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ORIGINAL PAPER

EFFECT OF ORGANIC AND INORGANIC STIMULANTS ON THE CONTENT OF GLUCOSINOLATES IN WINTER RAPE SEED*

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Abstract

The research was based on a three-year field experiment conducted in 2018-2021, at the Zawady Agricultural Experimental Station (52°03'N and 22°33'E), Siedlce, in the climatic and soil conditions of north-eastern Poland. The experiment was set up in a split-plot design with three replications. The research factors were: I - 3 morphotypes of winter oilseed rape: population (Chrobry), restored hybrid morphotype with the traditional type of growth (PT 271), restored hybrid morphotype with the semi-dwarf growth type (PX 113). II -4 ways to use growth stimulants: object 1 - control object, without the use of stimulators, object 2 - organic preparation containing microorganisms as well as micro- and macro-elements (UGmax), object 3 - biostimulant containing 13.0% $P_{2}0_5$ and 5.0% K_2O (Rooter), object 4 – biostimulant containing silicon (Optysil). The types of applied preparations significantly influenced the utility value of seeds by increasing or decreasing the concentration of glucosinolates. A significant reduction of harmful sulphur compounds compared to the control variant was noted only as a result of the use of a plant stimulator containing silicon. After the application of the stimulator containing phosphorus and potassium, an increase in the concentration of these compounds in seeds was noted. The thresholds of these compounds were not exceeded. In all the years of the research, after the application of the organic preparation Ugmax, the same content of these compounds was demonstrated as in the control object. The population morphotype was characterized by the lowest concentration of glucosinolates, and the highest one was determined in the restored hybrid with the traditional type of growth. The climatic conditions influenced the concentration of these compounds in the examined morphotypes.

Keywords: anti-nutritional substances, morphotype, *Brassica napus* L., growth stimulants, silicon, macro and microelements, glucosunolates

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INTRODUCTION

Wójtowicz and Jajor (2006) emphasize that the evaluation of the quality of a crop depends on the direction of use of the main and secondary yields of plants, and consists in determining the possibility of using the crop for consumption, fodder and technological purposes. The basic criterion in assessing the quality of rapeseed is the content of fat and protein as well as anti-nutritional substances called glucosinolates. The concentration of these components depends mainly on the genotype, as well as environmental and agrotechnical conditions.

Wielebski (2009) showed that the seeds of the composite hybrids Kaszub and Pomorzanin (17.1 and 16.8 μ M g⁻¹ of seeds) contained the highest content of glucosinolates, followed by the restored hybrids Extrem (15.4 μ M g⁻¹ of seeds), Kronos (11.0 μ M g⁻¹ of seeds), and the open-pollinated hybrid Bojan (12.2 μ M g⁻¹ of seeds). In turn, El-Beltagi and Mohamed (2010) obtained glucosinolate concentrations from 4.40 to 5.95 μ M g⁻¹ in seeds of the cultivars studied, Broniarz and Stroiwąs (2013) – from 7.1 to 16.4 μ M g⁻¹ on average, Jankowski et al. (2015) – 15.25 μ M g⁻¹ on average, Sikorska et al. (2018) – from 8.84 to 9.84 μ M g⁻¹ d.m on average, while in the studies by Bocianowski et al. (2020) the mean content in genotypes ranged from 4.13 (for PN66 × PN21) to 8.53 μ M g⁻¹ (Californium).

Since the 1960s, there has been a strong selection pressure to develop rapeseed with a very low erucic acid content and a significantly reduced glucosinolate content (GLS). Wittstock and Halkier (2002) and Śmiechowska et al. (2008) report that glucosinolates are synthesized from aliphatic (leucine, isoleucine, valine, methionine, alanine), aromatic (phenylalanine, tyrosine) and indole (tryptophan) amino acids. In the process of biosynthesis, oxidative decarboxylation of the amino acid (by N-hydroxyamino acid) to its aldoxime takes place, which is then converted to the glucosinolate structure by the intermediates: thiohydroxamic acid and desulfoglucosinate.

In Poland, the norm for the content of glucosinolates is the lowest in the world and amounts to 15 μ M g⁻¹ of seeds. This standard covers the sum of alkene and indole glucosinolates. Currently cultivated double enriched variates are a source of oil with high food value and meal with a significantly reduced content of glucosinolates (Patyra et al. 2016).

Despite the low content of glucosinolates in rape seeds, the possibility of lowering the concentration of these compounds is still a current breeding problem. Therefore, it is justified to conduct research aimed at reducing the content of glucosinolates in rapeseed.

In recent years, there has been an interest in and the use of various types of biostimulants, effective microorganisms, preparations and fertilizers enriching the soil with humus, improving the health and resistance of plants to stress conditions, facilitating the uptake of nutrients and production of good quality crops (Gajewski et al. 2011). Szczepanek et al. (2017) stated that a biostimulant increases the amount of plant dry matter in the period from flowering to ripening, while preventing a decrease in the Mg concentration in aerial and underground parts of oilseed rape.

The research hypothesis adopted in the paper was that natural growth stimulants of organic and inorganic origin may influence the content of harmful sulphur compounds in winter rape seeds. The aim of the research was to determine the effect of biostimulants containing microorganisms and micro- and macro-elements (object 2), phosphorus and potassium (3) and silicon (4) on the content of glucosinolates in the seeds of three morphotypes of winter oilseed rape (Chrobry, PT 271, PX 113).

MATERIAL AND METHODS

Location of the research area

The research was based on a three-year field experiment conducted in 2018-2021 at the Zawady Agricultural Experimental Station (52°03'N and 22°33'E), Siedlee, in the climatic and soil conditions of north-eastern Poland. The experiment was set up in a split-plot design with three replications. The area of a plot for harvesting was 18 m².

Research factor

The research factors were:

- I 3 morphotypes of winter oilseed rape:
 - population morphotype (Chrobry variety),
 - hybrid morphotype restored with a traditional type of growth (PT 271),
 - hybrid morphotype restored with a semi-dwarf growth type (PX 113);
- II 4 ways to use growth stimulants:
 - object 1 control object, without the use of biostimulants;
 - object 2 organic preparation containing microorganisms as well as micro- and macro-elements (UGmax) used in the following periods: I – in autumn before sowing rape seeds, II – in spring after the onset of the oilseed rape's vegetative growth: beginning of the side shoot formation (BBCH 21-36), in doses of 0.9 dm³ ha⁻¹;
 - object 3 biostimulant containing 13.0% P_2O_5 and 5.0% K_2O (Rooter) applied in: I in autumn in the 4-6 leaves phase (BBCH 13-15), II in spring after the onset of the vegetative growth (BBCH 28-30) in the doses of 1.0 dm³ ha⁻¹;
 - object 4-biostimulant containing silicon (Optysil) used: I term in autumn in the 4-6 leaf stage (BBCH 13-15), II term - in spring,

dense flower buds, the 'green bud' stage (BBCH 51), in doses of 0.50 dm³ ha⁻¹.

Soil conditions

The experiment was carried out on soil classified according to the World Reference Base for Soil Resources (2014) as representing the Haplic Luvisol group, sandy, belonging to the very good rye soil complex, of the IVa class, in the Polish soil taxonomy system. In the years of the experiment, the pH of the soil was slightly acidic and ranged from 5.68 to 5.75. The soil was characterized by a low content of available forms of phosphorus and average bioavailability of potassium, magnesium, boron, and sulfur. Chemical analysis of the soil was performed at the chemical laboratory of the University of Natural Sciences and Humanities in Siedlce.

Fertilization

Before sowing, phosphorus and potassium in doses of 40 kg P ha⁻¹ and 110 kg K ha⁻¹ and the first dose of nitrogen of 40 kg N ha⁻¹ were applied. Fertilization under oilseed rape was applied in the form of Lubofos at a dose of 600 kg ha⁻¹. Fertilization doses were supplemented with 55.9 kg ha⁻¹ ammonium nitrate, 29.6 kg ha⁻¹ triple superphosphate (13.6 kg P ha⁻¹) and 29 kg ha⁻¹ potassium salt. The second nitrogen dose of 100 kg ha⁻¹ was applied in the spring before the start of the crop's growth, using ammonium nitrate at a dose of 255.5 kg ha⁻¹ and ammonium sulphate at a dose of 62.5 kg ha⁻¹. The third nitrogen dose of 60 kg ha⁻¹ was applied at the beginning of budding, using ammonium nitrate at a dose of 176.5 kg ha⁻¹.

Sowing

Winter oilseed rape was sown at an inter-row spacing of 22.5 cm, keeping the planting density of 45 plants m⁻². Sowing was performed at the optimal date recommended for this region (from August 10 to 15).

Chemical protection

The preparation Command 480 EC ($0.25 l ha^{-1}$, active substance: clomazone) and Fusilade Forte 150 EC ($2.0 l ha^{-1}$, BBCH 13-14, active substance: fluazyfop-P-butyl) were used to control weeds. For pest control, Proteus 110 OD ($0.6 l ha^{-1}$, BBCH 30, 50-58, 60-69, active substance: thiacloprid, deltamethrin) was used three times. The fungicide treatments were carried out using Horizon 250 EW ($0.75 l ha^{-1}$, BBCH 14-18, tebuconazole), Propulse 250 SE ($1.0 l ha^{-1}$, BBCH 61, fluopyram, prothioconazole) and Mondatak 450 EC ($1.0 l ha^{-1}$, BBCH 65, prochloraz).

Chemical analysis of seeds

Four seed samples from each cultivar were collected for chemical analysis. The glucosinolate content was given in $\mu M g^{-1}$ of dry weight of seeds, and

determined using high pressure liquid chromatography (HPLC) with reversed-phase columns and gradient elution. The content of glucosinolates was determined at the chemical and technological laboratory of the Cultivar Testing Experimental Station at Słupia Wielka.

Statistical analysis

The significance of sources of variation was tested with the Fisher-Snedecor F test, and the significance of differences at the significance level α =0.05 between the compared means was assessed using the Tukey's multiple intervals.

Climatic conditions

The years in which the field experiments were conducted differed in climatic conditions (Table 1). The highest sum of precipitation, 419.0 mm on average, and the highest average air temp. 10.1°C on average, were recorded in the years 2019-2020. Although it was a very humid growing season in relation to the value of the calculated Selyaninov hydrothermal coefficient (K=2.68), the following months occurred: quite dry (September), dry (October), very dry (April), while the period from May to July was extremely humid.

Months Years 08 09 10 11 1201 02 03 04 0506 07 08-07 Precipitation (mm) sum 2018-2019 24.527.423.39.8 9.0 7.9 4.715.05.959.835.929.7244.02019-2020 29.167.743.917.49.517.812.926.85.96.0 63.5118.5419.02020-2021 52.722.6375.6 18.238.8 34.034.010.49.642.029.533.8 50.0Multiyear 20.233.6 total 59.942.324 2 18.6 19.016.018.358.359.657.54275(1996 - 2010)Air temperature (°C) mean 2018-2019 20.615.99.6 3.3 0.4-3.0 2.24.89.8 13.321.418.59.7 2019-2020 19.914.210.76.12.91.9 2.94.58.6 11.719.319.010.12020-2021 20.215.5-2.52.712.420.422.712.050-1.0-19 66 93 Multiyear 18.57.94.0-0.1 -3.2-2.38.013.517.019.78.2 mean 13.52.4(1996-2010)Selyaninov hydrothermal coefficient* VIII IX Х III IV V VI VII mean 2018-2019 1.19 1.722.423.130.60 4.491.681.602.102019-2020 2.201.220.891.310.706.14 3.562.685.422020-2021 2.202.990.90 2.504.393.556.36 2.381.66

Characteristics of climatic conditions in 2019-2021 (Zawady Agricultural Experimental Station, Poland)

* Coefficient value (Skowera 2014): extremely dry (ss) $k \le 0.4$, very dry (bs) 0.4-0.7, dry (s) 0.7-1.0, rather dry (ds) $1.0 \le 1.3$, optimal (o) $1.3 \le 1.6$, rather wet (dw) $1.6 \le 2.0$, wet (w) $2.0 \le 1.5$, very wet (bw) $2.5 \le 3.0$, extremely wet (sw) $k \ge 3.0$

Table 1

The lowest amount of precipitation, 244.0 mm on average, was recorded in the 2018-2019 growing season, and the average air temp. in this growing season was 9.7°C.

The lowest average air temperature, 9.3°C on average, was determined in the years 2020-2021. It was also a very wet year (K=2.99).

RESULTS AND DISCUSSION

Based on the research results, significant influence of the morphotype on the content of glucosinolates in the seeds of winter oilseed rape was demonstrated (Table 2). The lowest value of this parameter, 8.45 μ M g⁻¹ on average, was obtained in the population cultivar Chrobry, a significantly higher content was found in seeds of the semi-dwarf hybrid (11.59 μ M g⁻¹), and the highest one (12.78 μ M g⁻¹) was in seeds of the long-stem PT 271 morphotype. Similar research results were reported by Gugała et al. (2020), who showed the lowest content of GLS in seeds of the population cultivar Monolit, while the concentration of these compounds was higher by an average of 2.48 μ M g⁻¹ in the long-stemmed hybrid (PT248), and higher by an average of 1.24 µM g⁻¹ in the semi-dwarf form studied. Similarly, Wójtowicz and Jajor (2006) and Sikorska et al. (2018, 2021) recorded the lowest glucosinolate concentration in the population forms Monolit and Poznaniak, while the highest content of these compounds was found in seeds of the semi-dwarf morphotypes PR 44D06 and PX104. Ratajczak et al. (2017) obtained different research results, as seeds of the semi-dwarf hybrid PR45D03 were characterized by a significantly lower content of glucosinolates compared to a hybrid with the traditional type of growth and a population variety.

Statistical calculations showed the interaction of the research years and cultivars, which indicates varied responses of cultivars in the years of the experiment. In all the years of the study, the lowest concentration of gluco-sinolates was recorded in the population variety, where it ranged from 7.97 to 8.73 μ M g⁻¹ on average, but in the first two years of the study the differences were statistically insignificant, and the same value of this parameter was found in in the half-dwarf hybrid all the research years. Throughout the experiment, the highest concentration of these compounds was recorded in the PT 271 long-stemmed hybrid.

The analyses proved that the content of harmful sulphur compounds was the same after the application of the organic preparation UGmax as in the control object. On the other hand, growth stimulants containing phosphorus, potassium and silicon significantly influenced the concentration of glucosinolates (GLS) in seeds of the studied cultivars. The biostimulant containing phosphorus and potassium (Rooter) applied to object (3) caused a significant increase in the content of these compounds, by an average of 0.13 μ M g⁻¹ Table 2

| Mean | | | | 11.05 a | 11.09 a | $10.68 \ b$ | |
|------------------------------------|---------|----|---------------------------------|-------------|--------------|--------------|-------------|
| Ways to use growth stimulants | Objects | 4. | biostimulant Optysil | $10.81 \ b$ | 10.97 f | $10.58 \ d$ | $10.78 \ c$ |
| | | 3. | biostimulant Rooter | $11.24 \ c$ | $11.23 \ c$ | $10.77 \ b$ | $11.08 \ b$ |
| | | 2. | organic preparation UGmax | 11.04 af | $11.12 \ ac$ | $10.68 \ bd$ | 10.95 a |
| | | 1. | control variant | 11.11 a | $11.03 \ af$ | $10.71 \ b$ | 10.95 a |
| Cultivars Chrobry PT 271 PX 113 | | | 11.63 f | 11.60 f | 11.53 f | 11.59 c | |
| | | | PT~271 | 12.79 c | $12.99 \ d$ | $12.56 \ e$ | $12.78 \ b$ |
| | | | Chrobry | 8.73 a | 8.67 a | $7.97 \ b$ | 8.45 a |
| Years | | | | 2018-2019 | 2019-2020 | 2020-2021 | Mean |

Glucosinolate content ($(\mu \mathrm{M}~\mathrm{g}^{\text{-}})$ depending on the factors of the experiment

 $LSD_{0.05}$ for: years -0.07, cultivars -0.07, ways to use growth stimulants -0.07, interaction: years x cultivars -0.12, years x ways to use growth stimulants -0.12

compared to the control object, while in object 4, where a preparation containing silicon (Optysil) was used, there was a significant reduction in the concentration of these compounds. This value was lower by 0.17 μ M g⁻¹ on average than in the control variant. Gugała et al. (2020) found a significant increase in the concentration of these compounds compared to the control variant as a result of the application of a biostimulant containing amino acids and sulphur and boron supplementation. The above researchers showed the highest increase in the concentration of these compounds, higher by an average of 1.69 μ M g⁻¹ than in the control object, after the application of the amino acid, and the smallest one (by an average of 1.0 μ M g⁻¹) – in the object where sulphur and boron were used. An increase in the content of glucosinolates in rapeseed was also determined by Filipek-Mazur et al. (2019) after the application of sulphur-containing fertilizers, and by Davoudi et al. (2019), who tested the use of selenium.

In turn, Sikorska et al. (2018, 2021), who tested biostimulants containing titanium, sodium ortho-nitrophenol, sodium para-nitrophenol, sodium 5-nitroguajakol and silicon, potassium oxide, boron, and zinc, demonstrated a significant reduction in the glucosinolate concentration in rapeseed, emphasizing that the concentration of these compounds in the control variant was the highest and averaged to $10.15 \ \mu M \ g^{-1}$. Similarly, a decrease in the content of harmful sulphur compounds was observed by Kwiatkowski (2012) after applying a combination of foliar fertilizers, i.e. 75% of the NPK dose and autumn spraying with a urea solution with Plonovit R and MgSO₄ H₂O, and by Nawab et al. (2016), after a foliar application of ammonium sulphate.

The climatic conditions in the years of the experiment significantly influenced the content of glucosinolates. The analysis of variance applied to the data from the 2018-2019 and 2019-2020 growing seasons showed that the values of this parameter were the highest, but the differences were statistically insignificant. In those years, much less rainfall in April than the long-term average could have contributed to an increase in the glucosinolate concentration. A similar tendency was shown by Hu et al. (2007), Wielebski (2009), Ullah et al. (2012), Lääniste et al. (2016), and Ratajczak et al. (2017). The lowest concentration of these compounds was demonstrated, averaging 10.68 μ M g⁻¹ in the last year of the research.

Statistical calculations showed the interaction of years with the types of biostimulants used (Table 2), which indicates their operation in changing climatic conditions during the research. In the first and second growing season, the content of glucosinolates (GLS) was the same in the control object (1) and in the object with the organic preparation UGmax (2). A similar tendency was noted in the last year of the study, where the value of the tested feature was the same in the control object (1), with the organic preparation UGmax (2) and in the object where phosphorus and potassium were used (3). In all the research years, the lowest concentration of harmful sulphur compounds was demonstrated after the application of a silicon-containing biostimulant (4), while in the second and third research season, this value was statistically insignificantly different compared to the control variant.

CONCLUSIONS

1. The types of applied preparations significantly influenced the utility value of seeds by increasing or decreasing the concentration of glucosinolates. A significant reduction of harmful sulphur compounds compared to the control variant was noted only as a result of the use of a plant stimulant containing silicon. After the application of a stimulant containing phosphorus and potassium, an increase in the concentration of these compounds in seeds was noted. The permissible standards were not been exceeded.

2. In all the years of the research, the same content of these compounds was demonstrated as in the control object after the application of the organic preparation UGmax.

3. The population morphotype was characterized by the lowest concentration of glucosinolates, and the largest content of these compounds was determined in the restored hybrid with the traditional type of growth. The climatic conditions influenced the concentration of these compounds in the examined morphotypes.

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