RAMŪNĖ KOLOSEJ

INVESTIGATION OF SPRING RAPE GROWTH REGULATING PROPERTIES OF *N*-SUBSTITUTED *β*- AND *γ*-AMINO ACIDS BEARING AROMATIC AND AZOLES MOIETIES

SUMMARY OF DOCTORAL DISSERTATION

TECHNOLOGICAL SCIENCES, CHEMICAL ENGINEERING (05T)

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RAMŪNĖ KOLOSEJ

VASARINIŲ RAPSŲ AUGIMĄ REGULIUOJANČIŲ *N*-PAKEISTŲ *β*- IR *γ*-AMINORŪGŠČIŲ SU AROMATINIAIS IR AZOLŲ FRAGMENTAIS SAVYBIŲ TYRIMAS

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Summary of Doctoral Dissertation
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Introduction

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

The productivity and growth of plants is affected by various abiotic factors, such as high or low temperatures, salinity, etc. Oilseed rape growth and yield quality could be enhanced by fertilizing, treating with growth regulators or cobalt sulphate during growth. The number of pods per plant, the number of seeds per pod and the weight of seeds are important for the yield of oilseed rape. The development of pods is affected by nutrients as well as by hormones. The quantity of seeds in a pod can be increased by using growth regulator indole-3-acetic acid, while the quantity of pods can be increased by using 6-benzylaminopurine.

β-alanine is a non-protein amino acid found in all living organisms. The level of this amino acid increases under environmental stress. *β*-amino acids attract more and more attention not only for their role in the organisms but also because of their use in the synthesis of biologically active compounds. The most important *β*-amino acids are *β*-alanine, *β*-leucine, *β*-arginine, *β*-phenylalanine and *β*-tyrosine.

Researchers have been studying the influence of physiological analogues of auxin – TA-12 (2 mM) (1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid calcium salt) and TA-14 (4 mM) (1-[2-dimethylaminoethoxicarbonyl-methyl]naphtalene chlormethylate) on oilseed rape flowering and reproductive organ formation. It has been established that TA-12 shortens the duration of flowering and increases the number of pods per plant.

Objective of the thesis

To investigate the effect of various concentrations of *N*-substituted *β*- and *γ*-amino acids with aromatic and azole moieties on spring oilseed rape (*Brassica napus* L.) growth *in vitro*, select its two most active compounds and research the effect on spring oilseed rape growth *in vivo* as well as investigate the oilseed rape yield and chemical composition.

The main tasks of the research

1. To investigate the effect of *N*-substituted *β*- and *γ*-amino acids with aromatic and azole moieties on spring oilseed rape development *in vitro* and to select the compounds with the highest bioactivity.

2. To investigate the effect of the most active compounds on the biometric parameters, yield and the yield quality of spring oilseed rape grown *in vivo*.
3. To investigate the effect of the most active compounds on the composition and quality of rape seeds.
4. To investigate the effect of the most active compounds on the accumulation of bioactive substances in spring oilseed rape grown *in vivo*.
5. To determine whether the investigated compounds, i.e. *N*-substituted *β*- and *γ*-amino acids with aromatic and azole fragments, have an effect on spring oilseed rape DNA changes.

Scientific novelty of the dissertation

The effect of various concentrations of *N*-substituted *β*- and *γ*-amino acids with aromatic and azole moieties on the growth of oilseed rape (*Brassica napus* L.) *in vitro* was investigated for the first time; also, compounds having a positive effect on further field experiments have been determined.

The field experiments were carried out with the most active compounds from the two different classes by growing spring oilseed rape and estimating the effect on the biometric parameters of spring oilseed rape, its yield as well as the seed and oil chemical composition. It was determined that the investigated compounds do not exert influence on the spring oilseed rape DNA, hence these compounds can be used regarding the technologies for spring oilseed rape cultivation.

Practical significance of the dissertation

In the course of field experiments, it was determined that *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts improved the biometric parameters of spring oilseed rape during field experiments. *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine sodium salt increased the number of plant branches, the number of secondary branches and the average number of seeds per pod. The seed yield was increased together with the boost of the protein content in rapeseeds and the oil content obtained from rapeseeds. The number of spring oilseed rape branches increased, the number of secondary branches and the number of seeds per pod also grew under the impact of 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salt. Besides, the protein content in rapeseeds increased as well resulting in the higher amounts of obtained oil. Both investigated compounds did not exert any influence on the accumulation of glucosinolates or on the fatty acid quantity in rapeseeds. It can thus be assumed that 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salt could be used as a yield enhancer.

Approval and publication of research results

The results of the research have been presented in 8 scholarly publications: 2 of them have been published in journals which are included into the Thomson Reuters Web of Knowledge database; 1 article has been published in a reviewed

periodical scientific journal included into the list of other databases, and 5 articles have been delivered in conference proceedings papers.

Structure and content of the dissertation

The dissertation consists of the introduction, literature review and analysis, experimental, results overview and discussion parts, conclusions, a list of references and a list of publications on the dissertation topic. The list of references includes 233 bibliographic sources. The main results are discussed on 116 pages; the findings are illustrated in 37 tables and 65 figures.

Defended statements of the dissertation

1. *N*-substituted *β*- and *γ*-amino acids with aromatic and azole fragments increase the viability of spring oilseed rape, enhance its growth *in vitro* and induce the accumulation of photosynthesis pigments in spring oilseed rape biomass *in vitro* and *in vivo* conditions.
2. *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts improve the biometric parameters of spring oilseed rape and increase the yield of rapeseed oil, improve the chemical composition of rapeseed, i.e. increase the quantities of phenolic compounds, flavonoids, proteins and oil, yet they have no effect on the quantity of glucosinolates in rapeseeds. These salts increase DPPH radical scavenging in rapeseed extract and exert influence on the ash content.
3. *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts have no effect on the DNA of the spring oilseed rape.

Methods

Field experiments on the influence of *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts on spring oilseed rape (*Brassica napus* L.) were carried out in 2012–2013 at Rumokai Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry. The study involved the spring rape variety *Land Mark*. Oilseed rape plants were sprayed with compound solutions (concentrations 25–150 mg/L) before the flowering stage (BBCH 50). The soil of the experimental field is *Calcari-Epihypogleyic Luvisol* (*LVgp-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 from April to August. Samples for the count of branches and the pod per plant as well as the seed number per pod were taken from each plot before harvesting in four different places, 0.25 m^2 per plot. In the course of harvesting, the rapeseed yield collected from each plot was weighed separately, the rapeseed moisture content was determined, and seed

samples for the determination of quality parameters were collected. The seed yield of the spring oilseed rape was corrected to 8.5% standard moisture.

The protein content in rapeseed was measured by the Bradford method.

The 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. 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. 20 mL of the obtained solution was 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 a 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) 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 by 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 is the correction coefficient for hyperozide (k = 1.25), and m stands for the mass of the plant (g).

DPPH● (1,1-diphenyl-2-picrylhydrazyl) scavenging assay. The free radical scavenging capacity (RSC) of compounds was measured by DPPH using a fairly common method. Briefly, 1 mL of 1 mM DPPH solution in ethanol was added to the solutions of the tested compounds (1 mg/ml 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 stands for the absorbance in the presence of the samples.

The amount of glucosinolates in rapeseeds was investigated spectrophotometrically.

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

Chlorophyll a and b and carotenoids content in spring oilseeds rape leaves was investigated spectrophotometrically.

DNA analysis. Oilseed rape plants sprayed with 125 mg/L concentration of *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts were used for the random amplified polymorphic DNA (RAPD) analysis. Plant samples were taken at growth stage 64–69 according to the BBCH scale. The plant genomic DNA (gDNA) was extracted from the frozen leaf samples as described elsewhere. Approximately 0.5 cm^2 of 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 by 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 8). Polymerase chain reaction (PCR) settings (the annealing temperature) were set on the grounds of the analysis of gradient PCR results. An extension was carried out for 1 min, and 35 cycles were done in total.

Results and Discussion

Amino acids derivatives *in vitro* study

The compounds for the research were synthesized at the Department of Organic Chemistry, Faculty of Chemical Technology, Kaunas University of Technology. The compounds are listed in **Table 1**.

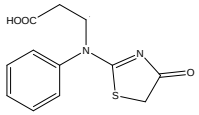
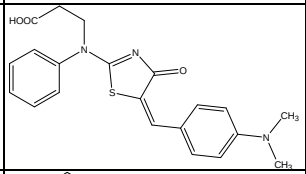
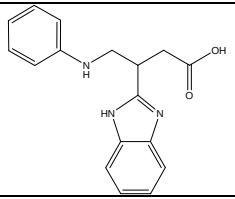
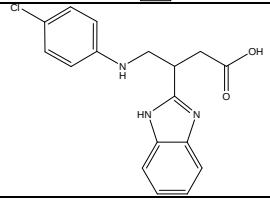
Table 1. Chemical compounds involved in the study

Compound group	Compound number in the research	Name of compounds
Group I	**1**	*N*-phenyl-*N*-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine
	2	*N*-phenyl-*N*-{4-[(4-dimetylaminophenyl)methyliden]-5-oxo-4,5-dihydro-1,3-thiazol-2-yl}-*β*-alanine
	3	*N*-phenyl-*N*-{4-[(4-bromphenyl)methyliden]-5-oxo-4,5-dihydro-1,3-thiazol-2-yl}-*β*-alanine
	4	*N*-phenyl-*N*-{4-[(4-chlorphenyl)methyliden]-5-oxo-4,5-dihydro-1,3-thiazol-2-yl}-*β*-alanine
	5	*N*-phenyl-*N*-{4-[(4-dimetylphenyl)methyliden]-5-oxo-4,5-dihydro-1,3-thiazol-2-yl}-*β*-alanine
	6	*N*-phenyl-*N*-{4-[(4-methoxyphenyl)methyliden]-5-oxo-4,5-dihydro-1,3-thiazol-2-yl}-*β*-alanine
	7	*N*-phenyl-*N* -(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*α*-methyl-*β*-alanine
	8	*N*-phenyl-*N*-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-methyl-*β*-alanine
Group II	**9**	3-(1*H*-benzimidazol-2-yl)-4phenylaminobutyric acid
	10	3-(1*H*-benzimidazol-2-yl)-4-(4chlophenylamino)butyric acid
	11	3-(1*H*-benzimidazol-2-yl)-4-(3chlorphenylamino)butyric acid
	12	3-(1*H*-benzimidazol-2-yl)-4-(4-phenoxyphenylamino)butyric acid
	13	3-(1*H*-benzimidazol-2-yl)-4-(4-metoxyphenylamino)butyric acid
	14	3-(1*H*-benzimidazol-2-yl)-4-(4-bromphenylamino)butyric acid
	15	3-(1*H*-benzimidazol-2-yl)-4-(4-dimetylphenylamino)butyric acid

The rapeseeds were grown in *Petri* dishes on filter paper wetted with 3 mL of the investigated compound solutions of various concentrations (ranging from 0.01 to 10 mg/L). The experiments were repeated two times with 40 rapeseeds. Plant development differences were determined by comparing the obtained results with the control treatment.

Two compounds with the highest activity were selected from the two investigated groups of compounds. Investigations were carried out repeatedly by using expedient concentrations. The rapeseeds were germinated repeatedly at one time with the most active compounds in compound groups. The results are presented in **Table 2**.

Table 2. The effect of the investigated compound concentrations on rapeseed germination *in vitro*

Compound	Compound structure	Concentration, mg/L	Germination, %	Developed root, %	Hypocotyls height, mm	Root length, mm	Plant weight, g
Control		0	75 ± 7	65 ± 7	27.0 ± 19.1	34.9 ± 30.4	0.060 ± 0.042
1		2	75 ± 14	70 ± 7	33.5 ± 16.9	78.9 ± 57.1	0.072 ± 0.036
		2.5	60 ± 0	60 ± 0	37.4 ± 27.0	54.9 ± 41.9	0.075 ± 0.055
		3	60 ± 7	50 ± 0	31.8 ± 24.4	55.5 ± 51.9	0.063 ± 0.047
5		2	80 ± 7	70 ± 14	28.7 ± 22.6	40.9 ± 38.3	0.067 ± 0.054
		2.5	40 ± 14	40 ± 14	31.9 ± 23.3	43.9 ± 40.8	0.066 ± 0.047
		3	80 ± 14	80 ± 14	27.8 ± 17.7	70.3 ± 63.2	0.068 ± 0.044
9		1.5	85 ± 14	85 ± 14	31.0 ± 18.2	79.1 ± 63.3	0.071 ± 0.041
		2	60 ± 14	60 ± 14	44.1 ± 22.9	63.8 ± 48.9	0.081 ± 0.042
		3	65 ± 21	65 ± 21	38.9 ± 22.9	73.5 ± 52.2	0.078 ± 0.046
10		3	80 ± 14	80 ± 14	24.7 ± 9.3	44.5 ± 31.5	0.064 ± 0.023
		3.5	85 ± 7	80 ± 14	27.1 ± 19.1	63.7 ± 57.3	0.065 ± 0.042
		4	70 ± 14	70 ± 14	29.9 ± 16.3	64.1 ± 32.3	0.071 ± 0.038

Repeated experiments were carried out with compounds **1**, **5**, **9** and **10**. The highest hypocotyls growth induction was observed in the samples which were grown in 2.5 mg/L solution of compound **1** (compound Group I) and in 2 mg/L solution of compound **9** (compound Group II). The growth of roots was induced by all the investigated compounds, and the longest roots were obtained with compounds from Group I (2 mg/L), and with compound **9** from compound Group II (1.5 mg/L). All the investigated compounds increased the biomass of spring oilseed rape plants, but the highest effect was produced with compounds **9** and **1**.

In another section of the experiment, rapeseeds were germinated in *Petri* dishes on MS medium for 2 weeks, and the research was replicated two times. Two compounds with the highest activity were chosen from different compound groups. The effect of the compounds on the development of the spring oilseed rape on MS medium is illustrated in **Table 3**.

Table 3. Effect of concentrations of compounds **1** and **9** on rapeseed germination *in vitro*

	Concentration, mg/L	Germination, %	Developed root, %	Hypocotyls height, mm	Root length, mm	Plant weight, g
Con-trol	0	75 ± 14	65 ± 14	21.6 ± 10.8	31.9 ± 16.1	0.068 ± 0.034
1	25	95 ± 3.5	95 ± 3.5	28.0 ± 13.9	49.2 ± 26.0	0.061 ± 0.031
	50	95 ± 3.5	95 ± 3.5	26.9 ± 6.8	55.5 ± 20.7	0.067 ± 0.017
	75	95 ± 3.5	95 ± 3.5	30.6 ± 6.4	24.2 ± 11.5	0.047 ± 0.009
	100	100 ± 0	100 ± 0	27.1 ± 8.5	44.7 ± 18.4	0.053 ± 0.018
	125	85 ± 7	85 ± 7	31.9 ± 8.9	33.8 ± 14.4	0.055 ± 0.015
	150	95 ± 3.5	95 ± 3.5	31.3 ± 7.0	46.9 ± 27.6	0.055 ± 0.012
9	25	100 ± 0	85 ± 14	25.1 ± 9.2	36.3 ± 14.9	0.059 ± 0.021
	50	90 ± 7	90 ± 7	34.9 ± 13.1	32.7 ± 15.7	0.053 ± 0.020
	75	85 ± 14	85 ± 14	36.5 ± 13.6	39.0 ± 27.2	0.064 ± 0.024
	100	65 ± 21	65 ± 21	28.1 ± 9.0	38.3 ± 18.6	0.053 ± 0.017
	125	85 ± 7	85 ± 7	26.1 ± 9.0	33.0 ± 14.3	0.057 ± 0.020
	150	90 ± 7	90 ± 7	31.8 ± 11.4	43.4 ± 20.7	0.069 ± 0.024

The highest effect on hypocotyls growth was observed with 50 mg/L and 75 mg/L solutions of compound **9**. Compound **1** had insignificant influence on hypocotyls height, but solutions of 125 mg/L and 150 mg/L influenced the growth of hypocotyls.

The selected compounds had a positive yet different influence on the root length: the highest effect was reached with 50 mg/L solution of compound **1**, while 75 mg/L showed a negative effect. Compound **9** had a weaker effect on spring oilseed rape root development in comparison with compound **1**. The highest effect on root growth was reached with the solution of compound **9** when using the concentration of 150 mg/L.

From the comparison of the results obtained in *Petri* dishes on filter paper and in *Petri* dishes on MS medium, it can be assumed that the higher concentration (100 mg/L) of compound **1** and the lower concentration (50 mg/L) of compound **9** should be used for spring oilseed rape growth in field conditions.

Compound 1 and 9 field studies

Biometric parameters

According to the data of *Dotnuvos projektai Ltd.*, *Land Mark* is a Swedish medium late linear and very high yielding spring oilseed rape variety. The experiment with the highest yield (4.14 t/ha) was carried out in 2007: the weight of 1000 seeds was 4.1 g, and the average plant height was 111 cm. This variety was the most popular in 2012, therefore it was chosen for the exploration in this work.

Field experiments were carried out twice during the first year of investigations. The concentrations to be used ranged from 25 mg/L to 150 mg/L. Field experiments were carried out three times during the second and the third year of research, the used concentrations were from 50 mg/L to 125 mg/L (**Tables 4** and **5**).

Table 4. Effect of various concentrations of compound **1** on the biometric parameters of the spring oilseed rape *in vivo*

	Compound **1** concentration in solution, mg/L				
	Control	50	75	100	125
Plant height, cm	121.7 ± 5.9	122.2 ± 6.4	124.1 ± 7.0	122.4 ± 5.1	121.4 ± 5.7
Number of branches per plant, unit	3.5 ± 1.0	3.6 ± 1.2	3.8 ± 1.0	3.4 ± 1.5	3.8 ± 1.1
Number of secondary branches per plant, unit	3.9 ± 2.0	4.7 ± 1.8	5.1 ± 1.8	5.4 ± 2.6	6.0 ± 2.2
Pod number per plant, unit	83.5 ± 31.3	85.5 ± 29.8	87.3 ± 29.1	90.9 ± 35.3	99.4 ± 33.8
Seed number per pod, unit	22.8 ± 2.2	24.4 ± 2.2	24.5 ± 2.6	24.3 ± 3.0	24.3 ± 3.1
Pod length, cm	7.5 ± 0.3	7.8 ± 0.3	7.6 ± 0.3	7.6 ± 0.3	7.6 ± 0.4
Seed yield, t/ha	1.94 ± 0.31	2.22 ± 0.19	2.25 ± 0.26	2.28 ± 0.15	2.39 ± 0.17
1000 seed weight, g	3.96 ± 0.14	4.01 ± 0.13	3.94 ± 0.18	4.00 ± 0.12	3.96 ± 0.15

The results showed that spring oilseed rape plants sprayed with 75 mg/L and 125 mg/L solutions of compound **1** were 0.3% lower in comparison with the control treatment. Spring oilseed rape plants sprayed with 125 mg/L solution of compound **1** had the highest number of branches and secondary branches (9% and 54%, respectively), the average number of pods per plant was 99.4.

The seed number per pod was higher throughout the entire experimental period in comparison with the control treatment. According to the results of three years, the seed number per pod varied from +1% to 12% compared with the

control treatment. Assuming the results from all the three years, the highest average seed number per pod (24.5) was obtained in the sample sprayed with 75 mg/L solution of compound **1**. It was determined that the highest seed yield can be obtained by spraying with 125 mg/L solution of compound **1** ($p < 0.05$).

The highest 1000 seed weight (4.17 g) was obtained in the first year of experiments when the spring oilseed rape plants were sprayed with 25 mg/L compound **1** solution. The highest 1000 seed weight (4.04 g) during the second and third years of the experiments was obtained by using 100 mg/L and 125 mg/L compound **1** solutions.

Table 5. The effect of various concentrations of compound **9** on the biometric parameters of the spring oilseed rape *in vivo*

	Compound **9** concentration in solution, mg/L				
	0	50	75	100	125
Plant height, cm	121.7 ± 5.9	124.7 ± 8.1	121.5 ± 11.0	123.2 ± 8.5	119.4 ± 9.3
Number of branches per plant, unit	3.5 ± 1.0	3.6 ± 1.3	3.5 ± 1.0	3.9 ± 1.2	3.7 ± 1.1
Number of secondary branches per plant, unit	3.9 ± 2.0	5.7 ± 2.4	4.1 ± 2.3	4.7 ± 1.8	5.3 ± 2.7
Pod number per plant, unit	83.5 ± 31.3	92.4 ± 32.0	82.0 ± 39.5	91.2 ± 38.9	89.8 ± 35.3
Seed number per pod, unit	22.8 ± 2.2	24.1 ± 2.8	23.8 ± 2.5	24.2 ± 2.7	23.9 ± 2.8
Pod length, cm	7.5 ± 0.3	7.7 ± 0.3	7.6 ± 0.3	7.6 ± 0.2	7,6 ± 0,3
Seed yield, t/ha	1.94 ± 0.31	2.18 ± 0.17	2.11 ± 0.40	2.34 ± 0.21	2,18 ± 0,29
1000 seed weight, g	3.96 ± 0.14	4.01 ± 0.18	4.14 ± 0.11	4.03 ± 0.11	4,19 ± 0,11

The shortest plants grew after spraying the spring oilseed rape with 125 mg/L compound **9** solution. They were 2.1% lower in comparison with the control treatment.

The highest number of branches and seed numbers per pod (24.2) were observed in the sample sprayed with 100 mg/L solution of compound **9**; in comparison with the control treatment, it had 11.4% more branches. In comparison with compound **1**, the lower concentration was sufficient to obtain the same number of branches. A slightly higher seed number per pod could be obtained by spraying spring oilseed rape plants before the flowering stage with 75 mg/L of compound **1** as it was noticed after analysis of three-year data.

The highest number of secondary branches, pods and the longer pods of the spring oilseed rape were grown by using 50 mg/L solution of compound **9**. The number of secondary branches increased by 46% in comparison with the control treatment. In comparison of three-year results shows that the highest

number of secondary branches and pods was obtained by spraying spring oilseed rape plants before the flowering stage with 125 mg/L solution of compound **1**, however, the quantity of the used compound was 2.5 times higher than that of compound **9**. No exceptional effect on the pod length was observed when using compounds **1** and **9**. The length of the pod in the analyzed samples varied insignificantly, in many cases it was the same as in the control treatment group.

The highest yield of the rapeseeds could be obtained by spraying them with 100 mg/L solution of compound **9** ($p < 0.05$). The application of compounds increased: in both cases, the rapeseed yield was 9% higher in comparison with the control treatment. In this case, the use of compound **9** is more beneficial because the lower concentration is sufficient; however, after treatment with compound **1**, a significantly higher rapeseed yield was obtained in comparison with the control treatment during all the experiment years.

The highest weight of 1000 rapeseeds (4.19 g and 4.25 g) was obtained during the first and second experiment years, when spring oilseed rape plants were sprayed with 125 mg/L solution of compound **9**. The highest weight of 1000 seeds (4.12 g) was obtained after using 75 mg/L and 125 mg/L solutions of compound **9** ($p < 0.05$) in the third year of the experiment.

Spring oilseed rape seed composition

Content of phenolic compounds

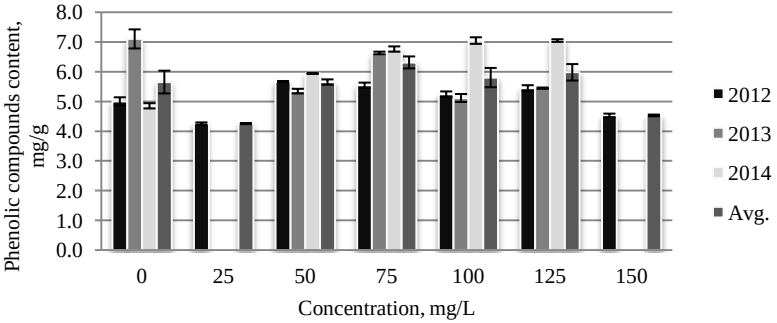


Fig. 1. The effect of various concentrations of compound **1** on the accumulation of phenolic compounds in rapeseeds

The content of phenolic compounds varied from 5.7 to 6.3 mg/g in rapeseeds (**Fig. 1**). The highest phenolic compound content (6.3 mg/g) was accumulated when spring oilseed rape plants were sprayed with 75 mg/L solution of compound **1** before the flowering stage.

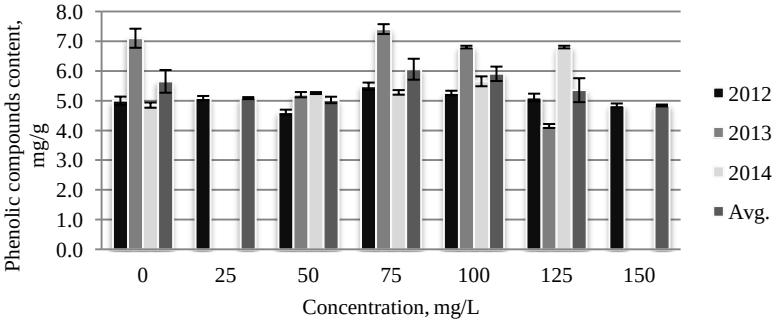


Fig. 2. The effect of various concentrations of compound **9** on the accumulation of phenolic compounds in rapeseeds

The content of phenolic compounds varied from 5.0 to 6.1 mg/g in rapeseeds (**Fig. 2**). When summarizing the results, the highest phenolic compound content (6.1 mg/g) was obtained when spring oilseed rape plants were sprayed with 75 mg/L solution of compound **9** before the flowering stage.

When comparing the effect of compounds **1** and **9** on the phenolic compound content in rapeseeds, the highest content was determined when spring oilseed rape plants were sprayed with 50 and 75 mg/L solutions of both investigated compounds. Compound **1** had a higher positive effect on the accumulation of phenolic compounds in rapeseeds.

Content of flavonoids

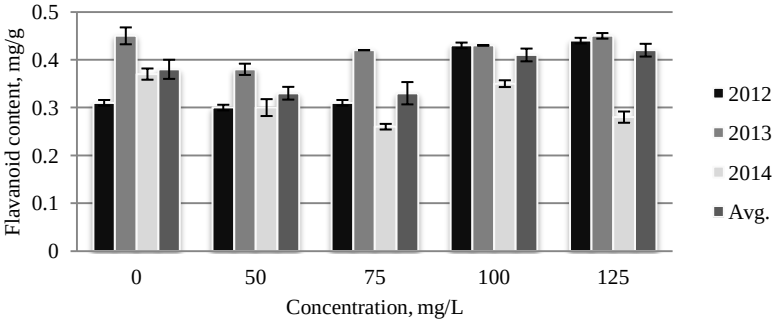


Fig. 3. The effect of various concentrations of compound **1** on the accumulation of flavonoids in rapeseeds

The effect of compounds **1** and **9** on the accumulation of flavonoids in rapeseeds was explored. The application of compound **1** solution flavonoids content in rapeseeds varied from 0.33 mg/g (50 and 75 mg/L) to 0.42 mg/g (125 mg/L). The highest flavonoid content accumulated in rapeseeds when plants

were sprayed with 125 mg/L solution of compound **1** before the flowering stage (**Fig. 3**).

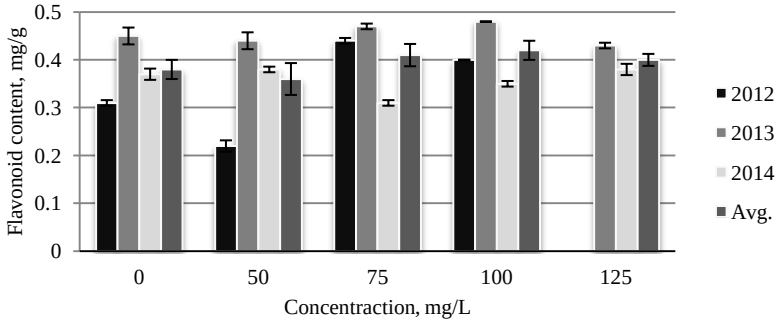


Fig. 4. The effect of various concentrations of compound **9** on the accumulation of flavonoids in rapeseeds

The effect of compound **9** on flavonoid content in rapeseeds was explored analogically (**Fig. 4**). It varied from 0.36 mg/g (50 mg/L) to 0.42 mg/g (100 mg/L). The highest flavonoid content accumulation was observed in rapeseeds when plants were sprayed with 100 mg/L solution of compound **9** before the flowering stage.

When summarizing the results of three years, it was found that the same effect of both investigated compounds on flavonoid accumulation in rapeseeds was observed. Considering the conclusion of this experiment, the use of compound **9** is more beneficial, as the lower concentration is sufficient to achieve the same content of flavonoids in comparison with compound **1**.

Glucosinolates

The highest amount of accumulated glucosinolates in rapeseeds was determined in the control treatment group and was at the level of 27.1 μmol/g as estimated in the samples of the three years of experiments. The amount of glucosinolates was slightly lower in the samples, in comparison with the control treatment group. The amount of glucosinolates in rapeseeds when plants were sprayed with investigated compounds **1** and **9** varied from 25.8 to 26.7 μmol/g.

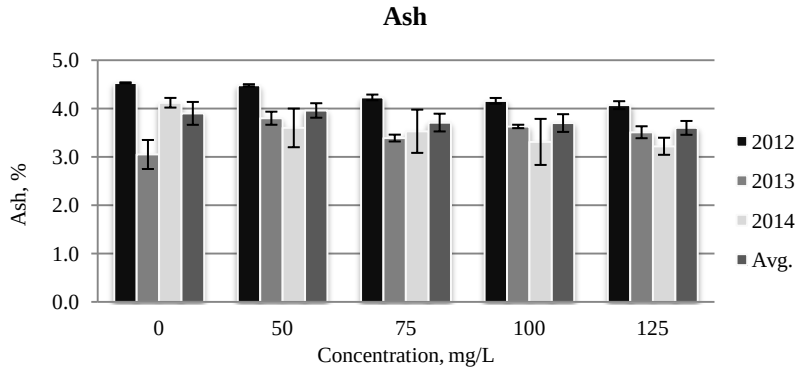


Fig. 5. The effect of various concentrations of compound **1** on the ash content in rapeseeds

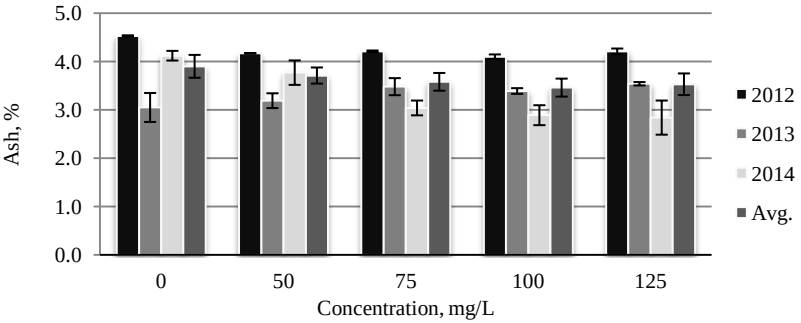


Fig. 6. The effect of various concentrations of compound **9** on the ash content in rapeseeds

Spring oilseed rape plants were sprayed with compounds **1** and **9** before the flowering stage, and, after the harvesting, the ash content of rapeseeds was investigated. The lowest ash content was determined in rapeseeds when the plants were sprayed with 125 mg/L solution of compound **1** and 100 mg/L solution of compound **9**. According to the average data of 2012–2014, compounds **1** and **9** had an effect of a decreased ash content in rapeseeds. The lower concentration of compound **9** was needed in comparison with compound **1**.

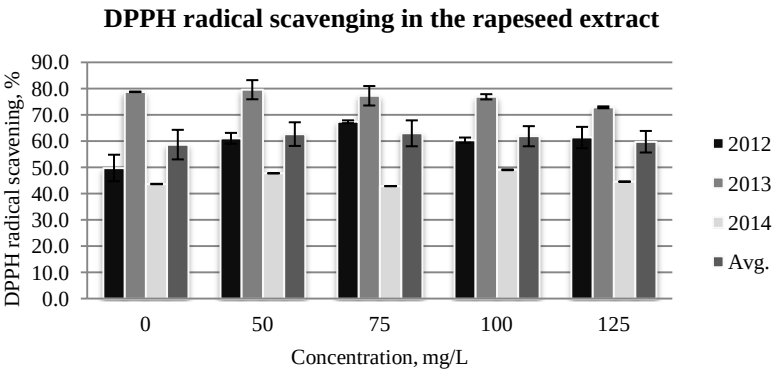


Fig. 7. The effect of various concentrations of compound **1** on the DPPH* radical scavenging in the rapeseed extract

The DPPH* radical scavenging in the rapeseed extract was explored. The highest DPPH* radical scavenging capacity was obtained in the rapeseed extract when spring oilseed rape plants were sprayed with 50 mg/L solution of compound **1** before the flowering stage (**Fig. 7**).

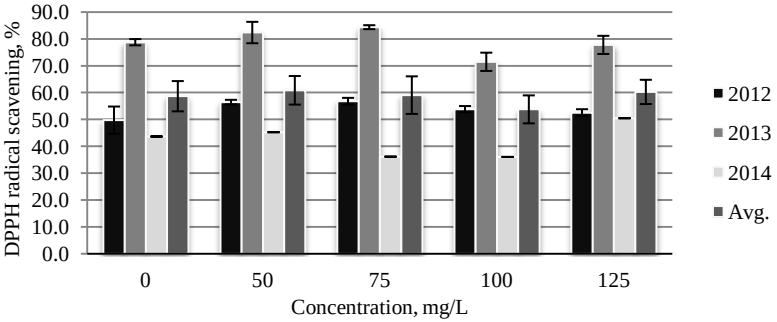


Fig. 8. The effect of various concentrations of compound **9** on the DPPH* radical scavenging in the rapeseed extract

When analyzing the DPPH* radical scavenging in response to compound **9**, the highest scavenging capacity was obtained in the rapeseed extract when spring oilseed rape plants were sprayed with 100 mg/L solution of compound **9** before the flowering stage (**Fig. 8**).

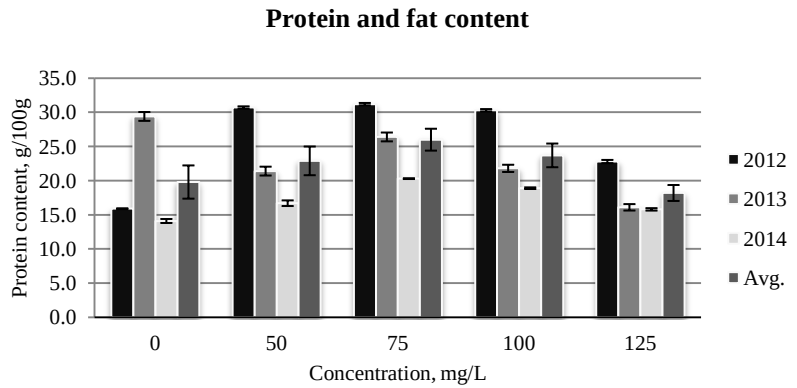


Fig. 9. The effect of various concentrations of compound **1** on protein accumulation in rapeseeds

The highest protein content in rapeseeds (26.0 g/100g) during the three years was obtained in samples when spring oilseed rape plants were sprayed with 75 mg/L solution of compound **1** before the flowering stage. When summarizing the data of the three experiment years, the content of proteins increased by 31% in comparison with the control treatment group (**Fig. 9**).

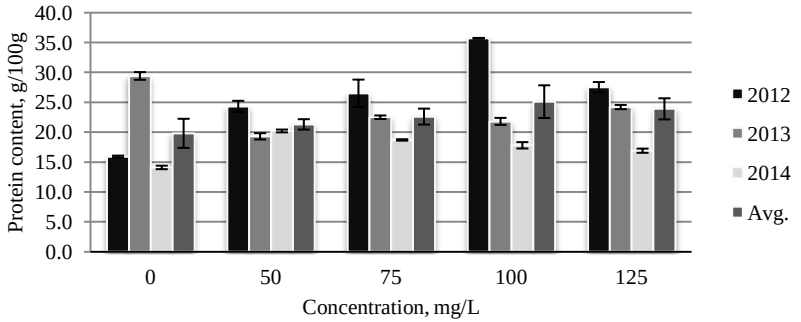


Fig. 10. The effect of various concentrations of compound **9** on protein accumulation in rapeseeds

The highest content of proteins in rapeseeds (25.1 g/100g) throughout the three experiment years under the same conditions was obtained in rapeseeds when spring oilseed rape plants were sprayed with 100 mg/L solution of compound **9** before the flowering stage. A summary of the data of the three experimental years showed that the content of proteins in rapeseed increased by 27% in comparison with the control treatment group.

The influence of compounds **1** and **9** on the protein content reveals that compound **1** had a higher influence on the content of protein. Since both compounds had a positive effect on the protein content in rapeseeds and on the

elongation of roots during germination – which corroborated the data of treating the rape with the same compounds in earlier experiments *in vitro* – it is possible to affirm that compounds **1** and **9** are osmoprotectants. Osmolites protect proteins at the cellular level and induce the lengthened growth of roots at the morphological level.

Oil content

The content of oil in kilograms which can be obtained per 1 ton of rapeseeds was determined during investigations. The obtained results are shown in **Figs. 11 and 12**.

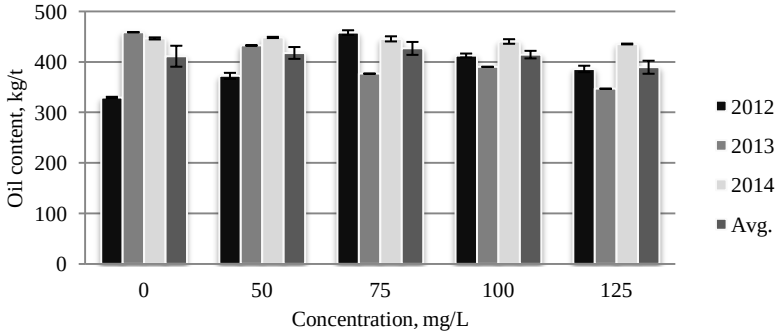


Fig. 11. The effect of various concentrations of compound **1** on the oil content in rapeseeds

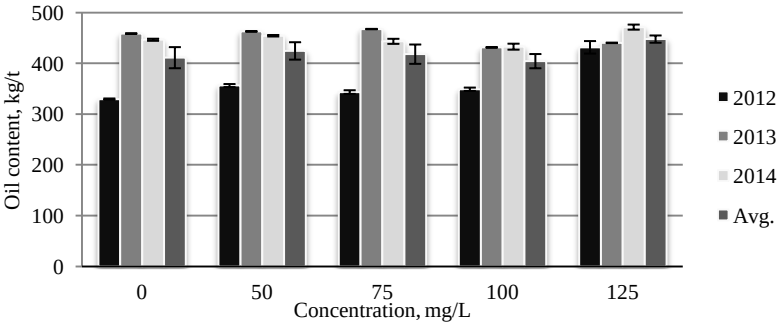


Fig. 12. The effect of various concentrations of compound **9** on the oil content in rapeseeds

The highest oil content was obtained in 75 mg/L of compound **1** treatment, the obtained oil content in rapeseeds was 4% higher, and after 125 mg/L of compound **9** treatment, it was 8.9% higher in comparison with the control treatment.

When summarizing the results of the three years, it is possible to affirm that both compounds have a positive effect on the oil content in rapeseeds.

Oil content per hectare

After evaluating the fact that different rapeseed yield per 1 hectare may be obtained, the content of oil obtained per hectare was calculated.

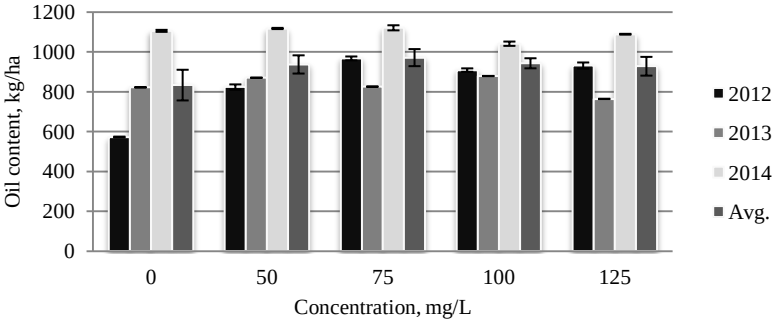


Fig. 13. The effect of various concentrations of compound **1** on the oil content obtained per 1 ha

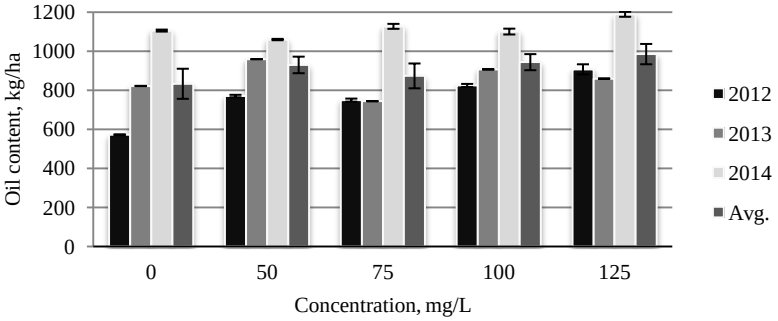


Fig. 14. The effect of various concentrations of compound **9** on the oil content obtained per 1 ha

The highest oil content was obtained in 75 mg/L of compound **1** treatment (970.5 kg/ha), and after 125 mg/L of compound **9** treatment (984.5 kg/ha). To sum up, the three-year results show that the yield was 17% and 18% higher in comparison with the control treatment.

Fatty acid composition in rapeseed oil

The content of fatty acids in rapeseed oil varied very insignificantly under the treatment with various concentrations of compounds **1** and **9**.

Table 6. The impact of various concentrations of compound **1** on the content of fatty acid in rapeseed oil

Fatty acid, %	Compound **1** concentration, mg/L						
	0	25	50	75	100	125	150
Palmitic	4.5 ± 0.5	4.6 ± 0.1	4.2 ± 0.5	4.3 ± 0.5	4.6 ± 0.6	4.4 ± 0.5	4.8 ± 0.0
Stearic	1.9 ± 0.2	2.0 ± 0.0	1.8 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.2	2.0 ± 0.0
Oleic	63.2 ± 1.8	61.1 ± 0.1	63.4 ± 1.5	63.3 ± 1.7	63.2 ± 1.7	63.4 ± 1.7	61.4 ± 0.0
Linoleic	19.6 ± 1.1	20.8 ± 0.1	19.4 ± 1.0	19.8 ± 1.3	19.8 ± 1.1	19.7 ± 1.1	20.9 ± 0.1
Eicosenoic	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	7.8 ± 0.7	8.8 ± 0.0	8.4 ± 0.8	7.9 ± 0.8	7.9 ± 0.8	7.7 ± 1.0	8.6 ± 0.0

Table 7. The impact of various concentrations of compound **9** on the content of fatty acid in rapeseed oil

Fatty acids	Compound **9** concentration, mg/L						
	0	25	50	75	100	125	150
Palmitic	4.5 ± 0.5	5.1 ± 0.1	4.8 ± 0.6	4.5 ± 0.7	4.7 ± 0.8	4.3 ± 0.7	4.9 ± 0.2
Stearic	1.9 ± 0.2	2.1 ± 0.1	1.8 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	2.0 ± 0.3	2.1 ± 0.0
Oleic	63.2 ± 1.8	61.0 ± 0.3	63.3 ± 1.8	63.4 ± 2.3	63.7 ± 2.3	63.3 ± 1.8	61.2 ± 0.0
Linoleic	19.6 ± 1.1	20.5 ± 0.1	19.4 ± 1.1	19.2 ± 1.5	19.2 ± 1.6	19.2 ± 0.9	20.8 ± 0.0
Eicozenoic	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.0 ± 0.0
Linolenic	7.8 ± 0.7	8.5 ± 0.0	7.9 ± 0.9	7.8 ± 0.9	7.8 ± 1.0	7.9 ± 1.0	8.7 ± 0.0

According to the averaged data of three years, a higher content of oleic acid in rapeseed oil could be further increased with compound **9** treatments. The higher content of linoleic acid was obtained after treatment with compound **1** (**Table 6**). The quantities of other fatty acids were virtually unchanged.

Chlorophyll and carotenoids

Chlorophyll and carotenoids in spring oilseed rape leaves *in vitro*

Investigations of chlorophyll and carotenoids content in spring oilseed rape cultivated for 2 and 4 weeks on MS medium *in vitro* with 75 mg/L solutions of compound **1** and **9** were carried out.

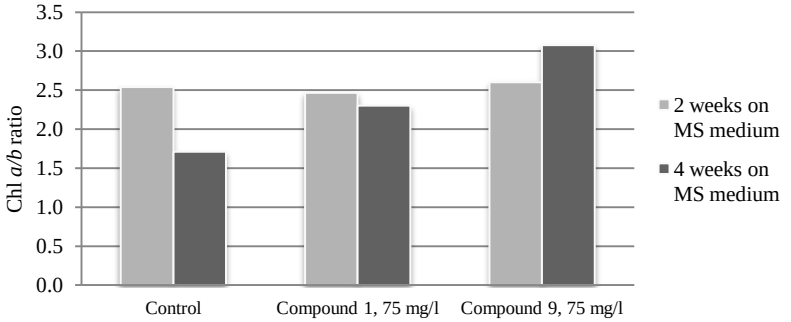


Fig. 15. Chl *a/b* ratio in spring oilseed rape leaves after 2 and 4 weeks cultivation on MS medium

Spring oilseed rape leaves had a higher level of Chl *a* and Chl *b* as well as carotenoid content if treated with 75 mg/L solution of compound **9** after germination for 2 weeks. The highest ratio of Chl *a*/*b* was obtained in the control group and in the group treated with compound **1**. The lowest content of carotenoids as well as Chl *a* and Chl *b* was noted in the control treatment group after 4-week cultivation on MS medium supplemented with the investigated compounds. The highest ratio of Chl *a*/*b* in spring oilseed rape leaves (3.1) was obtained in the case of treatment with compound **9**, which was 46% higher than in the control treatment group. Compound **1** treatment increased Chl *a*/*b* ratio by 26% in comparison with the control treatment group. Both compounds stimulated intensive photosynthesis *in vitro*.

Chlorophyll and carotenoids in oilseed rape leaves *in vivo*

For investigations of chlorophyll and carotenoids, spring oilseed rape leaves were collected 2 and 4 weeks after treating with compounds **1** and **9**, and the experiment was carried out over a period of 2 years.

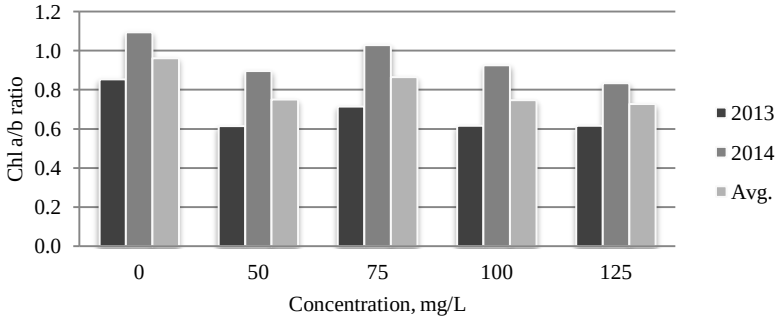


Fig. 16. Chl *a/b* ratio in spring oilseed rape leaves 4 weeks after spraying with compound **1**

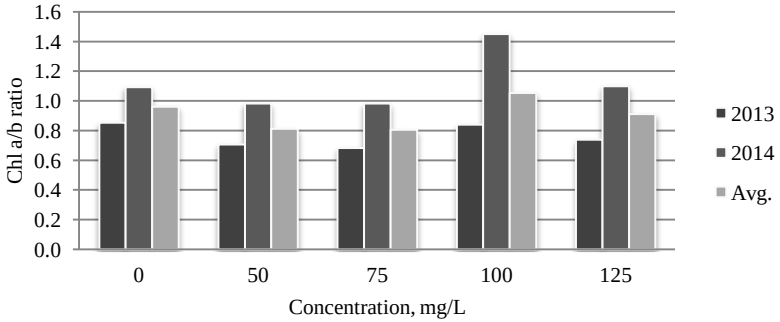


Fig. 17. Chl *a/b* ratio in spring oilseed rape leaves 4 weeks after spraying with compound **9**

Two weeks after spraying the samples with compounds **1** and **9**, a higher influence on Chl *a/b* ratio of compound **1** in comparison with compound **9** and the control treatment group was obtained. However, this influence disappeared after 2 weeks.

A lower Chl *a*/*b* ratio was obtained in samples which were sprayed with compound **1** in comparison with the control treatment group (**Fig. 16**). A higher Chl *a*/*b* ratio was detected in the sample which was sprayed with 100 mg/L solution of compound **9** in comparison with the control treatment group. Compounds **1** and **9** exhibited a decreased Chl *a*/*b* ratio and as well as a lower

content of carotenoids in spring oilseed rape plants in comparison with the control treatment group.

RAPD analysis of spring oilseed rape leaves DNA

Three primers (P-01, P-02 and P-03) used in the analysis involving spring oilseed rape produced 16 polymorphic amplification products. The amplification size ranged from 250 up to 1200 bp (**Table 8**). Each primer generated 4 to 8 individual bands per primer and provided a distinct and reproducible pattern of the amplified PCR fragments (**Fig. 18–20**).

Table 8. Primers used for oilseed rape DNA analysis

Primer	Sequence 5'→ 3'	Amplified product	Fragment size (bp)	Temperature
P-01	5'-AATCGGGCTG-3'	4	600 – 1200	42 ºC
P-02	5'-GGGTAACGCC-3'	8	250 – 1200	42 ºC
P-03	5'-CAATCGCCGT-3'	4	300 – 900	44 ºC

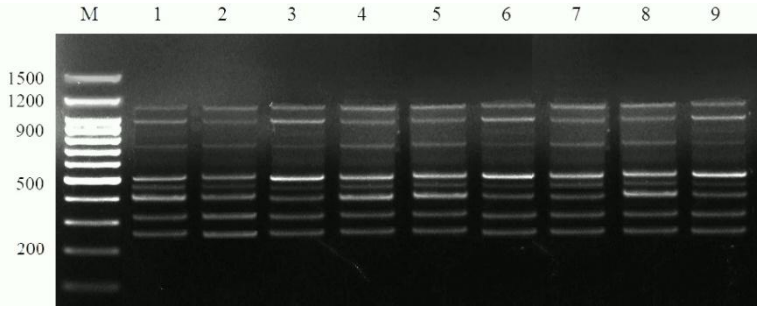


Fig. 18. Spring oilseed rape DNA analysis with primer P-01. Lines 1–3 represent control treatment, lines 4–6 show compound **1** treatment, lines 7–9 depict compound **9** treatment.

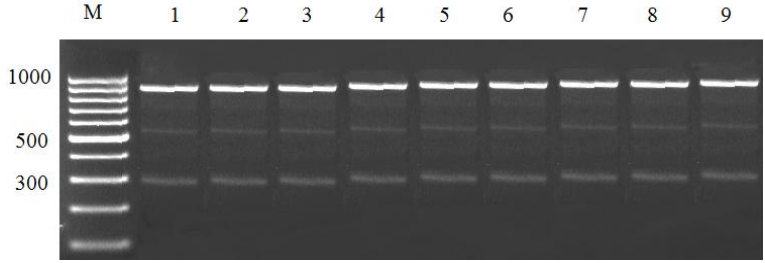


Fig. 19. Spring oilseed rape DNA analysis with primer P-02. Lines 1–3 represent control treatment, lines 4–6 depict compound **1** treatment, lines 7–9 show compound **9** treatment.

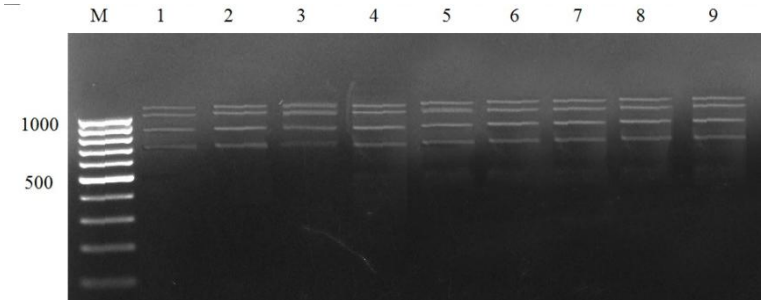


Fig. 20. Spring oilseed rape DNA analysis with primer P-03. Lines 1–3 represent control treatment, lines 4–6 depict compound **1** treatment, lines 7–9 show compound **9** treatment.

The results showed that in the leaf samples sprayed with 125 mg/L compounds **1** and **9,** the DNA was unchanged compared with the DNA of the control samples.

Conclusions

1. *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acids had a positive effect on spring oilseed rape *Land Mark* growth of hypocotyls and roots as well as on the accumulation of photosynthesis pigments in laboratory *in vitro*.
2. *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts increased the quantity of plant branches, secondary branches and pods, the average quantity of seeds per pod, the seed yield and the weight of 1000 seeds; these salts also increased the protein content in rapeseeds by 31% and 27% and the content of the obtained oil by 4% and 9%, respectively. The tested compounds had no significant impact on the chemical composition of rapeseed oil. The current study showed that 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salt effectively induces the growth of spring oilseed rape and that it might be used for spring oilseed rape yield enhancement.
3. Biochemical analysis of rapeseeds revealed that spraying of spring oilseed rape plants with *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts solutions:
   3.1. Increased the total content of phenolic compounds before the flowering stage;
   3.2. The compounds did not have influence on glucosinolates content in rapeseeds;
   3.3. The ash content of rapeseeds decreased after treatment with the investigated compounds. The lowest ash content was recorded in 3-(1*H*-benzimidazol-2-il)-4-phenyl amino butane sodium salt treatment;
   3.4. Increased DPPH radical scavenging capacity in the rapeseed extract. This indicates that the spring oilseed rape is resistant to oxidation.
4. The following statements have been proven true after the performance of photosynthesis pigments accumulation experiments *in vitro* and *in field*:
   4.1. The higher chlorophyll *a* and *b* ratio after 2 and 4 weeks were found in the spring oilseed rape cultivated *in vitro* on MS medium, supplemented with 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salt;
   4.2. 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salt influences the distribution of photosynthesis pigments more intensively.
5. *N*-phenyl-N-(5-oxo-4,5-dihydro-1,3-thiazol-2-yl)-*β*-alanine and 3-(1*H*-benzimidazol-2-yl)-4-phenylaminobutyric acid sodium salts had no effect on the DNA of the spring oilseed rape.

List of Scientific Publications of the Theme of the Dissertation
Publications in journals including into the *Thomson Reuters Web of Knowledge* database

1. Mickevičius, Vytautas; Voskienė, Aušra; Jonuškienė, Ilona; **Kolosej, Ramūnė**; Šiugždaitė, Jūratė; Venskutonis, Petras Rimantas; Kazernavičiūtė, Rita; Brazienė, Zita; Jakienė, Elena. (2015). Synthesis and biological activity of 3-[phenyl(1,3-thiazol-2-yl)-amino]propanoic acids and their derivatives // *Molecules*. Basel: Molecular Diversity Preservation International, 2013, vol. 18, pp. 15000–1018. ISSN 1420–3049. (ISI Web of Science, I.F. 2.465).
2. **Kolosej, Ramūnė**; Jonuškienė, Ilona; Venskutonis, Petras Rimantas; Kazernavičiūtė, Rita; Brazienė, Zita; Jakienė, Elena; Kvederavičiūtė, Kotryna; Kanopka, Arvydas; Vilys, Laurynas; Mickevičius, Vytautas. (2017). The influence of *β*-alanine derivative products on spring oilseed rape yield and oil quality // *Žemdirbystė-Agriculture*, 2017, vol. 104 (2), pp. 139–146. ISSN 1392–3196. (ISI Web of Science, I.F. 0.579).

Articles published in reviewed periodical scientific journals included into the list of other databases

1. **Kolosej, Ramūnė**; Mickevičius, Vytautas; Jonuškienė, Ilona; Brazienė, Zita; Jakienė, Elena. (2013). 3-(1*H*-Benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druskos poveikis vasarinių rapsų (*Brassica napus* L.) derliui ir kokybei // *Cheminė technologija*. 2013, vol. 1, no. 63, pp. 42–48. ISSN 1392–1231.

Publications based on presentations at internationals scientific conferences

1. **Gustevičiūtė, Ramūnė**; Jonuškienė, Ilona; Mickevičius, Vytautas. (2012). N-Fenil-N-tiazolil-β-alaninų poveikio vasarinių rapsų (*Brassica napus* L.) kultūrai tyrimas // *Organinė chemija*: mokslinė konferencijos pranešimų medžiaga. 2012, pp. 23–25. ISSN 2029–6401.
2. **Kolosej, Ramūnė**; Jonuškienė, Iona; Mickevičius, Vytautas. (2013). 3-(1H-benzimidazol-2-il)-4-fenilaminobutano rūgščių poveikis vasarinių rapsų (*Brassica napus* L.) vystymuisi *in vitro* // *Organinė chemija*: mokslinė konferencijos pranešimų medžiaga. 2013, pp. 78–80. ISSN 2029–6401.
3. **Kolosej, Ramūnė**; Mickevičius, Vytautas; Jonuškienė, Ilona. (2013). The %influence of 3-(1H-benzimidazol-2-yl)-4-phenylaminobutanoic sodium salt on spring rapeseed (*Brassica napus* L.) growth ant yield // 9-toji tarptautinė konferencija daRostim "Phytohormones, humic substances and other biologically active compounds for agriculture, human health and environmental protection." 2013, pp. 61–62.

4. **Kolosej, Ramūnė**; Mickevičius, Vytautas; Jonuškienė, Ilona. (2014). The influence of biological activity of 3-(1H-benzimidazol-2-yl)-4-phenylaminobutanoic sodium salt on summer rapeseed (*Brassica napus* L.) yield and seed quality // International Conference of Lithuanian Chemical Society "Chemistry and Chemical Technology 2014": mokslinės konferencijos pranešimų medžiaga. 2014, pp. 238–240. ISSN 2351–5643.
5. **Kolosej, Ramūnė**; Mickevičius, Vytautas; Jonuškienė, Ilona; Brazienė, Zita. (2014). The influence of 3-(*1H*-benzimidazol-2-yl)-4-phenylaminobutanoic acid sodium salt on summer rapeseed (*Brassica napus* L.) growth and yield // 8-oji tarptautinė mokslinė konferencija "The Vital Nature Sign": mokslinės konferencijos pranešimų medžiaga. 2014, p. 92. ISSN 2335–8653.
6. Jurgaitytė, Donata; **Kolosej, Ramūnė**; Žiauka, Jonas; Kuusienė, Sigutė. Growth of different aspen genotypes *in vitro* under water deficit stress // International conference "Smart Bio": mokslinės konferencijos pranešimų medžiaga. 2017, p. 84. ISBN 978–609–8104–42–4.

Curriculum Vitae

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REZIUMĖ

Vasariniai rapsai (*Brassica napus* L.) yra vieni iš svarbiausių aliejinių augalų, kuriuose daug (30–45%) aliejaus. Rapsų aliejus turi didelę maistinę vertę: jame mažai sočiųjų riebalų rūgščių, daug oleino rūgšties, palankus linolo ir linoleno rūgščių santykis, taip pat jo sudėtyje daug riebaluose tirpių vitaminų. Rapsų aliejuje omega-6 ir omega-3 rūgščių santykis yra 2:1 – šis santykis yra palankus žmogaus sveikatai.

Augalų augimą ir produktyvumą veikia įvairūs abiotiniai veiksniai, pavyzdžiui, aukšta temperatūra, druskingumas, žema temperatūra ir kt. Aliejinių rapsų augimą ir derliaus kokybę galima pagerinti tręšiant, apdorojant augimo reguliatoriais arba kobalto sulfatu augimo metu. Rapsų derliui svarbus yra rapsų ankštarų skaičius, sėklų skaičius ankštaroje, bei jų svoris. Ankštarų susiformavimą lemia tiek maistinės medžiagos, tiek hormonai. Sėklų kiekį ankštaroje galima padidinti naudojant augimo reguliatorių 3-indolilacto rūgštį, ankštarų kiekį – 6-benzilaminopuriną.

β-Alaninas yra nebaltyminė aminorūgštis, randama visuose organizmuose. Augaluose jos daugėja veikiant aplinkos stresams. *β*-Aminorūgštys sulaukia vis didesnio susidomėjimo ne tik dėl savo vaidmens organizmuose, bet ir dėl panaudojimo biologiškai aktyvių junginių sintezėje. Pačios svarbiausios *β*-aminorūgštys yra *β*-alaninas, *β*-leucinas, *β*-argininas, *β*-fenilalaninas, *β*-glutamatas, *β*-glutaminas ir *β*-tirozinas.

Lietuvos mokslininkai yra atlikę tyrimus su auksinų analogais (TA-12 (4-(2-chloretoksikarbonilmetil)-1-naftalinsulfo rūgšties kalcio druska) ir TA-14 (1-naftiletani rūgšties ω-trialkilamonioalkilesterio druska)). Tyrimų metu nustatyta, kad TA-12 skatina žiedų pumpurų susidarymą, sutrumpina žydėjimo trukmę ir padidina ankštarų kiekį.

Darbo tikslas

Ištirti *N*-pakeistų β- ir γ-aminorūgščių su aromatiniais ir azolų fragmentais įtaką vasarinių rapsų (*Brassica napus* L.) augimui *in vitro* bei, nustatyti aktyviausių junginių poveikį vasarinių rapsų augimui *in vivo*, sėklų derliui ir cheminei jo sudėčiai.

Pagrindiniai tyrimų uždaviniai

1. Ištirti *N*-pakeistų β- ir γ-aminorūgščių su aromatiniais ir azolų fragmentais poveikį vasarinių rapsų vystymuisi *in vitro* ir atrinkti didžiausiu bioaktyvumu pasižyminčius junginius.
2. Ištirti atrinktų aktyviausių junginių poveikį vasarinių rapsų, augintų *in vivo* sąlygomis, biometriniams rodikliams ir derliui.
3. Ištirti atrinktų aktyviausių junginių poveikį rapsų sėklų sudėčiai ir kokybei.
4. Ištirti atrinktų aktyvių junginių poveikį bioaktyvių medžiagų kaupimuisi vasarinių rapsų, augintų *in vivo* sąlygomis, sėklose.

5. Įvertinti junginių su *N*-pakeistais β- ir γ-aminorūgščių su aromatiniais ir azolų fragmentais poveikį rapsų DNR kitimui.

Darbo naujumas

Ištirtas *N*-pakeistų *β*- ir *γ*-aminorūgščių su aromatiniais ir azolų fragmentais skirtingų koncentracijų poveikis vasarinių rapsų (*Brassica napus* L.) augimui *in vitro*, teigiamą įtaką turėję junginiai atrinkti tolesniems lauko tyrimams. Panaudojant atrinktus dviejų skirtingų klasių junginius, *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazol-2-il)-*β*-alaniną ir 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano natrio druską, atlikti lauko tyrimai su vasariniais rapsais ir nustatytas jų poveikis rapsų biometriniams rodikliams, derliui, sėklų ir aliejaus cheminei sudėčiai. Palygintas *in vitro* ir lauko sąlygomis augintų rapsų žalios masės fotosintezės pigmentų kitimas. Nustatyta, kad tirtieji junginiai neturi poveikio rapsų DNR.

Darbo praktinė reikšmė

Atlikus lauko bandymus nustatyta, kad *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazol-2-il)-*β*-alaninas ir 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano natrio druskos pagerino vasarinių rapsų *„Land Mark“* biometrinius rodiklius *in vivo*: *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazol-2-il)-*β*-alaninas padidino augalo šakų skaičių, vidutinį šoninių šakelių skaičių, vidutinį sėklų skaičių ankštaroje. Padidėjo sėklų derlius, baltymų kiekis rapsų sėklose, išgaunamo aliejaus kiekis. 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgštis padidino augalo šakų kiekį, vidutinį šoninių šakelių kiekį, vidutinį sėklų kiekį ankštaroje, sėklų derlių. Be to, padidėjo baltymų kiekis rapsų sėklose, o aliejaus išgauta daugiau. Abu tirti junginiai poveikio gliukozinolatų kaupimuisi ir aliejaus riebalų rūgščių kiekiams rapsų sėklose neturėjo. Atlikus tyrimus galima teigti, kad 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgštį galima naudoti kaip rapsų derliaus didinimo priemonę.

IŠVADOS

1. *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazolil-2il)-*β*-alanino ir 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgštis teigiamai veikė rapsų hipokotilių ir šaknų vystymąsi ir skatino fotosintezės pigmentų susidarymą *in vitro*.
2. *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazolil-2il)-*β*-alanino ir 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druskos *in vivo* padidino augalo šakų, vidutinį šoninių šakelių, ankštarų kiekį, vidutinį sėklų skaičių ankštaroje, sėklų derlių ir 1 000 sėklų svorį, taip pat padidino baltymų kiekį rapsų sėklose atitinkamai 31 % ir 27 %, o išgaunamo aliejaus kiekį – 4 % ir 9 %. Aliejaus cheminiai sudėčiai tiriamieji junginiai poveikio neturėjo. Tyrimai parodė, kad 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druska efektyviai skatina vasarinių rapsų augimą ir ją galima naudoti rapsų derliaus didinimui.
3. Rapsus prieš žydėjimą nupurškus *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazolil-2il)-*β*-alanino ir 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druskos tirpalais ir atlikus rapsų sėklų cheminius tyrimus nustatyta, kad:
    3.1. padidėjo bendra fenolinių junginių koncentracija;
    3.2. gliukozinolatų kaupimuisi rapsų sėklose junginiai poveikio neturėjo;
    3.3. rapsus paveikus tirtaisiais junginiais jų sėklų peleningumas sumažėjo. Labiausiai peleningumą sumažino 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druska;
    3.4. padidėjo antioksidacinis rapsų sėklų ekstrakto efektyvumas, parodęs, kad rapsų sėklose esantis aliejus yra atsparesnis oksidacijai.
4. Fotosintezės pigmentų kaupimasis rapsų lapuose parodė, kad:
    4.1. *in vitro* sąlygomis, didesniu chlorofilo *a* ir *b* santykiu po 2 ir 4 savaičių pasižymėjo rapsai, auginant juos ant MS terpės, papildytos 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgštimi;
    4.2. 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druska intensyviau veikia fotosintezės pigmentų pasiskirstymą.
5. DNR tyrimai su pasirinktais pradmenimis parodė, kad *N*-fenil-*N*-(5-okso-4,5-dihidro-1,3-tiazolil-2il)-*β*-alanino ir 3-(1*H*-benzimidazol-2-il)-4-fenilaminobutano rūgšties natrio druskos nesukelia vasarinių rapsų DNR pakitimų.

PADĖKA

- Dėkoju savo darbo vadovui prof. habil. dr. Vytautui Mickevičiui už kantrybę ir vertingus patarimus visų studijų metu.
- Dėkoju Kauno technologijos universiteto Cheminės technologijos fakulteto Organinės chemijos katedros darbuotojai doc. dr. Ilonai Jonuškienei už pagalbą atliekant *in vitro* tyrimus ir patarimus metodiniais klausimais.
- Dėkoju Aleksandro Stulginskio universiteto Augalininkystės ir gyvulininkystės katedros doc. dr. Elenai Jakienei ir Lietuvos agrarinių ir miškų mokslo centro LŽI filialo Rumokų bandymo stoties darbuotojams, ypač dr. Zitai Brazienei, už pagalbą ir galimybę atlikti bandymus lauko sąlygomis Rumokų bandymų stotyje.
- Dėkoju Kauno technologijos universiteto Cheminės technologijos fakulteto Maisto chemijos ir technologijos katedros darbuotojams prof. dr. Rimantui Petrui Venskutoniui už galimybę atlikti rapsų sėklų aliejaus analizę ir jaunesniajai mokslo darbuotojai Ritai Kazernavičiūtei už vertingus patarimus šių tyrimų metu.
- Dėkoju Vilniaus universiteto Biotechnologijos instituto vyresniajam mokslo darbuotojui dr. Arvydui Kanoplai ir doktorantams Laurynui Viliui ir Kotrynai Kvedaravičiūtei už pagalbą atliekant rapsų DNR tyrimus ir vertingus patarimus.
- Nuoširdžiausia padėka savo šeimai už kantrybę, tikėjimą ir skatinimą rengiant disertaciją.

UDK 547.466+547.789+582.683.2+633.85](043.3)

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