ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LIX 2 Number 5, 2011

THE EFFECT OF NITROGEN AND SUPLHUR FERTILIZATION ON THE YIELD AND CONTENT OF SULFORAPHANE AND NITRATES IN CAULIFLOWER

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Received: March 31, 2011

Abstract

ČEKEY, N., ŠLOSÁR, M., UHER, A., BALOGH, Z., VALŠÍKOVÁ, M., LOŠÁK, T.: *The effect of nitrogen and sulphur fertilization on the yield and content of sulforaphane and nitrates in cauliflower.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 5, pp. 17–22

In the field experiment with cauliflower, we investigated the effect of four different variants of nitrogen and suplhur fertilization on quantity and quality of cauliflower in the term of sulforaphane content and nitrate accumulation.
The influence of fertilization was statistically significant between control variant and fertilization variants and in both experimental years within all parameters of cauliflower yield.
The highest yield of cauliflower was reached at the variant 4 when it was fertilized on the level of nutrients N:S = 250:60 $kg.ha^{-1}$. The increase of yield against control variant represented value 26.6%.
The applied fertilization positively affected on the accumulation sulforaphane in the cauliflower. Its highest content was determined at the variant 4 (N:S = 250:60 $kg.ha^{-1}$). In comparison with control variant, the sulforaphane content was increased about 18.4%. On the other side, applied nutrition resulted in increased accumulation of nitrates in the cauliflower. The most increase of nitrate content, compared to the control variant, was also ascertained at the variant 4 (about 31.4%).
The gathered data point towards to the possibility and way how we could effect on the increased accumulation of sulforaphane in cauliflower florets. This sphere of fertilization effect on the sulforaphane content is not sufficiently explored well. Our aim is to continue in this research subject and to find way how to cultivate vegetables with higher content of health-promoting compounds.

cauliflower, fertilization, nitrogen, sulphur, yield, sulforaphane, nitrates

The vegetables and fruits has got very important role in human nutrition and their health benefit is undoubted (Rope *et al.,* 2010). Nevertheless, we can observe a deficient level of annual vegetables and fruits consumption in this time. According to human nutrition specialists, recommended consumption of vegetable is on the level of 128 kilograms. But in Slovakia, we are deeply under this value – 85 kilograms (Valšíková and Uher, 2009). This fact is also confirmed by Kubicová and Fatrcová-Šramková (2010) who appeal to very low consumption of vegetables, especially by young people.

In comparison with other agricultural crops, vegetables require relatively high levels of nutrients for cultivation. It is well known that nitrogen nutrition markedly affects a quantity and quality of grown crops. But there is also very important not to forget to apply other macroelements (Fecenko and Ložek, 2000). Nitrogen and suplhur, as well as phosphorus and potassium, are determining nutrients for growing of broccoli and other *brassica* vegetables in relation to quality and quantity of harvested phytomass (Goodlass *et al.,* 1997; Lošák *et al.,* 2008).

A cruciferous vegetable contains sulphur compounds – glucosinolates in relatively high concentration. Glucosinolates content varies in dependence on species and cultivar (Sivakumar *et al.,* 2007), as well as different edible part of brassica vegetables (Kushad *et al.,* 1999). Broccoli, brassica group, contains sulforaphane in higher rate. It prevents against bacteria Helicobacter pylori which are responsible for stomach cancer (Fahey *et al.,* 2002; Hollósy, 2004).

Nitrates are present naturally in soils, water, plants – particularly in vegetables as a consequence of nitrogen uptake. The natural levels of nitrate in vegetables generally are high in comparison with other crops. It is estimated that 75–80% of the total daily intake comes from vegetables. Nitrate content varies considerably according to species (Mor *et al.,* 2010). Nitrate contamination in vegetables occurs when crops take up more nitrogen than they require for their sustainable growth. Spinach, lettuce, broccoli, radish, etc., possess the tendency to accumulate nitrates (Greenwood and Hunt, 2006). Nitrate is largely unreactive but can be reduced to nitrite. Nitrite ion can react with secondary or tertiary amines to form nitrosocompounds – some of elements in the etiology of cancers (Sebecic and Dragojevic, 1999). Another fact is that vegetables are an important part of most babies diets (Huarte-Mendicoa *et al.,* 1997). Young babies with low stomach acidity may suffer from infantile methemoglobinemia due to excessive nitrates in their diet where nitrite is substituted for oxygen in hemoglobin and death may occur (Ezeagu, 1996).

The objective of the research was to determine the effect of nitrogen and suplhur fertilization on the quantity and quality of cauliflower. Nutrition and fertilization are fundamental part of cultivation. Sulforaphane is very important health-promoting compound. Thus, we should search possibilities how we can positively affect on the increased accumulation of sulforaphane. But, there is also necessary to observe effect of nitrogen fertilization in respect of nitrates content.

MATERIAL AND METHODS

The small-plot field trial with cauliflower was established in the area of The Botanical garden of Slovak Agricultural University in Nitra in 2008 and 2009. In this trial, we used a middle-late cauliflower cultivar FLAMENCO F1.

In the field trial, we monitored the effect of four different fertilization variants on the quantity and quality of cauliflower yield:

1. variant – untreated control (without application of fertilizers),
2. variant – application of nitrogen at the level N = 200 kg.ha^{-1},
3. variant – application of nitrogen and suplhur at the level N:S = 250:50 kg.ha^{-1},
4. variant – application of nitrogen and suplhur at the level N:S = 250:60 kg.ha^{-1}.

Cauliflower seedlings were planted out at the experimental area on 24th June 2008 and 22nd June 2009. Each variant had four repeats. In every repeat, we planted nine seedlings into the plating space 0.5 × 0.5 m. The nutriments (nitrogen and sulphur) were applied at the level of particular variants on the basis of agrochemical soil analysis (Tab. I). At fertilization variants 2, we applied fertilizer LAD 27 (13.5% of ammonium nitrogen, 13.5% of nitrate nitrogen, 4.1% of MgO). At the variants 3 and 4, there were applied LAD 27 and DASA 26/13 (18.5% of ammonium nitrogen, 7.5% of nitrate nitrogen, 13% of sulphate sulphur). The fertilizer DASA 26/13 was applied three weeks before outplanting. The calculated dose of LAD 27 was applied in two terms - three (50%) and six weeks (50%) after planting.

The harvest of cauliflower was realized from 2nd September to 9th September 2008 and from 28th August to 10th September 2009. The content of monitored qualitative compounds was determined in average sample which was prepared from cauliflower harvested on 5th September 2008 and 2nd September 2009. The sulforaphane content was analyzed chromatographically (HPLC) according to slightly modified method by Sivakumar *et al.* (2007). The nitrate content was determined by nitrate electrode screening method.

Obtained results were evaluated by analysis of variance (ANOVA). Tukey tests were performed on confidence level 95%.

RESULTS AND DISCUSSION

Aplicating variation analysis (Tab. II, III and IV) on obtained results, we learnt statistically significant differences between control variant and fertilization variants and experimental years within all parameters of cauliflower yield. No significant differences were experienced among repeatings.

The cauliflower yield fluctuated in the range from 56.96 t.ha^{-1} to 72.13 t.ha^{-1} (Tab. V). The highest yield of cauliflower was attained at the variant 4 (72.13 t.ha^{-1}). This value, in comparison with control variant (56.96 t.ha^{-1}), introduced the statistically high-significant increase of cauliflower yield about 26.6%. At other variants, there was also showed

I: *Agrochemical characteristics of soil before the trial establishment at the depth of 0–0.30 m*

Year	pH/KCl	Content of nutrients in mg.kg^{-1}						Humus (%)
		N_{min}	P	K	S	Ca	Mg	
2008	7.13	16.8	130	570	67.5	6300	695	3.42
2009	7.20	10.5	200	580	34.0	6700	660	4.38

II: *Analysis of variance for Yield (t.ha⁻¹)*

Source of Variability	Sum of Squares	Degree of Freedom	Mean Squeres	F-ratio	P-value
Main Effects					
A: Years	2 354.35	1	2 354.35	1 086.50	0.0000*
B: Variants	1 091.90	3	363.97	167.97	0.0000*
C: Treatments	12.44	3	4.15	1.91	0.1980
Interactions					
A x B	25.77	3	8.59	3.96	0.0470*
A x C	8.41	3	2.80	1.29	0.3351
B x C	2.81	9	0.31	0.31	0.9960
Rezidual	19.50	9	2.17		
Total	3 515.18	31			

* P-value is statistically significant (P<0.05)

III: *Analysis of variance for sulforafan (mg.kg⁻¹ of fresh matter)*

Source of Variability	Sum of Squares	Degree of Freedom	Mean Squeres	F-ratio	P-value
Main Effects					
A: Years	10.35	1	10.35	1279.69	0.0000*
B: Variants	0.95	3	0.32	39.12	0.0000*
C: Treatments	0.01	3	0.01	0.58	0.6433
Interactions					
A x B	0.08	3	0.026	3.22	0.0753
A x C	0.03	3	0.01	1.35	0.3194
B x C	0.13	9	0.01	1.76	0.2071
Rezidual	0.07	9	0.01		
Total	11.62	31			

* P-value is statistically significant (P<0.05)

IV: *Analysis of variance for nitrates (mg.kg⁻¹ of fresh matter)*

Source of Variability	Sum of Squares	Degree of Freedom	Mean Squeres	F-ratio	P-value
Main Effects					
A: Years	13 427.50	1	13 427.50	26.92	0.0006*
B: Variants	79 828.00	3	26 609.33	53.35	0.0000*
C: Treatments	3 532.96	3	1 177.65	2.36	0.1393
Interactions					
A x B	610.76	3	203.59	0.41	0.7510
A x C	3 106.16	3	1 035.39	2.08	0.1738
B x C	2 277.60	9	253.07	0.51	0.8367
Rezidual	4 489.01	9	498.78		
Total	107 271.99	31			

* P-value is statistically significant (P<0.05)

statistically significant yield of cauliflower florets about 15.6% (variant 2) and 23.2% (variant 3). We experienced found significant difference of cauliflower yield also between experimental years (Tab. VI). The highest cauliflower yield was gained in 2009 – 74.86 t.ha⁻¹.

Thus, obtained results of our research confirmed fact that applied nitrogen fertilization has a fundamental effect on the yield of grown vegetable as it was demonstrated in previous research works by Biesada and Kolota (2008), Ahmadil *et al.* (2010) and Varga *et al.* (2004).

The content of sulforaphane in cauliflower florets varied in range from 2.50 mg.kg⁻¹ to 2.96 mg.kg⁻¹ of fresh mater and it increased in following variants order: 1 (control) < 2 < 3 < 4. The highest sulforaphane content was ascertained at the variant 4 (2.96 mg.kg⁻¹). It is statistically significant increase of sulforaphane content, compared to the control variant (2.50 mg.kg⁻¹) about 18.4%. At other variants with applied nutrition, we also reached

V: *Yield of cauliflower and content of sulforaphane and nitrates in cauliflower florets in dependence of fertilization variant (Tukey HSD, 95%)*

Variant	Yield (t.ha^{-1})		Sulforaphane (mg.kg^{-1})		Nitrates (mg.kg^{-1})	
	LS Mean	Homogeneous Groups	LS Mean	Homogeneous Groups	LS Mean	Homogeneous Groups
1	56.96	a	2.50	a	431.39	a
2	65.86	b	2.73	b	479.00	b
3	70.17	c	2.87	b c	531.11	b
4	72.13	c	2.96	c	566.84	c

Different letters between columns show statistically significant differences at the level α = 0.05

VI: *Yield of cauliflower and content of sulforaphane and nitrates in cauliflower florets in dependence of cultivation year (Tukey HSD, 95%)*

Year	Yield (t.ha^{-1})		Sulforaphane (mg.kg^{-1})		Nitrates (mg.kg^{-1})	
	LS Mean	Homogeneous Groups	LS Mean	Homogeneous Groups	LS Mean	Homogeneous Groups
2008	57.70	a	2.20	a	461.10	a
2009	74.86	b	3.33	b	527.07	b

Different letters between columns show statistically significant differences at the level α = 0.05

the significant increase of sulforaphane content in cauliflower (Tab. V). Thus, we learnt that applied fertilization tended to the increased accumulation of sulforaphane in cauliflower florets. We observed that higher sulforaphane content was found at the combined nitrogen-sulphur variants compared to the variant where nitrogen only was applied. We experienced statistically significant difference of sulforaphane content also between experimental years (Tab. VI). The highest sulforaphane content was observed in 2009 – 3.33 mg.kg^{-1}. The sphere of effect of fertilization is not sufficiently studied well and opinions are different about it. Aires *et al.* (2006) learnt that applied nitrogen and suplhur fertilization had detrimental effect of aliphatic glucosinolates (sulforaphane). On other side, Schonhof *et al.* (2007) state that it is possible to improve the level of aliphatic glucosinolates by optimalization of nitrogen and sulphur supply. De Pascale *et al.* (2007) also observed the positive effect of sulphur fertilization on the content of glucosinolates in research work with *Brassica rapa* L. subsp. *sylvestris*.

Cauliflower belongs to the brassica vegetable species which have tendency to accumulate nitrates in its edible parts. The content of nitrates ranged from 431.39 mg.kg^{-1} to 566.84 mg.kg^{-1} of fresh matter and it increased in following variants order: 1 (control) < 2 < 3 < 4 (Tab. V). The highest nitrate content was determined at the variant 4 (566.84 mg.kg^{-1}). At this variant, there was detected the statistically high-significant increase of nitrate content about 31.4% in comparison with control variant. We also observed the statistically significant accumulation of nitrates in cauliflower florets at variants 2 and 3. At these variants, the nitrates content was increased compared to the control variant about 11.0% and 23.1%. We also achieved statistically significant difference of nitrates content between experimental years (Tab. VI). The highest nitrate content was observed in 2009 – 527.07 mg.kg^{-1}. However, there was not exceeded the maximally highest acceptable amount of nitrates in cruciferous vegetable species (700 mg.kg^{-1} of fresh mater) according to Food codex of Slovak republic. Thus, obtained experimental results confirm fact that nitrate fertilization has cumulative effect on nitrate contents in edible parts of vegetable. This fact was showed in previous research works by Wang and Li (2004), Šlosár *et al.* (2009) and Ahmadil *et al.* (2010), who studied effect of nitrogen fertilization on the nitrates content in some vegetable species (broccoli, spinach, cabbage et al.).

SUMMARY

The objective of the research was to determine the quantity and quality yield of cauliflower in dependence on the nitrogen and sulphur fertilization. We learnt statistically significant differences among control variant and fertilization variants and both experimental years within all parameters of cauliflower yield.

The highest cauliflower yield (72.13 t.ha^{-1}) was reached at the combined nitrogen-sulphur variant where nutriments were fertilized at the level of N:S = 250:60 kg.ha^{-1}. It introduced the increase of cauliflower yield about 26.6% compared to the control variant. In the cauliflower quality, we focused on the effect of applied fertilization on the amount of sulforaphane and nitrates in cauliflower florets. The highest sulforaphane content was determined at the same nitrogen-sulphur variant (N:S = 250:60 kg.ha^{-1}). At this variant, we reached the increase of sulforaphane content about 18.4% compared to the control variant. The applied nitrogen fertilization tended to the increased accumulation of nitrates in cauliflower florets. The most increase of nitrate content was determined at the same variant as it

was in the term of sulforaphane content (N:S = 250:60 kg.ha^{-1}). There was detected the increase of nitrate content about 31.4% compared to the control-unfertilized variant. At all fertilized variants, there were not exceeded the maximally highest acceptable amount of nitrates in brassica vegetable species according to Food codex of Slovak republic (700 mg.kg^{-1} of fresh mater).

Findings of this study show that there is possible to effect on the sulforaphane content in cauliflower positively by applied fertilization. But we can not forget to be careful about nitrogen doses and not to exceed the nitrate content in cauliflower florets above admissible limit.

Acknowledgement

The study was supported by the Research plan No. MSM6215648905 "Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change", which is financed by the Ministry of Education, Youth and Sports of the Czech Republic.

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