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The effect of biostimulants on the glucosinolate content in winter oilseed rape (*Brassica napus* L.) seeds

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ABSTRACT

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The objective of the study was to determine the effect of biostimulants on the concentration of harmful sulphuric compounds called glucosinolates in the seed of three winter oilseed rape cultivars. An experiment was arranged as a split-split-plot design with three replications. The following factors were examined: I – three winter oilseed rape morphotypes: a population morphotype (cv. Monolit), a semi-dwarf restored cultivar (PR 44D06) and a standard restored cultivar (PT 205); II – two sowing methods: between-row spacing of 22.5 cm and 45.0 cm; III – four types of biostimulants: control, Tytanit®, Asahi®SL and Silvit®. The lowest concentration of glucosinolates was determined in the population cv. Monolit (on average 8.84 μmol/g) and the highest in the restored semi-dwarf cv. PR 44D06 (on average 9.84 μmol/g). Application of all of the biostimulants contributed to a significant decline in the concentration of harmful sulphuric compounds compared with control, the lowest concentration being recorded following the spraying with Silvit® (on average 8.88 μmol/g). Statistical calculations did not confirm a significant effect of two sowing methods on the characteristics examined.

Keywords: seed quality; biologically active compounds; environmental conditions; growth biostimulants

Modern agriculture pays more and more attention not only to yield quantity but also to its quality. According to Wielebski (2009), oilseed rape yield quality is determined by fat and protein content in seeds as well as by the presence of harmful sulphuric compounds called glucosinolates. Jankowski and Budzyński (2000), Tys and Jankowski (2002) and Malarz et al. (2006) claim that the chemical composition of rape seeds depends mainly on the genetic factor but can also be affected by environmental conditions and agrotechnology. Gawrońska and Przybysz (2011) stress the fact that high yields of good quality in crop production predominantly depend on the ability to prevent or reduce an occurrence of biotic and abiotic stresses as well as regeneration of injuries that they cause. As a result, more and more attention has been paid in recent years not only to application of fertiliser and pesticides, but also of a number of products known as plant development regulators, biostimulants, resistance stimulants or bacterial vaccines. Many authors (Matysiak et al. 2011, Calvo et al. 2014) demonstrated a positive effect of biostimulants on the qualitative and quantitative characteristics of

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agricultural plants. According to Grenda (2003), Pusz and Pląskowska (2008), Matysiak et al. (2011) as well as Bulgari et al. (2015), biostimulants applied in plant cultivation are a natural method of better utilisation of crop growth conditions (temperature, air and bed humidity, light quantity and intensity) and nutrients, all of which result in an increase in yield quantity and quality.

The research hypothesis assumed in the present work is that biostimulant application may contribute to better seed quality by reducing the concentration of harmful sulphuric compounds called glucosinolates. As few studies on the effect of this factor were undertaken and much interest in agricultural practice was expressed, this research was conducted to determine the effect of types of biostimulants on glucosinolate content in the seeds of three winter oilseed rape cultivars.

MATERIAL AND METHODS

Field studies were conducted in 2013–2016 at the Zawady Experimental Farm (52°03'N, 22°33'E) owned by the Siedlce University of Natural Sciences and Humanities. The experiment was designed as a split-split-plot arrangement with three replicates. The following three factors were examined:

I – three winter oilseed rape morphotypes: a population morphotype (cv. Monolit), a restored cultivar characterised by semi-dwarf type of growth (cv. PR 44D06) and a restored cultivar characterised by a standard type of growth (cv. PT 205);

II – two sowing methods: between-row spacing of 22.5 cm (seeds sown in rows at the sowing density of 60 seeds per 1 m^2) and 45.0 cm (seeds drilled at the sowing density of 40 seeds per 1 m^2);

III – four types of biostimulants applied:

1. control – no biostimulants applied;
2. biostimulant Tytanit® (active substance – titanium): date I – autumn application at the stage of 4–8 leaves (14–18 BBCH) at the rate of 0.20 L/ha; date II – spring application, after plants resumed growth and side buds started to develop (21–36 BBCH), at the rate of 0.20 L/ha; date III – application at the stage of flower bud development (budding) until the beginning of flowering (50–61 BBCH), at the rate of 0.20 L/ha;
3. biostimulant Asahi®SL (active substances: sodium ortho nitrophenol, sodium para nitrophenol, sodium 5-nitroguaiacolate): date I – autumn application at the stage of 3–5 leaves (13–15 BBCH), at the rate of 0.60 L/ha; date II – spring application after plants resumed growth (28–30 BBCH), at the rate of 0.60 L/ha; date III – two weeks following the second application, at the rate of 0.60 L/ha;
4. biostimulant Silvit® (active substances: active silicon, potassium oxide, boron – form of pure element, zinc – form of pure element): date I – application three weeks after emergence (12–14 BBCH), at the rate of 0.20 L/ha; date II – spring application after plants resumed growth (28–30 BBCH), at the rate of 0.20 L/ha; date III – two weeks after the second application, at the rate of 0.20 L/ha.

The studies were carried out on the soil classified according to WBR FAO (2014) to the Haplic Luvisols group, sandy, belonging to a very good rye soil complex, of the IVb botanical class. In the years of the experience, the soil reaction (pH) ranged from 5.68 to 5.75. The soil was characterised by a low total nitrogen content (average from 0.80 to 0.90 g/kg), phosphorous content (average from 0.33 to 0.55 g/kg), potassium content (average from 0.61 to 0.67 g/kg) and calcium content (average from 0.82 to 0.85 g/kg) and the average magnesium content (from 0.38 to 0.46 g/kg) and sulphur content (from 0.11 to 0.15 g/kg). It has a low abundance in assimilable forms of phosphorous (average from 75.0 to 80.0 g/kg) and the average assimilability of potassium (from 200.0 to 205.0 g/kg) and magnesium (average from 59.0 to 61.0 g/kg).

The phosphorous-potassium fertilization at the dose of 40.0 kg P/ha and 110.0 kg K/ha with the first dose of 40.0 kg N/ha was used before sowing. Fertilization was used in the form of Lubofos for rape at the dose of 600.0 kg, i.e. 21.0 kg N/ha, 26.4 kg P/ha, 92.1 kg K/ha, 34.8 kg S/ha, 1.2 kg B/ha. Fertilization rates were supplemented by 55.9 kg/ha of ammonium nitrate (19.0 kg N/ha), 29.6 kg/ha of triple superphosphate (13.6 kg P/ha) and 29 kg/ha of potassium salt (17.9 kg K/ha). The second nitrogen dose of 100.0 kg/ha was applied in spring before vegetation (BBCH 28–30) using ammonium nitrate at the dose of 255.5 kg/ha (86.9 kg N/ha) and ammonium sulphate at the dose of 62.5 kg/ha (13.1 kg N/ha + 15.0 kg S/ha). The third dose of nitrogen 60.0 kg/ha was applied at the beginning of budding (BBCH 50) using ammonium nitrate at the dose of 176.5 kg/ha (60.0 kg N/ha). Rape

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was collected in two stages, in the first and second decade of July (I year: 11.07.2014; II year: 17.07.2015; III year: 14.07.2016).

Glucosinolate content (μmol/g) in the dry matter of seeds was determined by means of reversed-phase high performance liquid chromatography (HPLC) with a gradient elution mode, following PN-EN ISO 9167-1. 1991.

Experimental results were statistically analysed by means of ANOVA. Significance of sources of variation was checked using the Fisher-Snedecor test and means separation was obtained using the Tukey's test at the significance level of P = 0.05 (Trętowski and Wójcik 1988).

Weather conditions varied during the study years (Table 1). In 2013, total precipitation was by 60.0 mm higher than the long-term mean sum of precipitation, the average air temperature being by 0.8°C higher. The highest annual precipitation sum, on average 599.2 mm, and the lowest average annual air temperature, on average 8.8°C, were recorded in the growing season 2014–2015. The annual total precipitation over this period was by 171.7 mm higher compared with the long-term mean. The third study year was the warmest and the driest, the annual total precipitation in this year being by 43.8 mm lower than the long-term mean, and the average annual air temperature being by 1.3°C higher than the long-term mean calculated across 1996–2010. The Sielianinov's hydrothermal coefficient indicated that there was no drought in all the study years although in some months with extreme values of this coefficient there was either strong drought or no drought.

RESULTS AND DISCUSSION

Glucosinolate content as affected by types of biostimulants applied (Table 2). Biostimulants applied in the experiment contributed to a decline in glucosinolate content compared with control. The lowest concentration of these compounds was recorded in seeds harvested in plots sprayed with Silvit® (on average 8.88 μmol/g), followed by Tytanit® (on average 9.08 μmol/g) and Asahi® SL (on average 9.65 μmol/g). The concentration of these harmful compounds was the highest in

Table 1. Characteristics of weather conditions in the years 2013–2016 (Zawady Meteorological Station, Poland)

	Month												
	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII–VII
Rainfall (mm)													sum
2013–2014	15.0	94.3	32.8	34.7	15.4	28.6	34.0	29.6	45.0	92.7	55.4	10.0	487.5
2014–2015	105.7	26.3	3.0	32.5	90.4	51.4	0.7	53.1	30.0	100.2	43.3	62.6	599.2
2015–2016	11.9	47.1	37.0	42.2	16.5	10.9	29.0	33.5	28.7	54.8	36.9	35.2	383.7
Multiyear sum (1996–2010)	59.9	42.3	24.2	20.2	18.6	19.0	16.0	18.3	33.6	58.3	59.6	57.5	427.5
Air temperature (°C)													mean
2013–2014	18.8	11.7	9.3	5.1	1.2	–4.5	0.7	5.8	9.8	13.5	15.4	20.8	9.0
2014–2015	18.1	14.1	8.5	3.4	0.1	0.6	0.7	4.6	8.2	12.3	16.5	18.7	8.8
2015–2016	21.0	14.5	6.5	4.7	3.7	–4.5	2.5	3.5	9.1	15.1	18.4	19.1	9.5
Multiyear mean (1996–2010)	18.5	13.5	7.9	4.0	–0.1	–3.2	–2.3	2.4	8.0	13.5	17.0	19.7	8.2

	VIII	IX	X	III	IV	V	VI	VII	mean
Sielianinow's hydrothermic coefficient*									
2013–2014	0.31	2.63	1.01	1.48	1.41	2.33	1.23	0.16	1.32
2014–2015	1.87	0.66	0.22	4.63	1.35	2.91	0.84	1.20	1.71
2015–2016	0.20	1.20	2.15	3.49	1.07	1.47	0.72	0.64	1.37

*Coefficient value (Bac et al. 1998); < 0.50 strong drought; 0.–0.69 drought; 0.70–0.99 weak drought; ≥1 no drought

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Table 2. Glucosinolate content (μmol/g) depending on factors of experience

Cultivar	Types of biostimulators used				Year			Mean
	control	Tytanit®	Asahi®SL	Silvit®	2013–2014	2014–2015	2015–2016	
Monolit	9.14	8.46	8.99	8.76	8.72	8.90	8.89	8.84
PR 44D06	10.82	9.45	10.60	9.05	9.58	10.00	9.95	9.84
PT 205	10.48	9.33	9.90	8.82	9.37	9.85	9.67	9.63
Mean	10.15	9.08	9.65	8.88	9.22	9.58	9.51	–

$LSD_{0.05}$ for: years – 0.08; cultivars – 0.08; types of biostimulators used – 0.07 interaction: years × cultivars – 0.14; cultivars × types of biostimulators used – 0.12

the unit where no biostimulants were applied (on average 10.15 μmol/g). In contrast, other authors (Gugała et al. 2016) who applied herbicides in combination with biostimulants (Harrier 295ZC 2.0 L/ha + Kelpak SL 2.0 L/ha and Sencor 70WG 1.0 kg/ha + Asahi®SL 1.0 L/ha) in potato observed increased glycoalkaloid concentrations in leaves and tubers compared with control.

Statistical calculations revealed an interaction between cultivars and kinds of biostimulants applied (Table 2). A significant decline in glucosinolate content was determined in the morphotypes examined following an application of natural growth stimulants. Regardless of the biostimulants used in the study, cv. Monolit accumulated less glucosinolates than the hybrid cultivars. Restored cultivars accumulated similar amounts of glucosinolates following an application of Silvit® and Tytanit®. Spraying with Asahi SL contributed to a significant decline in glucosinolate content in the cv. PT 205 compared with cv. PR 44D06.

An interaction of study years with biostimulants was confirmed, which indicates that biostimulants displayed a different mode of action under changeable weather conditions in the study period (Table 3). The lowest concentration of these compounds, ranging from 8.79 to 8.92 μmol/g, was recorded following the application of Silvit® in all the growing seasons, the quantity of glucosinolates in seeds being the same in the first study year after spraying with Silvit® and Tytanit®.

Glucosinolate content as affected by the genetic factor (Table 2). Broniarz and Stroiwąs (2013) say that the concentration of glucosinolates in winter rape seeds on average ranged from 7.1 to 16.4 μmol/g. Jankowski et al. (2015) received an average of 15.25 μmol/g while the average from 8.84 to 9.84 μmol/g dry matter was obtained in own studies. Variance analysis demonstrated a significant effect of the genetic factor on the concentration of glucosinolates in winter oilseed rape seeds. Of the examined cultivars, the lowest concentration of these compounds was observed in the population morphotype cv. Monolit (on average 8.84 μmol/g), which agrees with the findings reported by Wójtowicz and Wielebski (2000) as well as Wójtowicz and Jajor (2006) who found lower glucosinolate contents in the population

Table 3. Glucosinolate content (μmol/g) depending on ways of sowing

Types of biostimulators used	Year			Ways of sowing		Mean
	2013–2014	2014–2015	2015–2016	45.0 cm	22.5 cm	
Control	9.79	10.40	10.24	10.15	10.14	10.15
Tytanit®	8.87	9.17	9.20	9.12	9.03	9.08
Asahi®SL	9.44	9.84	9.66	9.64	9.65	9.65
Silvit®	8.79	8.92	8.92	8.87	8.88	8.88
Mean	9.22	9.58	9.51	9.45	9.43	–

$LSD_{0.05}$ for: ways of sowing – not significant; years – 0.08; interaction: ways of sowing × types of biostimulators used – not significant; years × types of biostimulators used – 0.12

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morphotype of the cv. Lisek. In the study discussed here, the highest concentration of glucosinolates (on average 9.84 μmol/g) was found in the seeds of the semi-dwarf restored cultivar (PR 44D06). Similar results were reported by Wielebski and Wójtowicz (2004) as well as Wielebski (2009, 2011). The authors obtained the highest concentrations of the sulphuric compounds in cvs. Kaszub and Pomorzanin. The content of harmful sulphuric compounds was also affected by weather conditions throughout the study years (Table 2). A similar trend was observed by Champolivier and Merrien (1999), Liersch et al. (2000), Wielebski (2009) as well as Wielebski (2011). Hu et al. (2007) and Wielebski (2009, 2011) reported that precipitation shortages during flowering and excess when seeds developed and matured contributed to an increase in the glucosinolate content. In the present study, the highest seed content of glucosinolates was recorded in the growing season 2014–2015 (on average 9.58 μmol/g) when precipitation shortages and a lower average daily air temperature were recorded in June compared with the long-term mean, whereas in July precipitation exceeded by 5.1 mm the long-term mean. A lower concentration of sulphuric compounds (on average 9.51 μmol/g) was determined in seeds harvested in 2015–2016 when precipitation in June and July was much lower and air temperatures were higher than the long-term mean. Glucosinolate content was the lowest (on average 9.22 μmol/g) in the first study year characterised by excessive precipitation in May followed by rainfall shortages in June and early July.

Statistical analysis revealed a significant interaction between cultivars and growing seasons (Table 2). The lowest glucosinolate content was recorded in the seed of the population morphotype and semi-dwarf restored cultivar in the first study year. In the remaining years, the cultivars accumulated similar amounts of these compounds. Cv. PT 205 and all the other cultivars had the lowest glucosinolate content in the first study year. Moreover, cv. PT205 contained more sulphuric compounds in the growing season 2014–2015 than 2015–2016. Contrasting findings were reported by Wielebski and Wójtowicz (2004) who found a similar response of the cultivars they studied to weather conditions in individual study years.

Glucosinolate content as affected by the sowing method (Table 3). Variance analysis demonstrated no significant effect of the two sowing methods on the concentration of glucosinolates in oilseed rape seeds, which agrees with the findings reported by Różyło and Pałys (2011). However, these authors obtained a slightly higher concentration – on average 0.4 μmol/g – when the between-row spacing of 25.0 cm was used. Similarly, Wielebski and Wójtowicz (2004) obtained much higher concentrations of alkene glucosinolates and total glucosinolates at the lowest crop density. In another study, Różyło and Pałys (2014) found than an increase in the between-row width from 33.0 cm to 44.0 cm and 55.0 cm contributed to a decline in the glucosinolate content.

Summarizing, under the influence of all natural growth stimulators applied and the plant development in the analysed morphologies, there was a significant reduction of the content of the harmful sulphur compounds compared to the control object depending on the climatic conditions during the research years.

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