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THE EFFECT OF PLANT BIOSTIMULATION WITH 'PENTAKEEP V' AND NITROGEN FERTILIZATION ON YIELD, NITROGEN METABOLISM AND QUALITY OF SPINACH

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Abstract. Foliar application of fertilizers containing biostimulators can influence yield and the uptake and accumulation of NO_3^- by plants. The aim of the research was to determine the influence of foliar nutrition with 'Pentakeep V' and diverse doses of nitrogen fertilization on yield, plant nitrogen metabolism and nutritional quality of spinach. The experiment design included two sub-blocks with and without foliar nutrition. Plants with foliar nutrition were sprayed twice with 0.02% w/v 'Pentakeep V' fertilizer (3000 dm^3 water per 1 ha). In each sub-block soil nitrogen fertilization (in the form of ammonium nitrate) was applied in following combinations: 1 – control (without N), 2 – 50% N dose prior to seed sowing (25 mg $N \cdot dm^{-3}$ of soil), 3 – 100% N dose prior to seed sowing (50 mg $N \cdot dm^{-3}$ of soil). The interaction between foliar nutrition and nitrogen fertilization had a significant effect on the content of: nitrates(V), soluble oxalates and ascorbic acid in spinach leaves. The effect of tested factors on the content of nitrates(V) and ascorbic acid was additionally modified by the weather conditions in both years of research. Foliar application of 'Pentakeep V' resulted in decreased concentration of soluble oxalates in control plants (without N) as well as higher amount of these compounds in plants fertilized with 100% of N dose. No significant effect of tested interaction was observed in the case of yield and the content of: dry matter, nitrates(III), ammonium ions, free amino acids, N-total, soluble sugars and phenolic compounds in spinach leaves.

Key words: 5-aminolevulinic acid, nitrates, oxalates, ascorbic acid, biological quality

INTRODUCTION

5-aminolevulinic acid (ALA) included, among others in Pentakeep® fertilizers, has been used in plant production for only few years [Tanaka et al. 2005]. ALA is a common precursor to tetrapyrrole compounds found in chlorophyll and hemes. Application

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of exogenous ALA improves plant growth, increases the concentration of photosynthetic pigments, affects the level of stomatal aperture opening and improves resistance to: stress under low-light low-temperature and salt conditions [Hotta et al. 1997, 1998, Tanaka et al. 2005, Zhang et al. 2006]. A positive relation was observed between ALA content in plants and photosynthetic activity [Tanaka et al. 2005, Yaronskaya et al. 2006, Memon et al. 2009] as well as antioxidative enzyme activities [Memon et al. 2009].

ALA affects processes involved in plant nitrogen metabolism. Exogenous application of 5-aminolevulinic acid (or Pentakeep® fertilizers) increases activity of nitrate reductase [Mishra and Srivastava 1983] as well as decreases the content of nitrates(V) in plants [Tanaka et al. 2005]. However, research conducted by Iwai et al. [2005] showed that application of fertilizer containing ALA, apart from increasing yield, caused intensified uptake of $N\text{-}NO_3$ by sweet pepper plants.

The aim of present study was to determine the effect of foliar nutrition with 'Pentakeep V' along with diverse doses of nitrogen fertilization on yield, nitrogen metabolism and the content of: oxalates, soluble sugars and phenolic compounds determining the nutrition quality of spinach plants.

MATERIAL AND METHODS

Spinach (*Spinacia oleracea* L.) 'Spinaker F_1' c.v. was cultivated in the 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 silty medium loam (35% sand, 28% silt and 37% clay – according to PTG PN-R-04033) with content of organic matter: 2.44–2.52% (in 2006 and 2007) and the following concentrations of the available nutrient forms soluble in 0.03 M acetic acid (respectively for 2006 and 2007): N ($N\text{-}NO_3$+$N\text{-}NH_4$) 16.6–86.3 mg, P 16.6–64.8 mg, K 37.6–53.1 mg, Mg 121.4–158.3 mg and Ca 1032.2–2342.9 mg in 1 dm^{-3} soil. In 2006 and 2007 soil $pH_{(H2O)}$ was 6.38–6.99, while general concentration of salt in soil (EC) 0.19–0.41 mS cm^{-1}, respectively. The content of assimilable nutrient forms of phosphorus and potassium was supplemented before cultivation to the following level: 60 mg P (in 2006) and 200 mg K dm^{-3} of soil (in 2006 and 2007). When soil humidity was not sufficient, plants in containers were watered with municipal water.

The research comprised two sub-blocks: with and without foliar nutrition. In the sub-block with foliar application plants were sprayed twice (on 5^{th} and 12^{th} September 2006 as well as 3^{th} and 14^{th} September 2007) with 'Pentakeep V' in a dose of 0.02% w/v (16 ml 100 dm^{-3}). The following combinations with soil fertilized with nitrogen were distinguished within each sub-block: 1 – control (unfertilized with nitrogen), 2 – 50% of N dose prior to seed sowing (25 mg N dm^{-3} of soil), 3 – 100% dose of N prior to seed sowing (50 mg N dm^{-3} of soil). Pre-sowing nitrogen fertilization was carried out in the form of ammonium nitrate (Zakłady Azotowe in Puławy). 'Pentakeep V' fertilizer contains (in gravimetric percent): 9.5% N (3.8% $N\text{-}NO_3$, 5.7% $N\text{-}NH_4$), 5.7% MgO, 0.14% B, 0.02% Cu, 0.6% Fe-DTPA, 0.23% Mn, 0.02% Mo and 0.16% Zn and

5-aminolevulinic acid in concentration not declared by the producer (Cosmo Seiwa Agriculture Co., LTD. Japan).

The experiment was carried out using a split-plot method in four replicates. Each replicate (one container) consisted of 4 rows with 10 plants per row. Seeds sowings were performed on 1^{th} August in both years of the study using 15 seeds in one row. After germination the plants were thinned out leaving 10 seedlings in one row (40 plants per one container). Spinach plants were harvested on 19^{th} and18^{th} September in the subsequent years.

The analysis of plant samples was performed in the fresh plant material immediately after harvesting. Dry matter content in spinach leaves was assessed at 105°C. Concentration of: nitrate(V) (NO_3^-), nitrate(III) (NO_2^-) and ammonium ions (NH_4^+) was determined by FIA technique [PN-EN ISO 13395: 2001, PN-EN ISO 11732: 2005 (U)] after extraction with 2% CH_3COOH [Nowosielski 1988]. The level of free amino acids was determined in the reaction with ninhydrin [Korenman 1973] and the amount of N-total was analyzed using Kjeldahl method [Persson and Wennerholm 1999]. The content of total soluble sugars was determined by the anthrone method [Yemm and Wills 1954] and soluble oxalates by titration with 0.02 M $KMnO_4$ in extracts prepared using 5% $CaCl_2$ and acetone [Wierzbicka 2004]. The phenolic compounds were analyzed with the use of Folin and Ciocalteu reagent [Swain and Hillis 1959] while the content of ascorbic acid was determined in extracts prepared with oxalic acid by titration with potassium iodide [Duliński et al. 1988].

After harvesting, soil $pH_{(H2O)}$ was assessed by potentiometer while total concentration of salt in soil (EC) was measured conductometrically. The content of N-mineral (N-NH_4, N-NO_3) was determined by FIA technique [PN-EN ISO 13395:2001, PN-EN ISO 11732:2005 (U)] after soil extraction with 0.03 M CH_3COOH [Nowosielski 1988].

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

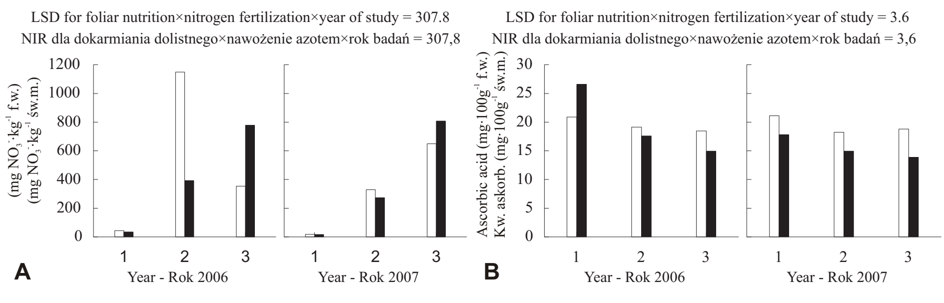
RESULTS AND DISCUSSION

Meteorological data gathered throughout the period of the research was shown in table 1. During spinach cultivation, year 2006 was characterized by lower values of average air temperature (measured daily) in August and higher in first two decades of September in comparison to 2007. Total rainfall amount in 2006 was 2.6 times lower than in 2007 but its distribution was more even throughout the cultivation period. In the first decade of August 2007 smaller amount of rain and almost two times higher number of sunshine hours was noted in comparison to the respective time in 2006. First decade of September 2007 was characterized by 4.6 times lower number of sunshine hours and 18.8 times higher amount of rainfall than analogous period in the year 2006. In 2006, during the first and the third decade of August higher air humidity was noted in respect of 2007. In both years of research, a comparable total number of sunshine hours were noted during spinach cultivation.

Table 1. Meteorological data from the spinach cultivation in 2006 and 2007
Tabela 1. Dane meteorologiczne w okresie uprawy szpinaku w latach 2006 i 2007

Year Rok	Month Miesiąc	Decade Dekada	Average air temperature Średnia temperatura powietrza ºC	Rainfall Opady mm	Sunshine Usłonecznienie h	Humid air RH, % Wilgotność powietrza WW, %
2006	August – sierpień	I	18.8	35.1	36.4	83.4
		II	20.2	10.3	63.1	73.7
		III	16.4	58.7	55.5	82.1
	September – wrzesień	I	16.8	15.3	68.4	76.5
		II	16.8	1.0	64.5	76.1
	sum – suma		-	120.4	287.9	-
2007	August – sierpień	I	20.9	0.2	71.0	61.0
		II	20.4	10.4	55.1	78.2
		III	19.2	14.0	81.0	68.2
	September – wrzesień	I	13.1	288.0	14.6	86.7
		II	12.1	0.8	56.7	79.9
	sum – suma		-	313.4	278.4	-

Yield and chemical composition of plants. With comparable yield in all combinations, a significant effect of interaction between foliar nutrition and nitrogen fertilization was found on the concentration of nitrates(V), soluble oxalates and ascorbic acid in spinach leaves (tables 2 and 3 – means for cooperation foliar nutrition × nitrogen fertilization). No interaction between foliar nutrition and nitrogen fertilization was found in reference to content of: dry matter, nitrates(III), ammonium ions, free amino acids, N-total, soluble sugars and phenolic compounds in spinach leaves. In both years of cultivation diverse climatic conditions had a modifying effect on interaction between foliar nutrition and N fertilization only in the case of the content of nitrates(V) and ascorbic acid (Test *F* in tables 2 and 3 as well as figure 1).



1 – Control / Kontrola, 2 – 50% dose of N / 50% dawki N, 3 – 100% dose of N / 100% dawki N.
□ – without foliar nutrition / bez dokarmiania dolistnego,
■ – with foliar application of 'Pentakeep V' / dolistna aplikacja 'Pentakeep V'.

Fig 1. The effect of 'Pentakeep V' application and nitrogen fertilization on the content of nitrates(V) (A) and ascorbic acid (B) in spinach in 2006 and 2007.
Ryc. 1. Zawartość azotanów(V) (A) i kwasu askorbinowego (B) w szpinaku w 2006 i 2007 roku w zależności od dolistnej aplikacji 'Pentakeep V' i doglebowego nawożenia azotem.

Table 2. The effect of ‘Pentakeep V’ application and nitrogen fertilization on yield and the content of dry matter, nitrates(V), nitrates(III), ammonium ions and amino acids in spinach leaves (means from 2006–2007)

Tabela 2. Wpływ dokarmiana dolistnego ‘Pentakeep V’ oraz nawożenia azotem na plon oraz na zawartość suchej masy, azotanów(V), azotanów(III), jonów amonowych oraz aminokwasów w szpinaku (średnie z lat 2006–2007)

Combinations Kombinacje			Yield Plon $kg \cdot m^{-2}$	Dry matter % d.m. Sucha masa % s.m.	$mg \cdot kg^{-1}$ f.w. – $mg \cdot kg^{-1}$ św.m. nitrate(V) azotany(V) NO_3^-	nitrate(III) azotany(III) NO_2^-	ammonium ions jony amonowe NH_4^+	Amino acids $N_2 \cdot 100g^{-1}$ f.w. Aminokwasy $N_2 \cdot 100g^{-1}$ św.m.
Means for cooperation foliar nutrition × nitrogen fertilization Średnie dla współdziałania dokarmiania dolistnego × nawożenie	without foliar nutrition bez dokarm. dolist.	control – kontrola	0.91 a	8.9 a	31.7 a	1.24 a	2.4 a	10.0 a
		50% dose of N 50% dawki N	1.75 a	8.0 a	739.6 c	1.29 a	6.0 a	13.1 a
		100% dose of N 100% dawki N	1.63 a	8.1 a	501.8 b	1.43 a	3.8 a	12.4 a
	‘Pentakeep V’	control – kontrola	0.94 a	8.8 a	26.4 a	1.16 a	2.5 a	12.3 a
		50% dose of N 50% dawki N	1.63 a	8.1 a	333.1 b	1.35 a	3.0 a	13.9 a
		100% dose of N 100% dawki N	1.86 a	7.9 a	793.3 c	1.34 a	3.4 a	14.0 a
Means for nitrogen fertilization Średnie dla nawożenia azotem	control – kontrola		0.92 a	8.8 b	29.1 a	1.20 a	2.4 a	11.2 a
	50% dose of N – 50% dawki N		1.69 b	8.1 a	536.4 b	1.32 a	4.5 b	13.5 b
	100% dose of N – 100% dawki N		1.74 b	8.0 a	647.5 b	1.38 a	3.6 ab	13.2 b
Means for foliar nutrition Średnie dla dokarmiania dolistnego	without foliar nutrition bez dokarm. dolist.		1.43 a	8.3 a	424.4 a	1.32 a	4.1 a	11.8 a
	‘Pentakeep V’		1.48 a	8.3 a	384.3 a	1.28 a	3.0 a	13.4 b
Test *F* for cooperation foliar nutrition × nitrogen fertilization × year of study Test *F* dla dokarmiania dolistnego × nawożenie azotem × rok badań			n.s. n.i.	n.s. n.i.	*	n.s. n.i.	n.s. n.i.	n.s. n.i.

Means followed by the same letters are not significantly different for $P < 0.05$. Test *F*: * – means are significantly different, n.s. – not significant.
Średnie oznaczone tymi samymi literami nie różnią się istotnie dla $P < 0,05$. Test *F*: * – średnie różnią się istotnie, n.i. – brak istotnego zróżnicowania.

Table 3. The influence of 'Pentakeep V' application and nitrogen fertilization on the content of N-total, soluble sugars, soluble oxalates, phenolic compounds and ascorbic acid in spinach leaves (means from 2006–2007)

Tabela 3. Wpływ dokarmiana dolistnego 'Pentakeep V' oraz nawożenia azotem na zawartość azotu ogółem, cukrów i szczawianów rozpuszczalnych, związków fenolowych oraz kwasu askorbinowego w szpinaku (średnie z lat 2006–2007)

Combinations – Kombinacje			N % d.m. N % s.m.	mg·100g^{-1} f.w. – mg·100g^{-1} św.m.			
				soluble sugars cukry rozpuszczalne	soluble oxalates szczawiany rozpuszczalne	phenolic compounds związki fenolowe	ascorbic acid kwas askorbinowy
Means for cooperation foliar nutrition × nitrogen fertilization Średnie dla współdziałania dokarmiania dolistnego × nawożenie azotem	without foliar nutrition bez dokarm. dolist.	control – kontrola	2.9 a	870.8 a	446.1 b	48.1 a	21.0 c
		50% dose of N – 50% dawki N	3.7 a	732.3 a	469.1 bc	39.7 a	18.7 b
		100% dose of N – 100% dawki N	3.9 a	692.1 a	470.3 bc	43.1 a	18.6 b
	'Pentakeep V'	control – kontrola	3.4 a	786.7 a	406.7 a	53.2 a	22.2 c
		50% dose of N – 50% dawki N	3.8 a	646.5 a	482.6 c	42.4 a	16.3 a
		100% dose of N – 100% dawki N	3.6 a	600.6 a	536.6 d	43.4 a	14.4 a
Means for nitrogen fertilization Średnie dla nawożenia azotem		control – kontrola	3.2 a	828.8 b	426.4 a	50.7 b	21.6 b
		50% dose of N – 50% dawki N	3.8 b	689.4 a	475.9 b	41.0 a	17.5 a
		100% dose of N – 100% dawki N	3.7 b	646.3 a	503.4 c	43.2 a	16.5 a
Means for foliar nutrition Średnie dla dokarmiania dolistnego		Without foliar nutrition Bez dokarm. dolist.	3.5 a	765.0 b	461.8 a	43.6 a	19.5 b
		'Pentakeep V'	3.6 a	677.9 a	475.3 a	46.3 a	17.6 a
Test *F* for cooperation foliar nutrition × nitrogen fertilization × year of study Test *F* dla dokarmiania dolistnego × nawożenie azotem × rok badań			n.s. n.i.	n.s. n.i.	n.s. n.i.	n.s. n.i.	*

Means followed by the same letters are not significantly different for $P < 0.05$. Test *F*: * – means are significantly different, n.s. – not significant.
Średnie oznaczone tymi samymi literami nie różnią się istotnie dla $P < 0,05$. Test *F*: * – średnie różnią się istotnie, n.i. – brak istotnego zróżnicowania.

Table 4. Content of mineral nitrogen in soil after spinach cultivation (means from 2006–2007)
Tabela 4. Zawartość azotu mineralnego w glebie po uprawie szpinaku (średnie z lat 2006–2007)

Combinations – Kombinacje			$mg \cdot dm^{-3}$ soil – $mg \cdot dm^{-3}$ gleby		
			$N\text{-}NH_4$	$N\text{-}NO_3$	$N\text{-}NH_4 + N\text{-}NO_3$
Means for cooperation foliar nutrition × nitrogen fertilization Średnie dla współdziałania dokarmiania dolistnego × nawożenie azotem	without foliar nutrition bez dokarmiania dolistnego	control – kontrola	1.07 a	1.78 d	2.85 a
		50% dose of N – 50% dawki N	1.36 a	0.54 ab	1.90 a
		100% dose of N – 100% dawki N	1.82 a	1.34 c	3.15 a
	'Pentakeep V'	control – kontrola	1.23 a	0.70 ab	1.93 a
		50% dose of N – 50% dawki N	0.86 a	0.28 a	1.14 a
		100% dose of N – 100% dawki N	1.24 a	0.96 bc	2.20 a
Means for nitrogen fertilization Średnie dla nawożenia azotem	control – kontrola		1.15 a	1.24 b	2.39 b
	50% dose of N – 50% dawki N		1.11 a	0.41 a	1.52 a
	100% dose of N – 100% dawki N		1.53 a	1.15 b	2.68 b
Means for foliar nutrition Średnie dla nawożenia dokarmiania dolistnego	without foliar nutrition – bez dokarmiania dolistnego		1.42 a	1.22 b	2.64 b
	'Pentakeep V'		1.11 a	0.65 a	1.76 a
Test *F* for cooperation foliar nutrition × nitrogen fertilization × year of study			n.s.	n.s.	n.s.
Test *F* dla dokarmiania dolistnego × nawożenie azotem × rok badań			n.i.	n.i.	n.i.

Means followed by the same letters are not significantly different for $P < 0.05$. Test *F*: * – means are significantly different, n.s. – not significant.
Średnie oznaczone tymi samymi literami nie różnią się istotnie dla $P < 0{,}05$. Test *F*: * – średnie różnią się istotnie, n.i. – brak istotnego zróżnicowania.

In both years of cultivation 'Pentakeep V' significantly reduced concentration of ascorbic acid in leaves of plants fertilized with 50% and 100% dose of nitrogen (tab. 3 and fig. 1). In control plants without N fertilization, 'Pentakeep V' application in 2006 contributed to significantly higher content of ascorbic acid while in the next year concentration of this compound was slightly reduced (with no statistical significance).

After application of 'Pentakeep V' in 2006 the content of nitrate(V) in spinach fertilized with 50% of N was lower while higher in plants fed with full dose of N in comparison to plants not treated with foliar nutrition (fig. 1). In the next year of cultivation (2007), 'Pentakeep V' had no significant effect on the content of these compounds in both combinations with N fertilization. Additionally, in both years of the research 'Pentakeep V' did not cause any significant changes in the concentration of nitrates(V) in control plants. It is worth to notice that presented results of nitrate(V) content were obtained when concentration of mineral nitrogen in soil was even and relatively low in both sub-blocks in 2006 and 2007– detailed data not presented (Table 4 – Test *F* and mean for cooperation foliar nutrition × nitrogen fertilization). A statistically significant diversity in the content of $N\text{-}NO_3$ in soil after spinach cultivation was observed. In control samples from the sub-block without foliar nutrition a significantly higher concentration of $N\text{-}NO_3$ in soil was found in comparison to control samples with 'Pentakeep V' application. Nitrogen fertilization (irrespectively of its dose) resulted in similar concentration of $N\text{-}NO_3$ in soil in both sub-blocks. Thus, increased content of nitrates(V) after 'Pentakeep V' application on spinach plants fertilized with full dose of nitrogen is difficult to interpret. Research by Iwai et al. [2005] showed that higher pepper yield could be partially correlated with increasing $N\text{-}NO_3$ uptake by plants resulting from application of fertilizer containing ALA. Current information indicate that ALA (or Pentakeep® fertilizers) can intensify nitrate reductase activity [Mishra and Srivastava 1983] as well as decrease nitrate(V) content in plants [Tanaka et al. 2005; Dwornikiewicz 2007, 2008]. Depleting effect of ALA on the level of nitrate(V) in plants is though ambiguous. No significant influence of 'Pentakeep V' foliar application on nitrate(V) content was found in leek plants [Babik and Babik 2007]. Pentakeep® fertilizers applied foliarly reduced the concentration of nitrates(V) in cabbage heads while increased its level in tomato leaves [Babik and Babik 2008]. Researches conducted by Sady and Smoleń [2007] as well as Smoleń and Sady [2009] showed that the time duration of effective reduction of nitrate(V) content depends on: species, variety as well as the level of plant nitrogen supply. In one-year field research with spinach 'Spiros F_1', decreased nitrates(V) concentration was found in leaves of plants fertilized with 50% and 100% of N dose on 7^{th}, 10^{th} and 14^{th} day after foliar application of 'Pentakeep V' [Sady and Smoleń 2007]. 'Pentakeep V' did not though influence nitrate(V) content in spinach plants not fertilized with nitrogen. In one-year study conducted by Smoleń and Sady [2009] in combination with 50% of N dose fertilization reduction of nitrates(V) concentration in carrot roots was noted after 3 days, while in combination with 100% N – on 7^{th} and 14^{th} day after foliar application of 'Pentakeep V'. On 3^{rd}, 7^{th} , 14^{th} and 21^{st} day after foliar treatment with 'Pentakeep V', increased nitrates(V) concentration (in comparison to control plants) was determined in carrot roots without N fertilization.

Adverse results obtained in both years of the study (in the case of 'Pentakeep V' effect on the content of nitrates(V) and ascorbic acid in spinach leaves) could have been caused by variable weather conditions throughout cultivation, especially in the last two decades of September 2007 in comparison to respective period in 2006 (tab. 1). First two decades of September 2007 were characterized by lower average daily temperature, greater amount of rainfall (only in first decade), higher air humidity as well as lower number of sunshine hours when compared to analogous time in 2006. These particular conditions could have then reduced the effectiveness of ALA activity. As a consequence, changes in biochemical and physiological responses to foliar application of 'Pentakeep V' in 2007 might have occurred with diminished intensiveness when compared to 2006. It can be thus hypothesized that ALA (or Pentakeep® fertilizers) interaction on uptake, reduction and assimilation of N-NO_3 in plants is dependent on: plant species, level of nitrogen supply, availability of mineral N in soil as well as weather conditions during cultivation.

Foliar application of 'Pentakeep V' resulted in decreased content of soluble oxalates in control plants as well as increased its concentration in plants fertilized with 100% of N dose (table 3 – mean for cooperation foliar nutrition × nitrogen fertilization). This observation can be positively verified by results obtained by Sady and Smoleń [2007] in field cultivation of spinach.

Statistical analysis of obtained results indicated that fertilization with both N doses contributed to higher yield as well as increased content of: nitrates(V), ammonium ions, free amino acids and soluble oxalates in spinach plants in comparison to control (tables 2 and 3 – mean for fertilization). The highest level of ammonium ions was found in plants fertilized with 50% dose of N, while oxalates accumulated the most in plants fed with 100% dose of N. In comparison to the control combination, nitrogen fertilization on both levels reduced the content of: dry matter, soluble sugars, phenolic compounds and ascorbic acid in spinach leaves. Still, it had no significant influence on the nitrate(III) concentration in spinach plants. The decrease in dry matter of plants fertilized with nitrogen could have been caused by higher yield (dilution effect). Nitrogen fertilization improved supply of this element in plants but also increased nitrate(V) level in spinach leaves. It should be also mentioned that the content of nitrates(V) in spinach leaves did not exceed the maximum level of 2500 mg $NO_3^-\cdot kg^{-1}$ f.w. for spinach plants harvested between 1st of April and 31st of October [Commission Regulation (EC) No 466/2001].

Reduction of the content of soluble sugars, phenolic compounds and ascorbic acid due to increasing level of nitrogen fertilization was previously observed in research conducted by Sady and Smoleń [2007]. Zhang et al. [2006] showed that exogenous application of ALA in the concentration range between 0.3, 3 and 30 $mg\cdot dm^{-3}$ (in the absence of NaCl), significantly increased the total soluble sugar concentrations in microtubers of potato in *in vitro* condition. Still, ALA caused reduction in accumulation of reductive sugars in microtubers. The effect of nitrogen fertilization on the oxalate content in spinach is dependent on various factors including: form and dose of nitrogen, plant cultivar and type of cultivation – soil [Stagnari et al. 2007, Sady and Smoleń 2007] or hydroponic system [Zhang et al. 2005].

In the present study foliar application of 'Pentakeep V (considered irrespective of N fertilization) contributed to higher concentration of free amino acids as well as reduced content of total soluble sugars and ascorbic acid in spinach leaves (tables 2 and 3 –

means for foliar nutrition). Plant biostimulation with this substance did not significantly affect spinach yield and the content of dry matter, nitrates(V), nitrates(III), ammonium ions, N-total, soluble oxalates and phenolic compounds in leaves. Influence of 'Pentakeep V' on yield and chemical composition of plants is confirmed by previous study by Sady and Smoleń [2007]. Still, these authors found no effect of 'Pentakeep V' on the level of free amino acids and ascorbic acid in spinach plants.

CONCLUSIONS

Interaction between foliar nutrition with 'Pentakeep V' and soil nitrogen fertilization had a significant effect on the content of: nitrates(V), soluble oxalates and ascorbic acid in spinach. Variable climatic condition throughout cultivation period additionally influenced the impact of tested factors on nitrates(V) and ascorbic acid accumulation.

Foliar application of 'Pentakeep V' contributed to reduced concentration of soluble oxalates in plants without nitrogen fertilization as well as increased level of these compounds in plants fertilized with 100% of N dose.

No significant interaction of foliar nutrition and soil nitrogen fertilization was observed in the case of yield and content of dry matter, nitrates(III), ammonium ions, free amino acids, N-total, total soluble sugars and phenolic compounds in spinach leaves.

In comparison to control plants, both levels of nitrogen fertilization resulted in: a) increased yield, b) higher accumulation of nitrates(V), ammonium ions, free amino acids, N-total and soluble oxalates, c) decrease in the content of: dry matter, total soluble sugars, phenolic compounds and ascorbic acid in spinach leaves. It had also no effect on the accumulation of nitrates(III) in plants.

Foliar nutrition with 'Pentakeep V' (considered irrespective of nitrogen fertilization) contributed to increased amount of free amino acids as well as reduced concentration of soluble sugars and ascorbic acid in spinach plants. 'Pentakeep V' did not though influence spinach yield and the content of dry matter, nitrates(V), nitrates(III), ammonium ions, N-total, soluble oxalates and phenolic compounds in leaves.

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WPŁYW BIOSTYMULACJI ROŚLIN NAWOZEM 'PENTAKEEP V' ORAZ NAWOŻENIA AZOTEM NA PLON, GOSPODARKĘ AZOTEM ORAZ JAKOŚĆ SZPINAKU

Streszczenie. Dolistna aplikacja nawozów zawierających biostymulatory może wpływać na plon oraz na pobieranie i akumulację NO_3^- przez rośliny. Celem badań było określenie wpływu dokarmiania dolistnego nawozem 'Pentakeep V' oraz zróżnicowanego pod względem dawki nawożenia azotem na plonowanie, gospodarkę azotem roślin oraz jakość odżywczą szpinaku. Badaniami objęto dwa podbloki z dolistnym i bez dolistnego dokarmiania roślin. Rośliny dokarmiano dolistnie dwukrotnie nawozem 'Pentakeep V' w dawce 0,02% m/o, stosując w przeliczeniu 3000 dm^3 wody na hektar. W obrębie podbloków zastosowano doglebowe przedsiewne nawożenie azotem (w formie saletry amonowej): 1 – kontrola (nienawożona azotem), 2 – 50% dawki N (25 mg $N \cdot dm^{-3}$ gleby), 3 – 100% dawki N (50 mg $N \cdot dm^{-3}$ gleby). Stwierdzono istotny wpływ współdziałania dokarmiania dolistnego z nawożeniem azotem na zawartość azotanów(V), szczawianów rozpuszczalnych i kwasu askorbinowego w szpinaku. Oddziaływanie tych czynników na zawartość azotanów(V) i kwasu askorbinowego w szpinaku uzależnione było jednak od przebiegu warunków klimatycznych. Dokarmianie dolistne roślin nawozem 'Pentakeep V' powodowało obniżenie zawartości szczawianów rozpuszczalnych w roślinach kontrolnych oraz wzrost zawartości tych związków w roślinach nawożonych 100% dawki N. Stwierdzono brak współdziałania dokarmiania dolistnego z nawożeniem azotem na plon oraz na zawartość suchej masy, azotanów(III), jonów amonowych, wolnych aminokwasów, N-ogółem, cukrów rozpuszczalnych i związków fenolowych w szpinaku.

Słowa kluczowe: kwas 5-aminolewulinowy, azotany, szczawiany, kwas askorbinowy, wartość biologiczna

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