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EFFECT OF FERTILIZATION ON THE CONTENT OF MACRONUTRIENTS IN FRUITS OF MILK THISTLE (*SILYBUM MARIANUM* L. GAERTN.)

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Abstract

Milk thistle was grown on a substrate with the texture of light soil, slightly acid in reaction and moderately abundant in available phosphorus, potassium and nitrogen. The effect of incremental nitrogen fertilization (0.0, 1.0, 2.0, 3.0 g N per pot) against the background of constant PK fertilization (P – 1.0, K – 1.5 g per pot) on the content of macronutrients in fruits of two forms of milk thistle, a cultivar called Silma and a population line bred in Poland, was tested in the first trial; the influence of boron fertilization was assessed in the second series and the impact of forms of magnesium fertilizers ($MgCl_2$ and $MgSO_4$) was analyzed in the third variant of the experiment. The variety-specific traits had a significant effect on the content of nitrogen, phosphorus and potassium, but did not affect the concentrations of calcium, magnesium and sodium. The content of nitrogen in achenes of the population plant was 26.38 g N kg^{-1} d.m., compared to 25.16 g N kg^{-1} d.m. determined for cv. Silma. Fruits of the population line also contained significantly more phosphorus and potassium (by app. 17%), whereas the levels of sodium, calcium and magnesium did not differ in a statistically significant way between the two types of the plant. The highest nitrogen (27.62 g kg^{-1} d.m.) and phosphorus (8.78 g kg^{-1} d.m.) concentrations were obtained by applying 2 g N per pot. Sulphur introduced to soil in the form of magnesium sulphate raised the content of nitrogen in achenes of both forms of milk thistle.

Key words: milk thistle, N doses, boron, magnesium fertilizers, macroelements.

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WPŁYW NAWOŻENIA NA ZAWARTOŚĆ MAKROELEMENTÓW W OWOCACH OSTROPESTU PLAMISTEGO (*SILYBUM MARIANUM* L. GAERTN.)

Abstrakt

Ostropest plamisty uprawiano na podłożu o składzie granulometrycznym gleby lekkiej, o odczynie lekko kwaśnym i średniej zasobności w przyswajalny fosfor, potas i magnez. W doświadczeniu pierwszym badano wpływ wzrastającego poziomu nawożenia azotem (0,0; 1,0; 2,0; 3,0 g N na wazon) stosowanego na tle stałego nawożenia PK (P – 1,0; K – 1,5 g na wazon), w drugim – boru, a w trzecim – formy nawozów magnezowych ($MgCl_2$ i $MgSO_4$) na zawartość makroelementów w owocach dwóch form ostropestu – odmiany Silma i populacji krajowej. Cechy odmianowe istotnie wpływały na zawartość azotu, fosforu i potasu, natomiast nie miały wpływu na koncentrację wapnia magnezu i sodu. Zawartość azotu w niełupkach roślin populacyjnych wynosiła 26,38 g N kg^{-1} s.m., a u odmiany Silma -25,16 g N kg^{-1} s.m. Owoce roślin populacyjnych zawierały również istotnie więcej fosforu i potasu (o ok. 17%), natomiast zawartości sodu, wapnia i magnezu nie różniły się statystycznie. Największą zawartość azotu (27,62 g N kg^{-1} s.m.) oraz fosforu (8,78 g P kg^{-1} s.m.) uzyskano stosując 2 g N na wazon. Siarka wprowadzona w postaci siarczanu magnezu wpłynęła na zwiększenie zawartości azotu w niełupkach obu form ostropestu.

Słowa kluczowe: ostropest plamisty, dawka azotu, bor, nawozy magnezowe, zawartość makroelementów.

INTRODUCTION

Milk thistle is a popular herbal plant. The medicinal substance silymarin is produced from seed shells, while defatted seeds become a by-product. In some countries, milt thistle sprouts or even whole new plants are consumed as a rich source of anti-oxidants (VAKNIN et al. 2008, HUSSAIN et al. 2010). A high content of protein (about 25%) indicates that defatted milk thistle seeds could be used for animal nutrition (POTKAŃSKI et al. 1991, ŁANGOWSKA et al. 2002, STOPYRA et al. 2006) or for production of functional food (BARANYK et al. 1995, SZCZUCIŃSKA et al. 2003).

Ever since animal meat and bone meals were banned from animal nutrition, new sources of fodder protein have been looked for. The use of genetically modified soybean and maize for production of animal feeds also raises many concerns. Defatted milk thistle seeds seem to be as good a good source of protein as canola meal. A small quantity of flavonolignands found in defatted seeds of milk thistle may – to some extent – replace antibiotics or growth hormones, whose use in animal feed is increasingly often prohibited (CYBULSKI, RADKO 2006, PASCHMA et al. 2010, URBAŃCZYK et al. 2002, SUCHY et al. 2008). Moreover, oil and some seed protein fractions possess the properties that resemble preservative agents used in the pharmaceutical and cosmetics industries (SZCZUCIŃSKA et al. 2003, 2006, 2007).

The total acreage of farmland dedicated to canola grown for diesel oil is limited to about 1 million ha because of certain ecological, organizational and economic considerations. It might be possible to fulfill the requirements on use of energy from renewable resources set by the Directive 2009/28/EC if we raised canola yields or promoted cultivation of other oil plants, for example milk thistle (Baranyk et al. 1995, Kozera, Nowak 2004, Andrzejewska, Sadowska 2007, Andrzejewska, Mielcarek 2011, Sadowska et al. 2011, Wierzbowska et al. 2012b), which has a short vegetative period and can be grown all over Poland. This plant does not require good farmland and can be cultivated on light soils, which are unsuitable for canola (Sadowska, Andrzejewska 2010).

The objective of this study has been to evaluate the effect of mineral fertilization on the content of macronutrients in milk thistle achenes.

METHODS

The research objective was achieved in three, two-factorial pot experiments, set up in a completely random design with four replications. Pots were filled with 10 kg of light soil, which was moderately rich in available forms of phosphorus (58 mg P kg^{-1}), potassium (97 mg K kg^{-1}) and magnesium (32 mg Mg kg^{-1}). The soil reaction was slightly acid (pH_{KCl} = 5.8).

The first experiment was established to investigate the effect of increasing doses of nitrogen (N_0–0.0, N_1–1.0, N_2–2.0, N_3–3.0 g N per pot in the form of urea), applied against the background of constant PK fertilization (1.0 g P as $CaHPO_4$ and 1.5 g K as KCl), on the content of macronutrients in milk thistle achenes. The second experiment focused on the influence of boron (5 mg B per pot in the form of H_3BO_3). The third series tested the effect of magnesium fertilizers (0.3 g Mg per pot in the form of $MgCl_2$ or $MgSO_4 \cdot 7H_2O$) applied together with the increasing nitrogen fertilization and constant PK nourishment. The PKMg fertilization as well as boron and 1 g of nitrogen (treatments N_1, N_2, N_3) were applied before sowing and the remaining part of nitrogen (treatments N_2, N_3) was supplied in a top-dressing treatment during the leaf rosette phase. Two forms of milk thistle were used in the experiments: a cultivar called Silma and a population line grown in Poland.

The plant material harvested during the technological maturity phase was ground and mineralized in concentrated sulphuric acid (IV) with added hydrogen dioxide as an oxidant. Total nitrogen was determined colorimetrically with the hypochlorite method (Baethen, Alley 1989); phosphorus was assayed using the vanadium molybdenum method, while potassium, calcium and sodium were determined with atomic emission spectrophotometry (AES) and the determination of magnesium was performed by atomic absorption spectrophotometry (AAS).

The results of chemical determinations were submitted to statistical analysis aided by a Statistica 10 software package. All statistical calculations were performed at the level of significance p=0.01. The Fisher's test was applied to verify the significance of differences.

RESULTS AND DISCUSSION

The content of mineral constituents in fruits of milk thistle depended on the variety-specific traits and fertilization (Tables 1-5). The characteristics of the two types of milk thistle significantly determined the content of nitrogen, phosphorus and potassium but had no effect on the concentration of the other elements (Tables 1 and 4). The average content of nitrogen in achenes of the population plant was 26.38 g N kg^{-1} d.m., thus being significantly higher than in achenes of cv. Silma (25.16 g N kg^{-1} d.m.). Fruits of the population plant also contained significantly more phosphorus and potassium (by about 17%) than cv. Silma, but there were no differences between the two types of milk thistle in the content of sodium, calcium and magnesium.

Table 1

Content of mineral components in milk thistle fruits depending on the variety

Variety	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma	25.16±0.68^{a}	8.41±0.15^{a}	6.42±0.15^{a}	0.33±0.01^{a}	9.69±0.39^{a}	3.68±0.06^{a}
Population	26.38±0.70^{b}	8.70±0.13^{b}	7.50±0.15^{b}	0.35±0.01^{a}	9.31±0.29^{a}	3.86±0.07^{a}

On average for the tested nitrogen fertilization doses, the highest content of nitrogen (27.62 g N kg^{-1} d.m.) and phosphorus (8.78 g P kg^{-1} d.m.) in milk thistle fruits was achieved when 2 g N per pot had been applied (Tables 2 and 4). Any further increase in the nitrogen level fertilization did not have significant influence on the content of nitrogen but decreased phosphorus in achenes of milk thistle. The different nitrogen fertilization levels did not have any significant effect on the content of potassium and magnesium in milk thistle fruits, whilst the content of calcium in achenes produced by plants fertilized with 3 g N per pot was over 21% less than in plants not fertilized with nitrogen. The analogous decline in the content of sodium was 14%.

The highest nitrogen concentration of nitrogen in achenes collected from the population line of milk thistle (28.11 g N kg^{-1} d.m.) was obtained after an application of 3 g N per pot; in the case of cv. Silma, the highest content of nitrogen in the fruits (28.13 g N kg^{-1} d.m.) was found in pots fertilized with a dose of 2 g (Tables 3 and 4). The highest dose of nitrogen depressed

Table 2

Content of mineral components in milk thistle fruits depending on nitrogen fertilization

N doses	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
N0	22.99±0.90^{b}	8.50±0.21^{a}	7.04±0.35^{a}	0.37±0.01^{c}	10.73±0.35^{c}	3.83±0.09^{a}
N1	25.51±0.79^{c}	8.61±0.15^{ab}	6.86±0.30^{a}	0.33±0.02^{ab}	10.18±0.38^{b}	3.78±0.10^{a}
N2	27.62±0.48^{a}	8.78±0.27^{b}	6.92±0.31^{a}	0.35±0.02^{bc}	8.68±0.22^{a}	3.76±0.11^{a}
N3	26.98±0.61^{a}	8.33±0.19^{a}	7.02±0.33^{a}	0.32±0.02^{a}	8.42±0.17*a*	3.73±0.10^{a}

Table 3

Content of mineral components in milk thistle fruits depending on the variety and level of nitrogen fertilization

N doses	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma						
N0	22.66±1.04^{c}	8.40±0.29^{ab}	6.43±0.29^{a}	0.34± 0.01^{b}	11.25±0.29^{c}	3.89±0.10^{ab}
N1	24.02±0.48^{d}	8.44±0.20^{ab}	6.59±0.42^{a}	0.35±0.03^{bc}	10.36±0.71*b*	3.60±0.10^{c}
N2	28.13±0.65^{b}	8.65±0.46^{ac}	6.38±0.32^{a}	0.31±0.01^{a}	8.76±0.22^{a}	3.66±0.11^{cd}
N3	25.85±0.45^{e}	8.18±0.32^{b}	6.35±0.20^{a}	0.30±0.03^{a}	8.38±0.22^{a}	3.59±0.15^{c}
Population						
N0	23.32±1.63^{cd}	8.60±0.33^{ac}	7.65±0.33^{b}	0.38±0.02^{cd}	10.20±0.50^{b}	3.77±0.17^{ad}
N1	26.99a±0.88^{a}	8.78±0.21^{ac}	7.20±0.37^{c}	0.31±0.02^{a}	10.00±0.37^{b}	3.96±0.11^{b}
N2	27.11±0.66^{ab}	8.95±0.32^{c}	7.46±0.30^{bc}	0.37±0.01^{d}	8.60±0.41^{a}	3.86±0.18^{ab}
N3	28.11±0.67^{ab}	8.49±0.20^{ab}	7.69±0.30^{b}	0.34±0.02^{b}	8.45±0.31^{a}	3.86±0.11^{ab}

the content of phosphorus in achenes of both forms of milk thistle. The level of nitrogen fertilization did not have any larger effect on the content of potassium and magnesium in fruits of both forms. The intermediate and high doses of nitrogen (N_2 and N_3) significantly decreased the content of calcium in both forms of milk thistle (by 22.1 and 25.5%, respectively, in cv. Silma and by 11.6 and 17.1% in the population line compared to the control).

The results of the analysis of variance, shown in Table 4, confirm that the variety-specific traits significantly differentiated the content of all analyzed macronutrients in achenes of milk thistle. The fertilization level affected the content of nitrogen, phosphorus, sodium and calcium but did not have any effect on the concentration of potassium and magnesium.

Table 4

Analysis of variance for the content of mineral components depending on the variety and nitrogen fertilization level

Components	Variety	N doses	Interaction
Nitrogen (N)	**	**	**
Phosphorus (P)	**	*	NS
Potasium(K)	**	NS	NS
Sodium (Na)	**	**	**
Calcium Ca)	**	**	*
Magnesium (Mg)	**	NS	**

* significant for $p \leq 0.05$; **significant for $p \leq 0.01$

High correlation coefficients prove that the content of nitrogen in achenes from both forms of milk thistle was significantly positively correlated with the dose of nitrogen (Table 5). Higher doses of nitrogen introduced as fertilizer significantly depressed the concentration of sodium, calcium and magnesium in cv. Silma fruits; they had the same effect on the content of calcium in achenes from the population line.

Table 5

Coefficient of correlation between the content of mineral components in milk thistle achenes versus the level of nitrogen fertilization

Components	Variety	
	Silma	population
Nitrogen (N)	0.664**	0.675**
Phosphorus (P)	-0.110	-0.040
Potasium(K)	-0.090	0.077
Sodium (Na)	-0.570**	-0.110
Calcium Ca)	-0.860**	-0.740**
Magnesium (Mg)	-0.440*	

* significant for $p \leq 0.05$; **significant for $p \leq 0.01$

Wierzbowska et al. (2012a) obtained a higher yield of achenes and green mass when growing a Polish population line rather than the cultivar Silma. Population plants responded better to higher doses of nitrogen fertilization than cv. Silma. They produced more fruits, which were also more robust. Fertilization composed of 2 g N per pot resulted in the highest contribution of achenes to the total biomass yield. Other experiments conducted by the same authors (Wierzbowska et al. 2012b) indicate that higher nitrogen fertilization levels result in a lower fat content in fruits, especially ones produced by cv. Silma. Andrzejewska and Skinder (2007) demonstrated that the content of potassium in the green matter of milk thistle increased proportionally to the dose of this element, but was on a constant level of about 6 g K kg^{-1} d.m. in achenes irrespective of potassium fertilization doses. The size of a potassium dose did not have any effect on the yield of achenes and their content of silymarin. In turn, KOZERA and NOWAK (2004) found that the level of NPK fertilization had no effect on the content of nitrogen in milk thistle achenes, but depressed their concentration of potassium. On the other hand, micronutrients applied in the form of Basofoliar 36 Ex raised the content of phosphorus and potassium in achenes compared to plants fertilized with NPK alone.

Overall, boron fertilization did not affect the content of macronutrients in fruits of milk thistle (Table 6), except for the content of magnesium in achenes of cv. Silma, which was higher in the boron-fertilized treatments. CWALINA-AMBROZIAK et al. (2012) found that the yield of milk thistle achenes grown without nitrogen fertilization or fertilized with a dose of 40 kg N ha^{-1} was higher when boron had been applied. However, boron fertilization caused a decline in yield from plants nourished with higher doses of nitrogen. More-

Table 6

Effect of boron on the content of macronutrients in fruits of milk thistle

Fertilization	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma						
Without B	25.25±0.73^{a}	8.47±0.13^{a}	6.61±0.34^{a}	0.33±0.04^{a}	9.40±0.62^{a}	3.63±0.14^{b}
B	25.72±0.70^{a}	8.59±0.20^{a}	6.46±0.34^{a}	0.34±0.03^{a}	9.82±0.51^{a}	3.82±0.16b
Population						
Without B	27.61±1.31^{b}	8.65±0.21^{a}	7.53±0.16^{b}	0.35±0.02^{a}	9.47±0.81^{a}	3.94±0.11^{b}
B	26.39±1.04^{ab}	8.45±0.13^{a}	7.63±0.33^{b}	0.34±0.03^{a}	9.19±0.56^{a}	3.88±0.13^{b}

over, boron applied together with nitrogen changed the structure of pathogenic fungi colonizing the stem base, decreasing the share of *Fusarium* spp. but increasing the number of *Alternaria alternate*. Experiments conducted by ŠKARPA (2013) showed that foliar application of boron increased the biomass production of sunflower plants. Early application of boron (stage of 4 developed leaves) also increased the uptake of macronutrients (particularly N). The achene yield increased significantly (by 8.3%) after the application of 300 g B ha^{-1} at the beginning of vegetation. Similarly in a study conducted by WRÓBEL (2009) boron fertilization increased the performance and content of this component in cereal grains.

Sulphur introduced together with magnesium raised the content of nitrogen in achenes of the population line of milk thistle (Table 7). The form of magnesium fertilizer did not have any significant effect on the content of other macronutrients. In another study by WIERZBOWSKA et al. (2012b), an

Table 7

Effect of the form of magnesium fertilizer on the content of macronutrients in fruits of milk thistle

Fertilization	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma						
$MgCl_2$	24.69±1.62^{a}	8.30±0.14^{b}	6.18±0.23^{a}	0.33±0.04^{a}	9.98±0.96^{a}	3.63±0.14^{a}
$MgSO_4$	25.25±0.73^{a}	8.47±0.13^{b}	6.61±0.34^{a}	0.33±0.04^{a}	9.40±0.62^{a}	3.63±0.14^{a}
Population						
$MgCl_2$	24.97±1.66^{a}	8.56±0.14^{a}	7.27±0.44^{a}	0.35±0.04^{a}	9.43±0.79^{a}	3.84±0.16^{a}
$MgSO_4$	27.61±1.31^{b}	8.65±0.21^{a}	7.53±0.16^{b}	0.35±0.02^{a}	9.47±0.81^{a}	3.94±0.11^{a}

addition of sulphur contributed to a decrease in the content of crude fat in fruits of cv. Silma. Also, some modifications appeared in the structure of fatty acids, namely there was more linoleic acid ($C_{18:2}$), which is the dominant fatty acid in oil from milk thistle fruits, but less oleic acid ($C_{18:1}$).

According to SADOWSKA et al. (2011), the weather conditions during the plant growing season had a stronger effect on the chemical composition of milk thistle fruits than agronomic factors. The content of total protein changed very little, whereas the percentages of crude fibre and macronutrients, especially calcium and magnesium, were modified most profoundly. ANDRZEJEWSKA et al. (2011) claimed that delayed sowing led to an increased content of protein in achenes of this plant. Other experimental data suggest that the content of protein and ash components was independent from the date of sowing (ANDRZEJEWSKA, SADOWSKA 2007).

The content of nitrogen in the storage material of achenes was 36-38 g N kg^{-1} (ANDRZEJEWSKA, SADOWSKA 2008). Experiments conducted by SADOWSKA et al. (2011) showed that fruits of milk thistle contained about 160 g kg^{-1} d.m. of total protein and the sum of exogenous amino acids was about 22 g 100 g^{-1} of total protein. Glutamic acid was the dominant amino acid while tryptophan was a limiting one. POTKAŃSKI et al. (1991) reported that the content of total protein in hulled milk thistle seeds, a by-product of silymarin production, was 215 g kg^{-1} d.m. Other authors (e.g. BARANOWSKA et al. 2003) suggest that such by-product can contain up to 310-360 g kg^{-1} of total protein while the content of crude ash in milk thistle fruits could reach 50-60 g kg^{-1} d.m. (POTKAŃSKI et al. 1991, BARANOWSKA et al. 2003). ANDRZEJEWSKA and SADOWSKA (2007) report that whole achenes compared to hulled seeds had about 80 g kg^{-1} d.m. less total protein and about 4 g kg^{-1} d.m. less phosphorus, but the content of potassium, calcium and magnesium was similar in both types of seeds. Differences in the content of protein and phosphorus were caused by a large share of the pericarp (48.5–55.8%) in the total mass of achenes (ANDRZEJEWSKA, SADOWSKA 2008). Finally, the content of silymarin was negatively correlated with the content of nitrogen in achenes.

CONCLUSIONS

1. Fruits of the population plants contained more nitrogen, phosphorus and potassium than achenes of the cultivar Silma.

2. The highest concentration of nitrogen was determined in achenes of the milk thistle cultivar Silma plants fertilized with 2 g N per pot (28.13 g kg^{-1} d.m.); among the population plants, most nitrogen was found in fruits growing on plants nourished with 3 g N per pot (28.11 g kg^{-1} d.m.).

3. The highest content of phosphorus in achenes from both types of milk thistle plants was achieved when the fertilization treatment consisted of 2 g N per pot.

4. Boron fertilization did not affect the content of macronutrients in fruits of either form of milk thistle.

5. Achenes from population plants fertilized with magnesium sulphate contained more nitrogen than from the ones treated with $MgCl_2$.

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