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SULPHUR NUTRITION AND ITS EFFECT ON YIELD AND OIL CONTENT OF OILSEED RAPE (*BRASSICA NAPUS* L.)

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Abstract

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The aim of the experiment was to study the importance of sulphur in oilseed rape (*Brassica napus* L.) nutrition as well as the effect of rising doses of sulphur in combination with nitrogen on yield, oiliness, oil production, nutrients content in seed and nutrients uptake by rapeseed. The plot–scale experiment was established in years 2013/14 and 2014/15 within the agricultural cooperative in Mojmírovce. There were four fertilization treatments on 600 m^2 experimental plots in three replications in this experiment. The first treatment was unfertilized control. Other three treatments were fertilized by the same nitrogen dose of 160 $kg.ha^{-1}$ and by increasing doses of sulphur. The second treatment was fertilized by a dose of 15 $kg.ha^{-1}$ S, the third by a dose of 40 $kg.ha^{-1}$ and a dose of 65 $kg.ha^{-1}$ S was applied at the fourth treatment. The highest average yield 3.96 $t.ha^{-1}$ was found when a dose of 40 $kg.ha^{-1}$ S was applied. The application of sulphur in a dose of 65 $kg.ha^{-1}$ was accompanied by a yield decrease by 11.4 % as compared to the treatment where a sulphur dose of 40 $kg.ha^{-1}$ was used. An average oil content of 45.1, 45.5, and 44.0 % was found in treatments in which the doses of sulphur of 15, 40 and 65 $kg.ha^{-1}$ were applied. No significant difference among the treatments fertilized by sulphur was found. The average oil production reached 1809, 1802 and 1595 $kg.ha^{-1}$ in cases of treatments fertilized by sulphur doses of 15, 40 and 65 $kg.ha^{-1}$.

Keywords: oilseed rape, sulphur, yield, oil content, oil production, nutrients uptake, nutrients content

INTRODUCTION

Oilseed rape is the second most common oil crop in the world, after soybean, and the European Union is the biggest producer and consumer of the crop worldwide (López-Mosquera *et al.*, 2015). In terms of nutrient consumption, rape is ranked among very demanding crops. To produce 1 ton of seeds and appropriate amount of straw, oilseed rape takes up from the soil approximately 50 kg of nitrogen, 11 kg of phosphorus, 50 kg of potassium, 35 kg of calcium, 6 kg of magnesium, and 0.3 kg of boron (Fecenko and Ložek, 2000). Oilseed rape has also high demand of sulphur. Approximately 16 kg of sulphur is required to produce 1 ton of seeds (McGrath and Zhao, 1996). Oil crops respond to the application of sulphur, moreover, the sulphur requirement is the highest in oil crops in comparison with other crops, which is related to the role of this nutrient in oil biosynthesis (Ahmad *et al.*, 2007).

Sulphur is a component of plant amino acids, proteins, vitamins, and enzyme structures. It has been observed that increasing sulphur application increases oil, protein, and glucosinolates of canola seeds (Haneklaus *et al.*, 1999). Sulphur is necessary for the synthesis of oil and proteins, as well as the vegetative growth of the plant (Parker, 2009). Sulphur has been reported to influence productivity of oil seed and total oil content (Singh *et al.*, 1999; Jankowski *et al.*, 2008; Egesel *et al.*, 2009).

Because oilseed rape has a high demand for sulphur, it is particularly sensitive to sulphur deficiency compared to other crops such as cereals or legumes (Zhao *et al.*, 1997). Visual symptoms of sulphur deficiency in cruciferous crops are very specific and can be addressed in the field throughout the whole vegetation period (Pierre *et al.*, 1999). During flowering, characteristic changes of macroscopic sulphur deficiency are to be seen in colour and shape of the petals (Haneklaus *et al.*, 1999). Sulphur deficiency leads to the accumulation of amino acids, which is assumed to down-regulate nitrogen uptake and assimilation, while processes that increase the turnover of organic sulphur compounds and stress defence responses are induced. Severe sulphur deficiency can ultimately result in a reduced growth, which is particularly associated with a reduced shoot root ratio (Hawkesford and DeKok, 2006). Sulphur deficiency also causes a reduction in the quality and quantity of oilseed rape by 40 % (De Pascale *et al.*, 2008; Abdallah *et al.*, 2010; D'Hooghe *et al.*, 2013).

We focused on monitoring the effect of increasing doses of sulphur on the yield, oiliness, oil production, nutrients content in seed, and nutrients uptake by rapeseed.

MATERIALS AND METHODS

Characterization of the locality and treatments

The plot–scale experiments were established on 2 September 2013 and on 22 August 2014 in Mojmírovce (48°12′25.9″N 18°03′50.4″E) near Nitra (Slovakia). The block method of experimental plots with a plot size of 600 m^2 in three treatments was used. Hybrid Artoga was sown. Quantity of seeds was 0.45 million of germinable seeds per 1 ha. In both experimental years, the previous crop was winter wheat (*Triticum aestivum* L.). Mojmírovce is a corn growing region situated at an altitude of 140 m a. s. l. The climate is very warm, dry with mild winters. The average annual temperature during the growing season is 11.9 °C. Average annual rainfall is 436.7 mm. More detailed characteristics of the climatic conditions are displayed in Tab. I, II.

The Luvic Chernozem (black-coloured soil) is the predominant soil type. The doses of nutrients were determined according to the results of the soil analysis. The agrochemical soil properties are displayed in Tab. III.

The effect of sulphur doses on the yield, oil content, oil production, nutrients content in seed, and nutrients uptake by rapeseed was studied in a plot-scale experiment. The experiment was comprised of four fertilization treatments. The first variant was the unfertilized control. Three other treatments were fertilized by the same dose of nitrogen 160 kg.ha^{-1} and by increasing doses of sulphur. The second treatment was fertilized by a dose of 15 kg.ha^{-1} S, the third by a dose of 40 kg.ha^{-1} and a dose of 65 kg.ha^{-1} S was applied in the fourth treatment. Nitrogen was applied in the form of dolomite–ammonium nitrate (DAN, 27 % N) and ammonium nitrate and ammonium sulphate (ANAS 26 % N, 13 % S) in the growth stage BBCH 20 in treatment 2. ANAS was also applied in the growth stage BBCH 20 at treatment 3, 4. Nitrogen in the form of urea ammonium nitrate (UAN, 39 % N) was used in the growth stage BBCH 30 at treatments 2 and 3. The fourth treatment was fertilized by ANAS in the growth stage BBCH 30. All treatments were fertilized by UAN in the growth stage BBCH 51. The doses of nitrogen and sulphur are displayed in Tab. IV.

Soil analyses were performed by routine analytical methods. The effect of treatments of sulphur nutrition on the yield, oil content, oil production,

I: *The average monthly precipitation in experimental years in Mojmírovce (the evaluation of monthly precipitation normality according to the long-term average of 1982–2013)*

Month	Long--term average	2013		2014		2015	
		Precipitation (mm)	Evaluation of normality	Precipitation (mm)	Evaluation of normality	Precipitation (mm)	Evaluation of normality
I.	32.9	67.3	very wet	38.2	normal	82.0	extraordinary wet
II.	29.2	70.1	very wet	39.5	normal	18.5	normal
III.	31.9	71.0	very wet	19.5	normal	31.5	normal
IV.	36.9	45.5	normal	51.5	wet	19.5	dry
V.	60.5	104.2	wet	84.7	wet	74.5	normal
VI.	59.0	21.5	very dry	34.6	dry	8.0	extraordinary dry
VII.	55.3	0.0	extraordinary dry	56.2	normal	19.0	very dry
VIII.	48.7	56.5	normal	116.1	extraordinary wet	74.4	wet
IX.	46.1	59.5	normal	107.2	very wet	63.5	normal
X.	35.9	31.4	normal	38.0	normal	-	-
XI.	45.4	89.5	very wet	21.5	dry	-	-
XII.	42.3	8.5	very dry	67.5	wet	-	-

II: *The average monthly temperatures in experimental years in Mojmírovce (the evaluation of monthly air temperature normality according to the long-term average of 1982–2013)*

Month	Long--term average	2013		2014		2015	
		Temperature (°C)	Evaluation of normality	Temperature (°C)	Evaluation of normality	Temperature (°C)	Evaluation of normality
I.	0.9	–0.7	normal	–0.5	normal	–0.6	normal
II.	0.5	2.3	normal	2.5	normal	–0.6	cold
III.	5.0	3.6	normal	3.6	normal	2.5	cold
IV.	10.9	11.7	normal	7.6	very cold	4.2	extraordinary cold
V.	15.9	17.2	normal	11.2	extraordinary cold	10.2	extraordinary cold
VI.	18.7	20.7	warm	14.2	extraordinary cold	14.9	extraordinary cold
VII.	20.9	23.6	extraordinary warm	17.2	extraordinary cold	17.4	extraordinary cold
VIII.	20.5	23.9	extraordinary warm	16.2	extraordinary cold	18.2	cold
IX.	15.6	17.5	warm	12.8	very cold	13.1	cold
X.	10.3	13.7	extraordinary warm	9.3	normal	-	-
XI.	4.8	7.0	very warm	5.5	normal	-	-
XII.	0.3	3.4	very warm	0.6	normal	-	-

III: *Agrochemical soil properties before the experiment (0–0.3 m)*

Type of soil analysis	Content of available nutrients ($mg.kg^{-1}$)	
	2013/14	2014/15
N_{an}–N_{min} = mineral nitrogen = N-NH_4^+ and N-NO_3^-	11.4	7.0
N–NH_4^+ (colorimetry, Nessler reagent)	4.8	3.8
N–NO_3^- (colorimetry, phenol acid 2.4-disulphonic)	6.6	3.2
P–available (Mehlich III–colorimetry)	18	28
K–available (Mehlich III–flame photometry)	165	233
Mg–available (Mehlich III–AAS)	393	353
Ca–available (Mehlich III–flame photometry)	5450	2170
S (ammonium acetate solution)	2.5	1.3
pH/KCl–exchangeable reaction (0.2 $mol.dm^{-3}$ KCl)	6.6	6.8

IV: *Treatments of oilseed rape nutrition*

Treatment	Fertilization level					The total dose of N ($kg.ha^{-1}$)	The total dose of S ($kg.ha^{-1}$)
	BBCH 20		BBCH 30		BBCH 51		
	N ($kg.ha^{-1}$)	S ($kg.ha^{-1}$)	N ($kg.ha^{-1}$)	S ($kg.ha^{-1}$)	N ($kg.ha^{-1}$)	Σ N ($kg.ha^{-1}$)	Σ S ($kg.ha^{-1}$)
1	0	0	0	0	0	0	0
2	80	15	50	0	30	160	15
3	80	40	50	0	30	160	40
4	80	40	50	25	30	160	65

and nutrients content in seed and nutrients uptake by rapeseed was monitored after harvesting. The harvest was carried out on 25 June, 2014 and on 7 July, 2015 by a harvester Claas Lexion 770.

The nitrogen content was determined by the Kjeldahl method, phosphorus by colorimetry, potassium by flame photometry, calcium and magnesium by AAS, and sulphur by colorimetry. Determination of phosphorus, potassium, calcium, magnesium, and sulphur was preceded by wet digestion of plant material, i.e. by using of concentrated nitric acid as an oxidizing agent. The surplus of nitric acid was removed by evaporation with perchloric acid. The ratio of the nitric and the perchloric acid was 2:1 (Koppová *et al.* (1955). The determination of the oil content was performed according to the standard STN 4610111-28. The determination was realized by the extraction for assistance to petroleum ether (50/70). The apparatus DET-GRAS N (P Selecta) was used for this determination. A superfluous extractant was distilled after the extraction. The obtained oil was drained and weighed. The following formula was used for the calculation of the oil content in rapeseed:

$$W = (m_1/m_2) * 100$$

m_1 = the amount of extracted oil (g)
m_2 = mass of the test sample (g)

The achievable yields (at 12 % moisture) and the oil content were evaluated statistically by the analysis of variance. The differences among treatments were analysed by the LSD test in the program Statgraphics Plus 5.1.

RESULTS AND DISCUSSION

Yield of oilseed rape

A lot of studies were focused on the optimization of nutrition of oilseed rape. The results of several of those confirm that the highest seed yield was reached at treatments where nitrogen was applied in combination with sulphur (Mansoori, 2012; Jackson, 2000). However, the researchers' opinion on the dose of sulphur is not unanimous. Wielebski (2008) found significantly higher yield after application dose of sulphur 10–30 kg.ha^{-1}. Several experiments proved that the seed yield increased at 40 kg.ha^{-1} of sulphur (Vaseghi *et al.*, 2013; Sattar *et al.*, 2011). Similar results were found in Mojmírovce. The highest average yield of 3.96 t.ha^{-1} was achieved in treatment 3 where the dose of sulphur 40 kg.ha^{-1} was used (Tab. V, VI). The lowest average yield of 2.38 t.ha^{-1} was observed in the unfertilized control treatment. It means a decrease by 66.4 % in comparison to treatment 3. There is a highly significant difference among these treatments. Among treatments fertilized by sulphur, there is a significant difference between treatment 2 and treatment 4 where the highest dose of sulphur (65 kg.ha^{-1}) was applied. The average yield in the experimental year 2013/14 was 4.66 t.ha^{-1} and the average yield in year 2014/15 was 2.31 t.ha^{-1}. The yield of seed was greatly influenced by the climatic conditions. Both experimental years were not equable in precipitation and temperatures. While during the experimental year 2013/14 there were optimal climatic conditions for achieving high yield, the climatic conditions of the experimental year 2014/15 were not as favourable. From the perspective of the achieved yield, there was a highly significant difference between the two experimental years.

V: *Effect of sulphur fertilization on rapeseed yield*

Treatment	Yield (t.ha^{-1})			
	2013/14	2014/15	Average	Relatively (%)
1	3.41	1.35	2.38 aA	100.0
2	5.20	2.66	3.93 bcB	165.1
3	5.24	2.69	3.96 cB	166.4
4	4.80	2.53	3.67 bB	153.8
LSD $_{0,05}$			0.29	
LSD $_{0,01}$			0.40	

Averages indicated by different letters are statistically significantly different on the significance level of $\alpha \leq 0.05$ (small letters) and $\alpha \leq 0.01$ (capital letters).

VI: *Yield of oilseed rape in experimental years*

Year	Yield (t.ha^{-1})	LSD test$_{0.05}$	LSD test$_{0.01}$
2013/14	4.66 bB	0.20	0.28
2014/15	2.31 aA		

Averages indicated by different letters are statistically significantly different on the significance level of $\alpha \leq 0.05$ (small letters) and $\alpha \leq 0.01$ (capital letters).

The results of other experiments proved that the highest seed yield (2,18 t.ha^{-1}) was obtained with the application of 100 kg.ha^{-1} S, in the experiment realized by Mirzashahi *et al.* (2010). It is in accordance with Jackson (2000).

Oil content

The most important factor of oilseed rape quality is its oil content that is in direct proportion to its protein content (Brennan *et al.* 2000). Protein content is in negative correlation with oil content, so the effect of sulphur on the oil content of winter rape seeds is ambiguous (Krauze and Bowszys, 2000). Ahmad *et al.* (2007) used a dose of 0, 10, 20, and 30 kg.ha^{-1} S. The oil content was significantly increased with the increasing doses of sulphur to 20 kg.ha^{-1}. The higher dose of sulphur had no significant effect on the oil content. Conversely, Subhani *et al.* (2003) reports, that the oil content is directly proportional to the doses of sulphur. In further experiments, the most effective dosage of sulphur in regard to the yield and oil content was 40 kg.ha^{-1} (Rehman *et al.*, 2013; Ahmad *et al.*, 2011). In other experiments, the optimal dose of sulphur was 60 kg.ha^{-1}. A beneficial effect of sulphur fertilization, namely the dose of 60 kg.ha^{-1}, on the oil content was also reported by Malarz *et al.* (2011). However, a higher dose of sulphur is not recommended. On the contrary, Walker and Booth (2003) state that in contrast to the yield, the oil content is not dependent on the dose of sulphur. This has been confirmed by the results of the experiment based in Mojmírovce in the experimental years 2013/14 and 2014/15 (Tab. VII, VIII). The lowest average oil content of 44.1 % was found at treatment 4, where the highest dose of sulphur (65 kg.ha^{-1}) was used. It means a decrease by 2.9 % in comparison to treatment 1. The highest average oil content of 45.5 % was reached at treatment 3 where the sulphur dose of 40 kg.ha^{-1} was applied. It means an increase by 0.4 % compared to the oil content of the unfertilized control treatment. Although there is some increase, no statistical difference among all treatment of the experiment and also between experimental years 2013/14 and 2014/15 was found.

Oil production

The oil production is often studied in experiments. The oil production is closely correlated with the yield and oil content. According to Fábry (1992), the oil production per hectare increases in parallel with the increasing yield of seeds. Orlovius (2003) reports increase in both the yield and the oil production per hectare as a result of sulphur fertilization in several experiments. In experiments where sulphur was not applied, the yield did not increase and the oil production was not affected. Also Richter and Hřivna (1999) found the highest oil production of 1650 kg.ha^{-1} at treatment fertilized by nitrogen and sulphur. The yield of this treatment was 3.74 t.ha^{-1} and the oil content was 44.2 %. This is in accordance with the findings by Sýkora (2006). The results of his experiments confirmed that compared to the treatment fertilized by calcium ammonium nitrate, there is an increase of oil content by 4.4 % and also oil production by 2.7 %, at treatment where ammonium nitrate and ammonium sulphate was applied. Similarly, the highest average oil production of 1809 kg.ha^{-1} was reached at treatment 2 fertilized by the lowest dose of 15 kg.ha^{-1} (Tab. IX, X). It means an increase by 64.4 % compared to the control treatment 1. Among the treatments fertilized by sulphur, the lowest average oil production of 1595 kg.ha^{-1} was observed at treatment 4 where the highest dose of sulphur was applied. A significant difference among treatments 1, 2, and 4 was found. On the contrary, Blake-Kalff *et al.* (1998) did not find any effect of

VII: *Effect of sulphur fertilization on oil content in seeds of oilseed rape*

Treatment	Oil content (%)			
	2013/14	2014/15	Average	Relatively (%)
1	47.4	43.3	45.4 aA	100.0
2	46.6	43.5	45.1 aA	99.3
3	45.3	45.8	45.5 aA	100.4
4	42.3	45.9	44.1 aA	97.1
$LSD_{0,05}$			2.88	
$LSD_{0,01}$			4.06	

Averages indicated by different letters are statistically significantly different on the significance level of α ≤ 0.05 (small letters) and α ≤ 0.01 (capital letters).

VIII: *Oil content of oilseed rape in experimental years*

Year	Oil content (%)	LSD $test_{0.05}$	LSD $test_{0.01}$
2013/14	45.4 aA	2.03	2.87
2014/15	44.6 aA		

Averages indicated by different letters are statistically significantly different on the significance level of α ≤ 0.05 (small letters) and α ≤ 0.01 (capital letters).

IX: *Effect of sulphur fertilization on oil production of oilseed rape*

Treatment	Oil production (kg.ha^{-1})			
	2013/14	2014/15	Average	Relatively (%)
1	1617	584	1100 aA	100.0
2	2420	1198	1809 cB	164.4
3	2372	1232	1802 cB	163.8
4	2030	1159	1595 bB	144.9
LSD $_{0,05}$			153.03	
LSD $_{0,01}$			215.94	

Averages indicated by different letters are statistically significantly different on the significance level of $\alpha \leq 0.05$ (small letters) and $\alpha \leq 0.01$ (capital letters).

X: *Oil production of oilseed rape seed in experimental years*

Year	Oil production (kg.ha^{-1})	LSD test $_{0.05}$	LSD test $_{0.01}$
2013/14	2110 bB	108.2	152.7
2014/15	1043 aA		

Averages indicated by different letters are statistically significantly different on the significance level of $\alpha \leq 0.05$ (small letters) and $\alpha \leq 0.01$ (capital letters).

the dose of sulphur on the yield of seeds and oil production of oilseed rape. The oil production was affected by climatic conditions. A negative effect of climatic conditions in experimental year 2014/15 was observed. So as far as the oil production is concerned, a highly statistically difference between experimental years was found.

Nutrients content in seed and uptake by rapeseed

Numerous authors have published data in regard to the optimum nutrient concentrations of different plant parts obtained by a plant analysis. The results of the experiment held in Mojmírovce in experimental years 2013/14 and 2014/15 indicate that the highest average nutrients content was in the seed of the control treatment 1 (Tab. XI.). Also, the highest average sulphur content of 0.24 % of dry matter was reached at unfertilized control treatment. The lowest average sulphur content of 0.18 % of dry matter was found at treatment 3 where the highest average yield was reached.

Oilseed rape has a high nutrients uptake capacity. Efficiency of its transport system is higher than that of other crops (Šrojtová, 2002). A significant part of nutrients removed from soil is returned in the form of crop residues. The organic matter is returned by fall of leaves and ploughing of crop residues. Due to this fact, oilseed rape belongs to crop that contribute to positive balance of organic matter. The results of the experiment in experimental years 2013/14 and 2014/15 in Mojmírovce are displayed in Tab. XII. The highest average sulphur uptake of 2.08 kg per 1 t of seed was found at the unfertilized control treatment. The lowest average sulphur uptake of 1.60 kg per 1 t of seed was observed at treatment 3 where the highest yield was reached. Overall, the highest average nutrients uptake by seed was recorded at the unfertilized control.

XI: *Content of nutrients in rapeseed*

Treatment	Nutrients content in rapeseed in % of dry matter					
	N	P	K	Ca	Mg	S
1	3.75	0.530	0.672	0.214	0.295	0.238
2	3.29	0.544	0.671	0.165	0.292	0.212
3	3.25	0.505	0.675	0.163	0.266	0.182
4	3.27	0.544	0.697	0.174	0.282	0.213
Relatively (%)						
1	100.0	100.0	100.0	100.0	100.0	100.0
2	87.9	104.8	99.9	77.1	99.0	89.1
3	86.8	97.2	100.6	76.2	90.3	76.6
4	87.2	104.8	103.7	81.0	95.7	89.7

XII: *Uptake of nutrients by rapeseed*

Treatment	Nutrients uptake in kg per 1 t of rapeseed					
	N	P	K	Ca	Mg	S
1	32.98	4.58	5.90	1.90	2.58	2.08
2	28.98	4.75	5.90	1.45	2.58	1.85
3	28.63	4.45	5.93	1.43	2.35	1.60
4	28.75	4.78	6.15	1.53	2.48	1.88
	Relatively in %					
1	100.0	100.0	100.0	100.0	100.0	100.0
2	87.9	103.7	100.0	76.3	100.0	88.9
3	86.8	97.2	100.5	75.3	91.1	76.9
4	87.1	104.4	104.2	80.5	96.1	90.4

CONCLUSION

Effect of sulphur fertilization on the yield, oil content, oil production, nutrients content in seed, and nutrients uptake by rapeseed was monitored in an experiment established in the experimental years 2013/14 and 2014/15 within the agricultural cooperative in Mojmírovce. Regarding the yield, the highest average yield of 3.96 t.ha^{-1} was reached at the treatment where a medium dose of sulphur (40 kg.ha^{-1}) was applied. Among treatments fertilized by sulphur, the lowest average yield of 3.67 t.ha^{-1} was found at the treatment where the highest dose of sulphur was used. A strong effect of climatic conditions on the yield was confirmed in this experiment. The results of the experiment indicate that the highest dose of sulphur (65 kg.ha^{-1}) in combination with nitrogen can have a hindering effect on yield.

The oil content reached 45.1 %, 45.5 %, and 44.1 % at treatments where the doses of 15, 40 and 65 kg.ha^{-1} were used. The difference among all treatments is not statistically significant. Based on this, it can be concluded that the oil production is closely dependent on the yield of seeds.

As for nutrients content and nutrients uptake, there was not any marked difference among treatments of fertilization.

The results of the experiment indicate that the sulphur dose of 40 kg.ha^{-1} in combination with nitrogen was the most effective from the perspective of the yield and the oil content in seed of oilseed rape. However, the the difference between effect of 40 kg of sulphur and 15 kg is almost negligible.

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