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ORIGINAL PAPER

EFFECT OF DIFFERENT SULFUR FERTILIZER DOSES ON THE GLUCOSINOLATE CONTENT AND PROFILE OF WHITE MUSTARD SEEDS

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Abstract

After nitrogen, phosphorus and potassium, sulfur is another essential element that is required for the optimal growth, development and yield of white mustard and other species of the family *Brassicaceae*. The effect of different pre-sowing sulfur fertilizer doses on the glucosinolate content and profile of three traditional white mustard cultivars that are widely grown in Poland was analyzed in a three-year, small-area field experiment. White mustard was grown in the experimental fields of the Wrocław University of Environmental and Life Sciences in south-western Poland in 2007-2009. The experiment had a split-plot design with two levels of experimental factors. White mustard cultivar (Metex, Nakielska, Radena) was the first experimental factor, and sulfur fertilizer dose (0, 10, 20, 30 S kg ha^{-1}) was the second experimental factor. The glucosinolate content of white mustard seeds was analyzed by gas chromatography. White mustard seeds were most abundant in sinalbin, an aromatic glucosinolate. The sinalbin content of seeds varied in response to different sulfur fertilizer doses. An increase in the sinalbin concentration was observed already in response to a sulfur dose of 10 kg ha^{-1} relative to the control treatment. The total content of aliphatic glucosinolates was significantly lowest in the treatment where sulfur fertilization was not applied. The content of aliphatic glucosinolates in white mustard seeds was significantly highest in the treatment fertilized with sulfur at 20 kg ha^{-1}. Weather conditions significantly influenced the total glucosinolate content and the proportions of the analyzed glucosinolate groups in white mustard seeds.

Keywords: glucosinolates, white mustard, S fertilization.

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INTRODUCTION

Sulfur is an essential macronutrient that is required for the optimal growth and development of plants (MAATHUIS 2009, SKWIERAWSKA et al. 2014). Sulfur belongs to the group of essential elements that determine high yields in oilseed crops (ABDALLAH et al. 2010, GRANT et al. 2012, JANKOWSKI et al. 2014, JANKOWSKI et al. 2015*ab*). Sulfur is also the primary component of glucosinolates, compounds that occur as secondary metabolites in plants. Glucosinolates are formed by biosynthetic pathways that are independent of primary metabolism. The shift between primary and secondary metabolism is genetically conditioned in plants. For this reason, glucosinolates are present only in selected taxonomic groups, mainly plants of the order Capparales, including the family *Brassicaceae* (HALKIER, DU 1997). Around 300 glucosinolates (FAHEY et al. 2001, MORENO et al. 2006), including 30 in the family *Brassicaceae* (KRZYMAŃSKI 1995), have been identified to date. Glucosinolates are classified into functional groups derived from one of eight amino acids (FAHEY et al. 2001) based on the chemical structure of the corresponding amino acid precursors. Aliphatic glucosinolates are derived from methionine (Met), alanine (Ala), leucine (Leu), isoleucine (Ile) and valine (Val); indole glucosinolates are derived from tryptophan (Trp); and aromatic glucosinolates are derived from phenylalanine (Phe) or tyrosine (Tyr) (HALKIER, GERSHENZON 2006). The quantitative and qualitative composition of glucosinolates varies depending on plant growth stages and plant organs (CISKA et al. 2000, BELOSTAS et al. 2007). Seeds and young seedlings are most abundant in glucosinolates, whereas inflorescences, siliques, roots and leaves are characterized by the lowest glucosinolate concentrations (PETERSEN et al. 2002, ZUKALOVÁ, VAŠAK 2002, BROWN et al. 2003). White mustard (*Sinapis alba* L.) seeds contain approximately 0.1-1.1% sinalbin. The remaining glucosinolates present in white mustard seeds are sinigrin, progoitrin, gluconapin and glucobrassicanapin (DROZDOWSKA 1994, MURAWA et al. 1999, PASZKIEWICZ-JASIŃSKA 2005, PIĘTKA et al. 2004).

Glucosinolates and their metabolites can reduce the risk of cancer. These biologically active substances are supplied with food or as isolated chemical compounds (KELLOFF 2000). Recent research has demonstrated that the chemopreventive properties of glucosinolates can slow down or even reverse carcinogenesis (KELLOFF 2000). However, the intake of glucosinolates should be controlled due to possible adverse effects. Because of their high glucosinolate content (approx. 180 μM g-1), white mustard seeds are widely used in the production of mustard and condiments, but they are not suitable for the production of edible oils (Jankowski et al. 2020). The seeds of traditional white mustard cultivars are not processed into meal and press cake as a source of protein for animals, either (Krzymański 1995). Glucosinolate metabolites contain toxic substances that reduce feed intake, retard growth and reproduction, and impair thyroid function in animals. Like oilseed rape, only double-low white mustard cultivars with the reduced glucosinolate content can be processed into meal (Piętka et al. 2014, Jankowski et al. 2020).

White mustard is a major spring oilseed crop in Central-Eastern Europe. Therefore, sulfur fertilizer doses in white mustard production have to be optimized to obtain high seed yields with satisfactory qualitative characteristics. The effect of sulfur fertilization on glucosinolate levels has been extensively researched in winter oilseed rape, but not in other species of oilseed crops. The present study aims to fill in this knowledge gap.

MATERIALS AND METHODS

In 2007-2009, a field experiment was conducted on the experimental fields of the Wrocław University of Environmental and Life Sciences (51°10′ N, 17°06′ E) to evaluate the effect of different sulfur fertilizer doses on the glucosinolate content of white mustard seeds. The experiment had a split-plot design with two variables: (i) traditional white mustard cultivars – Nakielska (Polish, grown for seeds), Metex (German, grown for seeds), Radena (Polish, grown for green forage), and (ii) sulfur fertilizer dose (S kg ha^{-1}) – 10, 20, 30 and 0 (control treatment).

Each year, the experiment was established on typical brown Luvisols developed from light loam underlain by medium loam, quality class IIIb, suitable for wheat production. Soil samples were collected before the experiment to determine nutrient concentrations. Plant-available phosphorus and potassium were extracted with calcium lactate acidified to pH 3.6 (Egner-Rhiem method). The content of potassium was determined with the BWB Flame Photometer, and phosphorus levels were measured with the AquaMate spectrophotometer (Thermo Electron Corporation). Magnesium was extracted with 0.01 M $CaCl_2$ solution and quantified with the AquaMate spectrophotometer. Total sulfur content was determined by nephelometry in soil digested with magnesium nitrate (AquaMate spectrophotometer). The pH of soil was determined by potentiometric titration in 1 mol KCl solution with a digital pH meter (Table 1).

In each year of the experiment, mineral fertilizers were applied at 10, 20, 30 S kg ha^{-1} (ammonium sulfate, 24% S + 21% N), 100 N kg ha^{-1}

Table 1

Chemical properties of the analyzed soil in 2007-2009

Year	S_{og}	P	K	Mg	pH 1 mol KCl
	available macronutrients (mg kg^{-1})				
2007	12.1	82.2	150.0	58.8	5.9
2008	10.8	97.4	158.9	111.1	5.7
2009	7.5	94.6	135.3	79.7	5.9

(34% ammonium nitrate), 26.5 P kg ha^{-1} (46% triple superphosphate) and 100 K kg ha^{-1} (60% potash salt). Fertilizers were applied directly before sowing and were incorporated mechanically into the soil to a depth of around 5 cm.

Winter wheat (*Triticum aestivum* L.) was the preceding crop in each year of the experiment. White mustard was sown on 14 May 2007, 15 April 2008 and 14 April 2009. The sowing was past the optimal seeding date due to high soil moisture content. One hundred certified seeds were sown per m^2. Plot size for harvest was 15 m^2. In each year of the experiment, weeds were controlled with 333 g ha^{-1} of metazachlor and 83 g ha^{-1} of quinmerac, applied after sowing. White mustard was protected with one neonicotinoid pesticide (acetamiprid at 24 g ha^{-1}) and two pyrethroid pesticides (lambda-cyhalothrin at 7.5 g ha^{-1}, and cypermethrin at 2.5 g ha^{-1}). Each year before harvest, white mustard plants were desiccated with diquat at 340 g ha^{-1}. The plants were harvested at full maturity in mid-August. Weather conditions in each year of the study are presented in Table 2.

The glucosinolate content of white mustard seeds (μM g^{-1} DM) was determined by gas chromatography on an Agilent 7890 system (GC column HP-5 15 m; carrier gas – hydrogen; internal reference standard: glucotropaeolin). The glucosinolate content of seeds was expressed on an air-dry weight basis (Raney, McGregor 1990, Michalski et al. 1995). The following glucosinolates were identified in white mustard seeds: progoitrin, napoleiferin, glucobrassicin, 4-hydroxyglucobrassicin (4-OH), sinalbin and sinigrin. The identified

Table 2

Weather conditions in experimental years

Month	Temperature (°C)			
	2007	2008	2009	1976-2005
March	6.5	4.6	4.6	3.7
April	10.9	8.9	12.0	8.3
May	16.2	14.3	14.2	14.1
June	19.2	18.8	15.8	16.9
July	19.3	19.8	19.6	18.7
August	18.9	18.9	19.4	17.9
Mean March-August	15.2	14.2	14.3	13.3
Month	rainfall (mm)			
	2007	2008	2009	1976-2005
March	48.8	33.0	48.3	31.7
April	2.7	87.1	30.9	30.5
May	50.3	37.3	67.6	51.3
June	69.2	36.5	141.7	59.5
July	92.4	65.6	134.2	78.9
August	52.8	94.0	53.5	61.7
Total March-August	316.2	353.5	476.2	313.6

glucosinolates were divided into groups based on their structure (Malarz et al. 2011, Doheny-Adams et al. 2017, Park et al. 2017). The results of the three-year experiment were analyzed with the use of Student's t-test and the least significant difference (LSD) procedure at α=0.05 in the Statistica 10 PL program.

RESULTS AND DISCUSSION

The interaction effects between the analyzed factors did not influence the content of individual glucosinolates in white mustard seeds (Table 3).

Table 3

Glucosinolate content (μM g^{-1} DM) of white mustard seeds (means for interaction effects in 2007-2009)

Cultivar	S dose (kg ha^{-1})	Glucosinolate content (μM g^{-1} DM)					
		aliphatic			indole		aromatic
		progoitrin	napoleiferin	sinigrin	glucobrassicin	4-OH glucobrassicin	sinalbin
Metex	0	2.800	0.033	1.000	0.200	0.533	124.27
	10	3.467	0.033	1.000	0.233	0.500	144.90
	20	4.033	0.067	1.000	0.333	0.567	133.43
	30	3.833	0.033	1.000	0.267	0.467	156.17
Nakielska	0	3.167	0.033	1.000	0.167	0.667	122.63
	10	3.367	0.067	1.000	0.267	0.333	156.20
	20	3.633	0.067	1.000	0.300	0.233	173.53
	30	3.800	0.033	1.000	0.233	0.467	158.63
Radena	0	2.967	0.067	1.000	0.233	0.533	145.23
	10	4.433	0.067	1.000	0.233	0.600	154.33
	20	4.467	0.033	1.000	0.200	0.600	147.17
	30	4.233	0.033	1.000	0.233	0.233	170.13
LSD, α=0.05		ns	ns	ns	ns	ns	ns

ns – not significant, DM – dry matter

The evaluated seeds were most abundant in sinalbin, which is typically found in *Sinapis alba.* The sinalbin concentration ranged from 122.63 to 173.53 μM g^{-1} DM. The glucosinolate content of white mustard seeds was affected mainly by weather conditions in each year of the experiment, and sinalbin and progoitrin levels were determined mostly by the sulfur fertilizer dose (Table 4). The highest concentrations of progoitrin and 4-OH glucobrassicin were noted in 2009, whereas the seeds harvested in 2007 were most abundant in glucobrassicin and sinalbin. In a study by Paszkiewicz-Jasińska (2005), the sinalbin content of white mustard seeds cv. Nakielska ranged from 75.8 μM g^{-1} to 147.1 μM g^{-1} DM and was influenced mainly by weather conditions, and not the nitrogen fertilizer dose (30 to 120 N kg ha^{-1}).

Table 4

Glucosinolate content (μM g^{-1} DM) of white mustard seeds (means for experimental factors)

Means for factors	Glucosinolate content (μM g^{-1} DM)					
	aliphatic			indole		aromatic
	progoitrin	napoleiferin	sinigrin	glucobras-sicin	4-OH gluco-brassicin	sinalbin
Cultivar						
Metex	3.533	0.042	1.000	0.258	0.517	139.7
Nakielska	3.492	0.050	1.000	0.242	0.425	152.7
Radena	4.025	0.050	1.000	0.225	0.492	154.2
LSD, α=0.05	0.357	ns	ns	ns	ns	ns
S dose (kg ha^{-1})						
0	2.978	0.044	1.000	0.200	0.578	130.7
10	3.756	0.056	1.000	0.244	0.478	151.8
20	4.044	0.056	1.000	0.278	0.467	151.4
30	3.956	0.033	1.000	0.244	0.389	161.6
LSD, α=0.05	0.412	ns	ns	ns	ns	18.64
Year						
2007	1.617	0.142	1.000	0.408	0.283	164.6
2008	5.600	0.000	1.000	0.100	0.442	158.2
2009	3.833	0.000	1.000	0.217	0.708	123.9
LSD, α=0.05	0.357	0.028	ns	0.067	0.220	16.15

ns – not significant, DM – dry matter

An increase in the sulfur fertilizer dose from 0 to 30 kg ha^{-1} increased the sinalbin content of seeds by 23.7% relative to the control treatment, whereas the progoitrin content did not increase significantly, but it was significantly higher than in control seeds (Table 4). Malarz et al. (2011) also reported an increase in progoitrin levels in the seeds of winter oilseed rape in response to sulfur fertilization. The enhancing effects of sulfur fertilizers on the accumulation of glucosinolates in the seeds of traditional and double--low cultivars of winter oilseed rape have been described by many authors (Horodyski, Krzywińska 1979, Wielebski, Wójtowicz 2003, 2004, Wielebski 2006), whereas a few studies have investigated the influence of sulfur fertilization on the glucosinolate content of white mustard seeds.

An analysis of the effects exerted by white mustard cultivars on glucosinolate levels revealed a significantly higher progoitrin concentration (4.025 μM g^{-1} DM) only in the seeds of cv. Radena, which is grown for green forage. Paszkiewicz-Jasińska (2005) demonstrated that variations in the progoitrin content of white mustard seeds cv. Nakielska resulted mainly from weather conditions, whereas varietal characteristics were a less influential factor.

The interaction effects between the experimental factors did not induce significant differences in the total glucosinolate content, the concentrations

Table 5

Total glucosinolate content (μM g^{-1} DM) of white mustard seeds and the proportions of different glucosinolate groups (means for interaction effects in 2007-2009)

Cultivar	S dose (kg ha^{-1})	Glucosinolate content (μM g^{-1} DM)				Proportion (%)		
		total	aromatic	aliphatic	indole	aromatic	aliphatic	indole
Metex	0	128.83	124.27	3.833	0.733	96.27	3.077	0.650
	10	150.13	144.90	4.500	0.733	96.43	3.060	0.510
	20	139.43	133.43	5.100	0.900	95.07	4.183	0.743
	30	161.77	156.17	4.867	0.733	96.56	2.980	0.460
Nakielska	0	127.67	122.63	4.200	0.833	96.16	3.180	0.660
	10	161.23	156.20	4.433	0.600	96.79	2.827	0.387
	20	178.77	173.53	4.700	0.533	96.96	2.733	0.303
	30	164.17	158.63	4.833	0.700	96.48	3.053	0.463
Radena	0	150.03	145.23	4.033	0.767	96.72	2.737	0.540
	10	160.67	154.33	5.500	0.833	96.03	3.430	0.537
	20	153.47	147.17	5.500	0.800	95.70	3.723	0.573
	30	175.87	170.13	5.267	0.467	96.72	3.017	0.267
LSD, α=0.05		ns	ns	ns	ns	ns	ns	ns

ns – not significant, DM – dry matter

of aromatic, aliphatic and indole glucosinolates, or the proportions of glucosinolate groups (Table 5). Aromatic glucosinolates, represented by sinalbin, had the highest percentage share of total glucosinolates. Regardless of the applied dose of sulfur fertilizer, the seeds of all white mustard cultivars were characterized by higher concentrations of aliphatic than indole glucosinolates. A varietal effect was noted only in the total content of aliphatic glucosinolates (Table 6), which was higher in the seeds of white mustard cv. Radena. The cultivars Metex and Nakielska did not differ significantly in the content of aliphatic glucosinolates.

Increasing sulfur fertilizer doses significantly modified the concentrations of different glucosinolate groups in white mustard seeds and their proportions in the total content of the identified glucosinolates (Table 6). The concentrations of aromatic (sinalbin) and aliphatic glucosinolates were significantly lower in white mustard seeds from the control treatment (without sulfur fertilization). The seeds from the treatment supplied with 20 S kg ha^{-1} were characterized by the highest content of aliphatic glucosinolates. Numerous studies have demonstrated that sulfur fertilization significantly increases the accumulation of aliphatic glucosinolates, which are regarded as more toxic than indole glucosinolates, in plants of the family *Brassicaceae* (Fismes et al. 2000, Wielebski 2006, 2011).

Weather conditions during the experiment had a varied effect on the proportions of different glucosinolate groups and the total glucosinolate content of white mustard seeds (Table 6). In 2009, the evaluated cultivars were

Table 6

Total glucosinolate content (μM g^{-1} DM) of white mustard seeds and the proportions of different glucosinolate groups (means for factors)

Means for factors	Glucosinolate content (μM g^{-1} DM)				Proportion (%)		
	total	aromatic	aliphatic	indole	aromatic	aliphatic	indole
Cultivar							
Metex	145.04	139.7	4.575	0.775	96.08	3.325	0.591
Nakielska	157.96	152.7	4.542	0.667	96.60	2.948	0.453
Radena	160.01	154.2	5.075	0.717	96.29	3.227	0.479
LSD α=0.05	ns	ns	0.364	ns	ns	ns	ns
S dose (kg ha^{-1})							
0	135.51	130.7	4.022	0.778	96.38	2.998	0.617
10	157.34	151.8	4.811	0.722	96.42	3.106	0.478
20	157.22	151.4	5.100	0.744	95.91	3.547	0.540
30	167.27	161.6	4.989	0.633	96.59	3.017	0.397
LSD, α=0.05	18.56	18.64	0.421	ns	ns	ns	ns
Year							
2007	168.07	164.6	2.758	0.692	97.90	1.667	0.432
2008	165.31	158.2	6.600	0.542	95.67	4.000	0.334
2009	129.63	123.6	4.833	0.925	95.41	3.832	0.757
LSD, α=0.05	16.07	16.15	0.364	0.197	0.625	0.451	0.189

ns – not significant, DM – dry matter

characterized by the highest concentrations of indole glucosinolates, but the lowest total glucosinolate content. The percentage of aliphatic glucosinolate in white mustard seeds was the lowest in 2007, when high air temperatures and high soil moisture levels were noted during the formation and maturation of siliques and seeds. These conditions supported the accumulation of aromatic glucosinolates, and the highest concentration of sinalbin was noted in 2007. In a study by Paszkiewicz-Jasińska (2005), weather conditions during the growing season also considerably affected the proportions of different glucosinolate groups in white mustard seeds.

CONCLUSIONS

1. White mustard seeds were most abundant in sinalbin, an aromatic glucosinolate. The sinalbin content of seeds varied in response to different sulfur fertilizer doses. An increase in the sinalbin concentration was observed already in response to a sulfur dose of 10 kg ha^{-1} relative to the control treatment.

2. Sulfur fertilization exerted a significant effect on glucosinolate levels in white mustard seeds. The total content of aliphatic glucosinolates was

significantly the lowest in the in the treatment where sulfur fertilization was not applied.

3. The content of aliphatic glucosinolates in white mustard seeds was significantly the highest in the treatment fertilized with sulfur at 20 kg ha^{-1}.

4. Weather conditions significantly influenced the total glucosinolate content and the proportions of the analyzed glucosinolate groups in white mustard seeds.

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