability to take up and accumulate relatively high amounts of Cd, Pb and Zn in roots with their further transfer to aboveground organs. Tested genotype of *Taxus baccata* proved to be inappropriate for this purpose. It can be only considered efficient to stabilize spoil shelves and slopes in order to prevent wind and water erosion.

Keywords: industrial wastes, zinc, lead, cadmium, phytoremediation, tree clones

Urban, industrial, and agricultural human activities are ever increasing source of environmental pollution, especially by both organic pollutants and heavy metals. In the Olkusz district, located on the border of the areas of Krakow-Czestochowa Jura and Silesia Upland, mining and metallurgical engineering have led to environmental degradation. During the exploitation of zinc and lead ore deposits the post-flotation tailings are formed, which constitute inappropriate substratum for plant growth because of low water capacity, the susceptibility to wind erosion, and elevated levels of lead, zinc and cadmium compounds. In settling ponds, with the surface of about 100 ha, 38 millions tons (38 Tg) of wastes are accumulated, containing about 1.0 % of Zn, 0.5 % of Pb, and 77 % of dolomite. They are the cause of dust pollution emission to the

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atmosphere. Presently, efficient technologies are needed that can provide the decrease of heavy metal content, mainly of lead and cadmium, up to the level allowing the use of stored dolomite in the agriculture [1–4]. The plant-assisted bioremediation of contaminants is environmentally friendly and cost-effective technique. The procedure, seen as a form of ecological engineering, depends on synergistic relationships among plants, microorganisms and the environment. Thus, phytoremediation is a promising pro-ecological method of air, soil or other substrata and groundwater detoxification. Actually it is tended towards exploiting vascular plants to increase effectiveness of *in situ* inorganic contaminants remediation. Tree species have range of features, which make them the possible candidates for application in phytoremediation techniques, especially *Salix* species, have the great potential for cadmium phytoextraction [5–9]. Another tree species that have been also shown to be promising plant material to clean-up toxic levels of metals are: *Acer pseudoplatanus* L., *Betula pendula* Roth., *Populus alba* L., *Populus deltoids* Bartr. ex Marsh, *Prunus virginiana* L. [10, 11].

In presented study candidate tree clones were selected to test remediation effectiveness from calamine waste heap rich in zinc, lead and cadmium compounds. Some deciduous trees, and the coniferous *T. baccata*, were included in the experimental design to assess their usefulness for zinc, lead and cadmium bioaccumulation.

Materials and methods

Plant material constituted clone B1of *Betula pendula* Roth (*Betula verrucosa* Ehrh.), clone E2 of *Prunus cerasus* L.'Tabel® Edabriz', clone WD3 of *Prunus domestica* L. 'Dabrowicka purple plum' and clone C5 of *Taxus baccata* L. Respective plant material was obtained *in vitro* from stabilized shoot cultures and acclimatized to *ex vitro* in the greenhouse conditions.

Experimental plots were established on the shelf of the settling pond formed from material disposed after zinc and lead ores flotation in Bukowno near Olkusz, at the altitude of 308 m a.s.l. (N 50° 16.856′ E 19° 30.204′), with eastern exposure. The three year experiment was carried out in three replications, using randomized blocks method. On the substratum, coming from the active settling pond, 30 two year old plantlets of each clone were planted without any preliminary treatments. A substratum control constituted the unplanted plot. Plants were spaced in the distance 1 × 1 m. They were placed into holes with the soil clod directly onto post-flotation substratum, covering with post-flotation materials. During the whole experiment plants were neither tended nor fertilized. At the end of the three vegetation periods experiment analyses of both plant material and substrata were performed.

From every replication one mixed plant sample, from 5 vigorously growing plants, separately for roots and shoots of each clone was taken. Plant samples, collected in May 2008, were washed with running water, and afterwards were rinsed thoroughly with distilled water, dried to constant mass, ground and dry-ashed at 450 °C. Simultaneously and from each replication, substratum samples were taken from 0–30 cm layer of a rooting zone of trees acquired for plant analyses, as well as from the same depth of the control plot. The substratum samples were air dried and the following analyses were

performed: pH potentiometrically, total nitrogen and carbon contents with the use of TOC-TN 1200 Thermo Euroglas apparatus. The content of organic carbon was calculated as a difference between total and inorganic carbon. The total contents of Zn, Pb and Cd were determined in plant and substratum samples, after digestion in the mixture of nitric(V) and chloric(VII) concentrated acids, with the use of an atomic emission spectrophotometer with inductively coupled argon plasma ICP-AES JY 238 ULTRACE using ICP multi-element standard solution IV (Merck). The accuracy of analytical methods was verified with the reference to the certified reference material GSS-8 (GBW 07408 – State Bureau of Meteorology, Beijing, China). Chemical analyses of plants and substrata were performed in three replicates. The results were subjected to STATISTICA 6.1, ANOVA analysis and a posteriori Fisher's test was used to study differences between respective variants at the significance level $\alpha = 0.05$.

Results and discussion

Selected characteristics of substrata taken from the rooting zone of tree clones are shown in Table 1. In all studied samples pH values were equal or higher than 7.5, and they revealed low organic carbon contents ranging from 0.68 to 1.001 $g \cdot kg^{-1}$. They were statistically differentiated by nitrogen content, which varied from 0.029 $g \cdot kg^{-1}$ (mean value from control plots) to 0.049 $g \cdot kg^{-1}$ (plots planted with birch). Studied substrata were characterized by a very strong contamination with cadmium, zinc and lead (Table 2). Determined contents of these elements exceeded limiting levels defined by the Ministry of the Environment in the directive for industrial lands issued on 9 September 2002 [12] about 5 fold for Cd, 8 fold for Zn and from 5 to 8 fold for Pb. Substrata taken from the rooting zone of respective tree clones, analyzed after the period of plant cultivation, were diversified considering zinc and lead contents whereas contents of cadmium were still on the same level likewise in the substratum taken from not planted, control plots. Definitely the highest Pb content was found in the substratum sample from *P. domestica* 'Dabrowicka purple plum' plots, while contents of all studied heavy metals determined in the sample from plots planted with *Taxus baccata* were equivalent to the level of their content in the control plots.

Table 1

Mean values of pH, organic carbon and total nitrogen contents in studied substrata

Substratum	pH in 0.01 $mol \cdot dm^{-3}$ $CaCl_2$	Organic C contents $[g \cdot kg^{-1}]$	Total N contents $[g \cdot kg^{-1}]$
B1*	7.6^{a} **	0.788^{a}	0.049^{d}
E2	7.7^{a}	0.967^{a}	0.041^{d}
WD3	7.6^{a}	0.680^{a}	0.035^{abc}
C5	7.7^{a}	1.001^{a}	0.033^{ab}
K6	7.5^{a}	0.796^{a}	0.029^{a}

* Sample of substratum taken from rooting zone of plants from plots planted with: B1 – *Betula pendula*, E2 – *Prunus cerasus*, WD3 – *Prunus domestica* 'Dabrowicka purple plum', C5 – *Taxus baccata*, K6 – sample taken from the control plot, not planted from the depth of 0–20 cm; ** The different letters indicate statistically significant differences among mean values.

Table 2

Total contents of heavy metals in plant organs of studied tree clones and in substratum analyzed after the period of plants cultivation (mean ± standard deviation of the mean)

Material	Cd	Pb	Zn
	[mg · kg^{-1}]		
Roots – B1*	11.53 ± 0.95^{a}	495.69 ± 9.31^{ab}	1695.3 ± 96.9^{b}
Roots – E2	12.40 ± 0.62^{a}	691.59 ± 0.70^{abc}	1750.7 ±52.7^{b}
Roots – WD3	21.63 ±0.66^{c}	1115.3 ± 0.82^{c}	3875.9 ± 53.4^{c}
Roots – C5	19.93 ± 1.16^{b}	296.0 ± 5.70^{a}	1148.9 ± 50.28^{a}
Shoots – B1*	13.67 ± 0.86^{c}	1365.1 ± 7.23^{d}	1470.4 ± 16.7^{d}
Shoots – E2	1.74 ± 0.55^{b}	372.6 ± 0.91^{c}	760.1 ± 1.57^{c}
Shoots – WD3	2.51 ± 0.46^{b}	212.5 ± 2.33^{b}	493.0 ± 3.31^{b}
Shoots – C5	0.16 ± 0.04^{a}	19.9 ±1.29^{a}	53.8 ± 1.93^{a}
Substratum – B1**	72.79 ± 062^{a}	3010.0 ± 40,2^{ab}	8267.7 ± 111.1^{b}
Substratum – E2	74.00 ± 2.35^{a}	3819.0 ± 26,5^{c}	8113.6 ± 59.1^{b}
Substratum – WD3	75.43 ± 1.20^{a}	4935.3 ± 53.3^{d}	7825.5 ± 31.1^{a}
Substratum – C5	73.62 ± 3.00^{a}	3158.5 ± 201.6^{b}	8197.0 ±112.1^{b}
Substratum – K6	73.79 ± 0.76^{a}	3179.2 ± 5.73^{b}	8155.4 ± 171.7^{b}

* Samples of plant organs taken from respective tree clones; **Sample of substrate taken from rooting zone of plants from plots planted with: B1 – *Betula pendula*, E2 – *Prunus cerasus*, WD3 – *Prunus domestica* 'Dąbrowicka purple plum', C5 – *Taxus baccata*, K6 – sample taken from the not planted control plot; The different letters indicate statistically significant differences among mean values.

The results of cadmium, lead and zinc contents of the roots and shoots of B1 clone of *B. pendula*, E2 clone of *P. cerasus* L.'Tabel® Edabriz', WD3 clone of *P. domestica* 'Dąbrowicka purple plum', and C5 clone of *T. baccata* are given in Table 2. Comparison of heavy metals contents in plant clones resulted in significant differences in both underground and aboveground organs. The most effective in the accumulation of studied heavy metals in roots proved to be *Prunus domestica* clone. Contents determined in roots amounted to 21.63 mg Cd · kg^{-1} d.m., 1115.3 mg Pb · kg^{-1} d.m. and 3875.9 mg Zn · kg^{-1} d.m.. The lowest content of lead and zinc contents were determined in roots of *Taxus baccata* (Table 2). Considering aboveground plant parts, the highest levels of cadmium (13.67 mg · kg^{-1} d.m.), lead (1365.1 mg · kg^{-1} d.m.) and zinc (1470.4 mg · kg^{-1} d.m.) were found in shoots of birch. There were also determined relatively high contents of lead and zinc in shoots of *Prunus cerasus*, that is: 372.6 and 760.1 mg · kg^{-1} d.m., respectively. Surprisingly, the lowest contents of all studied elements were determined in shoots of *Taxus baccata.* Summing up, Table 2 shows that apart from *B. pendula*, in aboveground parts of plant material used in the experiment contents of cadmium, lead and zinc were noticeably lower than in roots.

High-biomass crops, comprising trees, are promising as phytoextractors. The biotechnological approaches, in this respect, are aimed at obtaining GMO trees, with the combinated trait of high uptake of heavy metals or high tolerance to such pollutants, to enhance plant survival on contaminated sites [7, 11, 13, 14]. Generated transgenic lines up till now have been tested under artificial conditions. As far as the field experiments

with tree clones are concerned, only a material obtained in a traditional way is applied. Moreover, trees used in this kind of experiments belong to genera commonly used for reclamation such as: *Populus, Salix, Alnus*, *Acer, Betula, Fraxinus, Robinia, Quercus* [11, 15]. In order to check the usefulness of woody plants to heavy metal phytoextraction, in presented experiment were compared the effectiveness of *Betula,* frequently used for these purposes with E2 clone of *P. cerasus* and WD3 clone of *P. domestica*. As far representatives of *Rosaceae* family have not been tested under such extreme conditions. With the aim of obtaining different accumulation of heavy metals was also used *Taxus*, which, in contrast to the above-mentioned clones, is characterized by an insignificant biomass increase. It was proved that only the B1 clone of *Betula pendula* was efficient in transfer of cadmium and lead to aboveground parts. Rosselli et al [11], working with decidous tree clones on metal contaminated soil, have found that *Betula* transferred to leaves elevated amounts of zinc and cadmium. Whereas Meers et al [16] have determined as effective only the clones of *Salix dasyclados* 'Loden', *S. fragilis* 'Belgish Rood' and *S. schwerinii* 'Christina' out of five other tested. The other difficulty is how long period of time would be needed to get the ground purified. Nevertheless, even though the introduction of trees would not give immediately positive results in that respect, it is also of great importantce to stabilize in such a way spoil shelves and slopes in order to prevent wind and water erosion.

The great endanger is brought about by high cadmium levels, so this element is presently particularly studied, especially in respect of biological quality of edible crop organs [17–20]. Next to cadmium, lead is frequently reported to have the highest impact on organisms. According to Gaweda [21] lead accumulated in plant tissue significantly decreased the content of physiologically important components of cells. The mechanisms of this phenomenon are intensively studied [22, 23]. The presented data showed a certain potential for application of *Prunus domestica* and *P. cerasus* clones in phytoremediation. At the same time, it is worth underlined that the ability of heavy metals accumulation in aboveground organs poses a threat to transfer of metals to fruits. Thus there is a considerable risk that yield coming from orchard cultivations located within the range of the emitter impact needs to be carefully monitored.

Conclusions

1. *Betula pendula*, *Prunus cerasus* L.'Tabel® Edabriz', *Prunus domestica* 'Dabrowicka purple plum' and *Taxus baccata* varied widely when assessed for effectiveness of contaminant remediation.

2. The studied *Prunus* clones revealed ability to heavy metal accumulation in their organs therefore it is recommended to monitor of orchard farms located within the range of metalliferous dust impact.

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FITOREMEDIACJA ODPADÓW POFLOTACYJNYCH O DUŻEJ ZAWARTOŚCI CYNKU, OŁOWIU I KADMU Z WYKORZYSTANIEM KLONÓW ROŚLIN DRZEWIASTYCH

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Abstrakt: Testowano przydatność klonów *Betula pendula*, *Prunus cerasus* L.'Tabel® Edabriz', *Prunus domestica* 'Węgierka Dąbrowicka', i *Taxus baccata* do usuwania metali ciężkich z materiałów odpadowych zanieczyszczonych kadmem, ołowiem i cynkiem.

Uzyskane wyniki wskazują na pewien potencjał roślin z rodzaju *Prunus* w tym zakresie. Z tego względu należy prowadzić monitoring upraw sadowniczych zlokalizowanych w zasięgu oddziaływania metalonośnych pyłów zawierających metale ciężkie. Wykorzystany w badaniach klon brzozy wykazał zdolność pobierania i akumulowania względnie dużych ilości Cd, Pb i Zn w korzeniach i ich przemieszczania do pędów. Testowany klon *Taxus baccata* okazał się nieprzydatny do usuwania tych pierwiastków z odpadów poflotacyjnych i może być jedynie wykorzystany do stabilizacji półek i zboczy w celu zapobieżenia erozji eolicznej i wodnej.

Słowa kluczowe: odpady przemysłowe, cynk, ołów, kadm, fitoremediacja, rośliny drzewiaste

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NITROGEN FERTILISATION OF *Stewartia pseudocamellia* CULTIVATED IN SUBSTRATES OF DIFFERENT REACTION

NAWOŻENIE AZOTEM STEWARCJI KAMELIOWATEJ *Stewartia pseudocamellia* UPRAWIANEJ W PODŁOŻU O ZRÓŻNICOWANYM ODCZYNIE

Abstract: The study was conducted on Stewartia (*Stewartia pseudocamellia* Maxim.) plants, grown in containers under field conditions. The shrubs were fertilised with two nitrogen forms: ammonia NH_4^+ and nitrate NO_3^- at the substrate pH values of 3.9 and 5.5. The plants were top-dressed with reference to the analysis of the substrate. During the vegetation season the leaves were harvested twice in order to analyse the content of easily soluble forms and nutrients.

In the experiment a gradual increase of acidity and salt content in the substrate was observed, as compared with the original content. After the first and second year of cultivation, low content of mineral nitrogen in the substrates was noticed. In both years of cultivation, higher contents of phosphorus and lower contents of potassium in the substrates of shrubs fertilised with ammonia form were detected. The content of easily soluble forms of elements in Stewartia leaves remained practically unchanged throughout vegetation. The study revealed a very low content of mineral nitrogen in the leaves of Stewartia. The contents of easily soluble forms of nutrients (K, Ca, Mg) determined in the leaves varied with reference to both the substrate acidity and the form of nitrogen fertiliser used. The total nutrients contents, except of the total nitrogen content, were comparable in the first and second harvest. The content of total phosphorus and magnesium in the leaves was dependent on the nitrogen form, higher contents were noted in objects fertilised with ammonia form. However, Stewartia showed greater uptake of potassium, when fertilised with the nitrate form. The experiments also revealed the effect of substrate acidity on the uptake of potassium by the plants.

Keywords: nitrogen, pH, macroelements content, *Stewartia pseudocamellia*

Stewartia pseudocamellia Maxim., the *Theaceae* family genus [1], is rarely grown in Polish nurseries and gardens. In the USA, the Stewartia cultivars (including the interspecies hybrids) were available on the market as early as in 1992 [2]. The

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genus has been widely propagated and cultivated also in Japan [3]. The small size of the shrubs, characteristic flowering at the end of June and in July, as well as attractive colouring in the autumn, contributed to the wider popularity of the genus from Japan and Korea. What hinders its wider cultivation in Poland is the unavailability of seeds and the lack of knowledge as to the nutrition and fertilisation requirements of the plants.

The aim of the present study was to specify the effect of fertilisation with different forms of nitrogen on the macroelements nutrition of Stewartia plants grown on a substrate of pH values 3.9 and 5.5.

Materials and methods

The study was conducted in 1998–1999 on four- or five-year-old plants of Stewartia (*Stewartia pseudocamellia* Maxim. var. koreana), grown in 5 dm^3 containers in field conditions.

The two-factor experiment was conducted using the random block method in four replications. Each treatment included 10 plants per replication. Factor I was nitrogen form: ammonia NH_4^+ (using ammonium sulphate, ammonium phosphate) and nitrate NO_3^- (using calcium nitrate, potassium nitrate). Factor II was the substrate pH values: 3.9 and 5.5.

The pH = 3.9 substrate was a mixture of peat and fine composted pine bark, mixed in a 2:1 (v/v) ratio. The pH = 5.5 substrate was the substrate made on the basis of the first one deacidified with calcium carbonate. Before (1st year – April), during (May, June) and at the end of vegetation season (September) the substrate was analysed after extraction with 0.03 mol $\cdot$ dm^{-3} acetic acid. The following nutrient contents in the substrate were adopted: 200 mg N, 70 mg P, 160 mg K and 20 mg Mg $\cdot$ dm^{-3}. The substrate acidity was determined as pH with potentiometric method (at 1:2 substrate to water ratio) and the electrolytic conductivity (EC) was measured with a conductometer [4]. During the season the plants were top-dressed twice (May, June) according to substrate analysis, using ammonium sulphate, ammonium phosphate and potassium sulphate in the objects with NH_4-N form, as well as calcium nitrate, potassium nitrate and triple superphosphate in the rest of the objects. In the middle (July) and at the end of vegetation season (September, before foliage discolouration) the leaves picked of the middle part of the twigs were subjected to analysis. Following drying, the leaves were analysed for the content of easily soluble forms of nutrients (in 2 % acetic acid) and after dry combustion, they were examined for the total content of components (except N). Mineral nitrogen was measured with Bremner's micromethod (modified by Starck), and total nitrogen by means of automatic distillation with boric acid after wet mineralisation. The content of phosphorus was determined with molybdenic-vanadic method and the concentration of K, Ca and Mg by means of the spectrophotometry of atomic absorption [4]. An overhead microemitters system was installed to be applied during vegetation season, and the soil humidity was maintained at ca 70 %.

Results

During the experiment the substrate acidity level increased by 0.11 (at pH = 5.5 and the NH_4-N form) to 1.57 (at pH = 3.9 and the NO_3-N form) in the first year and by 0.03 to 1.8 in the second year, with NH_4-N at pH = 5.5 and NO_3-N at pH = 3.9, respectively, as compared with the initial levels (Table 1). During the two-year cultivation, a gradual increase in the electrolytic conductivity in the substrate was observed.

Table 1

Changes of substrate pH and total salt concentration measured as EC [dS · m^{-1}] during Stewartia cultivation

Object	Initial		After 1st year of cultivation		After 2nd year of cultivation	
	pH	EC	pH	EC	pH	EC
NH_4-N; pH = 3.9	3.9	0.15	4.52	0.21	4.53	0.56
NO_3-N; pH = 3.9	3.9	0.10	5.47	0.13	5.70	0.46
NH_4-N; pH = 5.5	5.5	0.15	5.61	0.16	5.53	0.62
NO_3-N; pH = 5.5	5.5	0.10	6.29	0.16	6.06	0.53

The content of mineral nitrogen balanced from 18.4 to 40.3 mg · dm^{-3} after the first year of cultivation and from 7.0 to 9.3 mg · dm^{-3} after the second year (Table 2). The highest nitrogen content was noticed in the object fertilised with the ammonia form at pH = 3.9, whereas the lowest content was found in the substrate where the nitrate form was used at pH = 5.5. After the two-year cultivation a greater amount of nitrogen was determined in the substrates of initial pH = 3.9.

The phosphorus content in the two years of cultivation was higher in the objects where the ammonia form was used.

Table 2

Changes of forms of mineral components contents [mg · dm^{-3}] in the substrate during Stewartia cultivation

Object	NH_4^+	NO_3^-	$N_{min.}$	P	K	Ca	Mg
Initial							
	24.5	14.0	38.5	20.1	66.6	192.5	41.3
After 1st year of cultivation (IX 1998)							
NH_4-N; pH = 3.9	19.3	21.0	40.3	29.1	48.2	466.8	63.7
NO_3-N; pH = 3.9	10.5	11.4	21.9	17.8	51.1	625.9	73.5
NH_4-N; pH = 5.5	12.3	13.1	25.4	26.1	44.3	813.4	74.8
NO_3-N; pH = 5.5	7.9	10.5	18.4	16.4	67.2	1096.2	80.1
After 2nd year of cultivation (IX 1999)							
NH_4-N; pH = 3.9	9.3	0.0	9.3	32.5	94.9	705.8	104.4
NO_3-N; pH = 3.9	5.8	3.5	9.3	21.0	105.2	1144.4	149.7
NH_4-N; pH = 5.5	7.0	0.0	7.0	32.0	88.0	949.1	80.3
NO_3-N; pH = 5.5	7.0	0.0	7.0	23.7	128.3	1632.4	164.3

Greater content of available potassium in the substrate was observed when NO_3-N form was used, although the content of potassium was greater at the higher pH value. When fertilised with ammonia form, greater contents of potassium were noted in less acidic substrate. The calcium content in the substrates after two-year cultivation presented the lowest value in the NH_4-N and pH = 3.9 object, and the highest value in the one of NO_3-N and pH = 5.5. The content of available magnesium after the first year of cultivation ranged from 63.7 to 80.1 mg · dm^{-3}, with respect to ammonia form at pH = 3.9 and nitrate form at pH = 5.5. After two years of cultivation the contents levelled higher at the values from 80.3 (NH_4^+ and pH = 5.5) to 164.3 mg · dm^{-3} (NO_3^- and pH = 5.5).

In the middle and at the end of the vegetation season, in the first and second year of cultivation, Stewartia leaves were subjected to analysis. Changes in the content of soluble forms of nutrients were presented with reference to the first year of the study (Table 3).

Table 3

Changes of mineral form of macroelements contents [% d.m.] in Stewartia leaves during 1st year of cultivation[1]

Object	NH_4-N	NO_3-N	K	Ca	Mg
In the middle of vegetation (VII 1998)					
NH_4-N; pH = 3.9	0.014c[2]	0.0063a	1.32ab	0.40a	0.48a
NO_3-N; pH = 3.9	0.006a	0.0060a	1.50ab	0.54b	0.48a
NH_4-N; pH = 5.5	0.010bc	0.0050a	1.26a	0.39a	0.46a
NO_3-N; pH = 5.5	0.008ab	0.0080a	1.64b	0.58b	0.40a
After vegetation (IX 1998)					
NH_4-N; pH = 3.9	0.0078b	0.0093a	1.30ab	0.47ab	0.47b
NO_3-N; pH = 3.9	0.0060a	0.0088a	1.27ab	0.52b	0.38ab
NH_4-N; pH = 5.5	0.0063ab	0.0065a	0.87a	0.44a	0.37ab
NO_3-N; pH = 5.5	0.0070ab	0.0088a	1.72b	0.53b	0.31a

[1] P was not determined, due to analytical difficulties; [2] means marked by the same letter do not differ significantly at p = 0.05.

The content of soluble nutrients in Stewartia leaves stayed at approximately the same level throughout vegetation. The content of NH_4-N and NO_3-N was very low. The greatest amount of ammonia nitrogen was noticed in the object fertilised with NH_4^+ form of nitrogen at pH = 3.9 value, whereas the smallest amount was found in the one where calcium nitrate was used in the substrate of the same acidity level. No statistical differences were shown in the content of nitrate nitrogen in the objects. Potassium content was greatest in the leaves of plants fertilised with nitrate form at the pH = 5.5 value, and lowest when ammonium sulphate was used in the substrate of the same acidity. Greater amounts of calcium, both in the middle and at the end of vegetation season, were observed in objects fertilised with calcium nitrate. In the middle of vegetation season no significant differences in the content of magnesium in Stewartia leaves were noticed. However, at the end of the season, the highest magnesium value

was registered in the leaves of plants grown in the substrate of pH = 3.9 value and fertilised with NH_4-N form of nitrogen. The smallest amount of magnesium was determined at NO_3-N used in the substrate of greater acidity.

Table 4

Changes of total macroelements contents [% d.m.] in Stewartia leaves during cultivation

Object	N	P	K	Ca	Mg
In the middle of 1st year of cultivation (VII 1998)					
NH_4-N; pH = 3.9	2.10a[1]	—[2]	1.85ab	0.50a	0.50a
NO_3-N; pH = 3.9	2.26a	—	1.95ab	0.58a	0.45a
NH_4-N; pH = 5.5	2.22a	—	1.53a	0.53a	0.50a
NO_3-N; pH = 5.5	2.11a	—	2.15b	0.56a	0.42a
After 1st year of cultivation (IX 1998)					
NH_4-N; pH = 3.9	2.17b	—	1.68ab	0.54ab	0.48b
NO_3-N; pH = 3.9	1.85a	—	1.64ab	0.58b	0.33a
NH_4-N; pH = 5.5	1.95ab	—	1.56a	0.47a	0.39ab
NO_3-N; pH = 5.5	1.77a	—	1.84b	0.59b	0.33a
After 2nd year of cultivation (IX 1999)					
NH_4-N; pH = 3.9	1.39a	0.24b	1.46ab	1.29a	0.38b
NO_3-N; pH = 3.9	1.31a	0.14a	1.24a	1.94b	0.24a
NH_4-N; pH = 5.5	1.48a	0.21b	1.12a	1.41a	0.40b
NO_3-N; pH = 5.5	1.47a	0.13a	1.80b	2.13b	0.21a

[1] Means indicated by the same letter do not differ significantly at p = 0.05; [2] not determined due to analytical difficulties.

The total nutrients contents, except of the total nitrogen, were comparable in the first and second leaves harvest (Table 4). In the middle of vegetation, the content of nitrogen balanced between 2.10 and 2.26 % d.m. and did not differ significantly in the objects. At the end of vegetation season, the content of total nitrogen in the leaves began to decrease. Moreover, a significant difference was noticed between the analysed objects. The greatest amount was noticed when the plants grown in the substrate of pH = 3.9 value were fertilised with ammonium form of the fertiliser, and the smallest amount of total nitrogen was present in the leaves of objects in which calcium nitrate was used at both levels of substrate acidity. After the second year of cultivation, no significant differences in the nitrogen content in leaves were noticed. At the end of the second vegetation season, the leaves of the objects where ammonium form was used showed nearly twice as high content of total phosphorus as those fertilised with the nitrate form. The total content of potassium in all three measurements was the lowest in the leaves of plants fertilised with ammonium form in the substrate of higher acidity. The greatest amount of total potassium was detected in the plants fertilised with the nitrate form at the pH = 5.5 level. In the middle of vegetation period, no statistically significant differences were noticed in the total Ca and Mg content in the leaves of all objects. After the first and second year of cultivation, the highest content of calcium and lowest

content of magnesium was noticed in the objects where the nitrate form was used. The leaves harvested in the second year of cultivation revealed the lowest content of total nitrogen with reference to the previous year, but no statistically significant differences were present between the objects. The content of total phosphorus and magnesium in the leaves was dependent on the nitrogen form used, higher contents were noticed in objects fertilised with ammonia form. The greatest content of total potassium – 1.80 %, was observed in the plants fertilised with $N\text{-}NO_3$. The content of total calcium in the leaves harvested in the second year of cultivation was significantly higher (by three times on average). A considerable effect of nitrogen fertilisation on calcium nutrition of Stewartia plant was also observed. The use of nitrate form led to the increase of total calcium content in the leaves.

Discussion

The water used for irrigation (neutral acidity – pH = 7.1 and hardness of 18 ºdH) might have caused the substrates to become less acidic. The differences in the pH values of the objects were induced by the types of fertilisers used. As a physiologically acidic fertiliser, ammonium sulphate, was able to partly neutralise the effect of water, as opposed to calcium nitrate which enhanced that effect. According to Tumilowicz [5], due to its origins, Stewartia should be grown in more acidic substrates, similar to those used in *Rhododendron* shrubs cultivation (pH = 4.5–5). Our studies confirmed the initial results [6] indicating that the plants prefer more acidic substrate, yet tolerate lower acidity and do not show any disorders even at the pH = 6.3 value.

Mineral fertilisers, used at the same doses, increase the total salt concentration in the substrate to a different extent. The increase of the salt concentration caused by $NaNO_3$ was considered as the value of 100. With reference to this value, relative increase in the salt concentration was 69 for ammonia sulphate, and 53 for calcium nitrate [7]. That might explain a higher concentration of the soil solution in the objects fertilised with ammonia sulphate. The increase might also have been caused by accumulation of ballast substances introduced during fertilisation with fertilisers of low mineral content.

During the two years of cultivation similar amounts of nutrients were introduced into all objects. After the first and second year of cultivation, low content of mineral nitrogen in the substrates was noticed. The nitrogen uptake was accompanied by high temperatures in the summer and greater watering of plants. The absence of nitrate form in the substrate can be indicative of leaching of that element as non-sorbed in the soil [8]. Low nitrogen content in the substrate can also result from passing too much time between the date of last fertilisation and taking substrate samples for analysis. The ratio of NO_3^- and NH_4^+ ions uptake by the plants depends on many factors, such as eg plant genus, kind and dose of nitrogen fertiliser, the rate of nitrification process, sorption capacity and substrate acidity, as well as on temperature and availability of oxygen and light [9–12]. The plants take greater amounts of nitrogen in the form of nitrate ions rather than ammonia ions, due to the greater content of nitrate ions in the substrate and possibility of immediate uptake by the roots. Greater amount of the ammonia form in the substrate after the second year of cultivation could have been caused by the limited

NH_4-N uptake due to the smaller range of optimum substrate acidity [8]. Nitric ions are taken faster in acidic environment whereas ammonia ions – in the neutral one [13]. During the two years of cultivation the same tendencies were observed. In the objects where ammonia form was used, higher phosphorus contents and lower potassium contents were detected in the substrate. High content of calcium and magnesium in the substrates, as compared with the initial value, was mostly caused by the high content of these elements in the water used for irrigation. Using calcium nitrate in fertilisation of the objects with nitrate form amounted to the increased calcium content in these objects.

The literature available so far has not confirmed the data concerning the content of nutrients in Stewartia leaves. The values determined are comparable to the optimum contents in the leaves of *Gardenia jasminoides*, a genus of the same *Theaceae* family [14]. Lower content of nutrients was observed in the leaves harvested at the end of vegetation season than in those harvested in the middle of the season, which can be explained with withdrawal of mineral components from the leaves to other parts of the plant before the resting period. In early phases of plant growth, the content of nitrogen, phosphorus and potassium is higher than in later phases [8]. The nitrogen contents in vegetative parts decline very quickly after having reached maximum concentration before blooming. That is connected with migration of the nutrients from vegetative parts to the seeds and with the dying process of the senescence leaves [15]. At the end of the first year's vegetation season, the highest content of total nitrogen (2.17 and 1.95 % d.m.) was detected in the leaves of shrubs fertilised with ammonia form of nitrogen. In the second year, a tendency of greater nitrogen content in the objects fertilised with ammonium form and in the objects of higher acidity was observed. All species from heathland had higher N content in the presence of ammonium than with nitrate as a sole nitrogen source [16]. It can signify that similarly to the *Ericaceae* family (acidophilic plants), Stewartia belongs to the plants that prefer ammonium ions as the source of nitrogen in the soil [8, 9, 17]. Optimum nitrogen content in plants is a broad value of 0.5 to 5 % N in the dry mass of vegetative organs. The content depends on the plant genus and age, as well as on the degree of nitrogen content of the plant [8]. The content of nitrogen in poinsettia shoots was lower than in the plants fertilised with nitrate form rather than with ammonia form [10]. However, Ganmore-Neumann and Kafkafi's [9] studies revealed higher content of nitrogen in the above-mentioned part of the nitrate-nourished plants. Limited uptake of nitrates and their low accumulation in the shoots may signify some disorders of these processes in the plant.

Our study exposed a very low content of ammonia and nitrate nitrogen in the leaves of Stewartia plant. Comparable contents of the element had been found in our previous research [6]. The pH value have been shown to affect N uptake rates and in some cases differentially affect NH_4^+ and NO_3^- uptake rates [12]. Depending on the species and conditions, uptake can be stimulated or inhibited by changing pH. Moreover, such low content can be a result of fast transformation of mineral nitrogen into organic nitrogen compounds [11]. Accumulation of nitrates in the plant depends on the amount of nitrate nitrogen in the environment, as well as on the rate of reduction process in the plant. In the mineral composition of plants fertilised with ammonia form of nitrogen, nitrate ions

can be traced as a result of nitrification process of ammonia ions in the soil. Yet, what should be accentuated is that the concentration of nitrate ions is lower in that situation than during fertilisation with nitrate fertilisers [10, 11].

Contents of soluble nutrient forms specified in the middle and at the end of the vegetation season show differences in the number of mineral components (K, Ca, Mg) with reference to substrate acidity, as well as the form of nitrate fertiliser [11]. As ammonia and nitrate ions make 80 % of the total content of ions taken by the plants, the nitrogen form has a significant effect on the uptake of other cations and anions. Ammonia form stimulates the uptake of PO_4^{3-} anions, whereas reduces the uptake of Ca^{2+} and Mg^{2+} cations [8–10, 12]. In this experiment, cations contents tended to decrease in the presence of NH_4-N, causing increased cation uptake limitation. Plants from acid soils often have lower cation contents than plants from less acidic habitats [16, 18].

The total nitrogen content in the leaves was higher at the end, than in the middle of the vegetation period. Concentration of N is usually higher in spring than in the summer [15]. With respect to what has been stated above, the plants fertilised with ammonia form revealed higher contents of total phosphorus. Presented studies have shown a better uptake of potassium by Stewartia plants in the presence of nitrate form in the substrate. The form in which nitrogen is introduced into the substrate plays a significant role in the relations between nitrogen and potassium. In general, potassium – similarly to other cations – is said to be taken more intensively in the presence of nitrates, while ammonia ions probably act quite contrary to K^+ [7]. The opponents of this thesis state that the content of potassium in the plants fertilised with ammonia nitrate is similar or higher than in those using nitrates. What should be highlighted is that the sum of potassium, calcium, magnesium and sodium cations taken by the plants nourished with ammonia nitrogen is lower than in those using nitrates, yet the content of phosphorus is higher [8]. The study also revealed the effect of substrate acidity on potassium uptake by the plants. Less acidic substrate allowed for better uptake of the component. Acidic reaction does not foster the uptake of potassium or other elements [16, 18].

The higher calcium content in the leaves of Stewartia was observed in case of nitrate nitrogen fertilisation, which complies with other reports [13] that ammonia ions generally reduce the uptake of calcium (because of competitive relations between NH_4^+ and Ca^{2+}), while nitrate ions stimulate the uptake of that element. Similarly, the increase in the content of calcium in the substrate leads to the increase of its content in the leaves [8].

In the case of magnesium, no statistically significant differences were noted in the effect of individual nitrogen form of fertiliser, or in the substrate acidity on the uptake of that element. The reports that the plants fertilised with ammonia nitrate take less Mg^{2+} cations when compared with the plants fertilised with nitrates [8, 10, 12], have not been confirmed, either.

Conclusions

1. The studied specimens used the ammonia form of nitrogen (NH_4-N) in a better and more effective way.

2. The shrubs took greater amounts of potassium and calcium when fertilised with nitrate form of nitrogen (NO_3-N).

3. The plants fertilised with ammonia form of nitrogen showed higher contents of phosphorus and magnesium.

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NAWOŻENIE AZOTEM STEWARCJI KAMELIOWATEJ *Stewartia pseudocamellia* UPRAWIANEJ W PODŁOŻU O ZRÓŻNICOWANYM ODCZYNIE

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Abstrakt: Badaniami objęto rośliny stewarcji (*Stewartia pseudocamellia* Maxim.), uprawiane w latach 1998–1999 w pojemnikach w warunkach polowych. Krzewy nawożono amonową lub azotanową formą azotu przy odczynie podłoża pH = 3,9 i 5,5. Rośliny nawożono pogłównie na podstawie analizy podłoża. W sezonie wegetacyjnym dwukrotnie pobrano liście do analiz na zawartość łatwo rozpuszczalnych form składników pokarmowych oraz ogólną ich zawartość.

W czasie trwania doświadczenia obserwowano stopniowy wzrost pH oraz ogólnego stężenia soli w podłożu w stosunku do wartości wyjściowych. Po zakończeniu każdego roku uprawy stwierdzono małą zawartość azotu mineralnego w podłożach. W obu latach uprawy obserwowano większą zawartość fosforu oraz mniejszą zawartość potasu w podłożu krzewów nawożonych formą amonową w porównaniu z nawożonymi formą azotanową. Zawartość łatwo rozpuszczalnych form składników w liściach stewarcji utrzymywała się na zbliżonym poziomie przez cały okres wegetacji. Badania wykazały bardzo małą zawartość azotu mineralnego w liściach stewarcji. Oznaczone zawartości łatwo rozpuszczalnych form składników pokarmowych (K, Ca, Mg) wykazały zróżnicowanie w zależności od odczynu podłoża, jak również od formy nawozu azotowego. Całkowite zawartości składników pokarmowych w obu terminach pobierania liści były porównywalne, z wyjątkiem azotu ogólnego. Ogólna zawartość fosforu i magnezu

w liściach była uzależniona od zastosowanej formy azotu, przy czym większe zawartości notowano w obiektach nawożonych formą amonową. Natomiast potas był lepiej pobierany przez stewarcje w obecności formy azotanowej w podłożu. W badaniach wykazano również wpływ odczynu podłoża na pobieranie potasu przez rośliny.

Słowa kluczowe: azot, pH, zawartość makroelementów, *Stewartia pseudocamellia*

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USEFULNESS OF NITROGEN-ENRICHED GLASSY FERTILISER IN PLANTS FERTILISATION

PRZYDATNOŚĆ SZKŁA NAWOZOWEGO WZBOGACONEGO W AZOT DO NAWOŻENIA ROŚLIN

Abstract: The aim of the present study was to assess the possibility of using composite type of fertiliser, containing glassy fertiliser as a source of P, K, Ca and Mg, as well as ammonium sulphate providing nitrogen, in horticulture. Potassium water glass was used as a binder.

The study evaluated moreover the influence of composition and granulation of fertiliser composites (two granulations: 1.5 mm and 5 mm) on their solubility in laboratory conditions, and therefore on the components release. The usefulness of composites in the cultivation of celeriac was also estimated on the basis of the analysis of substrate and plant material, as well as biometric parameters. The achieved composites were characterised by the limited usefulness in plants cultivation, as ammonium sulphate dissolved too fast.

Keywords: glassy fertilisers, nitrogen, solubility, usefulness

Glassy fertilisers are mineral fertilisers providing a plant with basic nutrients, such as phosphorus, potassium, calcium and magnesium, as well as with microelements (Cu, Fe, Mn, Zn, B, Co). The internal structure of glass is similar to the structure of silicate minerals, and has a form of a network composed of silicon, phosphorus and oxygen atoms. In the free spaces of this network other components are contained in their biologically active form. The structure of glass does not contain Cl^-, SO_4^{2-} and other anions usually hardly tolerated by plants. Glassy fertilisers are produced by melting (in 1300–1400 ^oC) the mixture of such materials as: apatite, phosphorite, serpentine marble, potash (anhydrous potassium carbonate), and oxides incorporating appropriate micro-elements. The glassy mass obtained this way is cooled and then crushed. Due to vitrification and appropriate chemical composition, glasses are difficult to dissolve in water, which counteracts the occurrence of losses by preventing nutrients leaching from the soil. They do not contaminate the groundwaters, which makes them ecologically

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safe. On the other hand, they dissolve in the solutions of organic compounds produced in the soil by the root system of plants. Through the roots, the plants are able to absorb the nutrients in the amounts needed for their growth without the risk of overdosage. The mechanism of releasing the components of glassy fertilisers resembles natural processes of erosion. In the soil environment, the crystallization of secondary minerals, such as calcium and potassium silicates, takes place on the surface of glass. They gradually decompose, and the cations contained in them are released. The cycle of these changes depends on the environmental conditions (pH, concentration of nutritive components, temperature, soil biological activity) [1, 2].

The aim of fertilisation is to create optimal conditions for nourishing the plants, and thus satisfying their demand for all nutrients. Nitrogen is one of the most important elements to the plants, therefore its use in fertilisation is essential. Treated as a slow release fertiliser, the glassy fertiliser tested so far did not contain nitrogen, as to introduce it into the structure of glass required a complicated technological process to be applied. Therefore, a new type of fertiliser had to be developed. The fertiliser had a form of a composite which was supposed to provide the plants with nitrogen.

The aim of the study was to assess the usefulness of nitrogen-enriched glassy fertiliser in plants fertilisation. The experiments included the analysis of the influence of the composite fertilisers' composition and granulations on their solubility in solutions and in the soil, as well as on the nutritional status of celeriac plants.

Material and methods

In the Department of Advanced Ceramics, AGH – University of Science and Technology composite fertilisers were prepared in different weight ratios and with constant amount of potassium waterglass (metasilicate of K_2SiO_3 composition) used as a binder. In horticulture potassium silicate can be used as a soluble source of potassium and silicon. The fertilisers were a mixture of glassy fertiliser (39.3 % of SiO_2, 15 % of P_2O_5, 10 % of K_2O, 22 % of MgO, 14.4 % of CaO) granulated to 0.1 mm and ammonium sulphate serving as a source of nitrogen (Table 1).

Table 1

Composition of composite fertilisers under study

Composite fertiliser	Glassy fertiliser [g]	Ammonium sulphate [g]	Potassium waterglass [cm^3]
1	75	25	8
2	50	50	8
3	25	75	8

The acquired mass was sieved into two granulations: 1.5 mm and 5 mm. Fertiliser pellets were dried in temperature of 100 ^oC for 60 minutes. The material obtained in this way was used in further studies that were conducted in three steps.

Evaluation of composite fertilisers' solubility in solutions

1 g of composite fertiliser was shaken out of 100 cm^3 of extractor by a rotary mixer for 30 minutes. As extractors, distilled water and solutions used for determining the content of mineral elements in substrates and in fertilisers, i.e. the solution of acetic acid of 0.03 mol · dm^{-3} as an extractor that is commonly used in horticultural cultivation [3], the solution of citric acid of 2 % applied in determining the content of phosphorus in phosphoric fertilisers, were used [4].

After they have been sieved, the samples were analysed for the content of such mineral elements as NH_4-N, NO_3-N, P, K, Mg and Ca. Mineral nitrogen was determined with Bremner's micromethod (modified later by Starck), and other components by means of ICP AES method [3].

Incubation of composite fertilisers

The experiments were conducted on a brown soil containing (in 1 dm^3): 3.8 mg NH_4-N, 6 mg NO_3-N, 77.7 mg P, 196 mg K, 84.4 mg Mg and 954.2 mg Ca. The soil acidity was expressed as pH = 7.33, and the total concentration of the salt (described by it EC) was 0.116 mS · cm^{-1}. Composite fertilisers granulated to 1.5 mm and 5 mm were introduced into the substrate. Constant temperature of 22–25 ^oC and constant humidity of 60–70 % of the substrate was maintained during incubation. In the 3^{rd}, 5^{th}, 7^{th} and 9^{th} day single composite granules were taken to analysis. The surface of the composite fertiliser was evaluated with the scanning electron microscope (SEM) equipped in an adapter to X-ray analysis conducted within the micro-area monitored with energy dispersive X-ray spectroscopy (EDS).

Pot cultivation of celeriac plant

In the second half of May, 3 g · dm^{-3} of composite fertilisers granulated to the diameter of 1.5 and 5 mm were added into the pots filled with a brown soil in which celeriac seedlings (*Apium graveolens* L. *var. rapaceum* (Mill.) Gaud.) were planted. Each of the seedlings had 3–5 leaves measuring around 10 cm in length. The reason of choosing celeriac as the study object was its great demand for nitrogen, phosphorus and potassium. Now the process assumes using half of the advisable dose of nitrogen before planting the seedling, and then applying the rest once or twice by top dressing [5]. The use of slow nitrogen release fertiliser could however limit the top dressing fertilisation. To grow optimally, celeriac plant needs to be cultivated in the substrate of pH = 6.5–7. The study conducted so far on the glassy fertiliser showed the increase in the substrate acidity, irrespective of using physiologically acidic fertilisers [6]. After 8 weeks of cultivation (bunch harvest [5]), biometric measurements were conducted: the number of leaves, the length of leaves and stalks and the mass of the aboveground parts were evaluated.

At the same time index parts (stalks) were taken to analysis for the nutritional status of plants. The mineral and total nitrogen, as well as other nutrients' content was

determined with common methods [3]. The analyses of the substrate for the content of basic mineral components were done twice, ie before and after cultivation.

Results and discussion

The solubility of composite fertilisers was evaluated in laboratory conditions in the analysis of the number of mineral elements released (leached) from these fertilisers. The content of NH_4-N, NO_3-N, P, K, Mg and Ca was measured in the samples. (Table 2, Fig. 1).

Table 2

Release of nitrogen from composite fertilisers by different extractors as shown by the example of 1.5 mm fraction

Composite fertiliser	Extractor	NH_4-N	NO_3-N	$N_{min.}$	N introduced in $(NH_4)_2SO_4$
		[mg · 100 cm^{-3}]			
1	Water	22.4	2.10	24.50	53
	Citric acid	22.4	2.45	24.85	53
	Acetic acid	26.3	3.65	29.90	53
2	Water	65.8	3.15	68.95	106
	Citric acid	61.3	3.15	64.40	106
	Acetic acid	73.85	4.03	77.88	106
3	Water	114.8	2.80	117.60	159
	Citric acid	114.5	3.15	117.65	159
	Acetic acid	131.9	4.38	136.33	159

The solubility analysis of individual composite fertilisers showed that it was ammonium sulphate and potassium water glass releasing nitrogen and potassium into the solution which dissolved first. The amount of mineral nitrogen available in solutions after shaking increased in line with the increase of ammonium sulphate in composite fertiliser. Both in citric acid and in water the amounts of nitrogen leached were similar. Acetic acid turned out to be the strongest extractor for this element. Greater amounts of ammonium nitrogen in comparison with nitrate nitrogen were noted in the solutions. Potassium is a component both of a glassy fertiliser and a binder. While dissolving in the extractors used, this element entered into the solution faster and in greater amount than the elements coming from the structure of glassy fertiliser alone.

Composite fertilisers showed poor mechanical resistance; the granules disintegrated during shaking.

In the second stage of dissolving, leaching of such elements as P, K, Mg and Ca originating from the structure of glassy fertilizer occurred. The more glassy fertilisers were contained in the composite fertilisers, the more elements originating from the

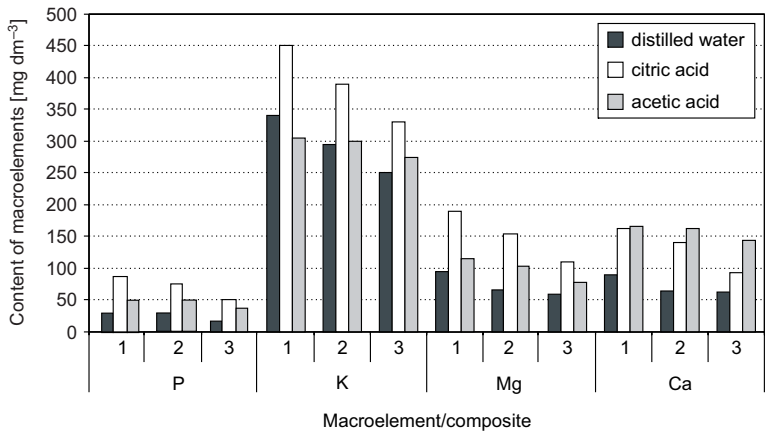

Fig. 1. Release of P, K, Ca and Mg from composite fertilisers by different extractors, as shown by the example of 1.5 mm fraction (1, 2, 3 – composite fertiliser)

structure of glass went into the solution. Citric acid turned out to be the strongest extractor for P, K and Mg, whereas the acetic acid for calcium (Fig. 1).

To assess the usefulness of composite fertilisers in horticulture, the processes of releasing nitrogen and the remaining mineral elements from the surface of glassy fertilisers during incubation in the soil were analysed. Dissolving of elements in the substrate went significantly slower than in the extraction solutions. However, after 3 days of composites incubation in the soil the presence of nitrogen was not detected. After 5 days, profuse occurrence of secondary calcium and magnesium phosphates were observed on the surface of glass. The size of composite granules had no effect on the occurrence of changes on the surface of glassy fertiliser. On the other hand, prolonged time of incubation caused dissolving of crystallised phosphates and gradual release of elements from them. After 9 days of soil environment's acting on the composite fertilisers, the total release of elements contained in the glassy fertiliser was noted, and the surface of the glassy fertiliser was enriched with silica.

Celeries intended to be sold in bunches are harvested when the plants have grown ca 10 leaves, which is between 6–8 weeks after planting of the seedlings [5]. Celeries collected after 8 weeks of cultivation had dispersed shape and week leaf stalks. The parameters of leaves were similar (Table 3), although there was a tendency of observing higher values for the composites granulated to the smaller size. With the greatest content of ammonium sulphate introduced into the composite, the longest leaves and leaf stalks, as well as the aboveground parts were noticed, irrespective of the granulation.

Table 4 presents the results of determining the content of mineral nitrogen in the material under study. The sufficient level of NO_3-N in the largest celeriac leaf stalks is 9000 mg · kg^{-1} [7]. It was only the plants fertilised with composite fertilisers of the smallest content of ammonium sulphate in which the amount of nitrates was similar to that value. In the rest of the object the concentration of NO_3-N exceeded that value very much.

Table 3

Selected parameters of the celeriac plant depending on the composition and granulation of the composite fertiliser

Composite fertiliser	Granulation [mm]	Average number of leaves/plant	Average length		Average mass of aboveground part [g]
			of leaf	of stalk	
			[cm]		
1	1.5	12.75	46.00	26.25	51.35
	5.0	12.25	46.00	25.50	49.84
2	1.5	12.50	46.25	26.25	55.02
	5.0	12.25	45.75	25.75	50.05
3	1.5	12.75	49.38	28.38	56.12
	5.0	12.75	47.50	27.00	55.46

Table 4

Average content of mineral N P, K, Ca and Mg in the celeriac leaf stalks

Composite fertiliser	Granulation [mm]	NH_4-N	NO_3-N	$N_{min.}$	P	K	Mg	Ca
		[mg · kg^{-1}]			[%]			
1	1.5	2162.48	9204.84	11367.32	0.419	8.601	0.290	1.274
	5.0	2011.18	8377.83	10389.01	0.371	8.333	0.293	1.098
2	1.5	2495.45	19892.51	22387.96	0.339	7.732	0.240	1.063
	5.0	2438.74	18222.02	20660.76	0.344	8.024	0.227	0.931
3	1.5	6986.86	22320.97	29307.83	0.247	7.471	0.202	0.869
	5.0	3741.03	21942.06	25683.09	0.272	6.548	0.190	0.716

The contents of soluble forms of the remaining elements in the stalks of celeriac leaves are shown in Table 4. In the middle phase, the level of the element sufficient for PO_4-P and K in celeriac stalks was 0.4 and 7 %, respectively [7]. The plants characterised by the optimal phosphorus content were only obtained when the ratio of glass to $(NH_4)_2SO_4$ in the composite fertiliser was 3:1. On the other hand, the phosphorus content in the celeriac plants (excluding object 3 with granulation of 5 mm) exceeded the advisable value.

The tendency of diminishing the content of phosphorus, potassium, calcium and magnesium in the plant material, as well as the decrease in the amount of glassy fertiliser into the composite was observed. The lower content of elements was noted also when the granulation of the composites was greater.

To grow optimally, celeriac plant needs to be cultivated in the soils of pH = 6.5–7 [5]. When the share of glassy fertiliser in the composite was greatest (irrespective of granulation), the lower acidity and the lowest total content of salt in the substrate was observed (Table 5). What was also noticed was a clear acidifying activity of ammonium nitrate (physiologically acid fertliser) [8]. The higher amount of ammonium sulphate was introduced into the composite, the lower was the acidity of the substrate. At the

same time, the total content of salt increased, as ammonium sulphate belongs to the group of fertilisers that have the greatest effect on the increase of the salt concentration in the substrate [8].

Celeriac is a plant of significant fertilisation needs. The soil used for cultivation must be rich in nutrients and humus. The optimal content of assimilable forms of nutrients amount to (in 1 dm^3): 100–130 mg N, 60–80 mg P, 200–250 mg K, 60–80 mg Mg and 1000–1500 mg Ca [5].

Table 5

Content of mineral components, pH and EC in the soil, after the cultivation of celeriac plant

Composite fertiliser	Granulation [mm]	pH	EC [mS · cm^{-1}]	NH_4-N	NO_3-N	$N_{min.}$	P	K	Mg	Ca
				[mg · dm^{-3}]						
1	1.5	6.99	0.49	42.0	14.0	56.0	204.1	137.9	290.7	1683.4
	5.0	7.17	0.54	24.5	17.5	42.0	183.8	119.6	288.0	1572.9
2	1.5	5.30	1.19	73.5	98.0	171.5	140.4	103.6	276.4	1533.4
	5.0	5.50	1.07	56.0	84.0	140.0	113.9	59.1	257.5	1470.4
3	1.5	5.25	1.32	94.5	115.5	210.0	111.2	55.7	235.7	1277.6
	5.0	4.95	1.33	112.0	168.0	280.0	92.9	49.9	231.2	1245.1

The greatest content of mineral nitrogen in the soil was observed in the objects with the highest value of ammonium sulphate in the composite fertiliser. The soil into which composites with glassy fertiliser and $(NH_4)_2SO_4$ in a ratio of 3:1 were introduced, the amount of nitrogen did not satisfy the needs of the celeriac plant. In the rest of the object the content of that element exceeded the advisable value. The increase in the amount of glassy fertiliser in the composite triggered the increase of its components in the soil. The amount of phosphorus and magnesium in the soil was high, whereas that of potassium was low. The concentration of calcium in the soil was within the range of standard values. The size of composite granules had little effect on the content of elements in the soil.

Conclusions

1. The effects of using ammonium sulphate as a compound which introduced nitrogen to the composites were far from satisfactory.

2. Potassium water glass used as a binder did not slow down the dissolving of ammonium sulphate in the soil environment. Therefore the time of nitrogen release could not be prolonged.

3. It seems that the study on how to enrich the glassy fertiliser in nitrogen by using other sources of that element (eg urea), as well as other kind of a binder that would limit dissolving of the nitrogen-containing compound, needs to be continued.

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PRZYDATNOŚĆ SZKŁA NAWOZOWEGO WZBOGACONEGO W AZOT DO NAWOŻENIA ROŚLIN

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Abstrakt: Celem badań była ocena możliwości wykorzystania w ogrodnictwie nawozu typu kompozytu nawozowego, zawierającego w swoim składzie nawóz szklisty jako źródło P, K, Ca i Mg, oraz siarczan amonu dostarczający azot. Jako lepiszcze zastosowano potasowe szkło wodne. Określano także wpływ składu i uziarnienia kompozytów nawozowych (dwie granulacje: 1,5 mm i 5 mm) na ich rozpuszczalność w warunkach laboratoryjnych, a tym samym uwalnianie składników. Oceniano również przydatność wytworzonych kompozytów w uprawie selera na podstawie analizy podłoża i materiału roślinnego, a także parametry biometryczne. Uzyskane kompozyty charakteryzowały się ograniczoną przydatnością do uprawy roślin, ponieważ zawarty w nim siarczan amonu zbyt szybko ulegał rozpuszczaniu.

Słowa kluczowe: szkła nawozowe, azot, rozpuszczalność, przydatność

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EFFECT OF ABSCISIC ACID, ETHYLENE AND INHIBITORS OF THEIR BIOSYNTHESIS (FLURIDONE AND SALICYLIC ACID) ON SOMATIC EMBRYOS CONVERSION IN TULIPS

WPŁYW KWASU ABSCYSYNOWEGO, ETYLENU ORAZ INHIBITORÓW ICH BIOSYNTEZY (FLURIDONU I KWASU SALICYLOWEGO) NA KONWERSJĘ ZARODKÓW SOMATYCZNYCH TULIPANA

Abstract: The experiments aimed to investigate the conversion of somatic embryos of the tulip 'Apeldoorn' variety in an *in vitro* culture supplemented with some chosen compounds. Tulip somatic embryos in torpedo stage, obtained by indirect somatic embryogenesis were placed for 1 week on media containing growth regulators (5 μM Picloram, 1 μM 6-benzylaminopurine (BAP) – control) and abscisic acid (ABA), abscisic acid + fluridone, Etephon and Etephon + salicylic acid (SA). Then, the embryos were maintained in the dark or under light for 10 weeks.

After time of experiment, the greatest percent of leaf-forming embryos (40 %) and the greatest number of leaves (3.6 leaves) were observed when the embryos were treated simultaneously with Etephon and salicylic acid. Light did not influence the number of newly developed leaves but significantly increased (vs dark) percentage of leaf-forming embryos on control media (from 0 to 24 %) and on Etephon-supplemented medium (from 4 to 24 %). Abscisic acid and abscisic acid + Fluridone supplementation inhibited organogenesis of leaves but only in the cultures maintained under light. Regeneration of leaves was not observed on the control media maintained in the dark and on the abscisic acid + fluridone-supplemented media under light.

Keywords: abscisic acid, ethylene, fluridone, salicylic acid, leaf formation

Reproduction of tulips, commercially important ornamental bulb plants, under natural conditions is a very slow process. Propagation *in vitro* using somatic embryos induction can shorten time required to obtain proper number of bulbs, which is especially valuable in introduction of new cultivars or elite-plant productions. However, this method require further studies because of low conversion of embryos to plants [1, 2], focussed on maturation, germination and conversion of somatic embryos [3]. The conversion of tulip somatic embryos to plants is decisive for efficacy of

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micropropagation. Plants obtained by somatic embryogenesis or shoot cultures [4, 5] are important for production of start material for further propagation. A possibility of cyclic multiplication of tulip auxiliary shoots can increase multiplication index in these plants [6].

The present experiment aimed to examine the effect of growth regulators, abscisic acid (ABA), ethylene (Etephon) and inhibitors of their biosynthesis (fluridone and salicylic acid – SA) on ability of somatic embryos to form leaves during embryo conversion to plants.

Material and methods

The studies were conducted on the tulip of the 'Apledoorn' variety, bulbs of which were maintained at 5 ^{o}C for 12 weeks. Ovaries isolated from bulbs, from flower buds were cut into 1–2 mm pieces and placed in Petri dishes on media containing mineral salts according to Murashige and Skoog [7], 3 % sucrose and growth regulators: Picloram (25 and 50 μM) and BAP (0.25–10 μM). Growth regulators stimulated development of embryogenic callus on explants. When Picloram and BAP concentration was lowered to 5 μM and 1 μM, respectively, the embryogenic tissue formed somatic embryos. Tulip somatic embryos in torpedo stage, 5–10 mm long, were placed on media containing 5 μM Picloram and 1 μM BAP (control) and: 10 μM ABA, 10 μM ABA + 30 μM fluridone, 25 μM Etephon, 25 μM Etephon + 10 μM SA, for 1 week. Then, the embryos were maintained on the medium enriched in 2.5 μM BAP and 0.25 μM NAA in the dark or under light for 10 weeks. Thereafter, the percentage of leaf-forming embryos and the number of leaves were recorded.

The experiment was performed in 5 repetitions, for 5 explants. The results were subjected to the analysis of variance. The means were compared using Duncan test at confidence level $\alpha = 0.05$.

Results and discussion

The embryo germinating under natural conditions first develops cotyledon (with apical bud at the base), root and stolon (an underground shoot). Then the cotyledon dries out and a leaf develops. Tulip seedlings require several years to bloom, until then, the apical bud forms only one leaf [8]. Conversion of somatic embryos consists in simultaneous development of the root and the shoot [9, op. cit. 3].

In present experiment tulip somatic embryogenesis progresses as follows: first, the cotyledon develops, which grows upright, then, it dries out from the top, while a leaf or stolon forms at the base of the embryo. Unfortunately, many embryos do not initiate growth nor develop cotyledon but undergo deformation or die. A majority of tulip somatic embryos do not form leaves or stolons, while those that develop do not have meristem. Similar observations in tulip culture *in vitro* were reported by Podwyszynska and Marasek [6]. The formation of stolons in tulip somatic embryo cultures has been observed sporadically, whereas no root formation was noted. According to Cavallini and Natali [10], problems with normal development and germination of somatic

embryos are observed frequently in monocotyledones. These embryos do not enter dormancy, prematurely germinate, and the plants having developed from them are characterized by low survival rate [11].

The conversion of tulip 'Apeldoorn' somatic embryos was observed on media containing (apart from growth regulators under study) low concentrations of auxins and cytokinins, like Picloram (5 μM), NAA (0.25 μM) and BAP (1 and 2.5 μM). According to Ptak and Bach [12], tulip embryos germinated forming normally developed plants when cultured with 5 μM BAP and 0.5 μM NAA. Picloram (1.4 μM) and BAP (13.3 μM) stimulated the formation of shoots in garlic cultures [13]. Only embryos that have accumulated a sufficient amount of storage reserves can undergo conversion to properly developed plants [3]. The accumulation of storage reserves, decisive for somatic embryo germination yield is stimulated by abscisic acid [14].

Exogenous ABA alone or in combination with fluridone (an inhibitor of its synthesis) inhibited leaf organogenesis of tulip cultures, in present experiment, but only under light (Figs. 1 and 2). According to Stasolla and Yeung [15], proper growth of somatic embryos requires exogenous ABA, concentration of which is species-dependent. ABA promoted the conversion of asparagus [16].

Supplementation of fluridone, an abscisic acid synthesis inhibitor which lowers ABA accumulation in plants [17], had no effect on leaf formation by tulip somatic embryos (Figs. 1 and 2). Probably, its effect was compensated by exogenous ABA, also present in the medium. Gabryszewska [18] noted that fluridone increased the number of leaves in peony cultured *in vitro*, but when it was used in combination with ABA, its action was abolished.

Medium supplementation with Etephon did not affect the percent of leaf-forming embryos or the number of leaves, in tulip 'Apeldoorn' either in the dark or under light (Figs. 1 and 2). Ethylene (released during Etephon breakdown) is considered to be mostly an inhibitor of cell division, but can stimulate some morphogenetic processes. In *Hemerocallis* cultures, ethylene caused transition from young to mature phase, while in

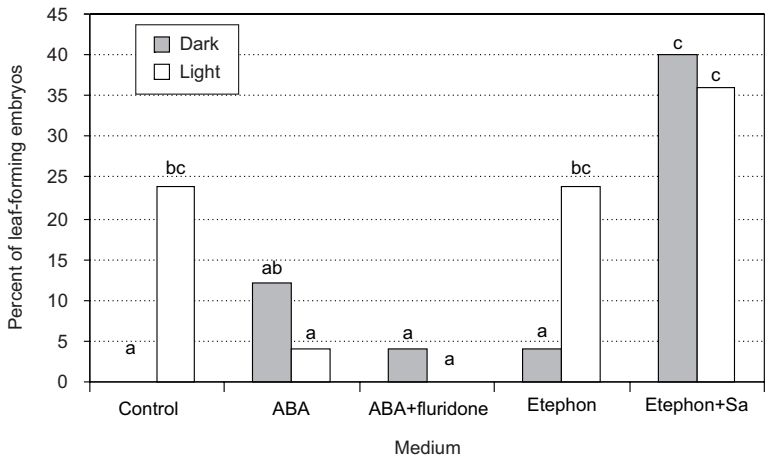

Fig. 1. The effect of growth regulators on leaf formation by somatic embryos of the tulip 'Apeldoorn' variety

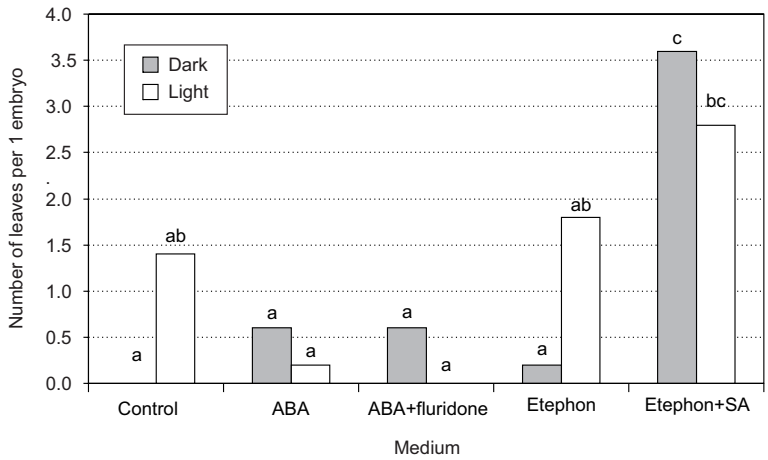

Fig. 2. The effect of growth regulators on the number of leaves formed by somatic embryos of the tulip 'Apeldoorn' variety

roses it stimulated flower bud formation [19]. Ethylene is also a stimulator of seed germination in many plant species [3]. According to Economou [20], ethylene can facilitate shoot formation in some species, however, it should be remembered that its supraoptimal concentrations are ineffective or even inhibitory.

In presented experiment, tulip embryos were exposed simultaneously to Etephon and SA, an inhibitor of ethylene biosynthesis. Medium enrichment in SA resulted in the greatest percentage of leaf-forming embryos (40 %) and the greatest number of leaves (3.6) in the dark. Under light, SA 1.5 times increased the share of leaf-forming explants and elevated the number of leaves, but the differences were not significant vs control medium (Etephon alone) (Figs. 1 and 2). Salicylic acid was shown to have a beneficial effect on cell growth [21]. Light significantly increased the percent of leaf-forming embryos on control medium (from 0 to 24 %) and on Etephon- -supplemented medium (from 4 to 24 %). Leaf regeneration was not observed on control medium in the dark and on medium supplemented with ABA and fluridone under light (Figs. 1 and 2).

Conclusions

1. The greatest share of leaf-forming embryos (40 %) and the greatest number of formed leaves (3.6) were obtained on medium containing Etephon in combination with SA maintained in the dark.

2. ABA and ABA + fluridone supplementation inhibited leaf organogenesis, but only when embryos were cultured under light.

3. Light significantly increased the percentage of leaf-forming embryos under control conditions (from 0 to 24 %) and after Etephon treatment (from 4 to 24 %) in comparison with cultures maintained in the dark.

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WPŁYW KWASU ABSCYSYNOWEGO, ETYLENU ORAZ INHIBITORÓW ICH BIOSYNTEZY (FLURIDONU I KWASU SALICYLOWEGO) NA KONWERSJĘ ZARODKÓW SOMATYCZNYCH TULIPANA

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Abstrakt: W przeprowadzonym doświadczeniu badano konwersję zarodków somatycznych tulipana ‘Apeldoorn’ pod wpływem wybranych czynników kultury *in vitro*. Zarodki somatyczne tulipana, w stadium torpedy, uzyskane drogą pośredniej embriogenezy somatycznej, wykładano na okres 1 tygodnia, na pożywki zawierające substancje wzrostowe (5 μM Picloram, 1 μM 6-benzyloaminopuryna (BAP) – kontrola) oraz: kwas abscysynowy (ABA), kwas abscysynowy+Fluridon, Etefon i Etefon+kwas salicylowy (SA). Następnie zarodki umieszczano na 10 tygodni w warunkach zaciemnienia lub światła.

Po upływie czasu trwania doświadczenia zaobserwowano, że pod wpływem jednoczesnego działania Etefonu i kwasu salicylowego uzyskano największy udział zarodków formujących liście (40 %) oraz największą liczbę wytworzonych liści (3,6 szt.). Światło nie miało wpływu na liczbę powstałych liści, natomiast istotnie zwiększyło udział tworzących je zarodków na pożywkach kontrolnych (z 0 do 24 %) i po zastosowaniu Etefonu (z 4 do 24 %) w porównaniu z zarodkami utrzymywanymi w ciemności. Dodatek kwasu abscysynowego oraz kwasu abscysynowego i Fluridonu hamował organogenezę liści, ale tylko w obecności światła. Nie obserwowano regeneracji liści na pożywkach kontrolnych w warunkach zaciemnienia oraz pod wpływem kwasu abscysynowego i Fluridonu na świetle.

Słowa kluczowe: kwas abscysynowy, etylen, Fluridon, kwas salicylowy, formowanie liści

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PROTECTION OF PEDUNCULATE OAK AGAINST POWDERY MILDEW AND ITS EFFECT ON PLANT GROWTH

OCHRONA DĘBU SZYPUŁKOWEGO PRZED MĄCZNIAKIEM PRAWDZIWYM I JEJ WPŁYW NA WZROST ROŚLIN

Abstract: Eleven synthetic and five biotechnical preparations were tested in experiments carried out in the experimental field of the Research Institute of Pomology and Floriculture in Skierniewice. The experiments were conducted in two series. After symptoms of powdery mildew had been noticed, oak plants were sprayed 4 times at 7-day intervals. Observations of the degree of intensity of the disease symptoms were carried out after 2 and 4 treatments, and additionally after 12 weeks from the beginning of the experiment.

The results have shown that the applied protection reduced the intensity and extent of infection. Among the 16 products tested, the best protection was achieved with the fungicides Falcon 460 EC, Bumper 250 EC and Systhane 12 EC, and also Domark 100 EC. Biotechnical preparations and plant extracts also reduced the degree of infection, but significantly less than the synthetic fungicides. The applied treatments also had an effect on plant growth rate. Measurements of plant height revealed statistically significant differences between the experimental combinations. The tallest plants were found in the combinations where Nimrod 250 EC and Bio-Blat 25 EC had been used, and the shortest ones in the control.

Keywords: powdery mildew, *Microsphaera alphitoides*, plant protection

Powdery mildew of oak (*Microsphaera alphitoides* Griff. et Maubl.) causes particularly extensive damage among young plants in nurseries. It affects European oak trees causing injuries to their leaves, buds, and the top, non-woody shoots [1]. A severe infection, manifesting itself in the form of a white coating of mycelium, lowers the decorative value on one hand, while on the other, by reducing assimilative processes and the total chlorophyll content, it can contribute to plant growth inhibition and defoliation.

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Under the influence of stress, to which plants infected with the fungus are subjected, many physiological processes in tree tissues become disrupted, and this in turn weakens photosynthesis and the transport and storage of assimilates [2–4].

Mildew-infected shoots are not generally able to reach the so-called winter maturity in time and for that reason succumb to autumn frosts [5].

So far, if there was a risk of infection, protection against this pathogen has consisted in spraying plants with preparations containing sulphur [6], which at the predominantly high temperatures in summer could result in phytotoxicity symptoms. For that reason, many other preparations are being tested at present, both synthetic fungicides and those based on plant extracts, as well as biotechnical preparations, which could be recommended for plant protection, especially in parks and gardens.

Materials and methods

The experiments with the means of controlling *Microsphaeria alphitoides* on oak trees were carried out in the experimental field of the Research Institute of Pomology and Floriculture in Skierniewice on one-year-old seedlings of pedunculate oak *Quercus robur*. In order to increase air humidity around the plants, and thus to guarantee near optimal conditions for the development of disease symptoms, the plants were covered with agro-fibre suspended at a height of about 1 m above ground. In addition, to make disease symptoms appear quickly, the plants were sprinkled with water, and were often left like that overnight. Also, a few shoots with symptoms of powdery mildew were put into containers with water and placed between the combinations of oak plants to serve as inoculum. The preparations tested included 11 synthetic preparations and 5 biotechnical preparations.

Two series of tests were carried out in 2005. As soon as the symptoms of powdery mildew of oak had appeared, the plants were sprayed 4 times at 7-day intervals. The amount of working solution used per 1 m^2 was about 100 cm^3. The treatments were carried out in the morning between 8 and 10 o'clock. During the course of the experiments there was a one-off application of Confidor 200 SL at 0.125 % to control aphids *Phylloxera coccinea*.

Observations of the degree of intensity of the disease symptoms were carried out before any control measures were taken, and after 2 and 4 spray treatments, and additionally after 12 weeks from the beginning of the tests to ascertain and measure the effect of the agents applied. The assessment of infection was carried out according to a 6-point scale given by Wojdyla [8], where: 0 – no symptoms, 5 – more than 20 % of shoot/leaf surface area covered by mycelium.

Results and discussions

The assessment of the extent of infection carried out after 2, 4, and 12 weeks from the beginning of the tests indicated a better health status of the plants protected by synthetic fungicides. The extent of infection in the protected combinations was significantly lower than in the control combination in both series of the tests (Tables 1 and 2).

As the final assessment of the degree of infection had shown, the best protective action was that of Falcon 460 EC, which gradually inhibited the development of mycelium on the leaves until it had been completely eliminated by the last date of the analysis (Table 1).

Table 1

Effectiveness of some compounds applied curatively in the control of *Microsphaeria alphitoides* on common oak *Quercus robur*; beginning of experiment – 18 July 2005, initial infection level – 0.4, height of plants before experiment = 25.1 cm

Treatments (Active ingredient)	Concentration [%]	Mean degree of plants infection after weeks			Height of plants after 4 weeks [cm]
		2	4	12	
Control	—	3.15 i	4.65 m	5.0 j	35.40 a
Control (water)	—	3.00 h	4.50 l	5.0 j	40.18 ab
Fungicides					
Amistar 250 SC (250 g azoxystrobin per 1 l)	0.1	1.15 d	0.35 de	0.35 c	35.75 a
Bumper 250 EC (250 g propiconazole per 1 l)	0.05	1.10 d	0.00 a	0.10 ab	37.68 a
Falcone 460 EC (167 g tebuconazole, 250 g spiroxamine, 43 g triadimenol per 1 l)	0.1	0.65 b	0.10 ab	0.00 a	38.15 a
Discus 500 WG (500 g kresoxim-methyl per 1 kg)	0.03	1.05 d	0.30 c–e	0.20 b	39.35 ab
Folicur Multi 50 WG (40 % tolylfluanid + 10 % tebuconazole)	0.1	0.85 c	0.25 cd	0.15 b	37.98 a
Dithane M 45 80 WP (80 % mancozeb)	0.2	1.50 ef	1.45 j	1.70 f	44.45 a–d
Domark 100 EC (100 g tetraconazole per 1 l)	0.05	0.50 a	0.25 cd	0.10 ab	40.38 ab
Nimrod 250 EC (250 g bupirimate per 1 l)	0.2	1.40 e	0.40 ef	1.55 e	50.85 cd
Score 250 EC (250 g difenoconazole per 1 l)	0.05	0.60 ab	0.20 bc	0.40 c	48.68 b–d
Sportak 450 EC (450 g prochloraz per 1 l)	0.05	0.85 c	0.50 f	0.55 d	43.15 a–d
Systhane 12 EC (125 g myclobutanil per 1 l)	0.03	0.80 c	0.30 c–e	0.20 b	44.15 a–d
Biotechnical preparations					
Bio Blatt 25 EC (25 % soybean lecithin)	0.15	1.60 fg	1.30 i	4.05 g	52.45 d
Biochikol 020 PC (20 g microcrystalline chitosan per 1 l)	1	1.40 e	0.80 g	4.20 h	37.35 a

Table 1 contd.

Treatments (Active ingredient)	Concentration [%]	Mean degree of plants infection after weeks			Height of plants after 4 weeks [cm]
		2	4	12	
Bioczos BR (10 g of crushed garlic coated with paraffin)	by instruc.	1.40 e	1.05 h	4.45 i	39.55 ab
Biosept 33 SL (33 % grapefruit extract)	0.1	1.65 g	1.50 jk	4.55 i	39.25 ab
Grevit 200 SL (200 g grapefruit extract per 1 l)	0.2	1.70 g	1.60 k	4.55 i	41.85 a–c

Explanation: Mean values marked with the same letter do not differ at the significance level p = 0.05 according to the Duncan's test; * Disease index: no symptoms, 1 – up to 1 % of shoot area covered with mycelium, 2 – 1.1 up to 5 %, 3 – 5.1 up to 10 %, 4 – 10.1 up to 20 %, 5 – over 20 % of shoot area covered with mycelium.

Table 2

Effectiveness of some compounds applied curatively in the control of *Microsphaeria alphitoides* on common oak *Quercus robur*; beginning of experiment – 01 August 2005, initial infection level – 0.7, height of plants before experiment = 27.3 cm

Treatments	Concentration [%]	Mean degree of plants infection after weeks			Height of plants after 4 weeks [cm]
		2	4	12	
Control	—	3.50 j	5.00 i	4.90 i	38.70 a–c
Control (water)	—	3.40 j	4.85 i	4.80 i	41.23 a–c
Fungicides					
Amistar 250 SC	0.1	1.45 e	0.65 de	0.25 b	33.85 a
Bumper 250 EC	0.05	1.15 d	0.50 b–d	0.15 ab	38.08 ab
Falcone 460 EC	0.1	0.65 a	0.25 a	0.10 a	38.45 ab
Discus 500 WG	0.03	1.40 e	0.70 e	0.20 ab	41.15 a–c
Folicur Multi 50 WG	0.1	0.85 b	0.35 ab	0.10 a	38.48 ab
Dithane M 45 80 WP	0.2	2.00 i	1.45 h	1.15 d	47.55 b–d
Domark 100 EC	0.05	0.80 b	0.25 a	0.15 ab	44.93 a–d
Nimrod 250 EC	0.2	1.00 c	0.60 de	0.55 c	56.18 d
Score 250 EC	0.05	0.75 ab	0.40 a–c	0.20 ab	49.43 b–d
Sportak 450 EC	0.05	1.40 e	0.70 e	0.55 c	46.20 b–d
Systhane 12 EC	0.03	0.80 b	0.55 c–e	0.10 a	50.15 cd
Biotechnical preparations					
Bio Blatt 25 EC	0.15	1.60 fg	1.15 f	3.55 e	46.83 b–d
Biochikol 020 PC	1	1.50 ef	1.20 fg	3.65 ef	39.45 a–c
Bioczos BR	by instruc.	1.60 fg	1.45 h	3.90 h	43.55 a–c
Biosept 33 SL	0.1	1.70 gh	1.35 gh	3.70 fg	38.05 ab
Grevit 200 SL	0.2	1.80 h	1.35 gh	3.80 gh	43.50 a–c

Explanation – see Table 1.

The results of the second series of tests also confirmed its high efficacy (Table 2). It is likely that this fungicide has a longer systemic action in plants. Liovic and Zupanic [7], while testing a few fungicides, had shown high effectiveness, of more than 90 %, of triazoles. And there are two such compounds in the composition of Falcon 460 EC – tebuconazole and triadimenol. Similarly high efficacy was also shown by the other two fungicides from the triazole group: Bumper 250 EC and Systhane 12 EC (Tables 1 and 2). Good effectiveness was shown by strobilurin fungicides, which had also been confirmed in the experiments by those authors. According to the above-mentioned authors, full protection can be achieved with the fungicides from this group only if the product is present on the leaves before infection has started, whereas in our experiments they were used only after infection had been detected.

Our study has shown that biotechnical preparations and those containing natural compounds do not protect as effectively as synthetic fungicides. The degree of infection in those cases was significantly lower than in oak from control combinations, but also higher than in the plants from other protected treatments (Tables 1 and 2). The tests have shown that the plant protection products used had an effect on plant growth rate. The largest height was achieved by the plants treated with Bio-Blatt 25 EC and Nimrod 250 EC. It turned out, however, that the fungicides Falcon 460 EC and Bumper 250 EC, showing the best effectiveness in controlling powdery mildew, can somewhat inhibit plant growth (Tables 1 and 2).

To sum up the obtained results it can be concluded that the fungicides used to spray the oak plants with reduce the surface area for the development of *Microsphaera alphitoides* mycelium.

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OCHRONA DĘBU SZYPUŁKOWEGO PRZED MĄCZNIAKIEM PRAWDZIWYM I JEJ WPŁYW NA WZROST ROŚLIN

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Abstrakt: Mączniak prawdziwy dębu (*Microsphaera alphitoides* Griff. et Maubl.) wyrządza szczególnie duże szkody wśród młodych roślin w szkółkach. Poraża dęby europejskie, powodując uszkadzanie liści, pąków oraz szczytowych, niezdrewniałych pędów. Silne porażenie widoczne w postaci białego nalotu grzybni z jednej strony obniża walory dekoracyjne, z drugiej natomiast może powodować zahamowanie wzrostu

roślin. Do tej pory ochrona przed tym patogenem ograniczała się do opryskiwania roślin w okresie zagrożenia preparatami zawierającymi siarkę, co przy wysokich temperaturach panujących latem mogło prowadzić do objawów fitotoksyczności. Stąd testuje się obecnie również inne preparaty, tak z grupy fungicydów syntetycznych, jak i z wyciągów roślinnych oraz preparaty biotechniczne, które mogą być wykorzystywane głównie do stosowania w ogrodach i parkach. W badaniach przeprowadzonych na polu doświadczalnym Instytutu Sadownictwa i Kwiaciarstwa w Skierniewicach testowano 11 preparatów syntetycznych oraz 5 biotechnicznych. Doświadczenia przeprowadzano w dwóch seriach. Po wystąpieniu objawów mączniaka prawdziwego rośliny opryskiwano 4-krotnie co 7 dni. Obserwacje stopnia nasilenia objawów chorobowych prowadzono po wykonaniu 2 i 4 opryskiwań oraz dodatkowo po 12 tygodniach trwania doświadczenia.

Wyniki wskazują, że ochrona zmniejsza intensywność oraz stopień porażenia roślin. Spośród 16 testowanych preparatów rośliny dębu były najlepiej chronione przez fungicydy Bumper 250 EC, Falcon 460 EC, Domark 100 EC. Preparaty biotechniczne i wyciągi roślinne ograniczały porażenie, lecz istotnie słabiej niż fungicydy syntetyczne. Prowadzona ochrona miała również wpływ na tempo wzrostu roślin. Pomiary wysokości roślin wykazały istotne statystycznie zróżnicowanie pomiędzy kombinacjami doświadczenia. Najwyższe rośliny stwierdzono w kommbinacjach, w których zastosowano Nimrod 250 EC oraz Bio-Blat 25 EC, a najniższe w obiektach kontrolnych.

Słowa kluczowe: mączniak prawdziwy dębu, *Microsphaera alphitoides*, ochrona

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EFFECT OF SUBSTRATES ON GROWTH, YIELD, AND PHENOL CONTENT IN LOWBUSH BLUEBERRY (*Vaccinium angustifolium* AIT.) FRUIT 'EMIL'

WPŁYW PODŁOŻY NA WZROST, PLONOWANIE ORAZ ZAWARTOŚĆ POLIFENOLI W OWOCACH BORÓWKI NISKIEJ (*Vaccinium angustifolium* AIT.) ODMIANY 'EMIL'

Abstract: The experiment was carried out in the years 2007–2008 in the Experimental Pomological Station at Rajkowo near Szczecin. In 2005 the plants of lowbush blueberry, 'Emil' cv. were planted in peat (acid muck soil), sawdust (previously composted), and cocoa husk substrate (a by-product from chocolate confectionary plant) at spacing 1.0 × 2.5 m. Plant growth, quantity, quality, and chemical composition of yield were assessed. No effect of substrate was observed regarding plant height tested in the substrates however, the bushes planted in peat and cocoa husk had bigger leaves and one-year shoots. Further, the bushes grown in these substrates yielded best and their fruits were largest, showed highest firmness as well as highest content of soluble solids, organic acids, and phenol compounds.

Keywords: *Vaccinium angustifolium* AIT., substrates, yield, fruit quality, phenols

Vaccinium angustifolium AIT., dubbed lowbush blueberry, Canadian blueberry occurs in the wild in north-east regions of North America [1]. The crossings of *Vac. angustifolium* with *Vac. corymbosum* yielded a few cultivars also named lowbush blueberry or half-highbush blueberry. Regarding the taste, appearance and chemical composition of hybrid berries they resemble wild berries of *Vac. angustifolium*. The half-highbush blueberry is relatively new orchard species but due to its low climatic requirements gets a growing interest in the countries of northern Europe [2]. The berries have high nutritional value [3] and both berries and other parts of plant exhibit health promoting properties [4]. Their high antioxidant activity [5] results from high concentration of flavonoids [6], and especially anthocyanins [7]. The lowbush blueberry has specific inhabitat requirements. Similarly to highbush blueberry [1], lowbush blueberry thrives and yields on light soils with optimum soil humidity, low pH, and

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high humus content [8]. The lack of suitable soils enforces usage of substrates matching the needs of the species.

The aim of the experiment was to test usefulness of selected organic substrates bedded in the alkaline reaction orchard soil for lowbush blueberry cultivation and estimation of effect of the substrates on the quality of the berries.

Material and methods

The experiment was carried out in 2007–2008 in the Pomological Experimental Station at Rajkowo, near Szczecin. The purpose of field trial was to evaluate suitability of the peat, a composted conifer sawdust (obtained from a local sawmill), and the cocoa husk (a by-product obtained from Chocolate Confectionary Plant 'Gryf' in Szczecin) (Table 1) for growing of lowbush blueberry, 'Emil' cv. In spring of 2005 the bushes were planted in the trenches 35 cm deep and 100 cm wide, and filled with the medium at spacing 1.0 m (in the row) × 2.5 m (between the rows).

The fertilization was limited to nitrogen supply only, because chemical analyses both of the soil and the substrates showed high and/or middle content of other nutrients. Each type of media was fertilized with the ammonium nitrate on three occasions at a total dose of 30 kg · N ha^{-1}. The fertilizer was spread evenly on the bed tops at the width of 1 m.

Table 1

Mineral composition, pH, and water capacity of the soil and substrates used in the experiment (mean of 2007–2008)

Bedding	pH	Field water capacity	Full water capacity	N	P	K	Ca	Mg
		[% v · v^{-1}]		[mg · 100 g^{-1}]				
Orchard soil	6.9	—	—	12	12	36	71	6
Peat	3.8	44.8	80.6	11	11	38	157	36
Cocoa husk	5.7	36.9	85.3	24	16	33	162	34
Sawdust	4.9	31.3	82.6	6	9	73	94	25

The supplemental irrigation was applied through the drip line type T-Tape with using water acidified with H_2SO_4 up to pH = 3.5 (as measured in H_2O). The intensity of water supply was adjusted to the substrate moisture by means of the tensiometer monitoring twice a week, according to pF units (the pF, soil suction being the common logarithm of water height in centimeters). Measuring tubes (30 cm) were installed 15 cm below the soil surface and pF 2.2 was used as a threshold value for irrigation. Having reached the threshold, the soil was irrigated to approximately pF 1.0.

Each year total yield, fruit size, fruit mass, and firmness was measured. The mass and firmness of berries was done with a FirmTech 2 apparatus (BioWorks, USA). The firmness of 50 randomly selected berries from each harvest was expressed as a gram-force causing fruit surface to bend 1 mm. Moreover, in fresh fruit soon after the harvest titratable acidity, soluble solids, and L-ascorbic acid content was measured. The titratable acidity was determined by titration of water extract of blueberry homogenate with 0.1 mol · dm^{-3} NaOH to the end point of pH = 8.1, according to PN-90/A-75101/04.

The soluble solids content was determined in the berry juice by means of the digital refractometer Atago (Japan). The L-ascorbic acid content was determined with the iodimetric method. The fruits meant for phenol analysis were kept each harvest. The HPLC analyses of polyphenols were carried out on combined samples of berries kept frozen after each harvest (–36 ^{o}C) prior to thawing. The HPLC apparatus consisting of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The separation was performed on a Synergi Fusion RP-80A 150 × 4.6 mm (5 mm) Phenomenex (Torrance, CA USA) column. Column oven temperature was set at 30 ^{o}C. Aliquots of 1 g fruit tissue were extracted with methanol acidified with 0.1 % HCl. The extraction was performed in an ultrasonic bath for 20 min. Next, the slurry was centrifuged at 19000 × g for 10 min and the supernatant was used for HPLC analysis. The supernatant was recovered and filtered through a 0.45 μm cellulose syringe filter before analysis. The mobile phase was composed of solvent A (2.5 % acetic acid, pH = 2.9) and solvent B (acetonitrile). The program began with a linear gradient from 0 % B to 25 % B (0–36 min), followed by washing and reconditioning the column. The flow rate was 1 $cm^3 \cdot min^{-1}$ and the runs were monitored at the following wavelengths: chlorogenic acid at λ = 320 nm, flavonol glycosides at λ = 360 nm, and anthocyanin glycosides at λ = 520 nm. The Photo Diode Array spectra were measured over the wavelength range λ = 200–600 nm in steps of 2 nm. Retention times and spectra were compared with those of pure standards within 200–600 nm.

The values were evaluated by the Duncan test and for phenolics by the t-Student test. The differences between the means at $p < 0.05$ were considered significant.

Results and discussion

Data referring to lowbush bluberry growth, yield and chemical composition of berries are presented in Table 2.

Table 2

The growth of bushes and fruit quality of lowbush blueberry 'Emil' in dependence on the substrates (mean of 2007–2008)

Parameter		Peat	Cocoa husk	Sawdust
Plant height [cm]		53.5 a	46.0 a	49.5 a
Mean length of one-year shoots [cm]		22.5 b	20.7 ab	18.5 a
Leaf area [cm^2]		5.4 b	5.6 b	4.8 a
Total yield per bush [g]		189 b	175 b	124 a
Mean mass of 100 fruits [g]		86.8 b	85.3 b	71.5 a
Fruit firmness [$G \cdot mm^{-1}$]	at fruit height	375 b	396 b	321 a
	at fruit diameter	147 ab	169 b	139 a
Soluble solids [%]		12.9 b	12.6 b	11.8 a
Titratable acidity [g citric acid · 100 g^{-1}]		0.98 b	0.94 b	0.85 a
Vitamin C [$mg \cdot 100\ g^{-1}$]		23.5 a	25.0 ab	26.5 b

Explanation: The means signed with the same letter do not differ significantly at the 5 % level of significance, according to Duncan t-test.

The substrates showed no significant effect on height of plants, however longer one-year shoots produced bushes grown in peat compared with that of sawdust. Three-year old bushes tested in the experiment of [9] were lower. The berries obtained from plants grown in peat and cocoa husk had larger leaf-area, yield per bush, mean weight of 100 fruits, and fruit firmness measured at fruit height compared with the berries originating from sawdust substrate. At full fruiting stage it is possible to obtain one kg of fruits per bush [2]. The highest soluble solids and titratable acidity was found in berries grown in peat and cocoa husk, whereas berries originating from peat showed higher vitamin C content than that of sawdust medium. In the finding of Starast et al [10] other cultivars showed similar amount of vitamin C, whereas acidity ranged from 0.2 to 2.3 g citric acid per 100 g of fruit and soluble solids content 11.5–14.9 %.

The data on phenolics composition of lowbush blueberry 'Emil' are presented in Table 3.

Table 3

Phenols pattern for lowbush blueberries, 'Emil' cv. [mg · 100 g^{-1}] – mean for 2007–2008

Phenols	Peat	Cocoa husk	Sawdust
Delphinidin 3-galactoside	30.29	36.50	18.71
Delphinidin 3-glucoside	4.92	29.17	24.84
Delphinidin 3-arabinoside	15.17	18.44	9.46
Cyanidin 3-arabinoside	8.43	7.11	4.98
Cyanidin 3-galactoside	8.41	10.34	4.90
Cyanidin 3-glucoside	10.76	8.00	6.44
Petunidin 3-galactoside	3.59	6.19	2.56
Petunidin 3-arabinoside	12.61	9.37	9.17
Petunidin 3-glucoside	9.30	7.17	5.47
Peonidin 3-galactoside	8.97	9.53	6.27
Peonidin 3-glucoside	4.01	4.06	3.05
Peonidin 3-arabinoside	0.30	0.94	0.79
Malvidin 3-galactoside	0.94	1.07	0.46
Malvidin 3-glucoside	1.25	0.40	0.26
Malvidin 3-arabinoside	0.90	0.66	0.73
Anthocyanins	**119.85 a**	**148.95 b**	**98.09 a**
Quercetin 3-galactoside	34.46	29.81	26.21
Quercetin 3-glucoside	5.28	5.16	4.04
Quercetin 3-ramnoside	3.92	3.70	4.90
Kaempferol 3-rutinoside	0.64	2.09	1.53
Flavonols	**44.30 a**	**40.76 a**	**36.68 a**
Chlorogenic acid	**79.03 c**	**40.86 b**	**28.21 a**
Total	**243.14 b**	**230.56 b**	**162.96 a**

The predominant anthocyanin identified in berries was delphinidin-3-galactoside followed by delphinidin-3-glucoside (for berries grown in cocoa husk and sawdust) and delphinidin-3-arabinoside. Further, the decreasing anthocyanin order was: cyanidin

glycosides > petunidin glycosides > peonidin glycosides > malvidin glycosides. Berries originating from plants grown in cocoa husk showed the highest content of total anthocyanin. Similar values were noted by Starast et al [10] for *Vac. angustifolium* and the hybrids of *Vac. corymbosum* × *Vac. angustifolium*. Quercetin-3-galactoside was predominat among identified flavonols and kaempferol-3-rutinoside content was lowest. However, no influence of substrate was observed regarding total flavonols content. Riihinen et al [11] determined 531 $\mu g \cdot g^{-1}$ quercetin for 'Northblue' (*Vac. corymbosum* × *Vac. angustifolium*) in berry peels, whereas no flavonols were detected in berry pulps. The substrates tested in this finding significantly affected chlorogenic acid content in 'Emil' berries (from ~28 mg · 100 g^{-1} for berries of bushes grown in sawdust to ~79 mg · 100 g^{-1} for peat-originating berries). Regarding total phenol, blueberries obtained from plants cultivated in peat and cocoa husk showed significantly higher amounts (>240 and 230 mg · 100 g^{-1}, respectively) compared with berries originating from sawdust bedding (>162 mg · 100 g^{-1}).

Conclusions

1. The plants of lowbush blueberry, 'Emil' cv., growning in peat and cocoa husk substrate had larger leaves and longer one-year shoots.

2. The lowbush blueberry, 'Emil' cv., cultivated in three organic substrates (peat, cocoa husk and sawdust) started to yield in the second year after planting. The best crop and the biggest berries were obtained from plants grown in peat and cocoa husk.

3. The bushes planted in peat and cocoa husk produced fruits of highest content of soluble solids, acidity, and phenol compounds.

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WPŁYW PODŁOŻY NA WZROST, PLONOWANIE ORAZ ZAWARTOŚĆ POLIFENOLI W OWOCACH BORÓWKI NISKIEJ (*Vaccinium angustifolium* AIT.) ODMIANY 'EMIL'

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Abstrakt: Doświadczenie przeprowadzono w latach 2007–2008 w Sadowniczej Stacji Badawczej Katedry Sadownictwa, gdzie w 2005 roku posadzono krzewy borówki niskiej odmiany 'Emil' w rozstawie 1,0 × 2,5 m w glebie murszowej (torf) o odczynie kwaśnym, przekompostowanych trocinach z drzew iglastych oraz w łusce z ziarna kakaowego, która jest odpadem przy produkcji czekolady. Określano wzrost roślin oraz ilość, jakość oraz skład chemiczny plonu.

Nie stwierdzono wpływu zastosowanych podłoży na wysokość roślin, jednak krzewy posadzone w torfie oraz łusce kakaowej miały większe liście oraz dłuższe pędy jednoroczne. Krzewy, które rosły w tych podłożach, również plonowały najlepiej a owoce z nich uzyskane były największe i najbardziej jędrne. Ponadto owoce z tych krzewów charakteryzowały się największą zawartością ekstraktu, kwasów organicznych oraz związków polifenolowych.

Słowa kluczowe: *Vaccinium angustifolium*, plon, jakość owoców, polifenole, podłoża

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USE OF CHEMICAL DRESSING AND POST-CULTURE LIQUIDS OF ANTAGONISTIC BACTERIA IN THE PROTECTION OF RUNNER BEAN (*Phaseolus coccineus* L.)

STOSOWANIE ZAPRAWY CHEMICZNEJ I PŁYNÓW POHODOWLANYCH BAKTERII ANTAGONISTYCZNYCH W OCHRONIE FASOLI WIELOKWIATOWEJ (*Phaseolus coccineus* L.)

Abstract: The purpose of the paper was to establish the protective effect of Zaprawa Oxafun T (active substances: carboxin 37.5 % + tiuram 37.5 %) and post-culture liquids of *Bacillus* sp. Bf 155 and *Pseudomonas* sp. Psf 47 against soil-borne fungi pathogenic towards *Phaseolus coccineus* L. Pre-sowing seed dressing with a chemical preparation or a post-culture liquid of bacteria considerably improved the emergences, healthiness and yielding of bean plants. Despite the pre-sowing seed dressing, plants and seeds obtained after the harvest were infected by *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The best protective effect against those plant pathogens was observed for the post-culture liquid of *Pseudomonas* sp. Psf 47.

Keywords: Zaprawa Oxafun T, runner bean, *Bacillus*, *Pseudomonas*, post-culture liquids

Excessive application of chemical preparations contaminates the environment and causes, for example, accumulation of harmful substances in the plants' yield. In the protection of crops, including leguminous plants, chemical methods are more and more frequently replaced by the biological method based on biopreparations [1, 2] or the microbiological material made of antagonistic microorganisms [3–6]. The literature provides information on the control of plant pathogens using antagonistic fungi (*Gliocladium* sp., *Trichoderma* sp.) and bacteria (*Bacillus* sp., *Pseudomonas* sp.), whose activity consists of antibiosis, competition or parasitism [3–6]. In practice, there is a possibility of using post-culture liquids of those antagonists, containing secondary

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metabolites. These metabolites inhibit the mycelium growth, germination of spores and their endospores, and they cause degradation of the cell walls of a number of fungi [7].

The purpose of the paper was to determine the protective effect of Zaprawa Oxafun T and post-culture liquids of *Bacillus* sp. Bf 155 and *Pseudomonas* sp. Psf 47 against soil-borne fungi pathogenic towards *Phaseolus coccineus*.

Material and methods

The object of the studies conducted in the years 2003–2005 on the field of the Experimental Station at Czeslawice near Naleczów were runner bean plants of 'Westa" cv. grown out of the seeds dressed directly before the sowing with post-culture liquids of *Bacillus* sp. Bf 155 and *Pseudomonas* sp. Psf 47 [8]. The bacteria used in the field experiment were from the soil environment of runner bean, and their antagonistic effect towards fungi pathogenic towards this plant was determined according to the method developed by Martyniuk et al [9]. The post-culture liquids of the used antagonistic bacteria was obtained as a result of the bacteria culture on a liquid medium PDB (Difco) at the temperature of 24 ^{o}C kept for 4 days [10]. The studies also considered a combination with chemical seed dressing with Zaprawa Oxafun T (active substances: carboxin 37.5 % + tiuram 37.5 %) in the quantity of 1 g · 100 g^{-1} seeds and a control combination, ie without any dressing. Each experimental combination included 4 plots (4 replications) with the area of 21 m^2, where 100 seeds were sown on each. In each year of studies observations were conducted twice – in the phase of seedlings and at plant anthesis – establishing the number of the grown plants and estimating their healthiness. Plants with distinct necrotic spots on the stem base and on the roots were sampled for a laboratory mycological analysis. After picking up the plants and drying up the seeds, the quantity of the seed yield and the proportion of seeds with spots were determined. The mycological analysis of the plant material and the seeds was conducted according to the method described by Pieta et al [11].

The obtained results concerning the number, healthiness and yielding of plants were analyzed statistically, and the significance of differences was established on the basis of Tukey's confidence intervals [12].

Results and discussion

Post-culture liquids of the applied antagonistic bacteria had a more positive effect on the emergences, healthiness and yielding of runner bean than Zaprawa Oxafun T. The most number of seedlings grew on the plots sown with the seeds soaked in a post-culture liquid of *Pseudomonas* sp. Psf 47 and *Bacillus* sp. Bf 155 (mean 89 and 87 seedlings, respectively) (Table 1). Slightly worse emergences were observed after the application of the chemical preparation (mean 79 seedlings).

The smallest number of seedlings grew on the plot of the control combination, without any seed dressing (mean 60 seedlings). Seedlings with inhibited growth and yellowing leaves occurred on all plots. The proportion of infected seedlings after dressing with Zaprawa Oxafun T or the post-culture liquids of *Bacillus* sp. Bf 155 and

Pseudomonas sp. Psf 47 was relatively small and it ranged from 3.0 % to 3.9 %, on average. A much higher proportion of infected seedlings occurred in the control (10.5 %, on average). In the period of bean anthesis, only small losses of plants and a slight increase of the proportion of infected plants with distinct necrotic spots on the stem base and the roots were noticed (Table 1).

Table 1

Number and healthiness of runner bean plants (mean from 2003–2005)

Experimental combination	Seedlings		Plants at anthesis	
	number of runner bean plants	mean share of infected runner bean plants [%]	number of runner bean plants	mean share of infected runner bean plants [%]
Seeds soaked in post-culture liquids of *Bacillus* sp. Bf 155	87^{bc}*	3.4^{a}	85^{bc}	4.0^{ab}
Seeds soaked in post-culture liquids of *Pseudomonas* sp. Psf 47	89^{c}	3.0^{a}	88^{c}	3.7^{a}
Seeds dresed with Zaprawa Oxafun T	79^{b}	3.9^{a}	77^{b}	5.0^{b}
Control	60^{a}	10.5^{b}	58^{a}	14.6^{c}

* Means in columns differ significantly ($p \leq 0.05$) if they are not marked with the same letter.

The quantity of the obtained seed yield was proportional to the number and healthiness of plants on the plots of individual experimental combinations (Table 2). The highest seed yield was collected from plants after the application of the post-culture liquid of *Pseudomonas* sp. Psf 47, slightly lower – in the combinations with the post-culture liquid of *Bacillus* sp. Bf 155 or Zaprawa Oxafun T. The smallest number of seeds was collected from the plants of the control combination. Small seeds, with spots on the seed cover occurred in the yield. The proportion of such seeds ranged from 4.5 % (in the combination with the post-culture liquid of *Pseudomonas* sp. Psf 47) to 12.0 %, on average (in the control) (Table 2).

Table 2

Yield and healthiness of runner bean seeds (mean from 2003–2005)

Experimental combination	Mean yield of runner bean seeds [g on the plot]	Mean share of infected seeds [%]
Seeds soaked in post-culture liquids of *Bacillus* sp. Bf 155	3320^{bc}*	4.75^{a}
Seeds soaked in post-culture liquids of *Pseudomonas* sp. Psf 47	3568^{c}	4.50^{a}
Seeds dresed with Zaprawa Oxafun T	2956^{b}	5.50^{a}
Control	2017^{a}	12.00^{b}

* Means in columns differ significantly ($p \leq 0.05$) if they are not marked with the same letter.

Table 3

Fungi isolated from infected of runner bean plants (total from 2003–2005)

Fungus species	Number of isolates										
	Seedlings					Plants at anthesis					Total
	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	
	of *Bacillus* sp. Bf 155	of *Pseudomonas* sp. Psf 47				of *Bacillus* sp. Bf 155	of *Pseudomonas* sp. Psf 47				
Acremonium roseum (Oud.) W. Gams	—	—	2	6	8	2	1	5	7	15	23
Alternaria alternata (Fr.) Keissler	3	3	5	8	19	6	5	7	10	28	47
Aspergillus niger van Tiegh	1	1	3	5	10	3	2	5	8	18	28
Botrytis cinerea Pers.	—	—	1	2	3	2	2	3	4	11	14
Cladosporium cladosporioides (Fres) de Vries	3	2	5	8	18	4	3	7	10	24	42
Epicoccum purpurascens Ehr. ex. Schl.	4	3	7	9	23	7	5	8	9	29	52
Fusarium culmorum (W.G. Sm.) Sacc.	3	2	6	7	18	4	3	7	9	23	41
Fusarium oxysporum Schl. f. sp. *phaseoli* Kend. Snyd.	5	4	9	12	30	6	5	10	17	38	68
Fusarium solani (Mart.) Sacc.	3	2	6	9	20	3	3	8	11	25	45
Gliocladium catenulatum Gilman Abbott	2	3	1	—	6	5	8	3	1	17	23

Table 3 contd.

Fungus species	Number of isolates										
	Seedlings					Plants at anthesis					Total
	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	
	of *Bacillus* sp. Bf 155	of *Pseudo-monas* sp. Psf 47				of *Bacillus* sp. Bf 155	of *Pseudo-monas* sp. Psf 47				
Gliocladium roseum Bainier	2	2	1	—	5	4	4	3	1	12	17
Mucor mucedo Fresenius	1	1	3	4	9	2	2	5	7	16	25
Penicillium nigricans (Bain.) Thom.	4	5	3	1	13	5	6	3	1	15	28
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	5	6	3	—	14	7	8	5	2	22	36
Phoma exigua Desm.	3	3	5	6	17	4	3	6	7	20	37
Pythium irregulare Buisman	2	2	7	11	22	—	—	—	—	—	22
Rhizoctonia solani Kühn	4	4	6	10	24	4	5	8	12	29	53
Sclerotinia sclerotiorum (Lib.) de Bary	3	2	5	7	17	3	4	7	10	24	41
Trichoderma harzianum Rifai	6	6	3	—	15	7	8	4	2	21	36
Trichoderma koningii Oud.	8	9	5	1	23	11	11	8	4	34	57
Trichoderma viride (Link ex Pers.) Rifai	8	8	4	—	20	9	10	6	2	27	47
Total	70	68	90	106	334	98	98	118	134	448	782

Table 4

Fungi isolated from infected seeds of runner bean (total from 2003–2005)

Fungus species	Number of isolates				
	Seeds soaked in post-culture liquids of *Bacillus* sp. Bf 155	Seeds soaked in post-culture liquids of *Pseudomonas* sp. Psf 47	Seeds dresed with Zaprawa Oxafun T	Control	Total
Acremonium roseum (Oud.) W. Gams	4	2	7	9	22
Alternaria alternata (Fr.) Keissler	6	5	8	14	33
Aureobasidium pullulans (de Bary) Arnaud.	3	3	5	8	19
Botrytis cinerea Pers.	2	2	6	15	25
Cladosporium cladosporioides (Fres) de Vries	4	3	7	11	25
Epicoccum purpurascens Ehr. ex. Schl.	2	—	4	7	13
Fusarium culmorum (W.G. Sm.) Sacc.	6	4	10	12	32
Fusarium oxysporum Schl.	11	10	16	24	61
Fusarium solani (Mart.) Sacc.	4	2	5	9	20
Gliocladium catenulatum Gilman Abbott	6	7	4	2	19
Gliocladium fimbriatum Gilman Abbott	6	4	3	—	13
Mucor hiemalis Wehmer	—	—	3	8	11
Mucor mucedo Fresenius	2	1	5	9	17
Penicillium expansum Link ex S.F.Gray	3	4	2	1	10
Phoma exigua Desm.	8	7	12	19	46
Rhizoctonia solani Kűhn	7	6	11	16	40
Rhizopus nigricans Ehrenberg	3	2	5	8	18
Sclerotinia sclerotiorum (Lib.) de Bary	10	8	14	20	52
Trichoderma harzianum Rifai	9	10	5	1	25
Trichoderma koningii Oud.	10	11	4	2	27
Total	106	91	136	195	528

The obtained results confirmed the information on the protective effect of antagonistic microorganisms against infection by plant pathogens [3, 5, 6, 11]. Post-culture liquids of the applied bacteria pointed to the positive effect on the emergences, healthiness and yielding of runner bean, and similar results were also obtained while studying other microorganisms in the biological protection of soybean [8, 11].

The species composition of fungi isolated from the infected seedlings, older plants and the collected seeds of bean as a result of the mycological analysis were similar. However, differences were observed in the quantitative composition of the isolated fungi (Tables 3 and 4). Totally, 334 fungi isolates were obtained from the infected seedlings, 782 – from plants at anthesis and 528 – from bean seeds. The least fungi were isolated after the application of post-culture liquids of *Pseudomonas* sp. Psf 47 or *Bacillus* sp. Bf 155. A little more fungi were obtained using Zaprawa Oxafun T, and the most – from plants of the control combination (Tables 3 and 4). Despite the pre-sowing dressing of the seeds, plants and seeds obtained after the harvest were infected by *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The protective effect against those plant pathogens was shown not only by Zaprawa Oxafun T but also by the post-culture liquids of the used antagonistic bacteria, with *Pseudomonas* sp. Psf 47 being the most effective. It should be supposed that the high effect of the post-culture liquid of those bacteria consisted in the activity of secondary metabolites such as antibiotics, enzymes, siderophores and many other substances inducing plants' resistance to infection by plant pathogens [4–7, 10]. In practice, therefore, the application of chemical preparations can be reduced for the benefit of antagonistic microorganisms and their post-culture liquids.

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STOSOWANIE ZAPRAWY CHEMICZNEJ I PŁYNÓW POHODOWLANYCH BAKTERII ANTAGONISTYCZNYCH W OCHRONIE FASOLI WIELOKWIATOWEJ (*Phaseolus coccineus* L.)

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Abstrakt: Celem pracy było określenie skuteczności ochronnego działania Zaprawy Oxafun T (substancja aktywna: karboksyna 37.5 % + tiuram 37.5 %) oraz płynów pohodowlanych *Bacillus* sp. Bf 155 i *Pseudomonas* sp. Psf 47 przeciwko patogenicznym dla *Phaseolus coccineus* grzybom przeżywającym w glebie.

Przedsiewne zaprawianie nasion, preparatem chemicznym lub płynem pohodowlanym bakterii, znacznie poprawiło wschody, zdrowotność oraz plonowanie roślin fasoli. Mimo przedsiewnego zaprawiania nasion, rośliny oraz uzyskane po zbiorze nasiona fasoli były porażane przez *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* i *Sclerotinia sclerotiorum.* Najskuteczniejszym ochronnym działaniem przed tymi fitopatogenami wyróżnił się płyn pohodowlany *Pseudomonas* sp. Psf 47.

Słowa kluczowe: Zaprawa Oxafun T, runner bean, *Bacillus*, *Pseudomonas*, post-culture liquids

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Elżbieta PATKOWSKA[1] and Danuta PIĘTA[1]

USE OF CHEMICAL DRESSING AND POST-CULTURE LIQUIDS OF ANTAGONISTIC FUNGI IN THE PROTECTION OF RUNNER BEAN (*Phaseolus coccineus* L.) FROM SOIL-BORNE FUNGI

WYKORZYSTANIE ZAPRAWY CHEMICZNEJ I PŁYNÓW POHODOWLANYCH GRZYBÓW ANTAGONISTYCZNYCH DO OCHRONY FASOLI WIELOKWIATOWEJ (*Phaseolus coccineus* L.) PRZED GRZYBAMI ODGLEBOWYMI

Abstract: The paper establishes the effectiveness of Zaprawa Oxafun T (active substance: carboxin 37.5 % + tiuram 37.5 %) and the post-culture liquids of *Trichoderma harzianum* F 79 and *Gliocladium catenulatum* F 135 in the protection of runner bean against soil-borne fungi. Post-culture liquids of antagonistic fungi used for the pre-sowing seed dressing had a more positive effect on the number, healthiness and yielding of *Phaseolus coccineus* L. plants as compared with the chemical preparation. The seedlings, plants at anthesis and the seeds were mainly infected by *Altenaria alternata, Botrytis cinerea, Fusarium* spp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Those fungi were more rarely isolated from plants after the use of post-culture liquids of *T. harzianum* F 79 and *G. catenulatum* F 135 than after using Zaprawa Oxafun T or in the control combination, ie without any seed dressing. A reverse relation was observed in the case of the occurrence of saprobiotic fungi such as *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. The obtained results point out the possibility of using post-culture liquids of *T. harzianum* F 79 and *G. catenulatum* F 135 in the protection of runner bean against soil-borne fungi, as an alternative method to the chemical protection.

Keywords: Zaprawa Oxafun T, *Phaseolus coccineus*, *Trichoderma harzianum*, *Gliocladium catenulatum*, post-culture liquids

Runner bean is commonly cultivated in south-eastern Poland, which is due to the optimum soil and climatic conditions. Soil-borne fungi, and especially *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani* f. sp. *phaseoli*, *Pythium* spp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, constitute a big danger for this plant [1, 2]. Because chemical protection of plants against plant pathogens

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creates a threat of contaminating agricultural products and the cultivation environment, more and more frequently it is replaced by alternative methods based on the use of antagonistic microorganisms since metabolites of fungistatic or fungicidal properties occur in post-culture liquids of antagonistic bacteria (*Bacillus* sp. and *Pseudomonas* sp.) and fungi (*Gliocladium* sp., *Penicillium* sp. and *Trichoderma* sp.) [3–7]. The purpose of the paper was to establish the effectiveness of Zaprawa Oxafun T and post-culture liquids of *T. harzianum* F 79 and *G. catenulatum* F 135 in the protection of runner bean from soil-borne fungi.

Material and methods

The studies were conducted in the years 2004–2006 on the field of the Experimental Station at Czeslawice near Naleczow on a field of a five-years' monocultivation of runner bean with naturally accumulated infection material in the soil. The object of the studies were runner bean plants of 'Westa' cv. grown out of the seeds dressed with post-culture liquids of *T. harzianum* F 79 and *G. catenulatum* F 135 for 5 min directly before sowing. The post-culture liquid of the enumerated antagonists was obtained after 8 days' fungi culture in a liquid medium PDB (Difco) at the temperature of 22 ^{o}C [8]. The studies also considered a combination with chemical dressing of the seeds with Zaprawa Oxafun T (active substance: carboxin 37.5 % + tiuram 37.5 %) in the quantity of 1 g · 100 g^{-1} seeds and a control combination, ie without any dressing. Each experimental combination included 4 plots (4 replications) with the area of 21 m^2, where 100 seeds were sown on each. In each year of studies observations were performed twice – in the phase of seedlings and at anthesis – determining the number and healthiness of plants. Plants with disease symptoms (5 plants from each plot) were sampled for a laboratory mycological analysis. After the harvest, the quantity and quality of the yield were determined. The mycological analysis of the plant material and the seeds was made according to the method described by Pieta et al [9].

The obtained results concerning the number, healthiness and yielding of plants were statistically analyzed, and the significance of differences was established on the basis of Tukey's confidence intervals [10].

Results and discussion

In the years 2004–2006 very similar results were obtained concerning the number, healthiness and yielding of *Phaseolus coccineus* L., which is why only their mean values are provided in the Tables. The most number of seedlings (90, on average), with the smallest proportion of infected ones (2.7 %, on average) grew out of the seeds soaked in a post-culture liquid of *T. harzianum* F 79, and the fewest (63, on average), with the biggest proportion of infected ones (9.4 %, on average), were obtained from the seeds that were not dressed (Table 1). During the second observation, conducted at full anthesis, the number and healthiness of plants were similar to the results obtained in the phase of seedlings. Only a small loss of plants on the plots of individual experimental combinations and a small increase of the proportion of infected plants were observed.

The protective effect of Zaprawa Oxafun T was similar to that of post-culture liquids of antagonistic fungi (Table 1).

Table 1

Number and healthiness of runner bean plants (mean from 2004–2006)

Experimental combination	Seedlings		Plants at anthesis	
	number of runner bean plants	mean participation of infected runner bean plants [%]	number of runner bean plants	mean participation of infected runner bean plants [%]
Seeds soaked in post-culture liquids of *T. harzianum* F 79	90^{b}*	2.7^{a}	89^{b}	3.6^{a}
Seeds soaked in post-culture liquids of *G. catenulatum* F 135	88^{b}	3.1^{a}	86^{b}	4.2^{ab}
Seeds dresed with Zaprawa Oxafun T	85^{b}	3.6^{a}	84^{b}	4.9^{b}
Control	63^{a}	9.4^{b}	60^{a}	12.7^{c}

* Mean in columns differ significantly ($p \leq 0.05$) if they are not marked with the same letter.

The mean yield of runner bean seeds from plot ranged from 3964 g (from plants in the combination with the post-culture liquid of *T. harzianum* F 79) to 1927 g (from control plants) (Table 2). Poorly formed seeds, with brown spots on the cover occurred in the collected yield. The highest proportion of such seeds (9.75 %, on average) occurred in the control combination, without any dressing (Table 2).

Table 2

Yield and healthiness of runner bean seeds (mean from 2004–2006)

Experimental combination	Mean yield of runner bean seeds [g on the plot]	Mean share of infected seeds [%]
Seeds soaked in post-culture liquids of *T. harzianum* F 79	3964^{c}*	2.75^{a}
Seeds soaked in post-culture liquids of *G. catenulatum* F 135	3601^{c}	3.00^{a}
Seeds dresed with Zaprawa Oxafun T	2753^{b}	4.50^{b}
Control	1927^{a}	9.75^{a}

* Mean in columns differ significantly (p 0.05) if they are not marked with the same letter.

The used post-culture liquids of *T. harzianum* F 79 and *G. catenulatum* F 135 as well as Zaprawa Oxafun T had a positive effect on emergences, healthiness and yielding of runner bean as compared with the control. As reported by Babu et al [3], De et al [4] and Roberti et al [6], it should be supposed that antagonistic fungi, through the enzymes (chitinase, chitobiase, protease and glucanase) exudated to the liquid medium, causing degradation of the cell walls of plant pathogens, effectively protected the seeds, roots and the stem base of older plants from infection.

Table 3

Fungi isolated from infected of runner bean plants (total from 2004–2006)

Fungus species	Number of isolates										
	Seedlings					Plants at anthesis					Total
	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	
	of *T. harzianum* F 79	of *G. catenulatum* F 135				of *T. harzianum* F 79	of *G. catenulatum* F 135				
Acremonium kiliense Grutz	—	—	2	4	6	2	2	5	8	17	23
Alternaria alternata (Fr.) Keissler	4	6	9	12	31	6	7	11	15	39	70
Aspergillus flavus Link	1	1	4	8	14	2	2	7	10	21	35
Cladosporium cladosporioides (Fres) de Vries	—	—	3	5	8	2	3	5	8	18	26
Fusarium oxysporum Schl.	8	9	12	15	44	10	11	15	22	58	102
Fusarium solani (Mart.) Sacc.	2	4	7	10	23	3	4	8	11	26	49
Fusarium sporotrichioides Sherb.	1	2	4	6	13	2	2	5	7	16	29
Gliocladium catenulatum Gilman Abbott	4	4	2	1	11	6	6	4	—	16	27
Gliocladium fimbriatum Gilman Abbott	3	3	2	—	8	5	5	3	1	14	22
Humicola grisea Domsch	—	—	2	3	5	1	1	4	6	12	17
Mucor mucedo Fresenius	2	2	4	6	14	3	4	5	7	19	33
Penicillium purpurogenum Stoll	3	3	2	—	8	4	4	3	1	12	20

Table 3 contd.

Fungus species	Number of isolates										
	Seedlings					Plants at anthesis					Total
	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	
	of *T. harzianum* F 79	of *G. catenulatum* F 135				of *T. harzianum* F 79	of *G. catenulatum* F 135				
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	6	5	3	1	15	7	7	4	2	20	35
Pythium irregulare Buisman	3	4	7	12	26	—	—	—	—	—	26
Rhizoctonia solani Kühn	5	5	9	15	34	6	7	11	18	42	76
Sclerotinia sclerotiorum (Lib.) de Bary	—	—	—	—	—	7	8	14	19	48	48
Talaromyces flavus (Ben.) Stolk et Samson	—	—	1	2	3	1	—	4	8	13	16
Trichoderma hamatum (Bon.) Bain	3	3	2	—	8	5	8	3	2	18	26
Trichoderma harzianum Rifai	1	1	—	—	2	3	2	—	—	5	7
Trichoderma koningii Oud.	5	5	4	1	15	6	6	5	2	19	34
Trichoderma pseudokoningii Rifai	4	4	3	2	13	5	5	3	—	13	26
Trichoderma viride Pers. ex S.F. Gray	8	7	4	1	20	10	11	8	3	32	52
Total	63	68	86	104	321	96	105	127	150	478	799

Table 4

Fungi isolated from infected seeds of runner bean (total from 2004–2006)

Fungus species	Number of isolates				
	Seeds soaked in post-culture liquids of *T. harzianum* F 79	Seeds soaked in post-culture liquids of *G. catenulatum* F 135	Seeds dresed with Zaprawa Oxafun T	Control	Total
Acremonium strictum W. Gams	—	1	3	5	9
Alternaria alternata (Fr.) Keisller	3	5	8	11	27
Aspergillus niger van Tiegh	2	2	4	5	13
Botrytis cinerea Pers.	2	4	6	9	21
Fusarium culmorum (W.G.Sm.) Sacc.	1	1	5	8	15
Fusarium equiseti (Corda) Sacc.	—	2	5	7	14
Fusarium oxysporum Schl.	6	9	14	25	54
Fusarium solani (Mart.) Sacc.	4	6	10	14	34
Gliocladium catenulatum Gilman, Abbott	7	5	3	1	16
Gliocladium roseum Bainier	3	5	2	—	10
Mucor hiemalis Wehmer	—	—	3	7	10
Penicillium chrysogenum Thom	4	3	1	—	8

Table 3 contd.

Fungus species	Number of isolates				
	Seeds soaked in post-culture liquids of *T. harzianum* F 79	Seeds soaked in post-culture liquids of *G. catenulatum* F 135	Seeds dresed with Zaprawa Oxafun T	Control	Total
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	6	4	3	1	14
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	8	6	4	2	20
Rhizoctonia solani Kühn	5	5	7	12	29
Rhizopus nigricans Ehrenberg	2	3	6	8	19
Sclerotinia sclerotiorum (Lib.) de Bary	4	6	9	18	37
Trichoderma koningii Oud.	10	8	3	1	22
Trichoderma viride Pers. ex S.F. Gray	11	10	4	—	25
Total	78	85	100	134	397

As a result of a laboratory mycological analysis of the infected roots and the stem base of seedlings, older plants (5 plants from each plot) and collected bean seeds, respectively, 321, 478 and 397 fungi isolates were obtained (Tables 3 and 4). The species composition of fungi isolated from the studied plant material and seeds was similar. On the other hand, differences in the quantitative composition of fungi isolated from individual experimental combinations occurred. The fewest fungi isolates were obtained after the application of post-culture liquids of *T. harzianum* F 79 and *G. catenulatum* F 135, slightly more – in the combination with Zaprawa Oxafun T, and the most – from the control combination (Tables 3 and 4). Seedlings, plants at anthesis and seeds were mainly infected by *Alternaria alternata*, *Fusarium* spp. and *Rhizoctonia solani.* Additionally, *Pythium irregulare* were isolated from the studied seedlings, *Sclerotinia sclerotiorum* – from older plants, and *Botrytis cinerea* – from seeds. A reverse relation was observed in the case of saprotrobiotic fungi of *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. (Tables 3 and 4). Results obtained from the present studies confirmed the protective effect of post-culture liquids of *Gliocladium* spp. and *Trichoderma* spp. as well as the chemical preparation in biological control of plant diseases, which was also shown by other authors [2, 4–7, 9].

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WYKORZYSTANIE ZAPRAWY CHEMICZNEJ I PŁYNÓW POHODOWLANYCH GRZYBÓW ANTAGONISTYCZNYCH DO OCHRONY FASOLI WIELOKWIATOWEJ (*Phaseolus coccineus* L.) PRZED GRZYBAMI ODGLEBOWYMI

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Abstrakt: W pracy określono skuteczność Zaprawy Oxafun T (substancja aktywna: karboksyna 37,5 % + tiuram 37,5 %) oraz płynów pohodowlanych *T. harzianum* F 79 i *G. catenulatum* F 135 w ochronie fasoli wielokwiatowej (*Phaseolus coccineus* L.) przed grzybami odglebowymi.

Płyny pohodowlane grzybów antagonistycznych, użyte do przedsiewnego zaprawiania nasion, wpłynęły korzystniej aniżeli preparat chemiczny na liczebność, zdrowotność i plonowanie roślin *Phaseolus coccineus* L. Siewki, rośliny w fazie kwitnienia oraz nasiona, porażane były głównie przez *Alternaria alternata*, *Botrytis cinerea, Fusarium* spp., *Rhizoctonia solani* i *Sclerotinia sclerotiorum.* Grzyby te wyosabniano rzadziej z roślin po zastosowaniu płynów pohodowlanych *T. harzianum* F 79 i *G. catenulatum* F 135 aniżeli po

zastosowaniu Zaprawy Oxafun T lub w kombinacji kontrolnej, tj. bez zaprawiania nasion. Odwrotną zależność stwierdzono w przypadku występowania grzybów saprotroficznych, jak: *Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp. Uzyskane wyniki wskazują na możliwość stosowania płynów pohodowlanych *T. harzianum* F 79 i *G. catenulatum* F 135, do ochrony fasoli wielokwiatowej przed grzybami odglebowymi, jako alternatywną metodę w stosunku do ochrony chemicznej.

Słowa kluczowe: Zaprawa Oxafun T, *Phaseolus coccineus*, *Trichoderma harzianum*, *Gliocladium catenulatum*, płyny pohodowlane

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Bożena PAWŁOWSKA[1] and Anna BACH[1]

EFFECT OF SALT STRESS ON *ROSA* 'NEW DAWN' IN *IN VITRO* CULTURE

WPŁYW STRESU SOLNEGO NA KULTURY *IN VITRO* RÓŻY 'NEW DAWN'

Abstract: Salt stress is one of the main causes of plant damage in horticulture and tree die back in cities. It can be of natural origin but most often is due to human activity, eg uncontrolled fertilization or the use excessive amounts of salt for winter maintenance of streets and pavements. Plants are selected for resistance to salt, and laboratory *in vitro* techniques proved very useful for this purpose. The present experiments aimed to determine tolerance of roses *Rosa* 'New Dawn' to salt. The effect of sodium and calcium chloride on plant growth and shoot development in 'New Dawn' rose in *in vitro* cultures was examined. The plants were cultured on the QL medium containing 5 μM BA, 0.05 μM NAA, 0.3 μM GA_3 and 20 g · dm^{-3} sucrose, pH = 5.6, supplemented with NaCl or $CaCl_2$ (at concentrations 0–400 mM). The media containing increasing concentrations of salts under study showed inhibitory effect on regeneration and multiplication of rose shoots. $CaCl_2$ was less toxic than NaCl. An increase in medium salinity, independently of the salt tested, reduced the height of newly grown shoots and the number of leaves. Moreover, the proportion of chlorotic, deformed or necrotic leaves increased with the increasing salt concentration. The media did not affect shoot fresh mass index or fresh-to-dry mass ratio, which remained at the same level independently of chloride concentration in medium.

Keywords: *in vitro*, multiplication, NaCl, $CaCl_2$, salt stress, *Rosa* 'New Dawn'

Salt stress is an important factor causing plant damage in horticulture and tree die back in cities. High salt concentration in soil suppresses growth and development of plants and decreases productivity. About 25 % of arable land worldwide shows excessive salinity caused mostly by NaCl. It can be of natural origin but most frequently is due to human activity, eg uncontrolled fertilization or excessive use of salt for winter maintenance of streets and pavements. *In vitro* techniques can be used as a tool for studying of the mechanisms of tolerance of plants to different environmental stresses, including salt stress. The advantages of tissue cultures include quick regeneration of plants and easiness of control of ambient conditions [1–9].

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This study aimed to determine the effect of salinity, examined by medium supplementation with NaCl or $CaCl_2$, on growth and development of *Rosa* 'New Dawn' in *in vitro* cultures.

Materials and methods

Microshoots of *Rosa* 'New Dawn', 5 mm high, with 2–3 leaves were placed in 250 cm^3 Erlenmeyer flasks on a basic medium according to Quorin and Lepoivre (QL) [10] supplemented with growth regulators: 5 μM BA, 0.05 μM NAA, 0.3 μM GA_3, pH 5.6. The tested media contained NaCl or $CaCl_2$ at concentrations: 12.5, 25, 50, 100, 200 and 400 mM. Tested salt-free media were used as the control medium. In each combination of the medium 6 replication (each flash with 5 explants) were tested.

Cultures were maintained in a growth room at 25/23 ^oC (day/night) at 80 % humidity, with 16-hour day for 6 weeks. The following values were recorded after 6 weeks: percentage of regenerating explants, shoot multiplication index (number of newly developed shoots per 1 explant), mean shoot height[mm], mean number of leaves per 1 newly formed shoot. Quality of regenerated plants was inspected visually and deformations and growth anomalies were recorded. Leaves on each shoot were classified into three groups: necrotic (brown, dying out), chlorotic (characterized by chlorosis) and/or deformation of the leaf blade and of good quality – properly developed, vividly green in color. Share of each class was calculated. Shoot fresh mass was measured and used for calculation of fresh mass growth index G_v according to the formula: $G_v = m_k – m_p/m_p$, where: G_v – fresh mass growth index, m_p – initial mass, m_k – final mass. Dry mass of plants was also determined after drying at 60 ^oC to constant mass.

Data were calculated using a two-factor statistical method for independent variables. Statistical significance was evaluated using Duncan test with confidence level $\alpha = 0.05$.

Results and discussion

Soil salinity is an increasingly important burden in all urban areas. It is mainly caused by uncontrolled use of salt for winter street maintenance [8]. Sodium chloride is most often used for this purpose because of its common commercial availability, efficaciousness and cost-effectiveness. Calcium chloride can be an alternative because it is even more effective but has milder effect on plants, however, it is more expensive, as well [11]. The mechanisms of plant tolerance to salinity can be investigated with the use of *in vitro* techniques which are a good tool for studies of plant reaction to salt and for selection of salt-tolerant lines [5].

Roses belong to the medium salt-sensitive plants [12].

Increasing concentrations of the chlorides under study, namely sodium chloride and calcium chloride in the QL medium (0–400 mM) showed an inhibitory effect on regeneration and multiplication of rose shoots (Tables 1 and 2).

Table 1

The effect of salt type in the medium on *in vitro* shoot cultures of *Rosa* 'New Dawn'

Treatment	Share of regenerating explants [%]	Shoot multiplication rate
Control	100.0 c*	2.6 c
NaCl	89.6 a	1.5 a
$CaCl_2$	93.8 b	1.7 b

* Means designated with the same letters do not differ significantly at $\alpha = 0.05$.

Table 2

The effect of salt type and concentration on a share of regenerating explants of *Rosa* 'New Dawn' [%]

Salt concentration [mM] (Average of NaCl and $CaCl_2$)	0 Control	12.5	25	50	100	200	400
NaCl	100 c	100 c	100 c	100 c	100 c	93.8 c	43.8 a
$CaCl_2$	100 c	100 c	100 c	100 c	100 c	100 c	62.5 b

Calcium chloride proved to be less toxic than sodium chloride, namely, rose shoots demonstrated better regenerative and multiplicative capacities (62.5 % of regenerating explants) but the result did not differ significantly vs NaCl-treated explants (43.8 %) (Tables 1 and 2).

The presence of 50 and 100 mM calcium chloride in the medium stimulated development of rose shoots but the difference did not reach statistical significance (1.9–2.5 shoots per 1 regenerating explants). In contrast, medium supplementation with NaCl significantly lowered rose shoot multiplication index (Fig. 1).

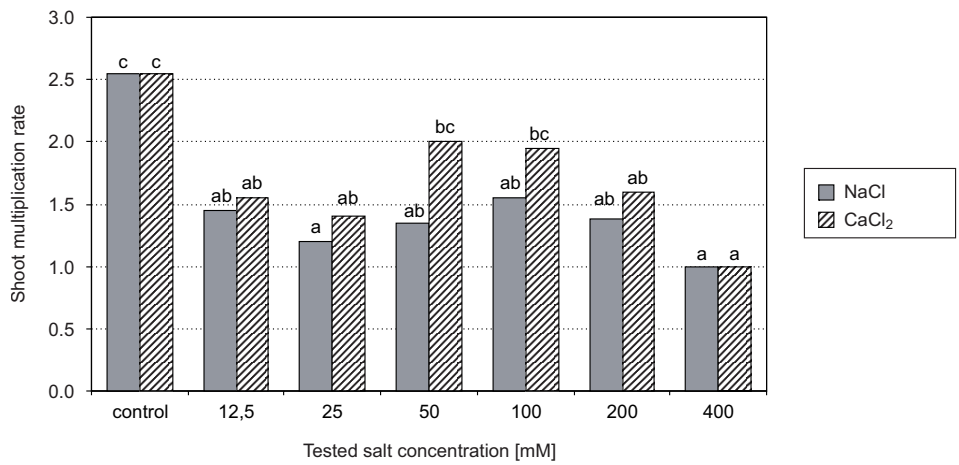

Fig. 1. The effect of salt type and concentration on multiplication rate of *Rosa* 'New Dawn' shoots

In *Populus euphratica in vitro* cultures, high NaCl concentrations (100–250 mM) inhibited callus growth but no die back was observed, however, the callus obtained on the high-salt medium differentiated very weakly and axillary buds did not develop [6]. Kuciakowski [13] in his studies on *Citrus limon* observed growth inhibition and die back of nucellar embryos on the medium containing 86 mM NaCl.

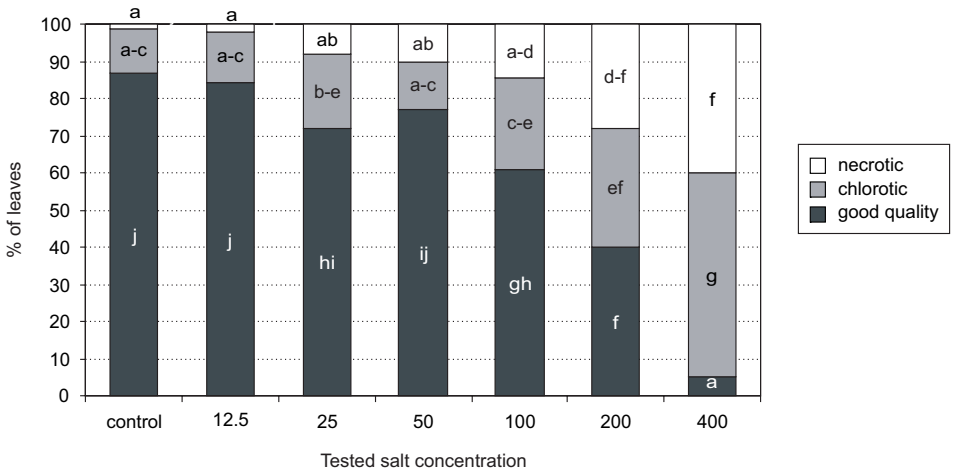

Fig. 2. The effect of average of tested salt concentration (NaCl and $CaCl_2$) on quality of leaves in *Rosa* 'New Dawn' regenerates

Zhang et al [6] observed maximal growth rate of *Populus euphratica* callus at 50 mM NaCl concentration. The difference in the reaction to salt between *Populus euphratica* plant and callus has not been explained. Vitagliano et al [14] investigated the effect of salt on *Cydonia oblonga* rootstock regeneration using shoot cultures, callus and cell suspension. The studies showed greater sensitivity of cell cultures, containing single cell suspensions, to salt than shoot and callus cultures.

Our studies on the effect of medium supplementation with NaCl or $CaCl_2$ on development of roses in *in vitro* cultures demonstrated a significant influence of salt concentration on mean length of newly formed shoots, independently of salt composition (Table 3). Shoots developed on the control tested salt-free medium were the longest (7.3 mm) in comparison with the growing on different concentration of NaCl (4.9–2.8).

Table 3

The effect of salt concentration in the medium on plant height and number of leaves in *in vitro* cultures of *Rosa* 'New Dawn'

Salt concentration [mM] (Average of NaCl and $CaCl_2$)	0 Control	12.5	25	50	100	200	400
Mean shoot height [mm]	7.3 c	4.9 b	4.4 b	4.9 b	3.2 a	2.5 a	2.8 a
Mean number of leaves/shoot	8.1 cd	8.9 d	8.4 d	7.2 c	5.9 b	5.2 b	4.1 a

Statistical analysis indicated that independently of salt type, its concentration affected the mean number of leaves per 1 regenerating shoot. The greatest number of leaves per regenerating shoot was observed on QL media containing 12.5 and 25 mM $CaCl_2$ (7.3, 8.4), and 12.5, 25 and 50 mM NaCl (8.5; 7.0; 7.7 leaves, respectively). The results obtained at these concentrations did not significantly differ from control (8 leaves) (Table 3).

The present experiment indicated worsening of quality of rose shoots with increasing chloride concentration in the medium. Visual analysis of rose leaves demonstrated a decrease in the fraction of good-quality, healthy, deep-green leaves with increasing salt concentration in the medium while the fraction of chlorotic, deformed and necrotic leaves rose was increased. The plants cultured on the control medium and medium containing 12.5 mM salt had the greatest number of healthy leaves (87–85 %). High NaCl concentration in the medium has been also shown to increase necrosis of poplar shoots [15].

Likewise, according to Wahone et al [4], an increasing salt concentration (5–30 mM) also elevated the number of damaged leaves in two varieties of *Rosa hybrida* 'Kiss' and 'Cardinal'. Those studies demonstrated high salt tolerance in the varieties of *Rosa hybrida*. The variety 'Kiss', was more salt resistant than 'Cardinal', since less than 50 % of its leaves were damaged on the medium supplemented with 0–10 mM salt while the variety 'Cardinal' had more than 50 % of necrotic leaves when cultured on the medium containing 5 mM NaCl. Increasing NaCl concentration in the medium can also lead to the accumulation of Na^+ and Cl^- ions, as observed in the varieties of *Rosa hybrida*: 'Cardinal' and 'Kiss' and in *Rosa chinensis* 'Major' and *Rosa rubiginosa*. The increased leaf damage can be attributed to the elevated chlorine concentration in these organs [4]. The damage of tissues, shoots and leaves in *Citrus limon* and tomato can be also caused by the excessive accumulation of Na^+ and Cl^- ions [13, 16].

Shoot growth index (Gv) did not significantly differ between plants cultured on media containing increasing salt concentrations and was similar as in control (Table 4).

Table 4

The effect of salt concentration in the medium on Gv and dry mass of regenerated plants of *Rosa* 'New Dawn'

Salt concentration [mM] (Average of NaCl and $CaCl_2$)	0 Control	12.5	25	50	100	200	400
Gv	3.3a	3.4a	3.0a	3.1a	2.9a	—	—
Dry mass [%]	12.9a	10.7b	10.0b	13.6a	12.6a	—	—

Wrochna et al [17] demonstrated that the presence of salt in medium stimulated fresh mass accumulation in *Amaranthus paniculatus*, *A. caudatus, Atriplex hortensis* and *Tamarix tetrandra.* This is a typical reaction of facultative halophytes, which need a low salt content for optimal growth [18].

The present experiments showed the effect of sodium and calcium chloride in medium on dry mass content. Tissues of roses cultured on the control medium contained 12.0 % of dry mass and did not significantly differ from tissues which were

cultured on the medium supplemented in 50 or 100 mM salt (12.6–13.6 %). However, dry mass content in plants cultured on these media was lower. Likewise, in *Cydonia oblonga* callus, the reduction of both fresh and dry mass was observed when cultured on the media supplemented with increasing NaCl concentrations, but fresh-to-dry mass ratio remained constant [14].

Conclusions

1. Increasing concentrations of sodium and calcium chloride (0–400 mM) in QL medium inhibited regeneration and multiplication of rose shoots, but $CaCl_2$ was less toxic than NaCl.

2. The salts under study reduced the mean length of newly developed shoots; the shoots were the longest on the control chloride-free medium.

3. Fraction of good-quality, healthy, deep-green leaves decreased and fraction of chlorotic, deformed and necrotic leaves rose enhanced with increasing both tested salt concentrations in the medium.

4. The present experiments demonstrated that an *in vitro* technique can be efficiently used for studies of the reaction of *Rosa* 'New Dawn' to sodium and calcium chloride.

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WPŁYW STRESU SOLNEGO NA KULTURY *IN VITRO* RÓŻY 'NEW DAWN'

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Abstrakt: Stres solny jest jednym z głównych czynników powodujących uszkodzenia roślin w uprawach ogrodniczych, a także obumieranie drzew w miastach. Może być wynikiem naturalnych procesów, ale najczęściej zachodzi pod wpływem działalności człowieka, np. nieracjonalnego nawożenia lub używania zimą dużych ilości soli w celu usuwania oblodzenia dróg i chodników. Przydatna w badaniu selekcyjnym reakcji roślin na zasolenie okazała się technika laboratoryjna *in vitro*. W przeprowadzonych doświadczeniach określano tolerancję róż *Rosa* 'New Dawn' na zasolenie. Oceniono wpływ chlorków: sodu i wapnia, na wzrost i rozwój pędów bocznych odmiany 'New Dawn' w kulturach *in vitro*. Rośliny były uprawiane na pożywce wg QL, zawierającej 5 µM BA, 0,05 µM NAA, 0,3 µM GA_3 oraz 20 g · dm^{-3} sacharozy, o pH = 5,6, wzbogaconej dodatkowo w NaCl lub $CaCl_2$ (w stężeniach 0–400 mM).

Wykazano hamujący wpływ pożywek ze wzrastającym stężeniem badanych soli na regenerację i namnażanie pędów róży, przy czym $CaCl_2$ działał mniej toksycznie w porównaniu z NaCl. Wzrost zasolenia pożywki, niezależnie od testowanej soli ograniczał wysokość nowopowstałych pędów i liczbę formujących się liści. Ponadto ze wzrostem stężenia soli zwiększała się liczba liści z chlorozą, a także zdeformowanych i nekrotycznych. Nie zaobserwowano wpływu badanych pożywek na współczynnik wzrostu świeżej masy pędów róży, podobnie stosunek świeżej masy do suchej masy pozostawał na tym samym poziomie, niezależnie od stężenia chlorków w pożywce.

Słowa kluczowe: *in vitro*, namnażanie, NaCl, $CaCl_2$, stres solny, *Rosa* 'New Dawn'

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EFFECT OF NITROGEN FERTILIZATION ON THE YIELD AND CONTENT OF NITRATES IN RED BEET STORAGE ROOTS

WPŁYW NAWOŻENIA AZOTEM NA PLON I ZAWARTOŚĆ AZOTANÓW W KORZENIACH SPICHRZOWYCH BURAKA ĆWIKŁOWEGO

Abstract: Field experiment with red beet 'Boro F_1' cv. was carried out in 2005–2007. The aim of the research was to determine the effect of the kind of nitrogen fertilizer (ammonium sulfate or nitrate urea solution – RSM) and the way of fertilizer application either broadcasting (liquid spreading) or localized, with emphasis on diversified (divided) doses of nitrogen and foliar nutrition on the plant yield and the content of nitrates, ammonium form and protein nitrogen in red beet storage roots.

The kind of nitrogen fertilizer and the way of its application did not significantly affect the total yield of the roots. In all years of the experiment there was no repeated effect of experiment factors of the quantity of marketable yield. The effect of the examined factors on the content of nitrates in beet root depended on the year of cultivation. In 2005 pre-sowing fertilization, both broadcasting and localized in the dose of 67.5 kg $N \cdot ha^{-1}$ combined with foliar nutrition resulted in obtaining roots with a slightly lower content of nitrates in comparison with other fertilization ways. Further years of the experiment did not reveal such a tendency. The kind of applied fertilizer did not affect nitrates content in any year of the experiment. The concentration of ammonium nitrogen and protein nitrogen in the roots was not dependent on the kind of nitrogen fertilizer or the way of its application.

Keywords: fertilization method, foliar nutrition, CULTAN method, biological value

Nitrogen fertilization has a significant effect on the quality and quantity of vegetable yield. An increase in the doses of nitrogen fertilizer resulted in the rise in the yield of red beet root, Chinese cabbage and spinach with a simultaneous increase in nitrates concentration in plant tissue and a decrease in dry weight [1–3]. Also foliar nutrition with nitrogen affects the growth in plant yield [4–6].

Nitrogen accumulation in plant cells depends not only on the quantity of nitrogen in soil but also on its form. Many authors [3, 7] noticed the growth in nitrates content in

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plants fertilized with nitrate form of nitrogen when compared to plants fertilized with reduced form.

Decreased content of nitrates in vegetables was observed with the application of localized fertilization (CULTAN method) [8, 9]. Del Amor et al [10] demonstrated that replacing a part of soil nitrogen dose with foliar nutrition in the reduced form (urea or ammonium sulfate) results in decrease of nitrogen contents in lettuce.

The aim of this experiment was to determine the effect of nitrogen fertilization type and way of its application (broadcasting/liquid spreading and localized) and the division of nitrogen dose into pre-sowing part in combination with soil top dressing fertilization or foliar nutrition on the quantity of total and marketable yield, and the contents of nitrogen compounds in red beet roots, 'Boro F_1' cv.

Material and methods

The experiment with red beet root, 'Boro F_1' cv., cultivation was conducted in field conditions in the years 2005–2007 in Mydlniki village near Krakow. The plants were cultivated in the second year after manure application in light silt loam, containing 2.7 % organic matter. The content of P, K, Mg and Ca was assessed on the basis of soil chemical analysis and supplemented pre-sowing to the level suitable for beet root requirements. The content of mineral nitrogen (NO_3-N + NH_4-N) in soil before the application of fertilizers was 3–6 mg · dm^{-3}, and pH_{H_2O} 7.10–7.20.

The effect of the following factors was determined:

– kind of nitrogen fertilizer:

1) ammonium sulfate (20.5 % NH_4-N),

2) nitrate-urea solution (RSM; 7.5 % NH_4-N, 7.5 % NO_3-N, 15 % NH_2-N),

– way of N fertilizers application – broadcasting (liquid spreading) or localized with different (divided) nitrogen doses and foliar nutrition of the plants:

1) 100 % dose of soil N, pre-sowing broadcasting (liquid spreading),

2) 75 % dose of soil N, pre-sowing, broadcasting (liquid spreading) + 25 % dose of N as top dressing,

3) 75 % dose of soil N, pre-sowing, broadcasting (liquid spreading) + foliar nutrition,

4) 75 % dose of soil N, pre-sowing, localized,

5) 75 % dose of soil N, pre-sowing, localized + 25 % dose of N as top-dressing,

6) 75 % dose of soil N, pre-sowing, localized + foliar nutrition.

90 kg · ha^{-1} was treated as 100 % nitrogen dose. The experimental design was shown in Table 1.

There were 48 plots of 9.6 m^2 each (4 × 2.4 m) in the experiment. Four plots were assigned to every variant of the experiment.

Pre-sowing N fertilization was conducted on the sowing day. Ammonium sulfate was broadcast on the surface of the field, while the RSM fertilizers was dissolved in water (determined dose of RSM was dissolved in dm^3 of water) and spread evenly. After fertilizers application, the soil was stirred with rake. In the sites with localized fertilization, fertilizer was applied just after sowing in every second row at 7–10 cm.

Table 1

Experimental design

Kind of nitrogen fertilizer	Fertilization method	N dose [kg · ha^{-1}]		
		Pre-sowing	Top-dressing	Foliar nutrition
Ammonium sulfate	1 broadcasting	90.0	—	—
	2 broadcasting	67.5	22.5	—
	3 broadcasting	67.5	—	14.1
	4 localized	67.5	—	—
	5 localized	67.5	22.5	—
	6 localized	67.5	—	14.1
Nitrate-urea solution (RSM)	1 broadcasting (liquid spreading)	90.0	—	—
	2 broadcasting (liquid spreading)	67.5	22.5	—
	3 broadcasting (liquid spreading)	67.5	—	14.1
	4 localized	67.5	—	—
	5 localized	67.5	22.5	—
	6 localized	67.5	—	14.1

Soil top-dressing fertilization was performed in designated sites at the initial stage of intensive plant growth (6 weeks after sowing – 6–8 leaves stage) in broadcasting manner with the use of ammonium sulfate or by liquid spreading with RSM solution (variants 2, 5). Plants in sites with foliar nutrition were sprayed three times (variants 3, 6). The date of first foliar nutrition treatment corresponded to the date of top-dressing fertilization, soil fertilization (17 July), and further spraying was conducted in two-week intervals (31 July and 14 August). In the first and third treatment the fertilization was conducted with 2 % urea solution, while in the second treatment 1 % solution of Supervit R (2.5 % NH_2-N, 1 % NO_3-N, 3.4 % K_2O, 0.6 % MgO + microelements) was applied, with the dose of 700–800 dm^3 solution per hectare. Total nitrogen dose applied in foliar nutrition was 14.1 kg · ha^{-1}.

The sowing of seeds in the rows every 30 cm was performed in the first decade of June with the help of hand seed drill. Rouging was conducted after 10 days keeping 7 cm distance between plants.

During harvesting the quantity of marketable yield was estimated (roots 4–10 cm in diameter) and yield out of the selection. Chemical analyses of the roots were carried out directly after harvesting. 10 pieces of roots were collected from marketable yield from every site, washed in distilled water and shredded in homogenizer. The content of NO_3^- and NH_4^+ was determined in the obtained material with the use of ion selective electrode after prior extraction with 0.02 mol · dm^{-3} $Al_2(SO_4)_3$. Protein nitrogen was assessed with Kjeldahl method.

Average mean temperature in the years of the experiments was 16–19 ^oC June––August, with average rainfall at the level of 75–150 mm. The only exception was July 2006, with average temperature of 21 ^oC and rainfall of 20 mm. The lowest mean temperature during the whole vegetation period was measured in 2005 (17 ^oC).

The obtained results underwent a two-factor variance analysis (Statistica 7). Differences in means were analyzed with LSD Fischer test. Differences significance was declared at $p = 0.05$.

Results

The results obtained from individual years of the experiment are presented in Tables 2 and 3. Mean total yield of the red beet root was 72.1, 55.4 and 68.6 Mg · ha^{-1} for 2005, 2006 and 2007, respectively (Table 2). The yield was not dependent on the kind of applied fertilizer (ammonium sulfate, RSM) in any year of the experiment. Only in 2006 the way of fertilizer application influenced the quantity of total yield; the highest yield was obtained in the sites with broadcasting fertilization (with the use of ammonium sulfate) or liquid spreading (with the use of RSM) in combination with top dressing fertilization in soil (59.2 Mg · ha^{-1} on average). Slightly lower yield was obtained in the sites with localized fertilization combined with foliar nutrition (58.5 Mg · ha^{-1} on average). The lowest total yield of red beet roots in 2006 was observed after pre-sowing fertilization with nitrogen in localized way linked with top-dressing (50.4 Mg · ha^{-1} on average).

The analysis of the results from three years of experiments shows a slightly higher total beet root yield from the sites fertilized with the dose of nitrogen divided into pre-sowing and top-dressing applied into soil as liquid spreading. Localized fertilization did not reveal such an interrelation.

The influence of experiment factors on the marketable yield quantity was diversified in the individual years of the experiment (Table 2). In 2005 and 2007 higher marketable yield was obtained from the sites fertilized with RSM, while in 2006 from the sites fertilized with ammonium sulfate. In 2005 and 2006 these differences were statistically significant. The use of nitrogen fertilization as pre-sowing only in the full dose, ie 90 kg N · ha^{-1} (100 % dose of N) and the division of the dose into pre-sowing (75 % dose of N) applied in a traditional way combined with top-dressing fertilization influenced the highest marketable yield in 2005 and 2006 irrespectively of the way of pre-sowing way of fertilizer application to the soil. In 2006 the level of the yield was equally high in the site where nitrogen was applied pre-sowing in the form of deposit in combination with top-dressing foliar nutrition. This year revealed a significant cooperation between the kind of nitrogen fertilizer and the way it was applied. To provide the plants with nitrogen in case of ammonium nitrate it seemed a better way to use broadcasting method on the whole surface, and in case of RSM to introduce it in the form of nitrogen deposit.

In the last year of the experiments (2007) the way of fertilizer application did not have any significant effect on the yield quantity, though there was a tendency of higher yield of plants after the fertilization with full pre-sowing N dose, ie 90 kg · ha^{-1}.

The kind of used fertilizer and the way of its application did not bring any statistically significant effect on the concentration of NH_4^+ in red beet storage roots (Table 3).

The content of nitrates in red beet storage roots was considerably diversified in the individual years of the experiment (Table 3). The highest level of NO_3^- (2047 mg · kg^{-1} fm) was assessed in 2005, while nitrates content in the following years were similar and reached 1260 and 1374 mg · kg^{-1} f.m., in 2006 and 2007, respectively.

Table 2

Effects of nitrogen fertilizer type and fertilization method on the total and marketable yield of red beet roots in 2005–2007

Fertilizer	Fertilization method	Total [Mg · ha^{-1}]				Marketable [Mg · ha^{-1}]			
		2005	2006	2007	Means	2005	2006	2007	Means
$(NH_4)_2SO_4$	1*	72.3	58.2	66.4	*65.6*	60.9	57.1	58.3	*58.7*
	2	76.2	63.0	71.9	*70.4*	57.5	62.0	50.2	*56.6*
	3	70.4	61.5	66.6	*66.2*	49.7	58.3	57.2	*55.1*
	4	68.1	52.2	78.5	*66.3*	56.0	48.4	60.9	*55.1*
	5	68.5	48.0	69.1	*61.9*	53.8	46.1	57.7	*52.5*
	6	77.3	56.4	65.3	*66.3*	53.2	54.4	52.8	*53.5*
RSM	1	78.2	52.4	72.2	*67.6*	65.5	50.1	62.6	*59.4*
	2	69.6	55.5	71.6	*65.6*	60.6	50.6	61.0	*57.4*
	3	75.5	51.7	66.2	*64.5*	62.9	45.7	60.5	*56.4*
	4	71.3	52.2	64.9	*62.8*	57.1	51.0	56.9	*55.0*
	5	66.9	52.9	66.0	*61.9*	52.3	47.4	58.2	*52.7*
	6	74.3	60.7	64.9	*66.6*	54.1	57.8	62.1	*58.0*
Means for year:		*72.1*	*55.4*	*68.6*		*57.0*	*52.2*	*58.2*	
Means for factors:									
fertilizer	$(NH_4)_2SO_4$	72.1	56.6	69.6	*66.1*	55.2	54.4	56.2	*55.2*
	RSM	72.0	54.2	67.6	*64.6*	58.8	50.4	60.2	*56.5*
fertilization method	1	75.2	55.3	69.3	*66.6*	63.2	53.6	60.4	*59.1*
	2	72.9	59.2	71.8	*68.0*	59.1	56.3	55.6	*57.0*
	3	73.0	56.6	66.4	*65.3*	56.3	52.0	58.8	*55.7*
	4	69.7	52.2	71.7	*64.5*	56.5	49.7	58.9	*55.0*
	5	67.7	50.4	67.5	*61.9*	53.0	46.8	58.0	*52.6*
	6	73.9	58.5	65.1	*65.8*	53.6	56.1	57.5	*55.7*
$LSD_{0.05}$ for:	fertilizer	ns	ns	ns		3.15	3.70	ns	
	fertilization method	ns	6.19	ns		5.45	6.42	ns	
	interaction	ns	ns	ns		ns	9.07	ns	

* 1 – 90 kg N · ha^{-1} pre-sowing, broadcasting (liquid spreading); 2 – 67.5 kg N · ha^{-1} pre-sowing, broadcasting (liquid spreading) + 22.5 kg N · ha^{-1} as top dressing; 3 – 67.5 kg N · ha^{-1} pre-sowing, broadcasting (liquid spreading) + foliar nutrition; 4 – 67.5 kg N · ha^{-1} pre-sowing, localized; 5 – 67.5 kg N · ha^{-1} pre-sowing, localized + 22.5 kg N · ha^{-1} as top-dressing; 6 – 67.5 kg N · ha^{-1} pre-sowing, localized + foliar nutrition; ns – non-significant differences.

Table 3

Effects of fertilizer type and fertilization method on the contents of ammonium and nitrates and protein-N in red beet roots in 2005–2007

Fertilizer	Fertilization method	NH_4^+ [mg · kg^{-1} f.m.]				NO_3^- [mg · kg^{-1} f.m.]				Protein-N [% dm]			
		2005	2006	2007	Means	2005	2006	2007	Means	2005	2006	2007	Means
$(NH_4)_2SO_4$	1*	242	224	173	*213*	2160	1332	1340	*1611*	1.91	2.30	2.96	*2.39*
	2	202	243	191	*212*	2052	1587	1717	*1785*	1.88	2.35	3.00	*2.41*
	3	222	238	159	*206*	1621	1249	1328	*1399*	1.73	2.47	2.78	*2.33*
	4	212	255	161	*209*	2340	1028	1031	*1466*	1.81	2.30	3.01	*2.37*
	5	253	220	160	*211*	2300	1035	1292	*1542*	1.81	2.26	3.13	*2.40*
	6	274	243	180	*232*	2028	1441	1283	*1584*	1.77	2.10	3.08	*2.32*
RSM	1	221	220	165	*202*	2215	1343	1369	*1642*	1.80	2.25	3.03	*2.36*
	2	243	255	167	*221*	1987	1011	1269	*1422*	1.71	2.00	2.89	*2.20*
	3	173	240	181	*198*	1477	1351	1342	*1390*	1.94	2.48	2.87	*2.43*
	4	245	220	186	*217*	2313	1427	1368	*1703*	1.94	2.46	3.13	*2.51*
	5	207	228	192	*209*	2186	1166	1580	*1644*	2.14	2.25	3.00	*2.46*
	6	253	242	179	*225*	1881	1152	1561	*1531*	2.02	2.40	3.06	*2.50*
Means for year:		*229*	*236*	*175*		*2047*	*1260*	*1374*		*1.88*	*2.31*	*3.00*	
Means for factors:													
fertilizer	$(NH_4)_2SO_4$	234	237	171	*214*	2083	1279	1332	*1564*	1.82	2.30	3.00	*2.37*
	RSM	224	234	178	*212*	2010	1241	1415	*1555*	1.93	2.31	2.99	*2.41*
fertilization method	1	231	222	169	*208*	2188	1337	1354	*1626*	1.86	2.27	2.99	*2.37*
	2	223	249	179	*217*	2019	1299	1493	*1604*	1.79	2.17	2.94	*2.30*
	3	197	239	170	*202*	1549	1300	1335	*1394*	1.83	2.48	2.83	*2.38*
	4	229	237	174	*213*	2327	1227	1199	*1584*	1.87	2.38	3.07	*2.44*
	5	230	224	176	*210*	2243	1100	1436	*1593*	1.98	2.25	3.06	*2.43*
	6	263	242	179	*228*	1954	1296	1422	*1557*	1.90	2.25	3.07	*2.41*
$LSD_{0.05}$ for:	fertilizer	ns	ns	ns		ns	ns	ns		ns	ns	ns	
	fertilization method	ns	ns	ns		186.3	ns	ns		ns	ns	ns	
	interaction	ns	ns	ns		ns	ns	ns		ns	ns	ns	

* See Table 2.

Means calculated from the three years of the experiment for the kind of fertilizer point to a slightly higher concentrations of nitrates in the beet root fertilized with ammonium sulfate (1564 mg · kg^{-1} f.m.). Rather than those fertilized with RSM (1555 mg · kg^{-1} f.m.). Such tendencies were observed in 2005 and 2006. Fertilization way influenced the content of nitrates in the roots in 2005 only (Table 3). The lowest NO_3^- content was assessed in the roots of plants fertilized with 75 % N pre-sowing (in traditional and localized way) in combination with foliar nutrition (1549 and 1954 mg · kg^{-1} fm respectively), with its highest concentration in plants fertilized pre-sowing in a localized way (2327 mg · kg^{-1} f.m.). Such a dependency did not reoccur in the following years of the experiment.

The analysis of mean data from the three-year period of the research demonstrates that pre-sowing (broadcasting or liquid spreading) fertilization with a lowered dose of nitrogen (75 % N) was the most favourable fertilization way with regards to limited accumulation of nitrates in storage roots in comparison with the 100 % dose and to combination with foliar nutrition. Slightly higher contents of nitrates were assessed in the plants with foliar nutrition but in combination with pre-sowing lowered dose of N in the form of deposit.

The highest content of protein nitrogen (3.00 %) was observed in the roots of plants cultivated in 2007 (Table 3). In 2005 red beet plants contained 1.88 % on average and in 2006 – 2.31 % of protein nitrogen. What seems interesting is the lower content of protein nitrogen with simultaneous highest content of nitrates in the roots collected in 2005, in comparison with the other years of the experiment.

Discussion

The results of conducted research show the lack of any significant effect of the way of fertilization on the quantity of total yield of red beet storage roots. Sites fertilized in localized way (CULTAN method) were characterized by the yield similar to the plants fertilized in a traditional way (broadcasting/liquid spreading). Sommer [11] points to high effectiveness of CULTAN method, which allows reducing the dose of mineral nitrogen fertilizers by 20 % without the decrease in the yield. This author reveals that better effectiveness of this method results from the reduction of waste in fertilizer nitrogen in the soil. Our research demonstrated a tendency to decrease marketable yield as a result of fertilization with ammonium deposit method; in 2005 and 2006 the highest marketable yield was obtained in the sites fertilized in a localized way (single application and divided dose). These results can show that CULTAN method is not efficient enough in the cultivation of red beet root.

The effect of foliar nutrition of the quantity of marketable yield of red beet root is also interesting. In 2005 and 2006 the replacement of nitrogen dose applied to soil with foliar nutrition caused similar results as on the sites with soil fertilization only. It is particularly favourable result with regards to the possibility of reducing environment burden with nitrogen while preserving similar yield. Only in 2005 the sites nourished foliarly were characterized by lower marketable yield than the plants fertilized pre-sowing with full N dose (100 % broadcasting/liquid spreading). It is possible that

rainfall and temperature conditions, on which effectiveness of foliar nutrition depends influenced this result [12]. The study by some authors [5, 6, 13] clearly demonstrated that the use of foliar nutrition, supplementing plant nourishment with N, with limited soil fertilization (reduces doses), influenced the increase in the yield quantity.

There were significant differences in yield quantity in individual years of the experiment. The lowest yield was obtained in 2006. This year was characterized by the period of high temperatures (mean temperature in July equaled 21 ^{o}C) and drought (rainfall in July amounted 20 mm), which could have had a negative influence on plant growth and development.

The greatest differences in the nitrates concentrations in beet root were observed between the years of the experiment which can indicate that environment conditions can have a greater effect on the contents of nitrogen rather than its kind and way of application to soil. Many authors [14, 15] revealed that nitrates content in plant tissue depends on diverse natural factors, including sunshine. In a study of Wang and Li [3] higher nitrates content was observed in vegetables fertilized with oxidized form of nitrogen in comparison with fertilization with ammonium nitrogen form. In our experiment, the use of ammonium sulfate containing reduced N form only, slightly influenced the decrease in NO_3^- ions in red beet storage roots in one year of experiment.

Localized fertilization did not affect decrease in nitrates content in red beet yield and in 2005 the plants fertilized with ammonium deposit belonged to the sites with their highest concentrations. Thus, it did not confirm the results obtained by others [9, 16] pointing to the reduction in nitrates content in the plants fertilized with CULTAN method. Del Amor et al [10] revealed that foliar nutrition with urea allows reducing the content of nitrates in vegetable yield. Our research corroborated such a dependency only in 2005, where the use of 67.5 kg · ha^{-1} dose of nitrogen as pre-sowing combined with foliar nutrition in the vegetation period resulted in plants with considerably lower nitrates content when compared with the plants fertilized pre-sowing with full nitrogen dose 90 kg · ha^{-1} N and divided dose, ie 67.5 kg · ha^{-1} N pre-sowing + 22.5 kg · ha^{-1} N as top-dressing, to the soil.

The research did not reveal the effect of the kind of nitrogen fertilizer or the way of its application on the content of protein-N in red beet storage roots. The increase in protein-N quantity after the application of foliar nutrition has already been observed by del Amor et al [10].

Conclusions

1. The kind of nitrogen fertilizer or the way of its application did not affect the quantity of total yield of red beet roots and the effect on marketable yield was diversified between the years of the experiment.

2. In comparison with traditional fertilization method (broadcasting/liquid spreading) the fertilization with CULTAN method did not result in the increase of biological quality of red beet root yield.

3. There were tendencies for decreasing the concentrations of nitrates in the roots of plants fertilized pre-sowing combined with foliar nutrition.

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WPŁYW NAWOŻENIA AZOTEM NA PLON I ZAWARTOŚĆ AZOTANÓW W KORZENIACH SPICHRZOWYCH BURAKA ĆWIKŁOWEGO

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Abstrakt: Doświadczenie polowe z burakiem ćwikłowym odm. 'Boro F_1' przeprowadzono w latach 2005–2007. Badano wpływ rodzaju nawozu azotowego (siarczan amonu lub roztwór saletrzano-mocznikowy – RSM) oraz sposobu ich stosowania – rzutowe (rozlewowe) lub zlokalizowane z uwzględnieniem zróżnicowanych (dzielonych) dawek azotu i dolistnego dokarmiania roślin na plonowanie oraz zawartość azotanów, formy amonowej i azotu białkowego w korzeniach buraka ćwikłowego.

Rodzaj nawozu azotowego oraz sposób jego stosowania nie miały wpływu na plon ogólny korzeni. Nie wykazano także powtarzalnego w latach badań wpływu czynników doświadczenia na ilość plonu handlowego.

Wpływ badanych czynników na zawartość azotanów w korzeniach buraka zależał od roku uprawy. W 2005 r. po zastosowaniu nawożenia przedsiewnego rzutowego i zlokalizowanego w dawce 67,5 kg N · ha^{-1} w połączeniu z dokarmianiem dolistnym uzyskano korzenie o mniejszej zawartości azotanów w porównaniu z pozostałymi sposobami nawożenia. W kolejnych latach uprawy nie wykazano takiej zależności. Rodzaj zastosowanego nawozu w żadnym roku badań nie miało wpływu na zawartość azotanów. Zawartość azotu amonowego oraz azotu białkowego w korzeniach buraka nie zależała od rodzaju nawozu azotowego oraz sposobu nawożenia.

Słowa kluczowe: sposób nawożenia, nawożenie dolistne, metoda CULTAN, wartość biologiczna

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EFFECT OF DIFFERENTIATED FERTILIZATION AND FOLIAR APPLICATION OF IODINE ON YIELDING AND ANTIOXIDANT PROPERTIES IN RADISH (*Raphanus sativus* L.) PLANTS

WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA I DOKARMIANIA DOLISTNEGO JODEM NA PLONOWANIE I WŁAŚCIWOŚCI ANTYOKSYDACYJNE RZODKIEWKI (*Raphanus sativus* L.)

Abstract: The aim of this research was an attempt to obtain radish plants enriched with iodine by means of foliar application and soil fertilization with two forms of iodine – KI and KIO_3. The effect of iodine on radish yielding and its antioxidant characteristic was determined.

It was demonstrated that the form of applied iodine and the way of its application had a meaningful effect on the quantity of biological yield and leaf mass. Although all plants accumulated greater amounts of iodine in comparison with the control, it was best manifested in case of foliar application, as well as combined foliar application and soil fertilization of radish with KI form as 34.17 and 29.30 mg $I \cdot kg^{-1}$ f.m., respectively. Although, the effect of the form of applied iodine as well as the way of its application on antioxidant characteristics of radish was not revealed, the content of phenolic compounds turned out to be significantly higher than in case of foliar application combined with soil fertilization with both forms of iodine.

Keywords: iodine, radish, phenolic compounds, antioxidant properties

The environmental iodine deficiency can cause a number of health problems known as iodine deficiency disorders (IDD) [1]. To develop properly, human being needs 100–150 μg of iodine per day, half of which can be of vegetable origin. [2]. Nowadays, over a billion people live in the areas with a significant deficiency of this element in the environment [3]. The most effective and most popular method of preventing iodine deficiency disorders is iodization of domestic salt. However, its excessive intake causes

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constant increase in the frequency of circulatory system disorders and hypertension occurrences. Hence, in accordance with the directive of WHO, one shall aim at decreasing the content of salt in human diet [4]. This resulted in the search for alternative sources of provisioning people with iodine, among others by attempts of enriching vegetables with this element. Despite the fact that iodine is an indispensable element for plants, they take in this element easily from the environment in both iodate and iodide forms. However, after its introduction into soil iodine rapidly undergoes the sorption process as well as volatization into the atmosphere and only its trace amounts are available for plants [5–7]. Iodine introduction into plants can happen by soil or foliar application [3, 8]. The research carried out by Strzetelski et al [9] on spinach plants revealed that foliar application is more effective than soil fertilization. Also Altmok et al [10] research on lucerne cultivation demonstrated higher content of this element in plants cultivated under field conditions with KI foliar application form in comparison with soil fertilization.

The aim of this work is to determine which of the applied forms of iodine (I^- or IO_3^-) and the way of application (soil fertilization or foliar application) in pot cultivation of radish have effect on yielding, antioxidant activity and the content of this element in radish roots.

Material and methods

Small radish (*Raphanus sativus* L. var. *radicula* Pers.), 'Opolanka' cv., was cultivated in the years 2006–2007 in open-work containers sized 60 × 40 × 20 cm, placed in the open field under a shade providing fabric. The containers were filled with silt loam (35 % sand, 28 % silt and 37 % clay) with mean content of organic matter 2.52 % and the following contents of available (extracted with 0.03 mol · dm^{-3} CH_3COOH) nutrients forms: 14.9 mg N (NO_3-N+ NH_4-N), 73.8 mg P, 92.4 mg K, 159.8 mg Mg and 1299.0 mg Ca in 1 dm^3 soil. Soil $pH_{(H_2O)}$ was 6.88 and $pH_{(KCl)}$ 6.20, while total concentration of salt in soil measured as EC was 0.15 mS · cm^{-1}.

The following treatments with soil and foliar application of iodine in experiments were applied: 1 – control (without iodine application), 2 – foliar application in KI form (FA-KI), 3 – foliar application in KIO_3 form (FA-KIO_3), 4 – soil fertilization in KI form (SF-KI), 5 – soil fertilization in KIO_3 form (SF-KIO_3), 6 – soil fertilization in KI form + foliar application in KI form (SF-KI+FA-KI), 7 – soil fertilization in KIO_3 form + foliar application in KIO_3 form (SF-KIO_3+FA-KIO_3). Soil fertilization in KI and KIO_3 was carried out before sowing radish seeds to the level of 15 mg I · dm^{-3} soil. Foliar application in KI and KIO_3 performed twice using a liquid with iodine concentration per pure element 0.2 %, in rate 0.4 $dm^3 \cdot m^{-2}$. The first foliar application with iodine was performed at two-leaf stage ie on 24 May 2006 and 15 May 2007, while the second application on 1 June 2006 and 18 May 2007, respectively.

Seeds sowing was performed on 4 May 2006 and 11 April 2007 in 5 rows with 25 seeds in one containers. After germination the plants were singled out leaving 12 seedlings in one row. The content of available nitrogen and potassium forms in soil before cultivation outset was supplemented to the level 100 mg · dm^{-3} of soil with the

use of solid fertilizers of ammonium nitrate and potassium sulphate. The level of other macroelements (K, Mg, Ca) in soil did not need supplementation. Harvesting combined with gathering leaf, root and soil samples was performed on 8 June 2006 and 22 May 2007, respectively. For analyses we used radish roots which were suitable for consumption. Their mass was 3.0–7.6 g.

Phenolic compounds contents were determined with the use of Folin reagent [11] in radish roots. The division of phenolic compounds into phenylpropanoids, flavonols and anthocyanins were conducted spectrophotometrically on the basis of absorption spectrum as per Fakumoto and Mazza method [12]. The content of ascorbic acid was assessed according to Dulinski et al method [13]. The activity of free radicals scavenging was evaluated on the basis of plant tissue reaction with diphenylpicrylhydrazyl (DPPH) being colour stable free radical according to Pekkarinen et al method [14].

In soil samples as well as radish roots the content of iodine was assessed with ICP-OES method with the use of Prodigy Teledyne Leeman Labs USA spectrometer. In soil samples iodine was evaluated after prior extraction with 0.03 mol · dm^{-3} CH_3COOH [15], while in radish roots after TMAH trials incubation according to standard project prEN 15111-R2-P5-F01 [16].

The obtained results were verified statistically with the ANOVA module of 'Statistica 8.0 PL' for $p < 0.05$. The significance of differences was estimated with Duncan test.

Results

It was demonstrated that both the way and the form of applied iodine for plant fertilization had a significant effect on the quantity of biological yield of radish plants (Table 1). It was noted definitely lowest biological yield after foliar application treatment with KI form when compared with other sites of the experiment. The highest and comparable yield (roots plus leaves) was observed in plants with soil fertilization with both iodide (KI) and iodate form (KIO_3). Interestingly, the yield of radish roots (usable parts) was independent of both form and way of iodine application.

Table 1

The effect of fertilization and foliar application with iodine on radish yielding – means from 2006–2007

Treatments	Yield (roots+leaves)	Roots yield	Leaves yield
	[g · m^{-2}]		
Control	2138.0 bc	1055.6 a	1082.4 ab
FA-KI	1622.5 a	784.7 a	837.7 c
FA-KIO_3	1979.2 abc	934.0 a	1045.2 abc
SF-KI	2274.7 c	1135.4 a	1139.2 a
SF-KIO_3	2219.7 c	1041.7 a	1178.0 a
SF-KI+FA-KI	1731.1 ab	819.4 a	911.7 bc
SF-KIO_3+FA-KIO_3	1937.6 abc	854.2 a	1083.4 ab

Means followed by the same letters are not significantly different for $p < 0.05$.

Demonstrated differences proved insignificant after statistical verification. It results from the evaluation of plant yielding that the revealed diversity in the quantity of biological yield was associated with considerable differences in leaf mass (Table 1). These differences most probably resulted from leaf impairment caused by foliar application with iodine. As in case of biological yield, the lowest leaf mass was noted in plants treated with foliar application with potassium iodide (KI), while the highest mass was observed after soil fertilization with both forms of iodine.

The analysis of iodine content in radish roots (Table 2) demonstrated a wide diversity among individual sites of the experiment. The highest content of this element was noted after foliar application with iodide form (I^-) and after combined foliar application and soil fertilization with potassium iodide (KI). The smallest amount of iodine was accumulated by the roots after soil fertilization with both forms of iodine. It is worth noting that foliar application with KI caused threefold increase in the content of iodine in radish, when compared with other sites with foliar application, with KIO_3 form. The analysis of residues in the soil conducted after the experiment demonstrated that the highest amounts of this element were assessed after the application of iodate form (IO_3^-) in plant fertilization, independently of the way of application (Table 2).

Table 2

The effect of fertilization and foliar application with iodine on the content of this element in soil after harvest, and on the content of iodine and ascorbic acid in radish – means from 2006–2007

Treatments	Content of iodine in soil [mg I · dm^{-3} soil]	Content of iodine in radish roots		Ascorbic acid [mg · $100g^{-1}$ f.m.]
		[mg I · kg^{-1} d.m.]	[mg I · kg^{-1} f.m.]	
Control	0.25 a	14.8 a	0.68 a	25.2 a
FA-KI	1.25 a	739.5 c	34.17 c	29.6 c
FA-KIO_3	5.18 b	263.1 b	11.41 b	27.1 b
SF-KI	1.21 a	27.0 a	1.20 a	24.3 a
SF-KIO_3	13.77 c	60.8 a	2.78 a	24.1 a
SF-KI+FA-KI	1.32 a	631.5 c	29.30 c	23.9 a
SF-KIO_3+FA-KIO_3	23.06 d	292.3 b	13.31 b	24.6 a

Means followed by the same letters are not significantly different for $p < 0.05$.

The highest level of ascorbic acid was observed in radish roots after foliar application of plants both with KI and KIO_3 forms (Table 2). We did not note any significant effect of iodine form and the way of its application on the capacity of free radicals scavenging (DPPH) or on the content of phenylpropanoids, flavonols and anthocyanins in radish (Table 3).

There were, however, considerable variations in the content of phenolic compounds. Their highest level was assessed in radish roots after foliar application with potassium iodide (KI) and combined foliar application and soil fertilization with I^- as well as IO_3^- form.

Table 3

The effect of fertilization and foliar application with iodine on the content of phenolic compounds in roots and leaves of radish, and free radical scavenging activity (DPPH) – means from 2006–2007

Treatments	Phenolic compounds	Phenyl propanoids compounds	Flavonoids	Anthocyanins compounds	DPPH [%]
	[mg · 100 g^{-1} f.m.]				
Control	42.1 ab	40.5 a	15.9 a	52.5 a	11.00 a
FA-KI	45.1 abc	40.3 a	16.0 a	48.2 a	11.53 a
FA-KIO_3	42.0 ab	38.1 a	15.7 a	46.7 a	10.54 a
SF-KI	41.0 a	35.7 a	14.7 a	45.6 a	10.96 a
SF-KIO_3	42.8 ab	36.9 a	15.4 a	48.1 a	10.83 a
SF-KI+FA-KI	46.1 bc	39.7 a	16.8 a	49.0 a	11.69 a
SF-KIO_3+FA-KIO_3	47.3 c	39.4 a	17.1 a	51.1 a	11.94 a

Means followed by the same letters are not significantly different for $p < 0.05$.

Discussion

The results obtained after assessment of iodine content in radish roots indicate that foliar application with this element is an undoubtedly better way of enriching plants with iodine in comparison with soil fertilization. Similar results in the search for efficient ways of plant bio-fortification with iodine were revealed by other authors [3, 9, 10]. Presented research allows to conclude that bioaccumulation of iodine in radish was threefold higher when the plants were treated with foliar application with iodide form (I^-) when compared with iodate form (IO_3^-). More rapid accumulation of iodine applied in the form of KI was also observed by Smith et al [17]. However, there is an opinion in contemporary literature that it is IO_3^- form which is taken by plants more efficiently, which was already confirmed by the research on spinach, carrot, celery and onion [18, 19] and lettuce [20]. Solving this problem would require further studies on the subject.

Demonstrated differences in the content of iodine in radish influenced the shape of biological yield and leaf mass of this vegetable (Table 1). High iodine level after foliar application with KI resulted in a significant decline in biomass of harvested plants, leaf mass and root yield (insignificant effect). A similar tendency was revealed when the plants were concurrently treated with foliar application and soil fertilization with iodide form (I^-). This is a very interesting interrelation, which was confirmed by Hong et al [21] in their research into iodine accumulation in celery, pepper and radish. It turned out that increasing content of iodine in soil and plants up to 150 mg I · kg^{-1}, decreased biomass of the examined plants by 40 % in pepper, 25 % in celery and by 10–15 % in radish.

Conducted assessment of ascorbic acid content demonstrated that foliar application of plants with iodine resulted in a significantly elevated level of this vitamin in radish, with iodide (KI) being the more efficient form. Similarly higher content of ascorbic acid in lettuce leaves after soil fertilization with potassium iodide was presented by

Ledwozyw et al [22]. Other authors also revealed a positive correlation between the content of iodine in plant and the content of ascorbic acid [23]. However, this problem needs further detailed studies.

Conclusions

1. Foliar application of radish plants with potassium iodide significantly decreased biological yield and leaf mass. The biggest biological yield was gathered after soil fertilization with both forms of iodine.

2. Foliar application and foliar application combined with soil fertilization with iodine, particularly in I^- form, caused the highest accumulation of this element in radish.

3. It was found an insignificant effect of the factors applied in the experiment on the content of ascorbic acid and phenols total in radish yield.

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WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA I DOKARMIANIA DOLISTNEGO JODEM NA PLONOWANIE I WŁAŚCIWOŚCI ANTYOKSYDACYJNE RZODKIEWKI (*Raphanus sativus* L.)

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Abstrakt: Celem podjętych badań była próba uzyskania roślin rzodkiewki wzbogaconych w jod poprzez zastosowanie dokarmiania dolistnego oraz nawożenia doglebowego dwoma formami jodu – KI oraz KIO_3. Określono również wpływ jodu na plonowanie rzodkiewki oraz jej właściwości antyoksydacyjne.

Wykazano, że forma użytego jodu oraz sposób jego aplikacji miały statystycznie istotny wpływ na ilość plonu biologicznego i masę liści. Chociaż wszystkie rośliny akumulowały większe ilości jodu w porównaniu z kontrolą to jednak najbardziej uwidoczniło się to przy dolistnym dokarmianiu, jak również przy łącznym dokarmianiu dolistnym i nawożeniu doglebowym rzodkiewki formą KI odpowiednio 34,17 oraz 29,30 mg $I \cdot kg^{-1}$ ś.m. Pomimo, iż nie wykazano statystycznie istotnego wpływu zarówno formy użytego jodu oraz sposobu aplikacji tego pierwiastka na właściwości antyoksydacyjne rzodkiewki, to zawartość sumy związków fenolowych okazała się istotnie większa w przypadku łącznego dokarmiania dolistnego jak i nawożenia doglebowego roślin rzodkiewki obiema formami jodu.

Słowa kluczowe: jod, rzodkiewka, związki fenolowe, właściwości antyoksydacyjne

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EFFECT OF STORAGE TIME ON CONTENTS OF POLYPHENOLIC COMPOUNDS IN SELECTED CULTIVARS OF PLUM (*Prunus domestica* L.)

WPŁYW CZASU PRZECHOWYWANIA NA ZAWARTOŚĆ ZWIĄZKÓW POLIFENOLOWYCH W WYBRANYCH ODMIANACH ŚLIWEK (*Prunus domestica* L.)

Abstract: The aim of the conducted investigations was to determine the effect of storage in selected cultivars of plum *Prunus domestica* L. on changes in contents of polyphenolic compounds, fruit mass as well as dry mass and solids contents. Plums harvested at the stage of harvesting maturity were stored for the period of 2 and 4 weeks in an ordinary refrigerator. During storage the total content of polyphenolic compounds, depending on the cultivar, increased by 16–88 % in comparison with the initial content in fruits after harvest. Fruits of 'Wegierka Dabrowicka' cv. exhibited a relatively low content of polyphenolics in comparison with the other analyzed cultivars, ie 'Wegierka Zwykla', 'Valor' and 'Cacanska Lepotica'. After storage an increase of dry mass and solids contents in fruits was observed, while fruit mass decreased.

Keywords: plum, polyphenolics, storage

Plums are fruits with high nutritional and dietary value, both when fresh and dried as prunes. They are a raw material rich in polyphenolic compounds, minerals (potassium, phosphorus, calcium and magnesium) and pectin substances and they are characterized by high antioxidant activity. According to literature data, in terms of their antioxidant properties, plums exceed such fruits as oranges, grapes or apples [1–3]. Diet rich in bioactive compounds found in plums *P. domestica* results, among other things, in reduced blood pressure, an improved blood lipid profile, a reduced risk of cancer and improved functioning of the alimentary tract [4–6].

The content of polyphenolics in plant raw materials, even within one species, varies considerably, since it depends on many factors, particularly the cultivar, maturation stage, environmental and agritechnical conditions during growth as well as conditions

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and time of storage after harvest [7]. Among polyphenolic compounds found in plums derivatives of caffeic acid (neochlorogenic, chlorogenic, cryptochlorogenic acids) predominate, while flavanols (catechin, epicatechin, proanthocyanins), flavonols (quercetin) and anthocyanins (derivatives of anthocyanidins: cyanidin and peonidin, for example cyaniding-3-glucoside) are found in smaller amounts [8, 9]. Contents of phenolic acids fall within a wide range of values, with neochlorogenic acid at 85–1300 mg · kg^{-1} d.m., chlorogenic acid at 13–430 mg · kg^{-1} d.m. and cryptochlorogenic acid at 9–56 mg · kg^{-1} d.m. [8, 10–12].

Cultivars of biggest importance in plum processing in Poland include 'Stanley', 'Wegierka Zwykla' and 'Wegierka Dabrowicka'. In recent years several new cultivars have been introduced, with advantageous sensory attributes and pomiculture characteristics, such as 'Bluefre' and 'Valor' [13]. Cultivars are to have attractive fruits, they should be resistant to diseases and productive, while another important trait is potential long cold storage of fruits, since for such fruits higher prices are obtained than during harvest. The longer the supply period for a given plum cultivar on the market, the more competitive in terms of prices they are in relation to cultivars with a short shelf-life. Consumer acceptance of plums is closely related with the date of harvest and maturation stage of fruits [14, 15].

The aim of the study was to determine the effect of storage time on changes in contents of polyphenolics in plums. During storage of plums changes in fruit mass and contents of dry mass and solids were also determined.

Materials and methods

Analyses were conducted on plums (*Prunus domestica*) of 'Wegierka Dabrowicka', 'Wegierka Zwykla', 'Cacanska Lepotica' and 'Valor' cultivars from season 2008. Plums were obtained from orchards of the Agricultural and Pomological Station in Przybroda, belonging to the Department of Pomology, the Poznan University of Life Sciences. Fruits came from trees in full fruiting, growing on Wagenheim rootstocks, on grey-brown podsolic soils. Fruits after harvest were sorted depending on their maturation stage. The primary criterion in the evaluation was fruit hardness. In the experiments plums were used at the stage of harvesting maturity. After fruits were sorted they were placed in cold storage at 0 ^{o}C and stored for 4 weeks. Fruits were analyzed in order to determine fruit mass, contents of dry matter, solids and polyphenolics, after harvest as well as after 2 and 4 weeks of storage.

Fruit mass was determined for fruits in two batches of 20 plums. Contents of dry matter were determined by gravimetry, while solids were determined by refractometry in three samples with five replications. The total content of polyphenolics was determined by spectrophotometry according to Folin-Ciocalteau at a wavelength of λ = 750 nm [16]. Polyphenolics were extracted using 80 % methanol solution for 24 h. Contents of polyphenolics were presented in terms of chlorogenic acid equivalents.

Statistical analysis of results was conducted using the analysis of variance (at the significance level $p \leq 0.01$), with the application of Statistica ver. 7.1 software.

Results and discussion

In analyzed plum cultivars the total content of polyphenolics was determined after harvest of fruits and during their storage. Results of measurements are presented in the chlorogenic acid equivalents, since it is one of the dominant phenolic acids found in plums (Fig. 1).

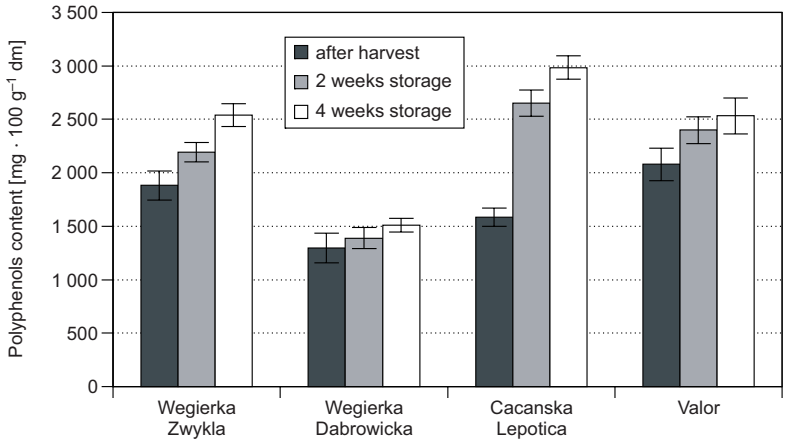

Fig. 1. Changes in polyphenolics contents during storage of plums depending on the cultivar; contents of polyphenolics are expressed in chlorogenic acid equivalents

The highest content of phenolics in fruits after harvest was recorded in plums of 'Valor' cv., 2080 mg · 100 g^{-1} d.m., while it was slightly lower in fruits of 'Wegierka Zwykla' cv. – 1882 mg · 100 g^{-1} d.m. (Fig. 1). The lowest amounts of polyphenolics in fruits immediately after harvest were determined in plums of 'Wegierka Dabrowicka' cv. at 1300 mg · 100 g^{-1} g d.m., while in fruits of 'Cacanska Lepotica' cv. it was 1583 mg · 100 g^{-1} d.m. In all cases an increase was recorded in contents of polyphenolics during storage of plums, both after 2 and 4 weeks of storage. The highest, 88 % increase in the content of phenolic compounds was found during storage of fruits of 'Cacanska Lepotica' cv., while it was lowest in fruits of 'Wegierka Dabrowicka' cv., amounting to only 16 %.

Fruits tested in this study were harvested in the first stage of maturation, ie harvesting maturation, and next placed in a cold storage. After storage they exhibited a higher maturation stage and higher contents of phenolic compounds, which shows that the process of ripening is accompanied by the synthesis of these compounds. In literature sources one may find examples of both an increase and a decrease in the polyphenolic contents during ripening of fruits. In case of sweet cherries the content of phenolic compounds was found to increase in the final stage of their ripening [17]. During ripening and storage of plums of different cultivars, peaches and nectarines both an increase and a decrease were observed in the levels of polyphenolics and either no definite direction of changes in the contents of these compounds was found or these

changes were statistically non-significant [12, 18]. When investigating changes in the content of selected phenolic compounds during 30-day harvest period of plums, Usenik et al [18] found a significant increase in the content of anthocyanins in the four investigated cultivars. When analyzing the results of this study it was found that during 4-week storage of plums gathered at harvesting maturation the total polyphenolics content increased significantly. Based on the multivariate analysis of variance it was stated that changes in the content of polyphenolics were significantly affected by the plum cultivar and storage time ($p \leq 0.01$).

The authors of this study conducted analogous investigations also in the previous vegetation season of 2007 for selected plum cultivars. Based on the analyses of results it was found that after 4-week storage of plums in case of 'Valor' cv. polyphenol content increased by 17 % (from 1594 to 1866 mg · 100 g^{-1} d.m.), while for 'Wegierka Zwykla' cv. this increase amounted to 42 % (from 728 to 1036 mg · 100 g^{-1} d.m.) [unpublished data; studies conducted at the Laboratory of Fruit and Vegetable Technology, the Poznan University of Life Sciences]. In the vegetation season of 2008 polyphenol content increased by 21 % in 'Valor' cv. and 35 % in 'Wegierka Zwykla' cv.

An increase in the contents of phenolic compounds during cold storage may be caused by physiological stress in plant material, such as eg mechanical damage, tissue decomposition or microbial infection [19]. On the other hand, during storage of fruit harvested at picking maturity, their further ripening occurs, including changes of many chemical compounds, such as saccharides, acids and polyphenols. Low storage temperature reduces the intensity of these changes, but they still occur, which leads to ripening and next overripeness of crops. Thus, frequently in the initial period of storage one may observe an increase in the contents of selected components, connected with the ripening process. Examples are also presented in literature showing increased polyphenol contents in stored processed fruit products, which may be explained by the decomposition of complex phenolic compounds (eg ellagitanin) and release of simpler phenols [20, 21].

During storage of tested plums fruit mass was found to decrease (Table 1). The highest weight loss was observed in fruits of 'Wegierka Zwykla' cv., while it was lowest in fruits of 'Valor' cv., by 15 % and 1 %, respectively. Mass loss during storage of fruits is related with the process of transpiration. It was observed that in small fruits mass loss was bigger (15 %) than in medium-sized fruits (8–11 %) and large ones (1 %). This may be explained by the ratio of fruit surface area to its volume, which is higher in case of small fruits, thus they are at risk of higher water losses. A similar level of mass losses during storage of plums was observed by Guerra and Casquero [22] and Serrano et al [23].

Fruit mass losses during storage were accompanied by an increase in the contents of dry mass and solids (Table 1). Statistical analysis showed a significant effect of the cultivar and storage time of plums on changes in fruit mass as well as contents of dry mass and solids ($p \leq 0.01$). Depending on the cultivar, contents of dry mass and solids increased from 3 to 18 %. This is probably connected with loss of water as well as an increase in the content of simple sugars as a consequence of ripening of stored fruits. This is confirmed by studies conducted by Ustnik et al [18], Guerra and Casquero [22]

Table 1

Changes in fruit weight, contents of dry mass and solids in plums during storage

Cultivar	Time of storage [weeks]	Fruit mass* [g]	Dry mass content** [%]	Solids content** [%]
Wegierka Zwykla	0	20.9 ± 3.12	19.8 ± 0.52	18.6 ± 0.69
	2	20.6 ± 3.07	19.9 ± 0.47	18.2 ± 0.79
	4	17.9 ± 2.68	21.7 ± 0.42	19.7 ± 0.52
Wegierka Dabrowicka	0	43.0 ± 5.04	12.9 ± 0.37	12.3 ± 0.71
	2	40.2 ± 4.05	12.4 ± 0.10	12.2 ± 0.90
	4	38.3 ± 4.48	13.3 ± 0.26	12.7 ± 0.78
Cacanska Lepotica	0	44.7 ± 5.23	14.3 ± 0.30	12.7 ± 0.15
	2	43.5 ± 5.11	14.9 ± 0.68	14.4 ± 0.49
	4	41.3 ± 4.84	15.0 ± 0.21	14.5 ± 0.37
Valor	0	77.3 ± 9.47	17.4 ± 1.02	16.7 ± 0.87
	2	74.3 ± 10.73	19.8 ± 0.18	18.6 ± 0.90
	4	76.9 ± 8.27	20.7 ± 0.79	19.3 ± 1.21

* ± standard deviation from 20 replications; ** ± standard deviation from 3 samples with 5 replications each.

and Serrano et al [23]. In contrast, in a study by Lysiak [24] no increase in the solids content was observed during fruit storage in plums of 'Valor' cv. or a decrease was recorded in the contents of solids compounds in the other analyzed cultivars.

Conclusions

1. During storage of selected plum cultivars *Prunus domestica*, harvested at the stage of harvesting maturation, their total content of polyphenolic compounds increased.
2. As a result of storage of analyzed plums fruit mass decreased.
3. Contents of dry mass and solids in plums increased during fruit storage.

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WPŁYW CZASU PRZECHOWYWANIA NA ZAWARTOŚĆ ZWIĄZKÓW POLIFENOLOWYCH W WYBRANYCH ODMIANACH ŚLIWEK (*Prunus domestica*)

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Abstrakt: Celem przeprowadzonych badań było określenie wpływu przechowywania owoców wybranych odmian śliw *Prunus domestica* L. na zmiany zawartości związków polifenolowych, masy owoców oraz zawartości suchej masy i ekstraktu. Śliwki zebrane w stadium dojrzałości zbiorczej, przechowywano przez okres 2 i 4 tygodni w chłodni zwykłej.

W czasie przechowywania ogólna zawartość związków polifenolowych, w zależności od odmiany, zwiększyła się o 16–88 % w porównaniu do początkowej zawartości w owocach po zbiorze. Owoce odmiany 'Węgierka Dąbrowicka' cechowały się stosunkowo małą zawartością związków polifenolowych w porównaniu do pozostałych badanych odmian, tj. 'Węgierka Zwykła', 'Valor' i 'Čačanska Lepotica'. Po okresie przechowywania stwierdzono wzrost zawartości suchej masy i ekstraktu w owocach oraz ubytek masy całkowitej.

Słowa kluczowe: śliwka, polifenole, przechowywanie

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APPLICATION OF THE PARTITION COEFFICIENT FOR ASSESSING HEAVY METALS MOBILITY WITHIN THE MIASTECZKO SLASKIE ZINC SMETLER IMPACT ZONE (POLAND)

ZASTOSOWANIE WSPÓŁCZYNNIKA PODZIAŁU DO OCENY RUCHLIWOŚCI METALI CIĘŻKICH W STREFIE ODDZIAŁYWANIA HUTY CYNKU W MIASTECZKU ŚLĄSKIM (POLSKA)

Abstract: The present paper outlines some approaches, which were suggested for assessing heavy metals (Cu, Zn, Pb and Cd) mobility in soils contaminated by a zinc smelter activity. The concept was based on the assumption that partition coefficients, as ratios of total (assayed by *aqua regia*) metal contents to exchangeable (extracted by 1 mol $CH_3COONH_4 \cdot dm^{-3}$, pH 7.0) or to bioavailable (extracted by 0.1 mol $NaNO_3 \cdot dm^{-3}$) metal contents, will be more predictive in assessing metals mobility as compared with single extractions. The calculated partition coefficients for exchangeable and bioavailable metal forms were respectively termed K_d-*Ex* and K_d-*Bio*, which in turn were intended to represent the *statico-dynamic* nature of heavy metal mobility.

It was found that metal mobility followed the sequence Cd > Zn > Pb > Cu, which implies that Cd and Zn will evoke more threat due to greater susceptibility for mobility. Regressions for the pairs logK_d *versus* pH were applied in order to exhibit the proton generating capacity of each metal. The average calculated proton coefficient in the case of exchangeable metal forms amounted to 0.16 mole of protons released for each mole of Cu, Zn and Pb. In contrast, the proton coefficient for Cd was about 81 % greater. The estimation made on the basis of bioavailable metal forms showed, that Cd and Zn retention by soils generated, respectively 77 and 131 % more protons as compared to Pb. In terms of environmental concern, it may be formulated, that the higher the proton generating capacity, the highest the metal mobility, and thus the weakest partition to the solid phase.

Keywords: partition coefficient, exchangeable, bioavailable forms, copper, zinc, lead, cadmium, zinc smelter

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Heavy metals, including micronutrients and toxic metals, exhibit a high affinity for naturally occurring adsorbents and the reactions between these metal ions and the adsorbents are observed to be weakly reversible. The magnitude of this process is generally estimated by chemical tests as well as speciation studies [1–3]. The mobility of heavy metals in contaminated soils is a notion of *static* nature (application of different chemical tests for heavy metals extractions), of *dynamic* nature (phytotests, batch studiem) and *statico-dynamic nature* (partition coefficients relating the content of heavy metals in the soil solid phase and soil solution concentration). The ratio of metals in the solid phase to those in solution at equilibrium is defined as partition coefficient K_d, which is reported as M_{ads}/M_{sol} where, M_{ads} – adsorbed/retained metal and M_{sol} – metal in solution [4–6]. High values of the partition coefficient are believed to indicate that metals have been retained by the soil solid phase through sorption reactions, while low values imply that most of metals are partitioned to the ambient soil solution, where they are potentially prone to transport and biological or geochemical reactions. The involved mechanisms are related to several physical and chemical soil properties of which soil reaction (pH), organic matter, clay and silt contents are mostly considered to control dynamic processes of metal geochemistry [7]. Therefore it seems targeted to apply the partition coefficient for assessing metal mobility, since this parameter involves intrinsically the buffering capacity, a core soils geochemical feature [8, 9].

The concept of the present paper is based on the assumption that bioavailable (assayed by 0.1 mol $NaNO_3 \cdot dm^{-3}$) and exchangeable (extracted by 1 mol $CH_3COONH_4 \cdot dm^{-3}$) metal fractions express a *static* approach in terms of metals mobility. The consideration of the partition coefficient (K_d) in assessing the potential mobility of these metal fractions in contaminated as well as polluted soils will provide more details, since the evaluation shifts from the *static* towards the dynamic approach ie, *statico-dynamic* nature of metal mobility-based processes.

The purpose of the paper is to outline some approaches in the evaluation of the partition coefficients-based heavy metals mobility in soils subjected to contamination by a Zinc Smelter activity.

Materials and methods

Location of the research area

The research area lies within the impact zone of the Miasteczko Slaskie Zinc Smelter, (N 51°41′03″ and E 15°57′12″, Poland) whose activity started since 1966. This zone is surrounded in the north, west and east by a large Lubliniec Forest complex, and in the south-east by the localities of Zyglin and Zygliniec, quarters of the Miasteczko Slaskie. A population of pine as part of artificial restoration, mainly of mixed forest, sporadically mixed wood grows in the impact zone. In the Miasteczko Slaskie region, the prevailing winds are from the south-westerly (21.4 %) and westerly (18.7 %) quarters, hence the emitted pollutants create the greatest threat to areas in the north-east and east zones of the Zinc Smelter.

Sample collection and analytical procedures

Five samples ordered like the five on a dice, with 15 m distance from the central point were collected (20 cm depth) at 8 selected sites (Table 1) on June, 2006.

Table 1

Selected physical and chemical properties of soils in the impact zone of the Miasteczko Slaskie Zinc Smelter (mean, n = 5)

Site	Particles [g · kg^{-1}]		$C_{org.}$ [g · kg^{-1}]	EC [μS · cm^{-1}]	pH 0.01 mol $CaCl_2$ · dm^{-3}	Ca	CEC
	Silt	Clay				[cmol(+) · kg^{-1}]	
A	90	90	5.5	34.7	5.7	1.0	1.4
B	150	90	9.1	41.6	4.9	1.4	1.8
C	490	260	15.5	189.0	7.2	24.1	26.8
D	240	260	20.0	128.5	7.5	13.3	15.1
E	270	130	11.2	68.3	5.6	4.3	5.4
F	290	80	7.5	72.3	6.0	4.9	5.9
G	410	90	31.1	65.7	4.6	2.2	2.9
H	180	60	66.4	134.6	3.4	1.8	2.5

Explanation: A, B – Experimental area 500 and 1100 m ESE, respectively; C – Cynkowa Street, 100 m SE from the Zinc Smelter (Miasteczko Slaskie); D – Dworcowa Street, 500 m W from the Zinc Smelter (Miasteczko Slaskie); E – Brynicka Street, 500 m E from the Zinc Smelter (Zyglin) F – Sw. Marka Street, 1500 m SE from the Zinc Smelter (Zyglin); G – Zyglinska Street, 4500 m E from the Zinc Smelter (Brynica); H – Staromiejska Street, 6000 m E from the Zinc Smelter (Bibiela); EC – Electrical Conductivity; CEC – Cation Exchange Capacity.

Prior to basic analyses soil samples were air-dried and crushed to pass through a 1 mm sieve. Granulometric composition was determined by the areometric method [10] and organic carbon by the Walkley-Black method as reported by Nelson and Sommers [11]. Soil pH at soil/solution ratio of 1:5 (0.01 mol $CaCl_2$ · dm^{-3}) was determined potentiometrically using a pH-meter [12], whereas the electrical conductivity was assayed in water extracts according to Jackson [13]. The cation exchange capacity (CEC) was obtained by summation of 1 mol KCl · dm^{-3} extractable acidity and exchangeable alkaline cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) extracted by 1 mol CH_3COONH_4 · dm^{-3} (pH 7.0) as described by Thomas [14]. The total content of heavy metals was determined by using the *aqua regia* procedure [15], whereas the bioavailable as well as exchangeable metals forms were assayed by 0.10 mol $NaNO_3$ · dm^{-3} [16] and 1 mol CH_3COONH_4 · dm^{-3} (pH 7.0) [17], respectively. All performed chemical tests were run in duplication and metals as well as other elements were determined by the FAAS method (*Flame Atomic Absorption Spectrophotometry*, Varian 250 plus). Computations and statistical evaluations were made by using the Excel® sheet.

Results and discussion

Soil properties versus exchangeable and bioavailable heavy metal content

Soils within the impact zone of the Miasteczko Slaskie Zinc Smelter are characterized by significantly different physical and chemical properties, summarized in Table 1. Soil reaction (pH) varied broadly from very acidic (pH_{CaCl_2} = 3.4) at the site H to slightly alkaline (pH = 7.5) for soils of the site D. Organic carbon (C_{org}) content fluctuated within a large range, ie, 5.5–66.4 g · kg^{-1}, whereas the cation exchange capacity (CEC) was in most cases markedly low (from 1.4 to 5.9 cmol(+) · kg^{-1}), except for sites C and D with CEC values of 26.8 and 15.1 cmol(+) · kg^{-1}, respectively. The amount of silt and clay fractions reveals that investigated soils are preponderatingly sandy, ca 75 % of soils exhibited silt + clay < 500 g · kg^{-1}. Such a soil texture may create a serious threat due to strengthened pollutants migration downward. The mean heavy metals content as reported in Table 2 showed significant variations related most specifically with both sites and type of metal.

Table 2

Total (*aqua regia*) metal contents within the impact zone of the Miasteczko Slaskie Zinc Smelter (mean ± SD, n = 5)

Site	Cu	Zn	Pb	Cd
	[mg · kg^{-1}]			
A	5.15 ± 0.45	614.50 ± 30.43	404.15 ± 46.96	5.75 ± 1.16
B	5.40 ± 1.29	368.50 ± 39.47	542.15 ± 61.44	5.05 ± 1.08
C	69.60 ± 12.40	4832.0 ± 517.48	2986.0 ± 147.88	52.00 ± 5.82
D	56.55 ± 9.69	1351.8 ± 247.69	1009.65 ± 95.60	16.35 ± 2.45
E	6.60 ± 0.80	246.0 ± 31.10	352.15 ± 14.65	5.52 ± 0.38
F	6.20 ± 1.36	649.0 ± 88.56	226.95 ± 12.34	5.05 ± 0.84
G	5.00 ± 0.40	202.0 ± 7.62	288.10 ± 24.04	4.50 ± 0.47
H	5.95 ± 0.86	240.0 ± 31.42	446.15 ± 30.35	4.35 ± 0.78
BLP	17.5	75.0	40.0	0.65

Explanation: SD – Standard deviation; Site – see Table 1; BLP – Background Level for Poland [18].

In the case of Zn, Pb and Cd, their levels exceeded ca 3 to 64 times; 6 to 75 times and 7 to 80 times, respectively, the Background Level for Poland (BLP) [18], (Table 2), in opposite to Cu with 75 % of its content not exceeding the BLP value. These orders of magnitude clearly show that cadmium and lead are the most threatening heavy metals in the impact zone. Zinc in turn occupies the intermediate position.

Linear correlation coefficients (only r ≥ 0.50 were considered) established for the pairs: soil properties (ie, CEC, Clay + silt, C_{org} and pH) *versus* exchangeable and bioavailable forms of Cu, Zn, Pb and Cd (Table 3) have outlined the occurrence of two groups of factors as determinants of metals geochemical dynamics of soils within the impact zone.

Table 3

Exchangeable (*Ex*) and bioavailable (*Bio*) Cu, Zn, Pb and Cd concentrations within the impact zone of the Miasteczko Slaskie Zinc Smelter (mean, n = 5)

Site	Cu		Zn		Pb		Cd	
	Ex	*Bio*	*Ex*	*Bio*	*Ex*	*Bio*	*Ex*	*Bio*
	[mg · dm^{-3}]							
A	0.034	t	19.71	14.25	3.63	0.424	0.212	0.568
B	0.025	t	10.13	13.83	7.11	0.129	0.175	0.410
C	0.086	t	16.77	10.65	7.33	0.304	0.386	0.257
D	0.046	t	15.04	6.33	6.30	0.049	0.292	0.093
E	0.022	t	3.74	2.09	0.33	0.071	0.073	0.064
F	0.027	t	4.96	1.15	1.09	0.062	0.066	0.060
G	0.023	t	1.65	0.15	0.70	0.065	0.045	0.062
H	0.024	t	2.90	0.22	2.56	0.074	0.056	0.074

Explanation: Site – see Table 1; Ex – extracted by 1.0 mol $CH_3COONH_4 \cdot dm^{-3}$, pH 7.0 (ratio *w:v* = 1:10); Bio – extracted by 0.10 mol $NaNO_3 \cdot dm^{-3}$ (ratio *w:v* = 1:2); t – traces (below detection limit).

These are the CEC and pH for exchangeable forms, with correlation coefficients (r) varying within the range: $0.62 \le r \le 0.86$, ($p < 0.01$). Organic carbon (C_{org}) content and pH were found to be the most significant determinants for bioavailable forms, since correlation coefficients fluctuated accordingly: $0.53 \le r \le 0.75$, ($p < 0.01$). This implies that the mobility of metals within the investigated ecosystem may be high due to decomposition of organic matter in one hand and to soil pH, which is potentially instable. More analyses are required to confirm such effects in detail, especially with respect to the activities of the soil microflora.

Partition coefficient, metal mobility estimation

The values of the partition coefficients (Table 4) varied widely accordingly to the particular metals and also depending on the chemically extracted metal forms (ie, extractable and bioavailable). Of these values, K_d-*Ex* for Cu as well as K_d-*Bio* both for Zn and Pb the most, spanned the widest ranges. On the basis of mean K_d values the following sequences may be established:

K_d-*Ex*: Cu > Pb > Zn > Cd
K_d-*Bio*: Pb > Zn > Cd

Interestingly the reported sequences follow the same trends, which implies that buffering capacities developed by soils within the impact zone control similarly exchangeable as well as bioavailable metals. This is particularly important in terms of mobility evaluation. The latter one follows the reverse sequence, ie, Cu < Pb < Zn < Cd,

which points out at the higher mobility of Cd and Zn over Pb and Cu as reported by Matos et al [19]. This is in line with the electronegativity of these metals and additionally with data reported by Christophi and Axe [20] and Fontes et al [21].

Table 4

Exchangeable (*Ex*) and bioavailable (*Bio*)-based partition coefficients (K_d) calculated for Cu, Zn, Pb and Cd

Heavy metals	Description	K_d-*Ex*	K_d-*Bio*
		$[dm^3 \cdot kg^{-1}]$	
Cu	R	154.2–1446.1	t
	M (*SD*)	465.5 (*143.7*)	t
Zn	R	31.3–288.6	14.6–4365.4
	M (*SD*)	106.5 (*13.4*)	1427.9 (*379.1*)
Pb	R	88.0–589.3	689.4–47869.8
	M (*SD*)	276.2 (*64.6*)	11643.0 (*2718.8*)
Cd	R	27.3–134.5	6.1–884.8
	M (*SD*)	72.8 (*12.7*)	176.3 (*33.0*)

Explanation: K_d-*Ex* – based on the ratio of total metal content (*Aqua regia*) to exchangeable metal content (*1 mol* $CH_3COONH_4 \cdot dm^{-3}$, *pH 7.0*); K_d-*Bio* – based on the ratio of total metal content (*Aqua regia*) to bioavailable metal content (*0.10 mol* $NaNO_3 \cdot dm^{-3}$); R – range, M(*SD*) – mean (n = 40) and standard deviation; t – traces (below detection limit).

A stepwise regression analysis was used to develop empirical models to estimate K_d for each metal. The relevant equations are reported below:

For exchangeable metal forms, K_d-*Ex for:*

$Cu = 312.8 \cdot pH + 91.9 \cdot C_{org} - 1474.9;$ $R^2 = 0.52$

$Zn = -24.2 \cdot pH + 10.2 \cdot CEC + 163.9;$ $R^2 = 0.71$

$Pb = 3.70 \cdot Clay + Silt + 133.5;$ $R^2 = 0.17$

$Cd = 1.08 \cdot Clay + Silt + 31.9;$ $R^2 = 0.43$

For bioavailable metal forms, K_d-*Bio for:*

$Zn = -208.6 \cdot C_{org} + 110.9 \cdot CEC + 991.2;$ $R^2 = 0.41$

$Pb = 1518.8 \cdot CEC - 82.9;$ $R^2 = 0.75$

$Cd = 30.8 \cdot CEC - 61.7;$ $R^2 = 0.86$

Although the coefficients of determination-based equations reported just above are useful for estimating the *statico-dynamic* nature of K_d, they are predictive only. They are not intended to describe adsorption mechanisms or even the relative contribution of the individual soil components to the adsorption/retention processes. In addition, if these equations are to be used to predict values of K_d, three or four characteristics must be specified for each soil considered. Clearly a simpler method for evaluating K_d-based

metal mobility would be useful. Therefore, consider the simplified metal sorption/retention reaction:

$$S - H_\alpha + M^{2+} \rightarrow S - M + \alpha H^+ \quad (1)$$

where: $S - H_\alpha$ – represents the surface binding sites,
M^{2+} – represents soluble metal species,
$S - M$ – represents surface-bound metal,
α – the proton coefficient represents the number of protons released when the metal binds.

An equilibrium constant can be rewritten for reaction (1) as:

$$K = \frac{(S-M)\cdot(H)^\alpha}{(M)\cdot(S-H)} \quad (2)$$

Substituting and solving for K_d in the logarithmic form yields the expression:

$$\log K_d = \alpha pH \log K \cdot (S - H) \quad (3)$$

A similar mass action approach was outlined by Kurbatov et al [22], and Honeyman [23] has discussed the limitations of this approach including the requirement for an excess of surface binding sites and the dependence of proton released on system pH.

In the present study we have assumed there was an excess of surface binding sites for each soil. If this is true and the site concentrations among the various soils are not significantly different, Eq. (3) would be valid not just for each soil independently, but for all soils, considered together. To test this assumption, exchangeable metals (Cu, Zn, Pb and Cd) extracted by 1 mol $CH_3COONH_4 \cdot dm^{-3}$, pH 7.0 as well as bioavailable ones, extracted by 0.10 mol $NaNO_3 \cdot dm^{-3}$ were plotted according to Eq. (3). The resulting slopes of the lines (Fig. 1) give a proton coefficient, which is an average over the soil pH ranges for the number of protons potentially generated by metals persistence in these sites (ie, contaminated soils).

The scatter in Fig. 1 may result because an equivalent concentration of high energy binding sites were not present in each of the soils. The stepwise correlations suggest that some of the most important sites involve the clay + silt pool and C_{org}. In soils characterized by low levels of these components, metals would be partitioned to lower energy binding sites, resulting in different parameters for Eq. (3).

The average calculated proton coefficient in the case of exchangeable metal forms amounts to 0.16 mol of protons released for each mole of Cu, Zn and Pb. In contrast, the proton coefficient for Cd is ca 81 % greater. The estimation made on the basis of bioavailable metal forms shows that Cd and Zn retention by soils has been generating 77 and 131 % more protons, respectively, as compared to Pb. The proton generating capacity of Zn was reported earlier by Leckie et al [24], who found that the retention of Zn produced ca 75 % more H^+ compared to Cd, Cu and Pb, whereas Anderson and Christensen [4] reported a value of 40 % for Zn with respect to Cd, Co and Ni. These

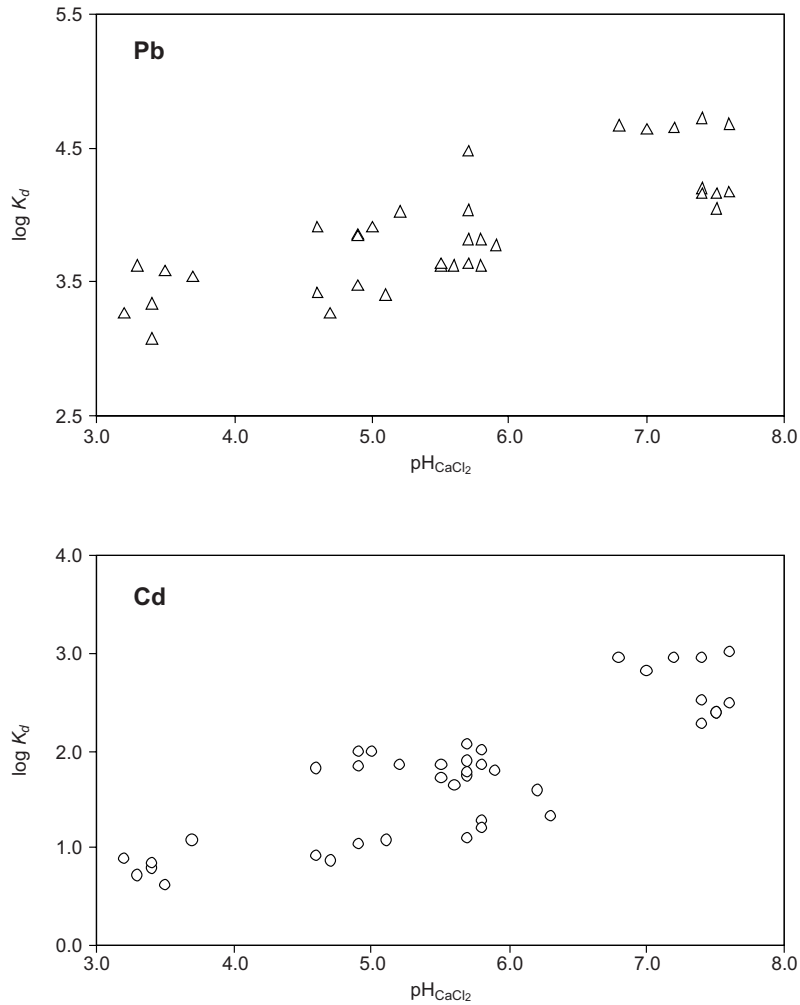

Fig. 1. Log K_d for bioavailable Pb and Cd as a function of pH

results suggest that for some surfaces, the sites involved in Zn and Cd retention in investigated soils are different from the sites involved for other metals. If these Zn and Cd binding sites are relatively more abundant, the assumptions made in developing these equations are more appropriate for Zn and Cd than for Cu and Pb. The equation also indicates that the partition coefficients for Zn and Cd are more sensitive to fluctuations in pH. In the event of reduced soil pH, Zn and Cd mobility would increase more rapidly than the other metals.

Heavy metals geochemistry is specifically related with protons generation, which in turn tend to lower the soil pH in order to increase solution metals activity. A sum up of data for all metals is reported in Table 5.

Table 5

Best values for proton coefficients (α) and intercepts for estimating log K_d from Eq. (3)

Metal	Partition coefficient	α [mol protons per mol of metal]	Intercept	R^2	*n*
Cu	*K_d-Ex*	0.18 ± 0.12	1.58	0.68	39
Zn		0.12 ± 0.05	1.46	0.47	36
Pb		0.18 ± 0.11	1.09	0.52	37
Cd		0.29 ± 0.01	0.02	0.64	37
Cu	*K_d-Bio*	nc	nc	nc	nc
Zn		0.60 ± 0.12	–1.34	0.79	38
Pb		0.26 ± 0.14	2.42	0.73	39
Cd		0.46 ± 0.11	–0.82	0.74	40

R^2 – coefficient of determination; n – number of samples, obvious outliers have been deleted; nc – not calculated (see Table 4: *K_d-Bio*).

Conclusions and statements

The consideration of the partition coefficient (K_d) in assessing the potential mobility of Cu, Zn, Pb and Cd in contaminated as well as polluted soils has revealed the intrinsic necessity for involving soil physical and chemical properties. This approach provided more details, which in turn set the basis of the *statico-dynamic* nature of metal mobility-based processes. Although the coefficients of determination-based equations were useful for estimating the *statico-dynamic* nature of K_d, they were predictive only. They were not intended to describe adsorption mechanisms, or even the relative contribution of the individual soil components to the adsorption/retention processes. Therefore log K_d *versus* pH regressions were applied in order to exhibit the proton generating capacity of each metal. The average calculated proton coefficient in the case of exchangeable metal forms amounted to 0.16 mol of protons released for each mol of Cu, Zn and Pb. In contrast, the proton coefficient for Cd was ca 81 % greater. The estimation made on the basis of bioavailable metal forms showed that Cd and Zn retention by soils generated, respectively 77 and 131 % more protons as compared to Pb. In terms of environmental concern, it may be formulated that the higher the proton generating capacity, the highest the metal mobility, and thus the weakest partition to the solid phase.

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ZASTOSOWANIE WSPÓŁCZYNNIKA PODZIAŁU DO OCENY RUCHLIWOŚCI METALI CIĘŻKICH W STREFIE ODDZIAŁYWANIA HUTY CYNKU W MIASTECZKU ŚLĄSKIM (POLSKA)

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Abstrakt: Praca przedstawia zagadnienia związane z oceną ruchliwości metali ciężkich (Cu, Zn, Pb, Cd) w glebach zanieczyszczonych w wyniku działalności huty cynku. Koncepcja opierała się na założeniu, że współczynniki podziału jako stosunki całkowitej zawartości metali (oznaczonej w wodzie królewskiej) do zawartości wymiennych (ekstrahowanych przez 1 mol $CH_3COONH_4 \cdot dm^{-3}$, pH 7.0) lub do zawartości biodostępnych (ekstrahowanych przez 0.10 mol $NaNO_3 \cdot dm^{-3}$) metali są bardziej przydatne do prognozowania ruchliwości metali ciężkich niż w przypadku pojedynczych ekstrakcji. Obliczone współczynniki

podziału przyjęto odpowiednio jako K_d-*Ex* i K_d-*Bio*, które z kolej miały odzwierciedlić *statyczno-dynamiczny* charakter ruchliwości metali ciężkich.

Uzyskane wyniki przedstawiają szereg Cd > Zn > Pb > Cu, z którego wynika, że Cd i Zn stanowią większe zagrożenie z uwagi na większą ich ruchliwość w glebie. Zależności między $\log K_d$ a pH wykorzystano w celu obliczenia zdolności każdego metalu do generowania jonów wodorowych. Średnie wartości obliczone dla wymiennych form metali wynosiły 0.16 mola protonów uwolnionych na każdy mol Cu, Zn i Pb w odróżnieniu od Cd, dla którego wartość ta była o 81 % większa. Oszacowanie dokonane na podstawie zawartości biodostępnych form metali ujawniło, że zatrzymywanie Cd i Zn przez gleby spowodowało generowanie odpowiednio o 77 i 131 % więcej protonów w porównaniu z Pb. Biorąc pod uwagę zagrożenie przyrodnicze, należy przyjąć, że im większa zdolność do generowania protonów, tym większą ruchliwość wykazuje dany metal i tym samym słabsze jest jego zatrzymywanie przez fazę stałą gleby.

Słowa kluczowe: współczynnik podziału, wymienne, biodostępne formy, miedź, cynk, ołów, kadm, huta cynku

Varia

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- full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.) ser. A or S.

Additional information one could find on the Conference website:

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The deadline for sending the Abstracts is **31.08.2010** and for the Extended Abstracts: **1.10.2010**. The actualised list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from 15.07.2010) on the Conference website.

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Further information is available from:
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Prof. dr hab. Maria Wacławek
Przewodnicząca Komitetu Organizacyjnego
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