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The influence of β-alanine derivative products on spring oilseed rape yield and oil quality

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Abstract

In 2012–2014, field experiments were conducted at Rumokai Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry with a view to estimating the effects of different concentrations of *N*-phenyl-*N*-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-β-alanine sodium salt (NPNDT) on spring oilseed rape.

Field experiments showed that 125 mg l^{-1} NPNDT solution had the greatest effect on plant growth. In this treatment, the number of secondary branches increased by 54%, the rapeseed yield by 23% compared with the control treatment. The highest seed number per silique (24.5 seeds, or 7.5% higher than in the control) was determined in the treatment where oilseed rape seedlings had been sprayed with 75 mg l^{-1} NPNDT solution. The highest oil and protein contents in rapeseed were determined in the 75 mg l^{-1} NPNDT treatment. Compared with the control, a statistically significant increase in rapeseed oil amounted to 137.9 kg ha^{-1} or 17% and protein content to 6.2 g 100 g^{-1} or 31%. The increase in rapeseed yield was statistically significant ($P < 0.05$) in all treatments. The increase of oil content was positively correlated with the seed yield increase ($P < 0.05$) when oilseed rape was sprayed with the 50 mg l^{-1} concentration of NPNDT solution.

The NPNDT had a positive effect on oil quality. It increased protein and flavonoid contents, reduced ash content in rapeseed and increased DPPH radical scavenging in rapeseed extract.

Key words: β-alanine derivative, *Brassica napus*, oil, RAPD, seed yield.

Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important oilseeds with the highest content (30–45%) of oil (Baux et al., 2008). Rapeseed oil, free of anti-nutritional substances, has a higher dietary value than many other vegetable oils due to the low content of saturated fatty acids, high oleic acid content, favourable ratio of polyunsatured linoleic and linolenic acids, as well as presence of sterols and fat-soluble vitamins (Cegielska-Taras, Pniewski, 2011). In addition, the ratio of omega-6:omega-3 fatty acids in rapeseed oil is 2:1, which is beneficial for human health, as possessing anti-inflammatory effects and protecting against heart diseases (Ion et al., 2010).

The plant growth and productivity are affected by various abiotic factors such as heat, salinity, cold, etc. (Pietryczuk et al., 2014). Oilseed rape crop growth and yield quality can be enhanced by increasing plant nitrogen uptake through the use of fertilizers, spray-application of growth regulators or cobalt sulphate during growth (Gad, 2010; Ullah et al., 2012). Plant growth regulators are important compounds for the rapeseed yield and quality (Matysiak, Kaczmarek, 2013; Huberman et al., 2014).

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The number of pods per plant, the number of seeds per pod as well as seed weight, as factors affecting both plant nutrition and hormones, influence the yield and quality of rapeseed. The number of seeds per pod may be increased by the growth regulators such as indole-3-acetic acid, whereas the number of pods per plant was increased by using 6-benzylaminopurine (Mohammadi et al., 2014).

Vegetable oils, depending on the content of unsaturated fatty acids, are sensitive to oxidation which finally renders the oil unusable for consumption. As a result, antioxidants are frequently added to inhibit lipid oxidation; however, the plants, including oilseed rape, biosynthesize numerous secondary metabolites, which may act as antioxidants or preservatives. These compounds may be lipophilic (tocols) and hydrophilic (ascorbic acid, flavonoids, phenolic acids). They may act both as primary (radical scavenging via single electron/hydrogen atom transfer) and secondary (metal catalyst chelators, single oxygen scavengers) antioxidants (Akoh, Min, 2002).

Flavonoids belong to a group of phenolic compounds and in food plants are responsible for biological factors, such as the defence system against viruses, bacteria, fungi, insects, protection against biotic and abiotic stresses, and can control plant hormones (Crozier et al., 2009; Pascual-Teresa et al., 2010; Amalesh et al., 2011; Cartea et al., 2011). Flavonoids absorb UV rays which are harmful to plant cells (Amalesh et al., 2011).

β-alanine is a non-protein amino acid found in all organisms (White et al., 2001); in plants it was found increasing in response to environmental stress. Various β-amino acids are present in the formula of different natural products, such as peptides, cyclopeptides, depsipeptides, glycopeptides, alkaloids, or terpenoids (Patočka, 2010). β-amino acids are currently of growing interest not only because of their roles, but also because of their use in the synthesis of peptide mimetics and of certain quite biologically active substances. Natural derivatives of β-amino acids are often characterized by potent pharmacological and toxicological activities crucially based on their β-amino acid substructures. The most important β-amino acids are β-alanine, β-leucine, β-arganine, β-glutamate, β-phenylalanine and β-tyrosine (Patočka, 2011).

The random amplified polymorphic DNA (RAPD) has been used to study the extent of genetic variation among the diverse groups of important crop species in the genus *Brassica*. The value of RAPD analysis for the efficient germplasm management in plants is already known. The technique is quick, easy and requires less time compared to other techniques. RAPD method detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Abdelmigid, 2012).

The present investigation aimed to determine the effect of *N*-phenyl-*N*-(4,5-dihydro-4-oxo-2-thiazolyl)-β-alanine sodium salt on spring oilseed rape (*Brassica napus* L.) seed yield and quality.

Materials and methods

Field experiments on the influence of *N*-phenyl-*N*-(4,5-dihydro-4-oxo-2-thiazolyl)-β-alanine sodium salt (NPNDT) on spring oilseed rape (*Brassica napus* L.) were carried out in 2012–2013 at the Rumokai Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry. The study involved the spring rape variety 'SW Landmark'.

Oilseed rape plants were sprayed with the compound solutions (concentrations 25–150 mg L^{-1}) before the flowering stage (BBCH 50). The soil of the experimental field is *Calcari-Epihypogleyic Luvisol* (*LVg-p-w-cc*) with the following characteristics: pH_{KCl} 6.7, 1.82% humus, 0.12% N_{total}, 308 mg kg^{-1} mobile P_2O_5 and 296 mg kg^{-1} mobile K_2O. Barley was the preceding crop.

The plot size was 2.7 × 11 m. The harvested plot size was 2.2 × 10 m. The experiments with oilseed rape were performed in April–August. Samples for counts of branches and siliques per plant and seed number per silique were taken from each plot before harvesting in four places 0.25 m^2 per plot. During harvesting the rapeseed yield collected from each plot was weighed separately, rapeseed moisture content was determined and seed samples for determination of quality parameters were collected. The seed yield of spring oilseed rape was corrected to 8.5% standard moisture. The protein content in rapeseed was measured by the Bradford (1976) method. Rapeseed ash content was determined by combustion at 500°C for 3 h.

The total amount of flavonoids was determined according to $AlCl_3$ colorimetric method (Singleton et al., 1999). Two grams of shredded plant material were diluted with 20 mL acetone and 2 mL of 28% hydrochloric acid and heated under reflux for 30 min in a round-bottom flask. After cooling, the hydrolyzate was filtered into a 100 mL volumetric flask, the remaining slurry was returned to the round-bottom flask and after adding 20 mL acetone was heated under reflux for 10 min. After cooling, the hydrolyzate was filtered into the same volumetric flask. The content of the flask was diluted with acetone up to 100 mL volume. Twenty mL of obtained solution were diluted with 20 mL water and extracted with ethyl acetate four times: 1 × 15 and 3 × 10 ml. The combined upper fractions were washed with 40 mL water, filtered into 50 mL volumetric flask and the filtrate was diluted with ethyl acetate up to 50 ml volume. The test solution was prepared by adding 2 ml of $AlCl_3$ solution (20 g l^{-1}) to 10 ml of the main solution and filling the flask up to 25 ml volume by solution of acetic acid and methanol (1:19). The reference solution was prepared by adding the same acetic acid – methanol (1:19) solution to 10 ml of the main solution up to 25 ml volume. After 30 min, the absorbance was measured at 415 nm in a spectrophotometer UV-200-RS using the reference solution. The amount of flavonoids (x, %) was calculated as follows: $x = (A \times k) / m$, where A is the absorbance of the reference solution, k – a correction coefficient for hyperozide (k = 1.25), and m – mass of the plant (g).

DPPH• (1,1-diphenyl-2-picrylhydrazyl) scavenging assay. Free radical scavenging capacity (RSC) of compounds was measured by DPPH using the widely used method (Madhu et al., 2011). Briefly, 1 mL of 1 mM DPPH solution in ethanol was added to the solutions of the tested compounds (1 mg ml^{-1} of dimethyl sulfoxide). The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. Afterwards, the absorbance was measured at 517 nm in

a spectrophotometer UV-200-RS (MRC Ltd., Israel). The RSC values were calculated according to the following equation: RSC (%) = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control reaction, and A_1 – the absorbance in the presence of the samples.

The oil content in rapeseed was measured by extraction with hexane for 3 hours in an apparatus Soxhlet (Behr Labor-Technik, Germany). Fatty acid composition was analyzed with a gas chromatograph HRGC 5300 Mega Series (Carlo Erba Strumentazione, Italy). The oil yield was determined from seed yield and oil content.

Oilseed rape plants sprayed with 125 mg l^{-1} concentration of NPNDT were used for the random amplified polymorphic DNA (RAPD) analysis. Plant samples were taken at growth stage 64–69 according to the BBCH scale. Plant genomic DNA (gDNA) was extracted from the frozen leaf samples as described elsewhere (Edwards et al., 1991). Approximately 0.5 cm^2 plant tissue was ground in a microcentrifuge tube with 400 µl of extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% w/v SDS), vortexed for 5 s and centrifuged at 16100 rpm for 1.5 min. 300 µl of supernatant was mixed with 300 µl of isopropanol to precipitate gDNA. gDNA was pelleted by centrifugation at 16100 rpm for 5 min and later dissolved in 100 µl TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0).

RAPD was carried out using Taq polymerase in 25 µl reaction mixture containing 2 µl of gDNA extract, 1 × Taq polymerase buffer (ThermoFisher Scientific, Lithuania), 2.5 mM $MgCl_2$ 0.24 µM primer, 0.2 µM dNTPs (Table 1). Polymerase chain reaction (PCR) settings (annealing temperature) were set by analysis of gradient PCR results. Extension was carried out for 1 min and 35 cycles were done.

For the separation of amplified products, agarose gel electrophoresis was performed using 1% agarose gels.

Table 1. Sequences of primers used for analysis, total number of bands counted and DNA fragment size (Nanodiagnostika, Lithuania)

Primer number	Primer sequence	Total number of bands	Fragment size range bp
P-01	5'-GGGTAACGCC-3'	8	250–1200
P-02	5'-CAATCGCCGT-3'	4	300–900
P-03	5'-AATCGGGCTG-3'	4	600–1200

Amplified DNA was stained using ethidium bromide and visualized under UV light. DNA amplification was carried out in PCR tubes; the total reaction volume was 25 µl. DNA amplification was performed using a thermocycler Veriti® 96-Well Thermal Cycler (Applied Biosystems, USA) programmed to 1 cycle at 94°C for 5 min, following 35 cycles at 94°C for 30 s, at 42–44°C for 1 min, at 72°C for 1 min, final extension at 72°C for 1 min, and kept at 4°C (Jodinskienė et al., 2008). After amplification, PCR products were separated by electrophoresis in 1% agarose gel. The agarose gel was stained with ethidium bromide and photographed under the UV light using the pro gel documentation system BioImaging MiniBis (ThermoFisher Scientific, Lithuania).

Statistical analysis. For the comparison of the obtained means, a two-tailed Welch's *t*-test intended for use with samples having possibly unequal variances was performed in the *Microsoft Excel 2010* (Žiauka et al., 2013).

Results and discussion

Before harvesting, biometric properties of oilseed rape plants were determined (Table 2). In all three experimental years, the oilseed rape plant height varied slightly in comparison with the control treatment. In the

Table 2. Effect of different NPNDT concentrations on the biometric properties of oilseed rape plants

Biometric properties	Year	NPNDT concentration mg l^{-1}						
		0 (control)	25	50	75	100	125	150
Plant height cm	2012	130.7 ± 0.1	133.5* ± 0.1	129.5 ± 0.2	130.9 ± 0.2	127.6 ± 0.3	124.4 ± 0.2	130.9 ± 0.3
	2013	117.0 ± 0.2	–	116.1 ± 0.2	116.5 ± 0.5	117.0 ± 0.3	115.4 ± 0.3	–
	2014	120.3 ± 0.1	–	123.5 ± 0.5	127.0* ± 0.2	124.3 ± 0.2	125.5 ± 0.3	–
	average	121.7 ± 0.6	133.5 ± 0.1	122.2 ± 0.6	124.1* ± 0.7	122.4 ± 0.5	121.4 ± 0.6	130.9 ± 0.3
Number of primary branches per plant	2012	5.4 ± 0.02	5.5 ± 0.0	5.5 ± 0.03	5.3 ± 0.02	5.8* ± 0.02	5.3 ± 0.01	5.4 ± 0.02
	2013	3.3 ± 0.04	–	3 ± 0.02	3.1 ± 0.02	2.9 ± 0.04	3.1 ± 0.03	–
	2014	3.1 ± 0.10	–	3 ± 0.07	3.4* ± 0.05	2.2 ± 0.02	3.4 ± 0.08	–
	average	3.5 ± 0.10	5.5 ± 0.0	3.6 ± 0.12	3.8* ± 0.10	3.4 ± 0.15	3.8 ± 0.11	5.4 ± 0.02
Number of secondary branches per plant	2012	3.2 ± 0.04	3.7 ± 0.05	4.2 ± 0.07	4.9 ± 0.06	5.0 ± 0.02	5.4* ± 0.00	3.1 ± 0.01
	2013	2.2 ± 0.02	–	3.0 ± 0.03	3.5 ± 0.07	3.1 ± 0.05	4.0* ± 0.05	–
	2014	6.1 ± 0.10	–	6.7 ± 0.11	6.8 ± 0.15	8.1 ± 0.19	8.3* ± 0.16	–
	average	3.9 ± 0.20	3.7 ± 0.05	4.7 ± 0.18	5.1 ± 0.18	5.4 ± 0.26	6.0* ± 0.22	3.1 ± 0.01
Number of siliques per plant	2012	131.8 ± 0.1	118 ± 0.6	128.2 ± 0.3	129.7 ± 0.6	145.9* ± 0.2	141.4 ± 0.3	131.8 ± 0.1
	2013	57.2 ± 0.3	–	57.2 ± 0.1	61.1 ± 0.2	62.4 ± 0.2	63.6* ± 0.4	–
	2014	77.7 ± 0.3	–	85.2 ± 0.9	85.3 ± 1.0	82.8 ± 0.4	107.3* ±1.4	–
	average	83.5 ± 3.1	118 ± 0.6	85.5 ± 2.9	87.3 ± 2.9	90.9 ± 3.5	99.4* ± 3.4	131.8 ± 0.1
Number of seeds per silique	2012	19.4 ± 0.01		21.3* ± 0.01	21.1 ± 0.09	19.8 ± 0.09	19.6 ± 0.03	20.2 ± 0.06
	2013	24.1 ± 0.06	–	26.4 ± 0.08	27.0 ± 0.10	27.0* ± 0.07	26.1 ± 0.11	–
	2014	23.8 ± 0.02	–	24.5 ± 0.02	24.3 ± 0.08	24.7 ± 0.07	25.7* ± 0.13	–
	average	22.8 ± 0.22	20.1 ± 0.06	24.4 ± 0.22	24.5* ± 0.26	24.3 ± 0.30	24.3 ± 0.31	20.2 ± 0.06
Silique length cm	2012	7.4 ± 0.00	7.4 ± 0.01	7.9* ± 0.05	7.4 ± 0.01	7.3 ± 0.03	7.5 ± 0.01	7.6 ± 0.01
	2013	7.3 ± 0.02	–	7.5* ± 0.01	7.5 ± 0.02	7.4 ± 0.01	7.3 ± 0.02	–
	2014	7.8 ± 0.01	–	8.1* ± 0.01	7.8 ± 0.02	7.9 ± 0.03	8.1 ± 0.02	–
	average	7.5 ± 0.03	7.4 ± 0.01	7.8* ± 0.03	7.6 ± 0.03	7.6 ± 0.03	7.6 ± 0.04	7.6 ± 0.01

* – the highest positive difference in comparison with the control; values are mean ± standard deviation (SD), $P < 0.05$

second year of study, the plants had a lower height.

In the first experimental year, the plant height ranged from 124.4 to 133.5 cm, in the second year – from 115.4 to 117.0 cm and in the third year – from 120.3 to 127.0 cm. The data averaged over the three experimental years showed that the lowest plant height was determined in the treatments sprayed with 125 mg l^{-1} of NPNDT solution.

The number of secondary branches per plant is an important biometric parameter. In the first experimental year, this number ranged from 3.1 to 5.4. The highest number of secondary branches was obtained in the treatments sprayed with 125 mg l^{-1} of NPNDT; it was by 69% higher than in the control treatment. In the second experimental year, the number of secondary branches was lower than in the first year, but higher than in the control sample. In the third experimental year, this number ranged from 6.1 to 8.3. The highest number of secondary branches was obtained in the treatments sprayed with 125 mg l^{-1} of NPNDT; it was by 36% higher than in the control treatment. Summarizing three experimental years, the highest number of secondary branches was obtained in the treatments sprayed with 125 mg l^{-1} of NPNDT. The average number of secondary branches increased by 54% compared with the control treatment. The highest number of siliques was obtained in the first experimental year in the 100 mg l^{-1} and in the second and the third years in the 125 mg l^{-1} of NPNDT treatments. In the first and the second year, the NPNDT solution increased the number of siliques by 11% in the third year by 38% compared with the control treatment. The researchers (Kazlauskienė et al., 2008) studied the influence of physiological analogues of auxin – TA-12 (2 mM) and TA-14 (4 mM) on oilseed rape flowering and reproductive organ formation. It has been found that TA-12 shortens the duration of flowering and increases the number of siliques per plant. Other researchers (Darginavičienė et al., 2011) have shown that the rapeseed cv. 'SW Landmark' sprayed with Ethephon (10 mM) formed more siliques on the primary and secondary branches as compared to the rapeseed hybrid cv. 'Terra'.

In the first experimental year, the length of siliques varied from −1% to +6%, in the second year from 0% to +3% and in the third year from 0% to +4% compared ith the control treatment. The longest siliques (7.8 cm) were produced in the treatment sprayed with 50 mg l^{-1} of NPNDT.

The NPNDT increased seed number per silique. In the first year, the number of seeds per silique increased by 1–10%, in the second year by 8–12% and in the third year by 2–8% compared with the control treatment. The highest number of seeds per pod was obtained in the treatment sprayed with 50 mg l^{-1} of NPNDT.

A previous study on the influence of N-(4-methoxy-2-nitrophenyl)-β-alanine sodium salt (380 μM) on oilseed rape showed, that the number of primary branches varied from +2% to +13%, secondary branches – from +11% to +155%, silique number per plant – from +3% to +75%, seed number per silique – from +2.5% to +5.3% compared with the control treatment (Žiaukienė et al., 2010).

The application of NPNDT increased the rapeseed yield in the first experimental year from 0.33 to 0.70 t ha^{-1}, in the second year from 0.22 to 0.46 t ha^{-1}, and in the third year from 0.10 to 0.30 t ha^{-1} (Table 3). The rapeseed yield ranged from +19% to +40% compared with the control treatment in the first experimental year, from +12% to +26% in the second year and from +4% to +9% in the third year. Average data showed that the highest seed yield was achieved in the treatments sprayed with 125 mg l^{-1} concentration of NPNDT solution. The increase in rapeseed yield was statistically significant ($P < 0.05$) in all treatments.

The highest 1000 seed weight (4.2 g) was determined in the 25 mg l^{-1} NPNDT treatment in the first experimental year; it reached 4.0 g in the 100 mg l^{-1} NPNDT treatment in the second year and 4.0 g in the 125 mg l^{-1} NPNDT treatment in the third year. Our results on the oilseed rape cv. 'SW Landmark' agree with those of Darginavičienė et al. (2011). The studies have shown that the 1000 seed weight of cv. 'SW Landmark' was by about 10% higher than that of the hybrid cv. 'Terra', but Ethephon (10 mM) increased the weight of cv. 'SW Landmark' only by 3–4%.

In 2012–2014, the oil content in rapeseed varied

Table 3. Effect of different NPNDT concentrations on rapeseed yield

Variable	Year	NPNDT concentration mg l^{-1}						
		0 (control)	25	50	75	100	125	150
Yield t ha^{-1}	2012	1.74 ± 0.06	2.07 ± 0.12	2.21 ± 0.06	2.11 ± 0.18	2.20 ± 0.06	2.41 ± 0.06	2.44* ± 0.12
	2013	1.79 ± 0.12	–	2.01 ± 0.06	2.19 ± 0.06	2.25* ± 0.12	2.20 ± 0.06	–
	2014	2.3 ± 0.06	–	2.4 ± 0.06	2.5 ± 0.06	2.4 ± 0.06	2.6* ± 0.06	–
	average	1.9 ± 0.18	2.1 ± 0.12	2.2 ± 0.12	2.3 ± 0.18	2.3 ± 0.12	2.4* ± 0.12	2.4 ± 0.12
1000 seed weight g	2012	3.9 ± 0.04	4.2* ± 0.01	4.0 ± 0.01	3.8 ± 0.04	4.0 ± 0.01	3.9 ± 0.02	3.9 ± 0.04
	2013	3.9 ± 0.01	–	4.0 ± 0.04	4.0 ± 0.01	4.0* ± 0.04	4.0 ± 0.01	–
	2014	4.0 ± 0.04	–	4.0 ± 0.01	4.0 ± 0.04	4.0 ± 0.01	4.0* ± 0.01	–
	average	3.9 ± 0.01	4.2 ± 0.01	4.0* ± 0.01	3.9 ± 0.04	4.0 ± 0.01	3.9 ± 0.04	3.9 ± 0.04
Oil content kg t^{-1}	2012	329.6 ± 1.4	388.2 ± 6.7	372.3 ± 6.1	458.1* ± 4.6	412.7 ± 3.9	386.1 ± 6.4	406.4 ± 5.2
	2013	458.8 ± 0.5	–	432.6** ± 0.3	376.7 ± 0.2	390.28 ± 0.3	346.9 ± 0.4	–
	2014	446.6 ± 2.1	–	448.5* ± 1.2	445.7 ± 5.1	440.5 ± 4.5	435.5 ± 0.8	–
	average	411.3 ± 36.6	388.2 ± 6.7	417.8 ± 20.7	426.8* ± 22.6	414.5 ± 13.2	389.5 ± 22.8	406.4 ± 5.2
Oil yield kg ha^{-1}	2012	571.8 ± 2.3	803.7 ± 13.9	822.8 ± 13.4	966.5 ± 9.8	907.9 ± 8.6	930.6 ± 15.5	991.7* ± 12.9
	2013	821.3 ± 0.9	–	869.5 ± 0.6	824.9 ± 0.5	878.1* ± 0.7	763.3 ± 0.9	–
	2014	1104.8 ± 5.1	–	1116.7 ± 3.0	1120.1* ± 12.8	1040.3 ± 10.8	1087 ± 2.1	–
	average	832.6 ± 135.9	803.7 ± 13.9	936.3 ± 80.8	970.5* ± 75.6	942.1 ± 44.5	927.2 ± 83.1	991.7 ± 12.9

* – the highest positive difference compared with the control; (values are mean ± standard deviation (SD), $P < 0.05$)

from −6% to +4% in comparison with the control sample. The highest oil content was obtained in the 75 mg l^{-1} of NPNDT treatment. The increase of oil content was positively correlated with the increase of rapeseed yield ($P < 0.05$) in the treatments sprayed with 50 mg l^{-1} concentration of NPNDT. Oil yield varied from +11% to +17% compared with the control treatment. Several authors have shown the influence of N-(4-methoxy-2-nitrophenyl)-β-alanine sodium salt (380 μM) on rapeseed and demonstrated that the yield varied from −7% to +13% (Brazienė et al., 2012) and 1000 seed weight increased to +9.4%, and the yield to +10.8% compared with the control treatment (Žiaukienė et al., 2010). According to Brazienė et al. (2012), plant growth regulating effects of β-alanine derivative can be linked to its osmolytic characteristics. Good results were shown by *N*-(4-methoxy-2-nitrophenyl)-β-alanine sodium salt (380 μM) together with the micronutrient fertilizers ARVI micro, the rapeseed yield, as compared with the control, significantly increased by 15.2%. Miliuvienė and Novickienė (2004) have shown that the influence of analogues of dimethylmorpholinium chloride 17-DMC increased seed number, 1000 seed weight and yield. This compound (17-DMC) is supposed to influence growth by changing the content of phytohormones, mainly IAA and GA_3. The compound tested in the current study may have similar properties.

Reaserch conducted in Germany with winter oilseed rape sprayed with triazole fungicides Harvesan (a.i. flusilazole 250 g l^{-1} + carbendazim 125 g l^{-1}) and Folicur (a.i. tebuconazole 250 g l^{-1}) reported that oil content in rapeseed significantly increased after triazole application compared with the control. The application of fungicides Ortiva (a.i. azoxystrobin 250 g l^{-1}) and Cantus (a.i. boscalid 500 g kg^{-1}) in combination with triazole fungicides enhanced oil content by extending the seed formation phase which led to increased oil accumulation in the seeds. Inclusion of the fungicide Caramba (a.i. metconazole 8.6 %) improved the yield-associated parameters (the number of siliques and the seeds per main stem) and seed yield, and also resulted in higher oil content (Ijaz et al., 2015).

The effect of NPNDT on the protein content, ash, flavonoids and DPPH radical scavenging in rapeseed is presented in Table 4.

The application of NPNDT increased protein content in the first experimental year from 15.9 to 31.2 g 100 g^{-1}, in the second year from 16.1 to 29.4 g 100 g^{-1} and in the third year from 14.4 to 20.3 g 100 g^{-1}. According to the average data of 2012–2014, the highest protein content (26.0 g 100 g^{-1}) was obtained in the 75 mg l^{-1} NPNDT treatments in all experimental years. The protein content increased by +31% compared with the control treatment. This increase was statistically significant ($P < 0.05$). Studies of other researchers (Brazienė et al., 2012) showed that the influence of *N*-(4-methoxy-2-nitrophenyl)-β-alanine sodium salt (380 μM) on rapeseed protein content varied from 0% to +3% compared with the control.

Table 4. Effect of different NPNDT concentrations on the composition of rapeseed

Rapeseed composition	Year	NPNDT concentration mg l^{-1}						
		0 (control)	25	50	75	100	125	150
Protein content g 100 g^{-1}	2012	15.9 ± 0.03	27.1 ± 0.10	30.7 ± 0.10	31.2* ± 0.01	30.3 ± 0.01	22.8 ± 0.01	22.5 ± 0.17
	2013	29.4 ± 0.37	–	21.4 ± 0.37	26.4 ± 0.37	21.8 ± 0.30	16.1 ± 0.27	–
	2014	14.1 ± 0.17	–	16.7 ± 0.23	20.3* ± 0.03	18.9 ± 0.07	15.8 ± 0.10	–
	average	19.8 ± 2.43	27.1 ± 0.10	22.9 ± 2.10	26.0* ± 1.60	23.7 ± 1.73	18.2 ± 1.17	22.5 ± 0.17
Ash %	2012	4.5 ± 0.00	4.5 ± 0.03	4.5 ± 0.03	4.2 ± 0.03	4.2 ± 0.03	4.1 ± 0.03	4.1 ± 0.03
	2013	3.1 ± 0.17	–	3.8* ± 0.07	3.4 ± 0.04	3.6 ± 0.03	3.5 ± 0.07	–
	2014	4.1 ± 0.07	–	3.6 ± 0.23	3.5 ± 0.14	3.3 ± 0.26	3.2 ± 0.10	–
	average	3.90 ± 0.24	4.5 ± 0.03	4.0* ± 0.17	3.7 ± 0.20	3.7 ± 0.20	3.6 ± 0.14	4.1 ± 0.03
Flavonoids mg g^{-1}	2012	0.31 ± 0.003	–	0.3 ± 0.003	0.31 ± 0.003	0.43 ± 0.003	0.44 ± 0.003	0.45* ± 0.003
	2013	0.45 ± 0.010	–	0.38 ± 0.001	0.42 ± 0.000	0.43 ± 0.000	0.45* ± 0.003	–
	2014	0.37 ± 0.007	–	0.30 ± 0.010	0.26 ± 0.003	0.35 ± 0.003	0.28 ± 0.007	–
	average	0.38 ± 0.02	–	0.33 ± 0.013	0.33 ± 0.023	0.41 ± 0.013	0.42* ± 0.013	0.45 ± 0.003
DPPH radical scavenging %	2012	49.8 ± 3.44	52.0 ± 1.16	61.1 ± 1.40	67.4* ± 0.36	60.4 ± 0.68	61.4 ± 2.76	65.9 ± 1.96
	2013	78.8 ± 0.08	–	79.6* ± 2.48	77.3 ± 2.52	76.9 ± 0.68	72.9 ± 0.24	–
	2014	43.7 ± 0.04	–	47.8 ± 0.04	42.9 ± 0.04	49.1* ± 0.08	44.6 ± 0.00	–
	average	58.7 ± 6.76	52.0 ± 1.16	62.7 ± 5.40	63.0* ± 5.92	61.9 ± 4.60	59.8 ± 4.92	65.9 ± 1.96

* – the highest positive difference in comparison with the control

With the application of NPNDT, ash content varied from 4.5% to 4.1% in the first experimental year, from 3.8% to 3.1% in the second year and 3.2% to 4.1% in the third year. The lowest ash content (4.1% and 3.2%) was recorded in the treatments sprayed with 125 mg l^{-1} of NPNDT in the first and in the third year, and it was 3.1% in the control treatment in the second year. According to the average data of 2012–2014, the lowest ash content was determined in the 125 mg l^{-1} NPNDT treatment.

In all seed samples in the first experimental year, the antioxidant activity was higher than in the control treatment. In the second year, the antioxidant activity in the seeds varied insignificantly. In the third year, the antioxidant activity in seeds varied from 42.9% to 49.1%. Summarizing three experimental years, the highest average antioxidant activity was obtained in rapeseed in the 75 mg l^{-1} NPNDT treatment.

Antioxidants include such compounds as flavonoids, ascorbic acid, carotenoids and other. The content of flavonoids in rapeseed was measured. The highest content of flavonoids (0.45 mg g^{-1}) was determined in the 150 mg l^{-1} NPNDT treatment in the first experimental year; it was 0.45 and 0.38 mg g^{-1} in the 125 mg l^{-1} NPNDT in the second and third year, respectively. According to the average data of three years, flavonoid content varied from −13% to +18% compared with the controltreatment. The increase in flavonoid content was

positively correlated with an increase in DPPH radical scavenging ($P < 0.05$).

Fatty acids were determined by gas chromatography. The effect of NPNDT on the composition of fatty acids in rapeseed is presented in Table 5.

In the first experimental year, the oleic acid content changed insignificantly and varied from −0.09% to +0.77% compared with the control treatment. In the 50 mg l^{-1} NPNDT treatment, the seeds contained the highest oleic acid content (61.5%). In the second year, oleic acid content in rapeseed was slightly lower than or equal to that in the control sample. In the third year, the oleic acid content changed insignificantly and varied from −0.6% to +0.6% compared with the control sample. In 2012–2014, the highest oleic acid content was determined in the 50 and 125 mg l^{-1} NPNDT treatments.

Table 5. Effect of different NPNDT concentrations on the content of fatty acids in rapeseed oil

Composition of fatty acids in %	Year	NPNDT concentration mg l^{-1}						
		0 (control)	25	50	75	100	125	150
Palmitic	2012	5.1 ± 0.1	4.6 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	5.2 ± 0.2	4.9 ± 0.1	4.8 ± 0.0
	2013	3.8 ± 0.1	–	3.7 ± 0.1	3.6 ± 0.0	3.8 ± 0.1	3.8 ± 0.2	–
	2014	4.5 ± 0.1	–	4.6 ± 0.1	4.6 ± 0.1	4.7 ± 0.0	4.6 ± 0.1	–
	average	4.5 ± 0.3	4.6 ± 0.1	4.2 ± 0.3	4.3 ± 0.3	4.6 ± 0.4	4.4 ± 0.3	4.8 ± 0.0
Stearic	2012	2.1 ± 0.1	2.0 ± 0.0	2.1 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	2013	1.7 ± 0.1	–	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	–
	2014	1.9 ± 0.1	–	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.0	2.0 ± 0.0	–
	average	1.9 ± 0.1	2.0 ± 0.0	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.2	1.9 ± 0.1	2.0 ± 0.0
Oleic	2012	61.0 ± 0.2	61.1 ± 0.1	61.5 ± 0.0	61.3 ± 0.0	60.9 ± 0.1	61.2 ± 0.1	61.4 ± 0.0
	2013	65.4 ± 0.1	–	65.2 ± 0.1	65.4 ± 0.1	65.3 ± 0.2	65.4 ± 0.1	–
	2014	63.2 ± 0.1	–	62.8 ± 0.1	63.1 ± 0.1	63.4 ± 0.1	63.6 ± 0.1	–
	average	63.2 ± 1.1	61.1 ± 0.1	63.4 ± 0.9	63.3 ± 1.0	63.2 ± 1.0	63.4 ± 1.0	61.4 ± 0.0
Linoleic	2012	20.6 ± 0.1	20.8 ± 0.1	20.6 ± 0.0	20.9 ± 0.1	20.9 ± 0.0	20.8 ± 0.1	20.9 ± 0.1
	2013	18.0 ± 0.0	–	18.3 ± 0.1	18.1 ± 0.1	18.3 ± 0.0	18.2 ± 0.1	–
	2014	20.3 ± 0.1	–	20.0 ± 0.1	20.4 ± 0.1	20.1 ± 0.0	20.0 ± 0.1	–
	average	19.6 ± 0.6	20.8 ± 0.1	19.4 ± 0.6	19.8 ± 0.8	19.8 ± 0.6	19.7 ± 0.6	20.9 ± 0.1
Eicosenoic	2012	1.0 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	0.9 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
	2013	1.2 ± 0.0	–	1.2 ± 0.1	1.2 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	–
	2014	1.2 ± 0.1	–	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	–
	average	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.0 ± 0.0
Linolenic	2012	8.5 ± 0.0	8.8 ± 0.0	8.5 ± 0.0	8.7 ± 0.0	8.7 ± 0.0	8.6 ± 0.0	8.6 ± 0.0
	2013	8.1 ± 0.1	–	8.2 ± 0.1	8.2 ± 0.1	8.2 ± 0.0	8.2 ± 0.1	–
	2014	6.8 ± 0.1	–	6.7 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	6.3 ± 0.1	–
	average	7.8 ± 0.4	8.8 ± 0.0	8.4 ± 0.5	7.9 ± 0.5	7.9 ± 0.5	7.7 ± 0.6	8.6 ± 0.0

Note. Values are mean ± standard deviation (SD), $P < 0.01$.

With the application of NPNDT, the content of linoleic acid in rapeseed oil in the first year of research slightly increased and remained unchanged in the second and third years. The highest content of linoleic acid (20.9%) was determined in the 75 mg l^{-1} NPNDT treatment in the first year; it was 18.3% in the 50 mg l^{-1} NPNDT treatment in the second year and 2.5% in the 75 mg l^{-1} NPNDT treatment in the third year.

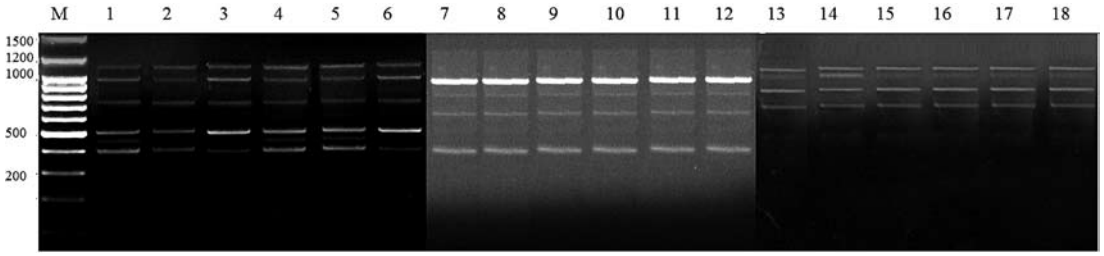
According to the averaged data of three years, the content of palmitic and stearic fatty acids in rapeseed oil changed insignificantly compared with the control treatment. Other researchers obtained similar data in the experiments with other β-alanine derivatives (Brazienė et al., 2012).

The results of Darginavičienė et al. (2011) demonstrated that Ethephon decreased the content of saturated fatty acids and increased the monounsaturated fatty acid levels in cv. 'SW Landmark'. The only observed change was the increase of the amount of linolic acid. In our study, the seeds of cv. 'SW Landmark' contained about 2% more oleic acid and about 2% less linolenic acid compared with Darginavičienė et al. (2011) findings.

Three primers (P-01, P-02 and P-03) used in the analysis with spring oilseed rape cv. 'SW Landmark' produced 16 polymorphic amplification products. Amplification size ranged from 250 up to 1200 bp (Table 1). Each primer generated 4 to 8 individual bands per primer and provided a distinct and reproducible pattern of the amplified PCR fragments (Fig.). The most informative primer was P-01.

The results showed that in the leaf samples sprayed with 125 mg l^{-1} NPNDT, the DNA was intact compared with the DNA of the control samples (C1, C2 and C3).

The main observation from these experiments is that NPNDT could be used as a possible growth regulator for increasing the yield of rapeseed. The further research of this compound will exhibit which stress resistance it will increase in plants. A wide range of metabolites have been identified, including mono-, di-, oligo- and polysaccharides such as glucose, fructose, sucrose, trehalose, raffinose, and fructans; amino acids such as proline, pipecolic acid; methylated proline-related compounds such as methyl-proline, proline betaine, and hydroxyproline betaine; sugar alcohols (polyols) such as sorbitol, mannitol, glycerol, inositol, and methylated inositols; other betaines, such as glycine betaine, β-alanine betaine, choline *O*-sulfate, and tertiary sulphonium compounds such as dimethylsulphoniopropionate (DMSP), in halophytes in response to salinity. Analogy to organic acids up and down regulation, the quaternary ammonium compounds also accumulate in plants subjected to salt stress. These include glycinebetaine, β-alanine betaine, proline betaine, choline *O*-sulfate, hydroxyproline betaine, and pipecolate betaine. Besides glycinebetaine, β-alanine betaine also



Lane 1–3, 7–9, 13–15 are C1–C3 samples, lane 4–6, 10–12, 16–17 affected samples (S1–S3); M-Gene Ruler 100 bp DNA Ladder Plus (Thermo Fisher Scientific, Lithuania)

Figure. DNA fragments from samples of oilseed rape leaves obtained by PCR with primers: P-01 (lane 1–6), P-02 (lane 7–12) and P-03 (lane 13–18)

acts as an osmoprotectant in saline conditions (Kumari et al., 2015). Our compound has a β-alanine fragment in its structure, and it could affect plant resistance when salinity is increasing. The β-alanine derivative could be used for the reinforcement of plants under the N/C imbalance. Studies of other researchers (Schwarz et al., 2014) from Germany showed that many metabolites of the N metabolism such as ornithine, arginine, β-alanine, and aspartate show a pattern that gives a clear indication for the N/C imbalance.

Conclusions

1. The research showed that 125 mg l^{-1} of *N*-phenyl-*N*-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-β-alanine sodium salt (NPNDT) had the greatest influence on spring oilseed rape plant growth. The highest number of secondary branches (6.0 ± 2.2 branches per plant) ($P < 0.05$), the lowest plant height (121.4 ± 5.7 cm) ($P < 0.05$) and the highest rapeseed yield (2.4 ± 0.2 t ha^{-1}) ($P < 0.05$) were determined in this treatment. The highest 1000 seed weight and silique length were established in the 50 mg l^{-1} NPNDT treatment. The highest seed number per pod ($P < 0.05$) was determined in the 75 mg l^{-1} NPNDT treatment.

2. The NPNDT solution increased oil and protein content in rapeseed. The highest oil (970.5 ± 128.6 kg ha^{-1}) ($P < 0.05$) and protein (26.0 ± 4.8 g 100 g^{-1}) ($P < 0.05$) content was obtained in the 75 mg l^{-1} NPNDT treatment. The tested compound had no significant impact on the chemical composition of rapeseed oil.

3. The highest content of flavonoids was obtained in the treatments sprayed with 125 mg l^{-1} NPNDT solution. The increase in flavonoid content was positively correlated with the increase in DPPH radical scavenging ($P < 0.05$).

4. The lowest ash content (3.6 ± 0.4%) was recorded in the 75 mg l^{-1} NPNDT treatment.

5. The 125 mg l^{-1} concentration of NPNDT solution had no effect on the DNA of spring oilseed rape (*Brassica napus* L.).

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β-alanino darinių įtaka vasarinių rapsų derliui ir aliejaus kokybei

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Santrauka

2012–2014 m. Lietuvos agrarinių ir miškų mokslų centro filiale Rumokų bandymų stotyje buvo tirta įvairių koncentracijų *N*-fenil-*N*-(4,5-dihidro-4-okso-2-tiazolil)-β-alanino natrio druskos (NPNDT) tirpalo įtaka vasariniams rapsams.
Lauko tyrimų duomenys parodė, kad augalų augimui didžiausią įtaką turėjo 125 mg l^{-1} NPNDT tirpalas. Panaudojus 125 mg l^{-1} koncentracijos tirpalą rapsų antrinių šakelių skaičius padidėjo iki 6,0 vnt., arba 54 %, lyginant su kontroliniu variantu ($P < 0,05$), derlius – iki 2,39 t ha^{-1}, arba 23 %, lyginant su kontroliniu variantu ($P < 0,05$). Didžiausias sėklų skaičius ankštaroje – 24,5 vnt., arba 7,5 % ($P < 0,05$) didesnis nei kontroliniame variante – gautas, kai rapsai buvo nupurkšti 75 mg l^{-1} koncentracijos NPNDT tirpalu. Didžiausi aliejaus ir baltymų kiekiai gauti, kai rapsai buvo nupurkšti 75 mg l^{-1} koncentracijos NPNDT tirpalu. Lyginant su kontroliniu variantu, išgaunamo aliejaus kiekis padidėjo 137,9 kg ha^{-1}, arba 17 %, baltymų kiekis – 6,2 g 100 g^{-1}, arba 31 %. Rapsų derliaus padidėjimas buvo esminis ($P < 0,05$) visuose laukeliuose, lyginant su kontroliniu variantu. Išgaunamo rapsų aliejaus kiekis teigiamai ir esmingai ($P < 0,05$) koreliavo su rapsų derliaus padidėjimu, kai augalai buvo apipurkšti 50 mg l^{-1} koncentracijos NPNDT tirpalu. NPNDT turėjo teigiamos įtakos aliejaus kokybei – padidėjo baltymų, flavonoidų kiekiai, rapsų sėklose sumažėjo pelenų kiekis, padidėjo DPPH radikalo slopinimas rapsų sėklų ekstrakte.

Reikšminiai žodžiai: aliejus, β-alaninų dariniai, *Brassica napus*, RAPD, sėklų derlius.