

## INFLUENCE OF IODINE FERTILIZATION AND SOIL APPLICATION OF SUCROSE ON THE EFFECTIVENESS OF IODINE BIOFORTIFICATION, YIELD, NITROGEN METABOLISM AND BIOLOGICAL QUALITY OF SPINACH

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**Abstract.** Iodine biofortification of vegetables can become an alternative (to iodized salt) method of introducing this element into human diet. Development of agronomic rules concerning its application requires detailed evaluation of iodine influence on plant physiological and biological processes including mineral nutrition and quality of yield. The aim of the study was to determine the effect of iodine and soil application of sucrose on iodine biofortification and nutritional quality of spinach plants. In 2009–2010, a pot experiment was carried out with spinach *Spinacia oleracea* L. ‘Olbrzym Zimowy’ cv. cultivation on mineral soil. The research included diverse combinations with pre-sowing iodine fertilization (in the form of KI) and soil application of sucrose: 1) – control (without iodine fertilization and sucrose application), 2) – 1 mg I dm<sup>-3</sup> of soil, 3) – 2 mg I dm<sup>-3</sup> of soil, 4) – 1 mg I + 1 g sucrose dm<sup>-3</sup> of soil and 5) – 2 mg I + 1 g sucrose dm<sup>-3</sup> of soil. In all tested combinations with iodine fertilization as well as simultaneous application of iodine and sucrose a significant increase in iodine, N-total and soluble oxalate content was observed along with reduced level of nitrate(V) and dry matter in spinach leaves (when compared to the control). The highest accumulation of iodine was noted in leaves of plants treated with 2 mg I + 1 g sucrose dm<sup>-3</sup>. Simultaneous application of iodine and sucrose diminished free amino acid content in comparison to the control. Additional introduction of sucrose along with both iodine doses decreased nitrate(V) and N-total level in spinach plants. Soil fertilization with both doses of iodine (1 and 2 mg I dm<sup>-3</sup> of soil) applied individually or together with sucrose did not significantly affect spinach yield and the level of nitrate(III), phenolic compounds and soluble sugars in plants as well as iodine content in soil after cultivation.

**Key words:** iodine, sucrose, nitrogen, nitrate, biological quality, spinach

## INTRODUCTION

In the last few years numerous studies have been carried out on iodine biofortification (enrichment) of plants [White and Broadley 2005, 2009, Yang et al. 2007, Zhao and McGrath 2009]. The basic objective of these works is to propose crop plants as an alternative (to iodized salt) source of this element in human diet. Excessive consumption of table salt is in many countries one of the main contributors to increased occurrence of cardiovascular diseases. For that reason, World Health Organization has developed “The Global Strategy on Diet, Physical Activity and Health” on years 2008–2013. One of the tasks presented in this strategy includes limitation of salt consumption with concurrent search for effective ways of introducing iodine into food chain, which is crucial due to numerous functions played by this element in human organism. In that aspect, studies presented in this work respond the goals proposed by the mentioned WHO program.

Iodine is not a mineral nutrient essential for plant growth and development. For that reason, evaluation of agronomic rules of its application requires thorough research focused not only on optimization of biofortification but also on iodine effect on the quantity and quality of yield. It is so important as iodine, most probably, affect nitrogen metabolism in plants which contributes the most to plant productivity. This assumption is indirectly confirmed by studies conducted by Tsugonai and Sase [1969] on *Escherichia coli* extracts as well as by Wong and Hung [2001] and Hung et al. [2005] on marine phytoplankton.

Iodine biofortification of plants through soil fertilization is relatively low effective, which is caused by strong iodine sorption in soil. Plant uptake of iodine depends on its availability, which is essentially governed by the adsorption-desorption characteristics of soils [Dai et al. 2009]. Three days after introduction to soil, approximately 90% of iodine is strongly bound by Al and Fe sesquioxides [Muramatsu et al. 1990, Yoshida et al. 1992]. The process of iodine desorption is very slow what results in low level of iodine in soil solution and, furthermore, limits its uptake by plants [Fuge and Johnson 1986, Muramatsu et al. 1996, Yamaguchi et al. 2005]. All attempts of enriching plants with iodine by fertilization with high doses bring risks of plant damage due to toxic effects of excessive iodine levels [Smith and Middleton 1982, Mackowiak and Grossl 1999, Mackowiak et al. 2005, Hong et al. 2009].

A significant issue undertaken in studies on iodine biofortification is how to increase soil level of iodine forms available for plants without applying too high iodine doses. Works presented by Muramatsu et al. [1996] propose the possible solution of enhancing iodine desorption from soil which is observed with negative values of soil redox potential (Eh). Most traditionally cultivated soils are characterized by positive Eh and negative values of this parameter are noted after prolonged flooding which induces anaerobic conditions in soils. Application of glucose can also decrease values of soil Eh. In previously mentioned report by Muramatsu et al. [1996] after 60-day soil incubation with 5 g dm<sup>-3</sup> soil dose of glucose (laboratory test), a decrease in soil Eh from positive (app. +580 mV) to negative values (app. -200 mV) was noted which was correlated with increased iodine desorption ratio. This model experiment with soil incubation with glucose (or rice straw) did not however document the influence of tested factors on

iodine content in plants. It should be mentioned that, prior to the ours study, a trial 13-day laboratory incubation with 5 g dm<sup>3</sup> of soil dose of sucrose and glucose was carried out on soil subsequently used for spinach cultivation. Sucrose application contributed to a decrease in Eh values from +238,5 to -116,3 mV, while glucose – to the level of -66,7 mV.

Changes in soil redox potential can affect solubility and availability of mineral nutrients, trace elements and heavy metals for plants [Calmano et al. 1993, Chuan et al. 1996]. As a consequence, level of mineral nutrition as well as accumulation of heavy metals in plants can be altered.

The aim of the study was to evaluate the influence of iodine fertilization (in diverse doses) and soil application of sucrose on yield, nitrogen metabolism as well as the content of dry matter, phenolic compounds, soluble sugars and oxalates in spinach.

## MATERIAL AND METHODS

Spinach (*Spinacia oleracea* L.) ‘Olbrzym Zimowy’ c.v. was cultivated in the 2009–2010 in open-work containers sized 60 × 40 × 20 cm, placed in the plastic tunnel. The containers were filled with silt loam (35% sand, 28% silt and 37% clay) with mean content of organic matter 2.76% and the following concentrations of the available nutrient forms soluble in 0.03 M acetic acid: N (N-NO<sub>3</sub>+N-NH<sub>4</sub>) 58.7 mg, P 39.3 mg, K 73.3 mg, Mg 151.5 mg, Ca 1245.2, S 17.2, Na 6.8 and Cl 0.0 mg in 1 dm<sup>3</sup> soil. Soil pH<sub>(H2O)</sub> was 6.97, the oxidation – reduction (redox) potential of the soil (Eh): +326.7 mV, while soil salinity (electrical conductivity – EC) 0.31 mS cm<sup>-1</sup>. The content of assimilable forms of nitrogen, phosphorus and potassium was supplemented before the cultivation to the following levels: 100 mg N, 60 mg P and 160 mg K dm<sup>-3</sup> of soil with the use of calcium nitrate, potassium phosphate and potassium sulfate. Plants in containers were irrigated with the same amount of tap water.

During spinach cultivation (in 2009 and 2010) the course of mean daily temperature and relative humidity remained at a comparable level. Mean daily temperature and air humidity throughout spinach cultivation were respectively: 13.7°C and 67.4% RH in 2009 while 15.5°C and 68.8% RH in 2010. Detailed meteorological data were presented in the previous publication – Smoleń and Sady [2011].

In the study, various combinations with pre-sowing fertilization with iodine (in the form of KI) and soil application of sucrose were used including: 1) – control (without iodine and sucrose application), 2) – 1 mg I dm<sup>-3</sup> of soil, 3) – 2 mg I dm<sup>-3</sup> of soil, 4) – 1 mg I + 1 g sucrose dm<sup>-3</sup> of soil and 5) – 2 mg I + 1 g sucrose dm<sup>-3</sup> of soil. Iodine and sucrose were applied pre-sowing as water solutions using 1 dm<sup>-3</sup> of solution per 1 container. In the control combination the amount of 1 dm<sup>-3</sup> of water per 1 container was used.

The experiment was carried out according to randomized method in three replications. Each replicate (one container) consisted of 4 rows with 10 plants per row. Seed sowing was performed with 20 seeds in a row on 20<sup>th</sup> and 23<sup>rd</sup> March in the subsequent years. After germination, the plants were singled out leaving 10 seedlings in one row (40 plants per one container). Spinach was harvested on 28<sup>th</sup> April 2009 and 4<sup>th</sup> May 2010.

Dry matter content in spinach leaves was assessed at 105°C. The content of nitrate(V) ( $\text{NO}_3^-$ ), nitrate(III) ( $\text{NO}_2^-$ ) and ammonium ions ( $\text{NH}_4^+$ ) in spinach fresh leaves was assessed by FIA technique [PN-EN ISO 13395:2001, PN-EN ISO 11732:2005 (U)] after extraction with 2%  $\text{CH}_3\text{COOH}$  [Nowosielski 1988]. The content of phenolic compounds were analyzed with the use of Folin and Ciocalteu reagent [Swain and Hillis 1959] while total soluble sugars – by the anthrone method [Yemm and Wills 1954]. The level of free amino acids in spinach samples were determined in the reaction with ninhydrin [Korenman 1973].

In order to assess the level of iodine, N-total and soluble oxalates, spinach leaves were air-dried at 70°C and subsequently ground. Iodine content was determined after sample incubation with 25% TMAH according to the standard method [PN-EN 15111 – 2008]. Total nitrogen content in spinach samples was determined by the Kjeldahl method [Persson and Wennerholm 1999], while soluble oxalates by titration with 0.02 M  $\text{KMnO}_4$  in extracts prepared using 5%  $\text{CaCl}_2$  and acetone [Wierzbicka 2004].

Prior to the experiment, organic matter concentration in soil was determined using Tiurin method modified by Oleksynowa [Komornicki et al. 1991]. The content of N-mineral ( $\text{N-NH}_4$ ,  $\text{N-NO}_3$ ), P, K, Mg, Ca, S, and Na was determined after sample extraction with 0.03 mol  $\text{CH}_3\text{COOH}$  [Nowosielski 1988]. Soil content of iodine and mineral nitrogen ( $\text{N-NH}_4$ ,  $\text{N-NO}_3$ ) after spinach cultivation was determined after extraction with 0.03 mol  $\text{CH}_3\text{COOH}$ . Nitrogen level in soil extracts was assessed using FIA technique [PN-EN ISO 13395:2001, PN-EN ISO 11732:2005 (U)], while K, Mg, Ca, S, Na and iodine – by ICP-OES method.

Obtained results were statistically verified by ANOVA module of Statistica 8.0 PL program for significance level  $P < 0.05$ . Changes of any significance were assessed with the use of variance analysis. In case of significant changes homogenous groups were determined on the basis of Duncan test.

## RESULTS AND DISCUSSION

**Spinach yield and effectiveness of iodine biofortification.** Iodine influence on plant growth and yield is related, among others, to its form and applied dose, as well plant response to iodine which is species-dependent [Borst-Pauwels 1961, Dai et al. 2006, Mackowiak and Grossl 1999, Zhu et al. 2003]. Application of excessive doses of iodine can be detrimental for plants leading to biomass reduction, chlorosis and, after exceeding toxic levels – leaf necrosis and whole plant death [Smith and Middleton 1982, Mackowiak and Grossl 1999, Mackowiak et al. 2005, Hong et al. 2009]. At low concentrations of iodine in soil (0.01–0.1 ppm), a positive influence of this element on plant growth and yield is observed [Borst-Pauwels 1961]. Kabata-Pendias and Mukherjee [2007] informed that no explanation have been found for stimulating action of low iodine doses on plant biomass increase. In the present study, 1 and 2 mg I  $\text{dm}^{-3}$  of soil iodine doses applied separately or together with sucrose did not significantly affect the yield of spinach leaves (fig. 1). In all combinations with iodine fertilization (introduced with or without sucrose) a significant increase of iodine content in spinach was found (when compared to the control). Introduction of the higher iodine dose (2 mg I  $\text{dm}^{-3}$  of

soil) contributed to the biofortification effect only when applied together with sucrose. Despite the eight-time increase of iodine level in plants from this combination (2 mg I + 1 g sucrose dm<sup>-3</sup> of soil – fig. 1), no visual symptoms of plant damage indicating toxic effect of iodine were observed. It is the more interesting as iodide form of iodine (I<sup>-</sup>) used in our studies was previously described as more harmful for plants than IO<sub>3</sub><sup>-</sup>. Zhu et al. [2003] revealed that iodine application in the form of IO<sub>3</sub><sup>-</sup> (in the following concentration range: 0, 1, 10, 50, 100 μmol) had no influence on the yield of spinach plants grown in solution culture. When iodide form was used, reduced root and leaf biomass spinach was noted along with increasing I<sup>-</sup> concentration in the nutrient solution. In the studies conducted by Gonda et al. [2007], symptoms of leaf necrosis were noted on spinach plants cultivated in hydroponic culture on nutrient solution containing 0.1 mmol I<sup>-</sup>. No such damages were observed on control plants as well as spinach grown with the presence of 0.05 mmol I<sup>-</sup> or 0.1 mmol IO<sub>3</sub><sup>-</sup> in solution. It should be mentioned that iodine content in spinach from our experiment was lower than obtained by Zhu et al. [2003] and Gonda et al. [2007]. This difference can be related to the fact that we carried out spinach cultivation in soil, not in hydroponics, in which iodine uptake is much easier than from soil due to lack of sorption complex.

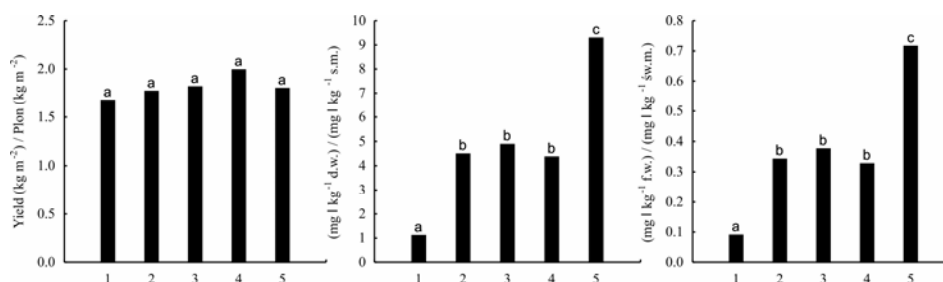


Fig. 1. Yield and iodine concentration in spinach leaves (expressed on fresh and dry weight basis) – means for 2009–2010: 1 – Control, 2 – 1 mg I dm<sup>3</sup> of soil, 3 – 2 mg I dm<sup>3</sup> of soil, 4 – 1 mg I + 1 g sucrose dm<sup>3</sup> of soil, 5 – 2 mg I + 1 g sucrose dm<sup>3</sup> of soil; means followed by the same letters are not significantly different for P < 0.05

Ryc. 1. Plon oraz zawartość jodu w szpinaku w przeliczeniu na suchą i świeżą masę liści (średnie z lat 2009–2010): 1 – Kontrola, 2 – 1 mg I dm<sup>3</sup> gleby, 3 – 2 mg I dm<sup>3</sup> gleby, 4 – 1 mg I + 1 g sacharozy dm<sup>3</sup> gleby, 5 – 2 mg I + 1 g sacharozy dm<sup>3</sup> gleby; średnie oznaczone tymi samymi literami nie różnią się istotnie dla P < 0,05

Despite increased iodine content in plants fertilized with KI (fig. 1), soil level of this element after spinach cultivation remained at a comparable level in all tested combinations (tab. 1). Enhanced uptake of iodine by cultivated plants could have been a possible explanation for the lack of influence of iodine fertilization on its content in soil (determined in 0,03 mol acetic acid). In the present study a relatively weak effect of simultaneous application of iodine and sucrose was observed in reference to soil pH and Eh (redox potential) – detailed data published previously [Smoleń and Sady 2011]. Values of soil pH varied from 6.78 in the control to 7.05 in soil from combination no. 4 (1 mg I

+ 1 g sucrose  $\text{dm}^{-3}$ ). The lowest value of Eh was found in combination no. 4, while the highest (+341.8 mV) – in soil fertilized with 2 mg I  $\text{dm}^{-3}$  of soil (combination no. 3). Lack of effect of sucrose on soil Eh could have resulted from measuring redox potential in soil after spinach cultivation – approximately 40 days after sucrose application. Additionally, it may have contributed to obtain no changes in soil concentration of iodine in combination with its application (combinations no. 2–4).

Table 1. Concentration of iodine and particular forms of mineral nitrogen in soil after spinach cultivation (means for 2009–2010)

Tabela 1. Zawartość jodu oraz azotu mineralnego w glebie po uprawie szpinaku (średnie z lat 2009–2010)

Combinations (iodine and sucrose doses per 1 $\text{dm}^3$ of soil) Kombinacje (dawki jodu i sacharozy na $\text{dm}^3$ gleby)	mg $\text{dm}^{-3}$ soil – mg $\text{dm}^{-3}$ gleby			
	I	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub>	N (N-NH <sub>4</sub> +N-NO <sub>3</sub> )
Control – Kontrola	3.7	0.5 a	49.7 b	50.2 b
1 mg I	3.9	0.5 a	62.0 c	62.5 c
2 mg I	4.3	0.8 a	76.3 d	77.1 d
1 mg I + 1 g sucrose – sacharoza	3.9	1.3 b	28.2 a	29.5 a
2 mg I + 1 g sucrose – sacharoza	4.1	0.7 a	52.8 bc	53.5 bc
Test <i>F</i> – Test <i>F</i>	n.s. – n.i.	*	*	*

Means followed by the same letters are not significantly different for  $P < 0.05$  – Średnie oznaczone tymi samymi literami nie różnią się istotnie dla  $P < 0,05$ .

Test *F*: \* – means are significantly different – średnie różnią się istotnie.

n.s. – not significant – n.i. – brak istotnego zróżnicowania.

**Nitrogen metabolism.** Soil application of iodine and sucrose had a significant effect on the content of: nitrate(V), ammonium ions, free amino acids as well as total nitrogen in spinach (tab. 2). No significant influence of tested factors was found in reference to nitrate(III) accumulation in spinach leaves. Iodine applied in both doses similarly decreased the level of nitrate(V) when compared to the control. Additional introduction of sucrose enhanced the above-mentioned relation. Simultaneous application of iodine and sucrose (in both iodine doses: 1 and 2 mg I  $\text{dm}^{-3}$  of soil) resulted in a decrease in the content of free amino acids as well as a slight increase of total nitrogen in spinach. Only after simultaneous application of 2 mg I and 1 g of sucrose per 1  $\text{dm}^3$  of soil, a reduced content of ammonium ions in spinach was noted. It is worthy to mention that increasing iodine dose from 1 to 2 mg I  $\text{dm}^{-3}$  soil was accompanied by an increase in the content of free amino acids as well as improvement in nitrogen nutrition of cultivated plants. Spinach plants fertilized with higher iodine dose (2 mg I  $\text{dm}^{-3}$  of soil) contained the highest amount of free amino acids and total nitrogen in leaves.

Lower content of nitrate(V) and total nitrogen in plants treated with iodine and sucrose (combinations 4 and 5) when compared to spinach fertilized only with iodine (in both tested doses: 1 and 2 mg I  $\text{dm}^{-3}$ ) could have resulted from reduced level of mineral

Table 2. Content of nitrate(V), nitrate(III), ammonium ions, free amino acids and N-total in spinach leaves (means for 2009–2010)

Tabela 2. Zawartość azotanów(V), azotanów(III), jonów amonowych, aminokwasów i N-ogółem w liściach szpinaku (średnie z lat 2009–2010)

Combinations (iodine and sucrose doses per 1 dm <sup>3</sup> of soil) <sup>1</sup> Kombinacje (dawki jodu i sacharozy na dm <sup>3</sup> gleby) <sup>1</sup>	mg kg <sup>-1</sup> f.w. – mg kg <sup>-1</sup> św.m.				N (% d.w.) N (% s.m.)
	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	amino acids (in calculation on N) aminokwasy (w przeliczeniu na N)	
Control – Kontrola	3 586.9 c	0.79	13.1 b	187.4 b	5.47 a
1 mg I	3 425.8 b	0.65	13.3 b	191.8 b	5.57 b
2 mg I	3 544.1 bc	0.46	13.1 b	204.8 c	5.85 c
1 mg I + 1 g sucrose – sacharoza	2 672.4 a	0.65	12.6 b	174.4 a	5.51 ab
2 mg I + 1 g sucrose – sacharoza	2 610.3 a	0.16	10.0 a	183.4 ab	5.53 ab
Test <i>F</i> – Test <i>F</i>	*	n.s. – n.i.	*	*	*

1 – See table 1 – Opis jak w tabeli 1.

nitrogen in soil – particularly in the case of N-NO<sub>3</sub> (tab. 1). It should be underlined that soil application of only iodine contributed to increase in N-NO<sub>3</sub> level in soil what was furthermore reflected in improved nitrogen nutrition of spinach. Iodine fertilization (especially in a higher dose – 2 mg I dm<sup>-3</sup>) could have stimulated growth and development of microorganisms responsible for mineralization of soil organic matter. As an effect, the amount of mineral nitrogen released in this process may have increased and nitrogen uptake by plants improved (higher content of total nitrogen) without the negative effect of elevated level of nitrate(V) in spinach. It can be therefore stated that iodine most probably affects nitrogen metabolism in plants. Comparison of our findings with those obtained by other authors concerning iodine influence on N metabolism in higher plants gives ambiguous information. In the studies conducted by Gonda et al. [2007], iodine applied as IO<sub>3</sub><sup>-</sup>, when compared to I<sup>-</sup>, contributed to an increase in nitrate(V) content in tomato and spinach cultivated in hydroponics. Blasco et al. [2010] revealed that iodide introduction (in the concentration of: 20, 40, 80 μmol KI) reduced NO<sub>3</sub><sup>-</sup> accumulation with accompanying diminished activity of nitrate reductase (NR) in lettuce. Application of iodate form in a dose of: 20, 40, 80 μmol as KIO<sub>3</sub> increased NR activity but had no influence on nitrate(V) content in lettuce. Ledwożyw et al. [2009] as well as Ledwożyw et al. [2010] presented a significant effect of foliar nutrition with KIO<sub>3</sub> on nitrate(V) content and nitrate reductase (NR) and nitrite reductase (NiR) activity in lettuce heads. In the studies conducted by Smoleń et al. [2009] soil application of KIO<sub>3</sub>, when compared to KI, led to elevated content of nitrate(V) in storage roots of carrot fertilized with Ca(NO<sub>3</sub>)<sub>2</sub> as well as not treated with nitrogen. Such relations were not found in the case of fertilization with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Results obtained in the above-mentioned works indicate that iodine fertilization with IO<sub>3</sub><sup>-</sup> in the condition of low N supply in soil can contribute to increased level of nitrate(V) in carrot. On the basis of presented data as well as results of our studies it can be assumed that iodine influence on nitrogen metabolism depends on: iodine form, cultivation conditions (soil and hy-

droponics), physico-chemical properties of soil as well as species and cultivar characteristics of plants.

In the context of presented information it should be underlined that additional application of sucrose decreased the content of nitrate(V) and total nitrogen in spinach when compared to fertilization with iodine only. Lower level of plant nitrogen nutrition could have resulted from the interaction between iodine and sucrose and its effect both on plants and soil environment. In combinations with sucrose application, soil content of N-NO<sub>3</sub> was significantly lower than in soil after cultivation of plants fertilized with iodine only. Particularly interesting is the fact that addition of sucrose into soil diminished the content of nitrates(V) and free amino acids in spinach – as well as the level of ammonium ions in plants fertilized with higher dose of iodine. In the combination with 2 mg I + 1 g sucrose dm<sup>-3</sup> a similar tendency was noted (though not statistically significant) of decreasing NO<sub>2</sub><sup>-</sup> content in spinach. Obtained results indirectly suggest that higher levels of iodine can negatively affect the rate of NO<sub>3</sub><sup>-</sup> uptake as well as impair its reduction to NO<sub>2</sub><sup>-</sup>. Consequently, iodine applied in high concentration could have diminished the amount of NO<sub>2</sub><sup>-</sup> reduced to NH<sub>4</sub><sup>+</sup>, as well as lower the level of free amino acids due to less effective incorporation of ammonium ions to organic compounds in GS/GOGAT cycle. In plants grown in the presence of 2 mg I + 1 g sucrose dm<sup>-3</sup> of soil no changes in yield as well as N-total content were noted. This fact indicates that previously described relations between iodine and nitrogen may have occurred primarily in the final period of the study or could have resulted from the effect of sucrose on nitrogen processes in soil environment. Sucrose could have been an additional source of



Phot. 1. White fungi filaments on soil surface appearing approximately 10 days after sucrose application

Fot. 1. Biały nalot grzybni na powierzchni gleby pojawiający się około 10 dni od aplikacji sacharozy



energy for soil microorganisms. Studies conducted by Kelliher et al. [2005] revealed that soil application of sucrose had a statistically significant effect on microbial respiration rate in soil. It is likely that in our studies, introduction of sucrose into soil could have stimulated the development of microorganisms which were taking up mineral nitrogen from soil. It is worth to mention that in both years of the experiment white fungal filaments appeared on soil surface approximately 10 days after sucrose application (Photo 1). During the next couple of days its development was gradually reduced along with spinach growth. No microbiological (species) analysis was carried out as it exceeded the main aim of the study. It should be underlined that no negative impact of this microorganisms was noted on plant health as well as spinach yield. This particular observation can be an additional support for the presented hypothesis concerning obtained changes in mineral nitrogen content in soil. It is worth to note that the development of mentioned microorganisms in the combination with the application of 2 mg I + 1 g sucrose  $\text{dm}^{-3}$  could have enhanced iodine desorption from soil, and thus increase its availability for spinach plants. Transient decrease in redox potential may have occurred in the initial stage of spinach cultivation due to appearance of these microorganisms. Yet, measurement of Eh values was not conducted at that stage.

**Other parameters of nutritional quality.** A significant influence of iodine and soil application of sucrose was found in relation to the content of dry mass and soluble oxalates; tested factors did not significantly affected the level of phenolic compounds and soluble sugars in spinach (tab. 3). Fertilization only with iodine as well as simultaneous application of I and sucrose reduced dry matter content as well as increased the level of soluble oxalates in spinach. It should be underlined that obtained differences in dry matter content were relatively small, ranging from 0.1% in plants fertilized with 1 mg I  $\text{dm}^{-3}$  to 0.44% in plants treated with 1 mg I + 1 g sucrose  $\text{dm}^{-3}$ . The effect of iodine application on the content of dry matter is considered to be related to its dose and method of introduction as well as cultivated plant species. In a pot experiment, iodine concentrations higher than 4 ppm KI applied in growth solution increased dry matter

Table 3. Content of dry matter, phenolic compounds, soluble sugars and soluble oxalates in spinach leaves (means for 2009–2010)

Tabela 3. Zawartość suchej masy, związków fenolowych oraz cukrów i szczawianów rozpuszczalnych w liściach szpinaku (średnie z lat 2009–2010)

Combinations (iodine and sucrose doses per 1 $\text{dm}^3$ of soil) <sup>1</sup> Kombinacje (dawki jodu i sacharozy na $\text{dm}^3$ gleby) <sup>1</sup>	Dry matter (% d.m.) Sucha masa (% s.m.)	mg 100 $\text{g}^{-1}$ f.w. – mg 100 $\text{g}^{-1}$ św.m.		
		phenolic compounds związki fenolowe	soluble sugars cukry rozpuszczalne	soluble oxalates szczawiany rozpuszczalne
Control – Kontrola	8.09 c	79.5	404.6	912.3 a
1 mg I	7.99 b	73.7	388.1	955.1 b
2 mg I	7.96 b	72.7	376.5	930.4 ab
1 mg I + 1 g sucrose – sacharoza	7.65 a	77.2	420.2	1020.2 c
2 mg I + 1 g sucrose – sacharoza	7.92 b	75.5	415.5	953.5 b
Test F – Test F	*	n.s. – n.i.	n.s. – n.i.	*

1 – See table 1 – Opis jak w tabeli 1.

content of tomato [Hageman et al. 1942]. In the studies conducted by Strzetelski et al. [2010] soil fertilization with iodine in a dose of  $15 \text{ mg I dm}^{-3}$  of soil as well foliar nutrition with 0.2% solution of iodine (in the amount of  $0.4 \text{ dm}^3 \text{ m}^{-2}$ ) had no statistical effect on dry matter content in radish leaves and roots.

Basically, increased accumulation of soluble oxalates in spinach treated with iodine (along with additional application of sucrose) could have been related to higher level of total nitrogen. Nitrogen fertilization as well as the level of plant nutrition with this macro element significantly affect the content of oxalates in spinach [Stagnari et al. 2007, Zhang et al. 2005, Smoleń et al. 2010]. Sucrose, or products of its decomposition, could have been taken up from soil by plant roots. For that reason, a relatively slight increase in the content of soluble oxalates in plants from both combinations with sucrose application (when compared to plants fertilized only with iodine) could have been caused by sucrose influence on physiological and biochemical processes occurring in plant tissues.

## CONCLUSIONS

In comparison to the control, in all combinations with iodine fertilization as well as simultaneous application of iodine and sucrose a significant increase was noted in the content of: iodine, total nitrogen and soluble oxalates as well as a reduction in the level of nitrate(V) and dry mass in spinach.

The highest accumulation of iodine was found in leaves of plants fertilized with  $2 \text{ mg I} + 1 \text{ g sucrose dm}^{-3}$  of soil.

Simultaneous application of iodine and sucrose, in comparison to the control, decreased the level of free amino acids. Increase of iodine dose from 1 to  $2 \text{ mg I dm}^{-3}$  (combination 2 and 3) contributed to higher content of free amino acids as well as improved plant nitrogen nutrition.

Reduction in the content of nitrate(V) and N-total in plants from combination with iodine and sucrose application (when compare to plants fertilized with iodine only) was related to lower level of N-NO<sub>3</sub> in soil due to sucrose introduction.

Simultaneous application of  $2 \text{ mg I} + 1 \text{ g sucrose dm}^{-3}$  of soil decreases the content of ammonium ions in spinach.

Fertilization with both doses of iodine ( $1$  and  $2 \text{ mg I dm}^{-3}$ ) applied with or without sucrose did not significantly affect spinach yield, the content of nitrate(III), phenolic compounds and soluble sugars in plants as well as iodine level in soil after spinach cultivation.

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**WPLYW NAWOŻENIA JODEM I DOGLEBOWEJ APLIKACJI  
SACHAROZY NA EFEKTYWNOŚĆ BIOFORTYFIKACJI W JOD, PLON,  
GOSPODARKE AZOTEM ORAZ JAKOŚĆ BIOLOGICZNĄ SZPINAKU**

**Streszczenie.** Biofortyfikacja warzyw w jod może być alternatywnym do jodowania soli kuchennej sposobem wprowadzenia tego pierwiastka do diety człowieka. Opracowanie agrotechnicznych zasad jego aplikacji wymaga określenia wpływu jodu na procesy fizjologiczne i biochemiczne roślin w tym na gospodarkę mineralną i jakość biologiczną plonu. Celem badań było określenie oddziaływania jodu oraz doglebowej aplikacji sacharozy na efektywność biofortyfikacji w jod, plon, gospodarkę azotem oraz jakość biologiczną szpinaku. W latach 2009–2010 przeprowadzono doświadczenie wazonowe z uprawą szpinaku *Spinacia oleracea* L. odmiany ‘Olbrzym Zimowy’ na glebie mineralnej. Badaniami objęto zróżnicowane kombinacje z przedsięwziętym nawożeniem jodem (w formie KI) i doglebową aplikacją sacharozy: 1) – kontrola (nienawożona jodem i bez aplikacji sacharozy), 2) – 1 mg I dm<sup>-3</sup> gleby, 3) – 2 mg I dm<sup>-3</sup> gleby, 4) – 1 mg I + 1 g sacharozy dm<sup>-3</sup> gleby i 5) – 2 mg I + 1 g sacharozy dm<sup>-3</sup> gleby. W porównaniu z kontrolą we wszystkich badanych kombinacjach z nawożeniem jodem oraz jodem z dodatkową aplikacją sacharozy stwierdzono istotne zwiększenie zawartości jodu, azotu ogółem i szczawianów rozpuszczalnych oraz zmniejszenie zawartości azotanów(V) i suchej masy w szpinaku. Najwyższą zawartość jodu stwierdzono w liściach roślin nawożonych 2 mg I + 1 g sacharozy dm<sup>-3</sup> gleby. Łączna aplikacja jodu i sacharozy w porównaniu z kontrolą powodowała obniżenie zawartości wolnych aminokwasów. Dodatkowa aplikacja sacharozy przy obydwu dawkach nawożenia jodem powodowała zmniejszenie zawartości azotanów(V) oraz azotu ogółem w szpinaku. Nawożenie jodem w obydwu dawkach (1 i 2 mg I dm<sup>-3</sup> gleby), aplikowanym oddzielnie oraz łącznie z sacharozą nie miało istotnego wpływu na plon, zawartość azotanów(III), związków fenolowych i cukrów w szpinaku oraz na zawartość jodu w glebie po uprawie roślin.

**Słowa kluczowe:** jod, sacharoza, azot, azotany, jakość biologiczna, szpinak

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