The effect of mineral fertilization on the content of biologically active compounds and mass of roots of *Salvia miltiorrhiza* Bunge

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S u m m a r y

The research of mineral fertilization effect of *Salvia miltiorrhiza* Bunge has been carried out in 2004–2006. It was found that potassium fertilization had the biggest influence on the amount of dry root mass changeability. The evaluation of mineral fertilizer effect of biological active compounds gave ambiguous results. In the raw material of Polish cultivation origin the phenolic acids and tanshinones, which determine the healing properties of *Salvia miltiorrhiza*, were noticed. In the roots the tanshinone IIA occurred to be the dominating substance from diterpenes group. The weather conditions significantly influenced the amount of biological active compounds.

Key words: Salvia miltiorrhiza, mineral fertilization, tanshinones, phenolic compounds

INTRODUCTION

Salvia miltiorrhiza Bunge (*Labiatae*) is a valuable medicinal plant well known in Asian countries. This perennial plant grows in the wild in China and Japan [1]. It is also mentioned in the flora of Vietnam and Korea. *Salvia miltiorrhiza* Bunge was also introduced to cultivation in China [2]. In available literature investigations on its cultivation and cropping conditions have not been found. The major components of raw material are diterpenes with phenanthrenequinone structure (so-called tanshinones) and phenolic compounds [3]. *Salvia miltiorrhiza* products are recommended especially in cardiovascular diseases [4].

The present study continues the research on cultivation of *Salvia miltiorrhiza* carried out in the Garden of Medicinal Plants, Research Institute of Medicinal Plants in Plewiska near Poznań [5].

MATERIAL AND METHODS

The studies were carried out in 2004–2006. The field cultivation was established by vegetative propagation in autumn. The two-three-node cuttings were rooted in perlite with the use of stimulating agent (Ukorzeniacz B). The plants obtained by vegetative reproduction were planted into the soil in spacing of 30x30cm.

The experimental field cultivation was established on the lessivé soil developed from boulder clay of ground moraine. The upper layer was filled with clayey sand. The humus thickness was 30 cm, pH about 7.06. The contents of mineral components in the arable layer were: NH_4 – 7.0; NO_3 – 7.0; P – 116.3, K – 41.3; Ca – 3236; Mg – 252; Cl – 13.9; SO_4 – 47.6; Na – 11.8.

The experiments were established in randomized complete block design in four repetitions. The area of each plot was 2.25 m^2. Seven combinations of fertilizers were applied compared to control plots (without fertilization).

The doses of fertilizers (pure component in $kg \cdot ha^{-1}$) were as following:

– N: 0, 70, 140 in form of ammonium nitrate 34 %, divided into two doses and applied in spring;
– P_2O_5: 0, 50, 100 in form of triple superphosphate 46% before planting;
– K_2O: 0, 90, 180 in form of potassium salt 60% before planting.

In November after one year of cultivation 3 randomly chosen plants from each plot were taken to determine the root mass and content of biologically active compounds. The roots of one-year-old plants were harvested and dried in natural conditions in shaded and well ventilated place. On average, 65% of roots withered.

Data concerning weather conditions in 2005–2006 come from the Poznań-Ławica Meteorological Office (tab. 1).

Table 1.

Atmospheric conditions in vegetation seasons (May–October) in 2005 and 2006. Data from the Poznań-Ławica Meteorological Office

years	average air temperature [^{0}C]	precipitation, total [mm]	insolation, total [h]
2005	15.7	270.7	1451.6
2006	16.5	268.4	1479.8

Contents of tanshinones and rosmarinic acid were indicated using HPLC method with UV detector. For HPLC analysis samples of 100 mg were extracted with 5 ml methanol for 30 min. in ultrasonic bath and filtered through a membrane filter (nominal pore size 0.45 μm).

HPLC analysis was performed on Agilent 1100 HPLC system, equipped with photodiode array detector. For all separations a Lichrospher 100 RP18 column (125 x 4 mm, 5 μm, Merck) was used. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (A) and acetonitrile (B), applied in the different gradient elution (tab. 2)

Table 2.

Scheme of gradient elution

time [min]	0.1% TFA_{aq} [%]	acetonitrile [%]
0.00	95.0	5.0
5.00	95.0	5.0
15.00	40.0	60.0
30.00	25.0	75.0
35.00	22.5	77.5

The following rate was adjusted to 0.5 ml/min, the detection wavelength set to DAD at λ=250.4 nm, and 20 μL of samples was injected. All separations were performed at the temperature of 25^0C. Peaks were assigned by spiking the samples with standard compounds and compared to UV-spectra and retention times. The contents of biological active compounds are expressed in percentage value of dry weight. The sum of tanshinones was calculated by adding the contents of particular diterpenes of each raw material. The research on the content of sum of phenolic compounds was carried out with use of colorimetric method [6].

The results of the experiments were statistically evaluated with use of the analysis of variance („F" Fischer – Snedecor test). The significance of differences was proved with t-Student test at a 5% level of significance.

RESULTS AND DISCUSSION

The results concerning the root mass, percentage and sum of particular tanshinones and phenolic acids contents obtained from a single plant are gathered in tables 3–5. The changes of raw materials mass sum and tanshinones and phenolic acids amounts obtained from a single plant are also shown in figures 1–3.

Table 3.

Effect of mineral fertilization on the mass of roots of *Salvia miltiorrhiza* Bunge (per 1 plant)

fertilization	dry mass of roots [g]		
	2005	2006	mean
O	10.77	10.85	10.81
N0P1K1	12.67	11.83	12.25
N1P1K1	14.83	12.62	13.72
N2P1K1	13.75	10.87	12.31
N1P0K1	12.58	11.07	11.82
N1P2K1	13.10	14.28	13.69
N1P1K0	13.60	11.58	12.59
N1P1K2	17.33	15.49	16.41
LSD 0.05	2.42	3.09	2.00

Table 4.

Effect of mineral fertilization on the content of tanshinones in *Salvia miltiorrhiza* roots

fertilization	content [%]														
	tanshinone I			tanshinone IIA			dihydrotanshinone			cryptotanshinone			sum of tanshinones		
	2005	2006	mean	2005	2006	mean	2005	2006	mean	2005	2006	mean	2005	2006	mean
O	0.023	0.027	0.025	0.083	0.100	0.092	0.030	0.053	0.042	0.020	0.027	0.024	0.156	0.207	0.182
N0P1K1	0.037	0.043	0.040	0.123	0.123	0.123	0.053	0.048	0.051	0.030	0.020	0.025	0.243	0.234	0.239
N1P1K1	0.043	0.053	0.048	0.137	0.097	0.117	0.047	0.043	0.045	0.030	0.023	0.027	0.257	0.216	0.237
N2P1K1	0.020	0.067	0.044	0.075	0.090	0.083	0.023	0.070	0.047	0.018	0.017	0.018	0.136	0.244	0.190
N1P0K1	0.020	0.040	0.030	0.083	0.143	0.113	0.028	0.063	0.046	0.023	0.023	0.023	0.154	0.269	0.212
N1P2K1	0.047	0.057	0.052	0.170	0.143	0.157	0.073	0.050	0.062	0.020	0.020	0.020	0.310	0.270	0.290
N1P1K0	0.030	0.065	0.048	0.110	0.105	0.108	0.040	0.053	0.047	0.030	0.023	0.027	0.210	0.246	0.228
N1P1K2	0.033	0.050	0.042	0.093	0.160	0.127	0.038	0.067	0.053	0.018	0.020	0.019	0.182	0.297	0.240
LSD 0.05	0.017	0.024	0.011	0.055	0.034	0.035	0.028	0.015	0.013	n.s.	n.s.	n.s.	0.141	0.048	0.062

Table 5.

Effect of mineral fertilization on the content of phenolic compounds in *Salvia miltiorrhiza* roots

fertilization	content [%]					
	rosmarinic acid			sum of phenolic acids		
	2005	2006	mean	2005	2006	mean
O	4.45	4.28	4.37	5.46	5.22	5.34
N0P1K1	4.71	3.62	4.17	6.26	4.63	5.45
N1P1K1	5.02	5.09	5.06	7.27	5.57	6.42
N2P1K1	4.20	3.62	3.91	7.16	4.97	6.07
N1P0K1	4.50	4.93	4.72	6.31	5.11	5.71
N1P2K1	4.88	4.51	4.70	7.20	6.13	6.67
N1P1K0	3.89	3.80	3.85	5.55	4.77	5.16
N1P1K2	5.14	4.89	5.02	6.24	5.64	5.94
LSD 0.05	n.s.	1.13	0.71	1.31	0.88	0.67

The root masses obtained from control plots are both similar in 2005, when the average mass per 1 plant was 10.77 g, and 2006, when mass came to 10.85 g (tab. 3, fig. 1).

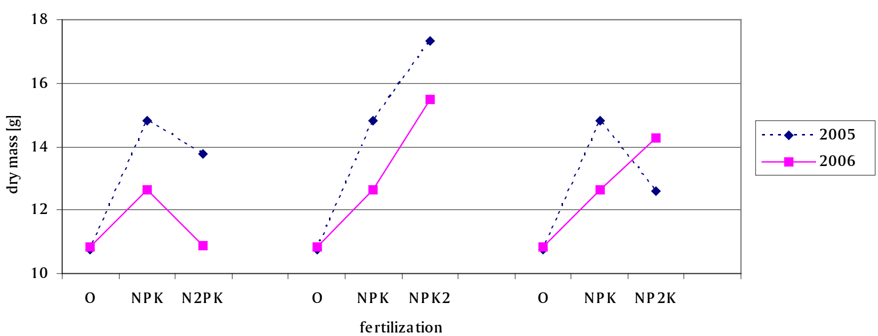

Figure 1. The effect of mineral fertilization on the mass of roots of *Salvia miltiorrhiza* Bunge (per 1 plant)

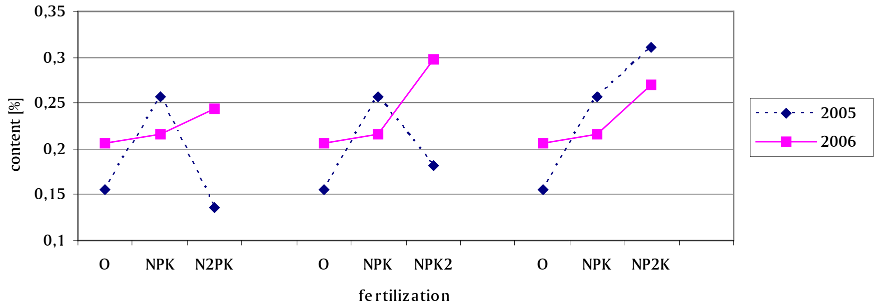

Figure 2. The effect of mineral fertilization on the content of sum of tanshinones in *Salvia miltiorrhiza* roots

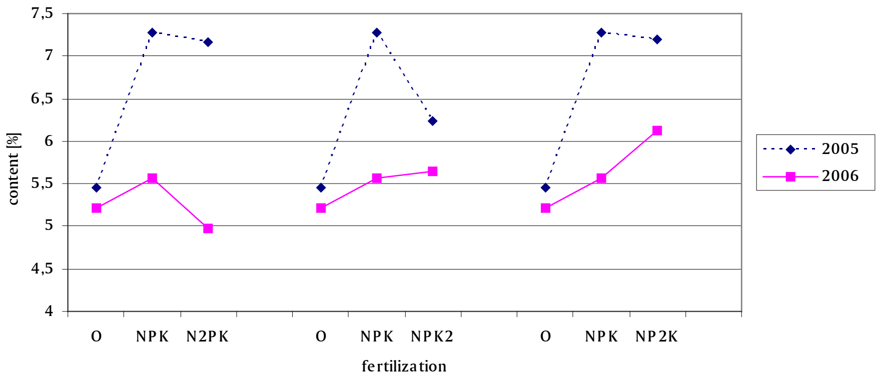

Figure 3. The effect of mineral fertilization on the content of sum of phenolic compounds in *Salvia miltiorrhiza* roots

There was a stronger effect of applying mineral fertilizer to root mass in 2005. The root mass, obtained from single plant oscillated from 12.58 g to 17.33 g, while in 2006 from 10.87 g to 15.49 g. The above mentioned results differed due to various weather conditions. Lower temperatures and sunless weather were observed in 2005, though, the amount of rain was similar to that of 2006 (tab. 1). In 2005, except from applying double dose of phosphorus fertilizer, all fertilization variants obtained larger amount of root mass then in 2006. Analysing the results of two-year study, the higher mass of dry raw material was obtained by applying double dose of potassium fertilizer. The average mass was 16.41 g per single plant. Potassium fertilizers had a beneficial influence on root mass, which is characteristic for root crops [7]. A significant influence on these results was the low content (41.3 mg/dm) of potassium elements in cultivated soil. Only increasing amount of root mass after the use of double dose of potassium fertilizer is statistically proved. However, applying other mineral elements cause a positive and clear result (fig. 1-3).

The evaluation of mineral fertilizer effect of biological active compound is ambiguous. The changeability of cryptotanshinon (in both study years) and rosemarinic acid compounds (in 2005) were not statistically significant. The differences between results of other substances were not statistically confirmed. However, it is possible to state certain regularities.

The most important factor determining the value of raw material is the amount of tanshinones and phenolic acids. As compared to raw material cultivated without fertilization, the percentage of tanshinones sum was higher after applying the basic dose of mineral substances (N1P1K1; see tab. 4, fig. 2). The maximum amount of diterpenes sum is found after using fertilizer dose of N1P2K1 (in 2005) and dose of N1P1K2 (in 2006). In 2005 the double dose of potassium and nitrogen fertilizer reduced the amount of tanshinones contents. Then, in 2006 all the variants of fertilization were profitable. The resulting amount of root mass changeability as well as various effect of mineral fertilization on percentage tanshinones sum contents were caused by different weather conditions in each year. In 2006 the cultivated plants accumulated higher amount of tanshinones, oscillating from 0.207 to 0.297%. In 2005 tanshinones sum contents oscillated from 0.136 to 0.310%. Taking into account the results achieved in two-year research, the maximum amount of tanshinones sum (average 0.290%) was obtained by applying double dose of phosphorus elements. Tanshinone IIA was predominated with diterpenes compounds (approx. 50% of all tanshinones sum). The other substances belonging to diterpenes appeared in lower frequency. The maximum amount of tanshinone IIA was found after the appliance fertilizer dose of N1P2K1 fertilizer in 2005 and use of N1P1K2 variant in 2006.

The changes of the phenolic acids sum were similar to those obtained during the analysis of changeability raw material masses. The plant material originated from control plots contains similar amount of phenolic acids in both years of investigation (5.46% in 2005 and 5.22% in 2006, see tab. 5, fig. 3). In 2005 (in all fertilization variants) the raw material contained larger amount of phenolic acids (5.46–7.27%) than in 2006, while content of these substances oscillated from 4.63 to 6.13%. Also in this case the differences were caused by various weather conditions in both years.

Comparing to control plots, the N1P1K1 fertilization increased the percentage of phenolic acids content. The double dose of certain mineral substances decreased on the amount of the substances (2005). In 2006 only applying fertilizer dose N1P2K1 fertilizer resulted in increasing content of phenolic acids. Taking into consideration both years, the highest sum of phenolic acids (6.67%, on average) was obtained by applying the double dose of phosphorus fertilizer.

The conclusion is that applying all of the fertilizer variants gave a positive effect on the increasing raw material masses. However, the strongest effect was observed after the use of potassium fertilizer. The evaluation of the effect of mineral fertilizer on biologically active substances has shown that no matter which variant is used, in roots cultivated in Polish climate and soil conditions there are two main groups of biologically active substances: phenolic acids and tanshinones. These compounds decide on healing properties of raw material. Tanshinone IIA was a predominating substance in the diterpenes group.

Compared to earlier investigation (where the cultivation was established by sowing seeds in the greenhouse and planting seedlings into the ground) [5], the vegetative propagation of *Salvia miltiorrhiza* Bunge is cheaper and easier. It also gave better results in obtaining root mass as well as in the amount of phenolic acids.

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WPŁYW NAWOŻENIA MINERALNEGO NA ZAWARTOŚĆ ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH I MASĘ KORZENI *SALVIA MILTIORRHIZA* BUNGE

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S t r e s z c z e n i e

W latach 2004–2006 przeprowadzono badania z zakresu nawożenia szałwii czerwonokorzeniowej. Stwierdzono, że najsilniejszy wpływ na suchą masę korzeni omawianego gatunku ma nawożenie potasowe. Ocena wpływu nawożenia mineralnego na zawartość związków biologicznie czynnych dała niejednoznaczne wyniki. W korzeniach pochodzących z upraw w polskich warunkach klimatyczno-glebowych stwierdzono występowanie polifenolozwiązków i diterpenów (tanszinonów) decydujących o właściwościach leczniczych surowca. Dominującym składnikiem grupy diterpenów występującym w korzeniach uprawianych roślin był tanszinon IIA. Warunki atmosferyczne miały istotny wpływ na zmienność masy surowca i zawartość badanych związków biologicznie czynnych.

Słowa kluczowe: Salvia miltiorrhiza, nawożenie mineralne, tanszinony, związki fenolowe