ISSN 1644-0692
www.acta.media.pl

ACTA Acta Sci. Pol. Hortorum Cultus 14(2), 2015, 109-121

EFFECT OF FERTILIZATION ON ROSEROOT (*Rhodiola rosea* L.) YIELD AND CONTENT OF ACTIVE COMPOUNDS

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Abstract. Roseroot (*Rhodiola rosea* L.) is an important medicinal plant with well-documented adaptogenic activity. The decrease in its natural resources induces to undertake research on the introduction of this species into cultivation. The aim of the present work was to determine the effect of organic and mineral fertilization on increasing the biomass of underground parts of *Rh. rosea* and the level of biologically active compounds. Throughout the study period, the highest raw material yield was obtained after the application of the following doses of mineral fertilization: N – 60.0 kg·ha^{-1}, P – 35.2 kg·ha^{-1}, K – 83.0 kg·ha^{-1}, without manure. The use of manure caused a decrease in the average rhizome and root weight by more than 20%. Mineral fertilization application increased the average raw material yield by about 30–40% and allowed obtaining the highest weight of underground organs of the species in question already in the fourth year of cultivation. Furthermore, the present study demonstrates that mineral fertilization does not affect substantially the level of the individual groups of compounds in the raw material, but the use of manure may significantly reduce the content of phenylpropanoids. The level of active compounds is also influenced by crop age. Two-year-old plants were characterized by the highest content of phenylpropanoids as well as by the lowest content of phenylethanoids and phenolic acids. In the following years of cultivation, no statistically significant changes were observed in the level of the analysed groups of compounds.

Key words: roseroot, organic and mineral fertilization, yield, quality of raw material

INTRODUCTION

Roseroot (*Rhodiola rosea* L.) grows in the Arctic regions and in the mountains of Europe, Asia, and North America. In Poland it is found in the Sudetes and the Carpa-

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thian Mountains on moist rocks, screes, and rocky grassland as well as in tall herb communities and on stream banks [Pawłowska 1955, Bykov et al. 1999, Krukowski et al. 2009]. The underground organs of the species in question (*Rhodiolae roseae rhizoma et radix*) are used in modern phytotherapy as adaptogens and antidepressants. This plant raw material exhibits cardioprotective, antioxidative, antibacterial, antitumor, and immunomodulatory effects [De Sanctis et al. 2004, Khanum et al. 2005, Krajewska-Patan et al. 2005, Siwicki et al. 2007, Skopińska-Różewska et al. 2008, Wolski et al. 2008, Panossian et al. 2010, Skopińska-Różewska et al. 2011, Zdanowski et al. 2014]. The underground plant parts are strongly developed rhizomes with numerous roots. Its aerial shoots grow to a height of 30–40 cm and are characterized by large variation in colour as well as in leaf size and shape. This plant is dioecious: male flowers are yellow, often with a pink tinge, whereas female flowers are usually devoid of petals. The described taxon blooms from June to July and bears fruit in autumn [Kozłowski and Szczyglewska 2001, Przybył et al. 2004, Galambosi et al. 2009, Kołodziej and Sugier 2012, Adamczak et al. 2014].

Laboratory investigations revealed the presence of three major groups of compounds that are responsible for the main pharmacological activity of roseroot. These are phenylpropanoids, phenylethanoids, and phenolic acids. Phenylpropanoids (rosavin, rosin, and rosarin) are esters of cinnamic acid with saccharides. Phenylethanoids are derivatives of phenylethanol and they primarily include the following: *p*-tyrosol, occurring as aglycone and its glycoside – salidroside. The following are mentioned among phenolic acids: gallic, chlorogenic, ferulic, salicylic, and caffeic acids [De Sanctis et al. 2004, Khanum et al. 2005, Krajewska-Patan et al. 2005, Wolski et al. 2008]. Essential oil was also found to be present in the raw material of *Rh. rosea*. More than 80 constituents were identified in it, mainly n-decanol, but also geraniol, 1,4-p-menthadien-7-ol, limonene, a-pinene, and dodecanol [Rohloff 2002].

There is rich literature on phytochemical variability of *Rh. rosea* associated with the type of raw material and harvest time, plant age and origin as well as climatic and soil conditions [Kurkin et al. 1986, Buchwald et al. 2006, Altantsetseg et al. 2007, Dreger et al. 2007, Platikanov and Evstatieva 2008, Węglarz et al. 2008, Kołodziej and Sugier 2013]. This work, however, relates to the still little known issue of the effect of agrotechnical factors on the quantity and quality of raw material yield in successive years of cultivation. The aim of the present study was to evaluate the effect of organic and mineral fertilization on increasing the biomass of underground parts of *Rh. rosea* and the level of biologically active compounds in raw material obtained from crops established by rhizome division.

MATERIAL AND METHODS

An agrotechnical experiment on roseroot was conducted at the Institute of Natural Fibres and Medicinal Plants (INF&MP) in Plewiska near Poznań (western Poland) over the period 2007–2012. The taxonomic status of plants was confirmed on the basis of the Soviet Union flora [Borisova 1939] and flora of Poland [Pawłowska 1955]. A voucher specimens are kept in the herbarium of Department of Botany, Breeding and Agricultural Technology in Plewiska near Poznań.

Experimental crops were grown using conventional tillage on lessivé soil formed from ground moraine with the granulometric composition of light loamy sands and with medium nutrient availability. The soil was characterized by the following properties – pH in H_2O: 7.6–8.0, $N\text{-}NO_3$ content: 7–21 $mg \cdot dm^{-3}$, P: 136–155 $mg \cdot dm^{-3}$, K: 100–150 $mg \cdot dm^{-3}$, Ca: 1224–2299 $mg \cdot dm^{-3}$, Mg: 55–69 $mg \cdot dm^{-3}$, NaCl: 0.14–0.17 $g \cdot dm^{-3}$ (analysis were performed in Regional Agrochemical Station in Poznań).

Rhizome cuttings (about 4–5 cm long) were planted at a spacing of 45 × 45 cm in autumn (October) twice – in 2007 and 2008. We used our own propagation material obtained from a maternal plantation (the Medicinal Plant Garden of INF&MP) by division of several-year-old rhizomes. This was a two-factor experiment set up in a randomized block design with three replicates. The first-order factor was the plant position differentiated by fertilization regime: 1) after phacelia ploughed in as green manure; 2) after phacelia ploughed in as green manure with the addition of cattle manure at a rate of 33 $t \cdot ha^{-1}$ (= 33 $Mg \cdot ha^{-1}$). Annual doses of mineral fertilization were used as the second-order factor in the following combinations: 1) N – 60 $kg \cdot ha^{-1}$ in the form of nitro-chalk, P_2O_5 – 60 $kg \cdot ha^{-1}$ (P – 26.4 $kg \cdot ha^{-1}$) as a granulated triple superphosphate, K_2O – 80 $kg \cdot ha^{-1}$ (K – 66.4 $kg \cdot ha^{-1}$) as a potassium salt; 2) N – 60 $kg \cdot ha^{-1}$, P_2O_5 – 80 $kg \cdot ha^{-1}$ (P – 35.2 $kg \cdot ha^{-1}$), K_2O – 100 $kg \cdot ha^{-1}$ (K – 83.0 $kg \cdot ha^{-1}$) and 3) without mineral fertilization (control). The plot with an area of 12 m^2 was a replicate in this experiment. A multi-row weeder was used for mechanical weed control, whereas in-row weeding was done by hand. Raw material was harvested from the second to the fifth year of plant growth. Fresh and air-dry matter yield of *Rh. rosea* underground parts was determined. Rhizomes with roots were harvested in autumn (October) and then they were washed, cut into small pieces, and dried in a GoBest UZ–108 drying oven at a temperature of 40ºC and a relative humidity of 20% [Jezierska 1995, Peschel et al. 2013, Adamczak et al. 2014].

Chemical analysis was performed by the HPLC-DAD method [Buchwald et al. 2006, Altantsetseg et al. 2007, Kołodziej and Sugier 2013]. The following groups of compounds were determined: phenylpropanoids (expressed as the sum of rosavin, rosin and rosarin), phenylethanoids (salidroside and *p*-tyrosol) as well as phenolic acids (gallic and caffeic acids). The content of investigated compounds was given in $mg \cdot g^{-1}$ dry matter (*DM*) of underground plant parts (rhizomes with roots). The dry weight of the raw material was measured after drying it at 105°C in a HR73 Halogen Moisture Analyzer (Mettler, Toledo).

For the HPLC analysis, approximately 1.0 g of air-dried and powdered rhizomes with roots of *Rh. rosea* was weighed out and placed in a 100 ml round-bottom flask. 20 ml of 70% (*v/v*) methanol was added and the solution was heated under a reflux condenser at the boiling point of the solvent for 15 min. Then, the sample was cooled, filtered and the filter with the sample was returned to the flask. Extraction of the sample was repeated three times with 15 ml of 70% (*v/v*) methanol for 15 min. The combined hydroalcoholic extracts were evaporated to dryness in a rotary evaporator in vacuum. The dry residue was dissolved in 5.0 ml of 70% (*v/v*) methanol. The sample was filtered through a membrane filter with a diameter of 0.45 μm. The assay of bioactive compounds was performed using a high performance liquid chromatograph with a photodiode array detector (HPLC-DAD, Agilent 1100). The separation of analytes was performed on a LiChrospher C18 column, 5 µm, 4.0 × 250 mm (Merck) at a temperature of 24ºC. The mobile phase consisted of 0.2% (*v/v*) phosphoric acid solution in water (A) and acetonitrile (B) with flow rate: 1.0 $ml \cdot min^{-1}$. The assay was performed in gradient elution: 0 min – 95% of phase A, 30 min – 80% of phase A, 35 min – 80% of phase A, 40 min – 20% of phase A, 56 min – 20% of phase A, 60 min – 95% of phase A. The detection wavelength was set at 205 nm (phenylpropanoids and phenylethanoids) as well as at 220 nm (gallic acid) and 330 nm (caffeic acid).

Pillai's trace test (MANOVA) and then F-test of ANOVA were used to evaluate the significance of differences between means. For multiple comparisons, Tukey's test was applied at a significance level of $p \leq 0.05$. Statistica 7.1 software was used for these calculations [StatSoft 2005].

The results of the study of two- and three-year-old plants were described in an earlier publication [Kucharski et al. 2011]. This article discusses the data obtained from the fourth and fifth year of cultivation and describes the general trends and relationships observed throughout the study period.

RESULTS AND DISCUSSION

In the conducted field experiment, large differences were observed in the average fresh and air-dry matter yield of *Rh. rosea* rhizomes with roots, depending on the applied mineral and organic fertilization treatment (tab. 1). Both, in the fourth and fifth year of cultivation, the highest average raw material yield was noted after the application of the higher rate of phosphorus and potassium fertilization, without manure. We

Table 1. The yield of rhizomes with roots of *Rhodiola rosea* in dependence on fertilization (means ±SD)

	Fertilization ($kg \cdot ha^{-1}$)	4th year of cultivation		5th year of cultivation	
		fresh weight ($t \cdot ha^{-1}$)	air-dry matter ($t \cdot ha^{-1}$)	fresh weight ($t \cdot ha^{-1}$)	air-dry matter ($t \cdot ha^{-1}$)
With manure	0 NPK	10.10 ±5.85	2.90 ±1.49	12.21 ±1.63	3.51 ±0.47
	N – 60.0, P – 26.4, K – 66.4	16.23 ±6.32	4.64 ±1.75	16.25 ±1.75	4.70 ±0.51
	N – 60.0, P – 35.2, K – 83.0	11.91 ±5.99	3.45 ±1.38	17.00 ±1.69	4.92 ±0.48
	total	12.75 ±6.27 A	3.66 ±1.63 A	15.15 ±2.67 A	4.38 ±0.78 AB
Without manure	0 NPK	13.58 ±7.23	4.04 ±1.96	13.67 ±1.58	4.01 ±0.41
	N – 60.0, P – 26.4, K – 66.4	20.92 ±12.36	6.39 ±3.68	17.42 ±0.85	5.08 ±0.19
	N – 60.0, P – 35.2, K – 83.0	22.90 ±16.30	7.05 ±4.61	19.33 ±1.69	5.83 ±0.51
	total	19.13 ±12.47 A	5.83 ±3.62 B	16.81 ±2.78 A	4.97 ±0.87 AB

Pillai's trace test (MANOVA) – for manure: 0.20, p = 0.0104, p > 0.05 for mineral fertilization, year of cultivation and interactions between factors. F-test for manure: 4.16, p = 0.0476 (air-dry matter), p > 0.05 (fresh weight of raw material). Values with the same letter are not significantly different (Tukey's test, p > 0.05). SD – standard deviation, 0 NPK – without mineral fertilization also found the same relationship earlier in the case of two- and three-year-old plants [Kucharski et al. 2011]. In turn, in agrotechnical experiments carried out in southern Finland, the use of organic fertilization caused an increase in height of plants and in the fresh weight of roseroot underground parts, and only a slight increase in dry matter of raw material. Depending on the combination, the average yield of *Rh. rosea* rhizomes with roots was from 1.26 to 1.37 $kg \cdot m^{-2}$, and after conversion: from 12.6 to 13.7 $t \cdot ha^{-1}$ [Galambosi 2006].

The data collected throughout the entire experiment (2–5 year of cultivation) allowed us to show important regularities in roseroot yielding. The analysis of variance found statistically significant differences for the studied main effects: organic and mineral fertilization as well as year of cultivation (tab. 2). On the other hand, the interactions between the analysed factors proved to be not significant (fig. 1–4). A pronounced increase in the fresh and air-dry weight of underground organs of the species in question was observed in the successive years of the experiment. It was only in the fifth year of cultivation that no significant change was found for the average yield calculated together for all combinations (tab. 2), though various trends were observed in individual fertilization treatments (fig. 1–2).

Table 2. Effect of fertilization and cultivation year on the yield of *Rhodiola rosea* rhizomes with roots (means ±SD)

Variables	Fresh weight ($t \cdot ha^{-1}$)	Air-dry matter ($t \cdot ha^{-1}$)	F-test *p*-level
With manure	10.67 ±4.42	3.13 ±1.33	**
Without manure	13.64 ±5.61	4.10 ±1.85	
0 NPK	9.54 ±3.55 a	2.86 ±1.20 a	**
N – 60.0, P – 26.4, K – 66.4 ($kg \cdot ha^{-1}$)	13.42 ±5.39 b	3.95 ±1.74 b	
N – 60.0, P – 35.2, K – 83.0	13.50 ±5.88 b	4.03 ±1.92 b	
2nd year of cultivation	6.04 ±1.21 a	1.45 ±0.26 a	***
3th year	10.66 ±2.56 b	3.60 ±0.68 bc	
4th year	15.94 ±5.08 c	4.74 ±1.65 d	
5th year	15.98 ±2.61 c	4.68 ±0.82 cd	

Pillai's trace test (MANOVA) – for manure: 0.11, p = 0.0029, for mineral fertilization: 0.11, p = 0.0183, for year of cultivation: 0.84, p < 0.0001, for interactions between factors: p > 0.05. F-test: ** – p < 0.01, *** – p < 0.001. Values with the same letter are not significantly different (Tukey's test, p > 0.05). SD – standard deviation, 0 NPK – without mineral fertilization

In the experiment under discussion, organic and mineral fertilization had a significant effect on roseroot yield. The application of manure caused an average decrease in yield of *Rh. rosea* rhizomes with roots by over 20%, whereas the use of mineral fertilization increased the average raw material yield by about 30–40% (tab. 2). Furthermore, a more detailed analysis showed that manure use had a modifying influence on the

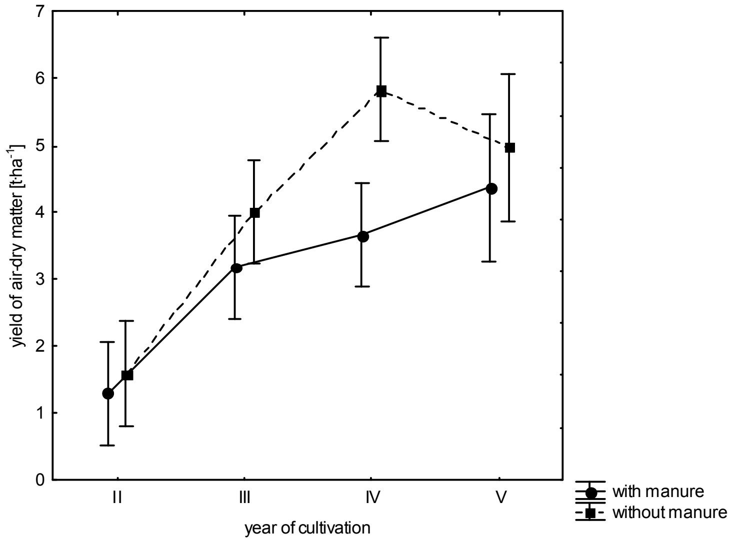

Fig. 1. Effect of organic fertilization on the yield of *Rhodiola rosea* rhizomes with roots in the different years of cultivation means ±0.95 confidence intervals); F-test for interaction effect: 2.25, p = 0.0875

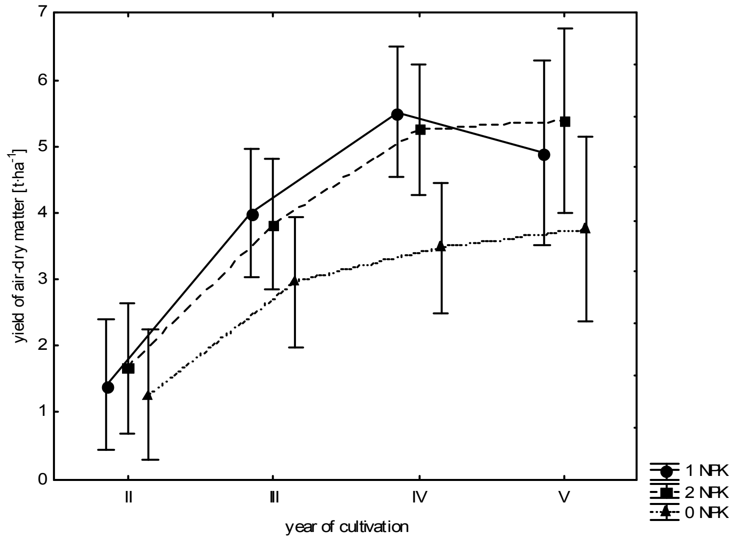

Fig. 2. Effect of mineral fertilization on the yield of *Rhodiola rosea* rhizomes with roots in the different years of cultivation (means ± 0.95 confidence intervals); F-test for interaction effect: 0.82, p = 0.5595, 1 NPK: N – 60.0, P – 26.4, K – 66.4, 2 NPK: N – 60.0, P – 35.2, K – 83.0 ($kg \cdot ha^{-1}$), 0 NPK – without mineral fertilization

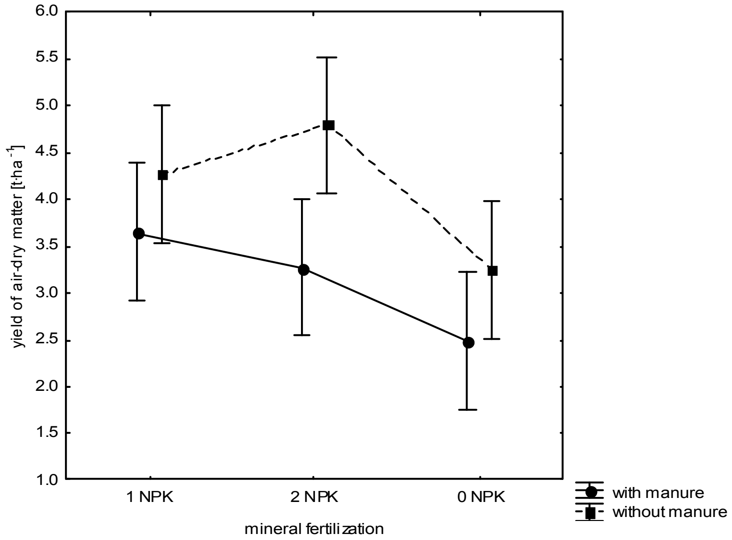

Fig. 3. Mutual effect of mineral and organic fertilization on the yield of *Rhodiola rosea* rhizomes with roots from the 2nd to 5th year of cultivation (means ±0.95 confidence intervals); F-test for interaction effect: 0.87, p = 0.4240, 1 NPK: N – 60.0, P – 26.4, K – 66.4, 2 NPK: N – 60.0, P – 35.2, K – 83.0 ($kg \cdot ha^{-1}$), 0 NPK – without mineral fertilization

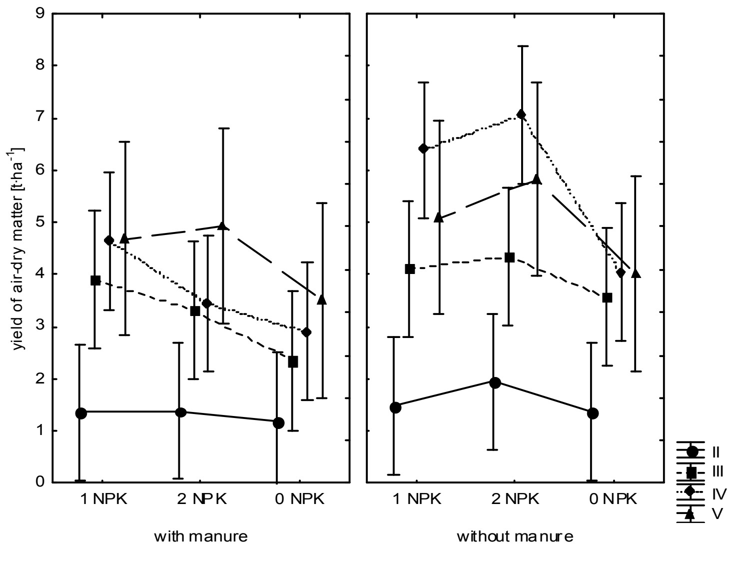

Fig. 4. Mutual effect of mineral and organic fertilization on the yield of *Rhodiola rosea* rhizomes with roots in the different years of cultivation (means ±0.95 confidence intervals); F-test for interaction effect: 0.41, p = 0.8683, 1 NPK: N – 60.0, P – 26.4, K – 66.4, 2 NPK: N – 60.0, P – 35.2, K – 83.0 ($kg \cdot ha^{-1}$), 0 NPK – without mineral fertilization, II–V – year of cultivation

yield-increasing effect of the different doses of phosphorus and potassium fertilization (fig. 3). Hence, the highest average raw material yield in the manured plots was recorded for the lower rates of mineral fertilization, whereas in the plots without manure – for the higher level of phosphorus and potassium fertilization. The above described relationship in the treatments without manure persisted throughout the experiment period, while in the combinations with manure it underwent some fluctuations and probably that is why the study found no statistically significant interaction between both types of fertilization (fig. 4).

In a field study of Kołodziej and Sugier [2012], the air-dry weight of roseroot underground organs systematically increased in successive years of observation, reaching its maximum value in 5-year-old plants, on average 120.96 g per plant at a spacing of 50 × 30 cm (after conversion: 8.06 $t \cdot ha^{-1}$). In our experiment, the increase in the average raw material yield in the fifth year of cultivation was found in the combination with manure and the higher dose of phosphorus and potassium fertilization (up to 4.92 $t \cdot ha^{-1}$) as well as after the application of manure without mineral fertilization (up to 3.51 $t \cdot ha^{-1}$). In the plots without organic fertilization, plants have achieved the maximum yield (up to 7.05 $t \cdot ha^{-1}$) already in the fourth year of cultivation (tab. 1, fig. 1).

Węglarz et al. [2008] conducted extensive research on climatic and soil conditions and plantation age on yield quantity and quality. In the sixth year of cropping, the above cited authors found symptoms of plant senescence (the decomposition of rhizomes into many smaller parts) and a large decline in yield. The average air-dry weight of rhizomes with roots increased with plant age reaching its maximum in the fifth year of cultivation (120.4 $g \cdot plant^{-1}$), and decreased to 44.3 g per plant in the next year. Roseroot cultivated in central and north-eastern Poland was characterized, on average, by a higher weight of underground parts but a lower content of phenylpropanoids and phenylethanoids compared to this species grown in southern Poland (the mountainous region). The average raw material yield for central Poland (alluvial soils) was twice higher than for north-eastern Poland (sandy soils) and more than four times higher than in the mountainous regions (loamy soil).

The phytochemical analysis of the raw material obtained from 4- and 5-year-old cultivations of roseroot reveals relatively large differences in the average content of the investigated groups of compounds in the particular mineral and organic fertilization treatments. Depending on the experimental combination used and plant age, the average content of phenylpropanoids ranged from 1.70 to 2.73 $mg \cdot g^{-1}$, phenylethanoids: from 0.77 to 1.85 $mg \cdot g^{-1}$ and for phenolic acids: from 0.49 to 0.92 $mg \cdot g^{-1}$ *DM* (tab. 3). A similar level of variability was earlier observed for 2- and 3-year-old plants [Kucharski et al. 2011]. Kołodziej and Sugier [2013] found a nearing content of phenylethanoids which, depending on plant age, ranged between 0.81 and 1.84 $mg \cdot g^{-1}$, and a clearly higher amount of phenylpropanoids: from 6.06 to 14.24 $mg \cdot g^{-1}$ rhizome dry matter. In turn, Węglarz et al. [2008] reports similar values for phenylethanoids and an even higher content of phenylpropanoids. Nevertheless, the above-mentioned authors indicate the high phytochemical variation in raw material of different geographical origin, in particular in the case of phenylpropanoids: from 9.75 to 40.53 $mg \cdot g^{-1}$, and to a lesser extent also for phenylethanoids: from 0.53 to 1.66 $mg \cdot g^{-1}$ *DM*. Likewise, Kurkin et al. [1986] reports very large differentiation of the content of the main constituent of phenylpropanoids – rosavin, in the plant material originating from various natural sites: from 0.5 to 25 mg·g^{-1} air-*DM*. Przybył et al. [2004] describes very high intrapopulation variability of roseroot, in particular evident in the case of salidroside (1.30–19.00 mg·g^{-1}) and rosavin (18.00–37.00 mg·g^{-1} *DM*). The amount of active compounds of roseroot is also significantly affected by raw material harvest time and plant growth stage [Buchwald et al. 2006, Platikanov and Evstatieva 2008].

Table 3. The content of main active compounds in rhizomes with roots of *Rhodiola rosea* in dependence on fertilization (means ±SD)

	Fertilization (kg·ha^{-1})	4th year of cultivation			5th year of cultivation		
		phenyl-propanoids	phenyl-ethanoids	phenolic acids	phenyl-propanoids	phenyl-ethanoids	phenolic acids
		(mg·g^{-1} *DM*)					
With manure	0 NPK	1.83 ±0.63	1.78 ±1.29	0.81 ±0.12	1.72 ±0.26	1.67 ±0.68	0.76 ±0.07
	N – 60.0, P – 26.4, K – 66.4	1.91 ±0.68	1.34 ±0.68	0.73 ±0.37	2.19 ±0.51	0.87 ±0.24	0.68 ±0.22
	N – 60.0, P – 35.2, K – 83.0	2.50 ±1.20	1.67 ±0.47	0.68 ±0.28	1.70 ±0.53	1.55 ±0.45	0.92 ±0.20
	total	2.08 ±0.88	1.60 ±0.86	0.74 ±0.27	1.87 ±0.46	1.36 ±0.57	0.79 ±0.19
Without manure	0 NPK	2.73 ±0.92	1.85 ±0.89	0.72 ±0.14	2.59 ±0.28	0.98 ±0.57	0.49 ±0.04
	N – 60.0, P – 26.4, K – 66.4	2.24 ±1.20	1.60 ±0.74	0.79 ±0.31	2.07 ±0.69	1.32 ±0.76	0.73 ±0.23
	N – 60.0, P – 35.2, K – 83.0	2.48 ±0.96	1.63 ±0.99	0.81 ±0.30	2.68 ±0.60	0.77 ±0.14	0.73 ±0.16
	total	2.49 ±1.00	1.69 ±0.83	0.78 ±0.25	2.45 ±0.56	1.02 ±0.53	0.65 ±0.19

Pillai's trace test (MANOVA): $p > 0.05$ for year of cultivation, manure, mineral fertilization and interactions between these factors. SD – standard deviation, 0 NPK – without mineral fertilization, *DM* – dry matter, phenylpropanoids – the sum of rosavin, rosin and rosarin, phenylethanoids – the sum of salidroside and *p*-tyrosol, phenolic acids – the sum of gallic and caffeic acids

The statistical analysis conducted for the entire period of the experiment showed significant effect of manure fertilization on the content of phenylpropanoids in *Rh. rosea* rhizomes with roots (tab. 4). An average higher level of the above-mentioned group of active compounds was recorded in the raw material harvested from the plots without organic fertilization (tabs 3–4). But no statistically significant differences (F-test) were found for the particular mineral fertilization treatments, though in the case of phenylpropanoids the significance level *p* was close to the accepted threshold value of 0.05, and Pillai's trace test (MANOVA) gave statistically significant value (tab. 4). The results of the research conducted by Galambosi [2006] also demonstrate that fertilization has an effect on the content of active compounds. In the above referenced article, the amount of salidroside and rosavin in roseroot rhizomes evidently increased after compost application and reached its highest value at a rate of 10 t·ha^{-1}, and then it decreased significantly at 20 t·ha^{-1}.

Table 4. Effect of fertilization and cultivation year on the content of main active compounds in rhizomes with roots of *Rhodiola rosea* (means ±SD)

Variables	Phenylpropanoids (mg·g^{-1} *DM*)	Phenylethanoids (mg·g^{-1} *DM*)	Phenolic acids (mg·g^{-1} *DM*)	F-test *p*-level
With manure	2.42 ±0.81	1.29 ±0.69	0.69 ±0.21	*[1] N.S.[2]
Without manure	2.64 ±0.81	1.29 ±0.65	0.70 ±0.19	
0 NPK	2.49 ±0.76	1.39 ±0.76	0.66 ±0.15	N.S.[3]
N–60.0, P–26.4, K–66.4 (kg·ha^{-1})	2.35 ±0.86	1.21 ±0.67	0.70 ±0.21	
N–60.0, P–35.2, K–83.0	2.75 ±0.79	1.28 ±0.58	0.73 ±0.23	
2nd year of cultivation	3.26 ±0.33 a	0.86 ±0.31 a	0.59 ±0.11 a	***[4] **[5]
3th year	2.24 ±0.66 b	1.41 ±0.57 b	0.72 ±0.18 b	
4th year	2.28 ±0.95 b	1.65 ±0.83 b	0.76 ±0.25 b	
5th year	2.16 ±0.58 b	1.19 ±0.56 ab	0.72 ±0.19 ab	

Pillai's trace test (MANOVA) – for year of cultivation: 0.49, $p < 0.0001$, for mineral fertilization: 0.16, $p = 0.0098$, for manure and interactions between factors: $p > 0.05$. F-test: N.S. – not significant, * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$; 1 – *p*-level for phenylpropanoids, 2 – for phenylethanoids and phenolic acids, 3 – for phenylpropanoids $p = 0.0541$, 4 – for phenylpropanoids and phenylethanoids, 5 – for phenolic acids. Values with the same letter are not significantly different (Tukey's test, $p > 0.05$). SD – standard deviation, 0 NPK – without mineral fertilization, *DM* – dry matter, phenylpropanoids – the sum of rosavin, rosin and rosarin, phenylethanoids – the sum of salidroside and *p*-tyrosol, phenolic acids – the sum of gallic and caffeic acids

A comparison of the average contents of the investigated groups of active compounds in particular years of cultivation provided interesting conclusions (tab. 4). The raw material from 2-year-old plants was characterized by the highest average amount of phenylpropanoids and at the same time the lowest content of phenylethanoids and phenolic acids. In the following years of cultivation, no statistically significant differences were observed, though in the fifth year of the experiment there was a decrease in the content of the analysed groups of compounds. The obtained results (tab. 4) and the literature data [Przybył et al. 2008, Kołodziej and Sugier 2013] indicate that the level of the main active compounds in *Rh. rosea* rhizomes with roots changes with plant age, though the pattern of these changes is not unambiguous. In our study, the content of phenylethanoids decreased quite clearly in the fifth year of cultivation, in the research by Przybył et al. [2008] such a decrease occurred not earlier than in 6-year-old plants, whereas according to Kołodziej and Sugier [2013] already in the fourth year of cropping. Additionally, the results reported in the article by Kołodziej and Sugier [2013] show a gradual increase in the level of phenylpropanoids in roseroot rhizomes until the fifth year of cultivation, whereas our data (tab. 4) and those presented by Przybył et al. [2004] demonstrate a clear reduction in the content of this group of compounds already from the second year of cultivation.

CONCLUSIONS

The obtained results indicate significant changes in roseroot yield quantity and quality depending on fertilization and cultivation age. Throughout the experiment period, the highest raw material yield was obtained after the application of the higher dose of mineral fertilization (N – 60.0 kg·ha^{-1}, P – 35.2 kg·ha^{-1}, K – 83.0 kg·ha^{-1}), without manure. The use of manure caused a decline in the average weight of *Rh. rosea* underground parts by more than 20%. The application of mineral fertilization had an effect on increasing the average raw material yield by about 30–40% and allowed obtaining the highest weight of underground organs of the species in question already in the fourth year of cultivation. Furthermore, the present field experiment demonstrates that mineral fertilization does not affect substantially the level of the individual groups of compounds in the raw material, but the use of manure may significantly reduce the content of phenylpropanoids. The level of active compounds is also influenced by crop age. 2-year-old plants were characterized by the highest content of phenylpropanoids and the lowest amount of phenylethanoids and phenolic acids. In the following years of cultivation, no statistically significant differences were observed in the level of these components.

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WPŁYW NAWOŻENIA NA PLON RÓŻEŃCA GÓRSKIEGO (*Rhodiola rosea* L.) I ZAWARTOŚĆ ZWIĄZKÓW CZYNNYCH

Streszczenie. Różeniec górski (*Rhodiola rosea* L.) jest ważną rośliną leczniczą o udokumentowanym działaniu adaptogennym. Zmniejszanie się jego zasobów w stanie naturalnym skłania do podejmowania badań nad wprowadzeniem tego gatunku do uprawy. Prezentowana praca miała na celu określenie wpływu nawożenia organicznego i mineralnego na przyrost masy części podziemnych *Rh. rosea* i poziom związków biologicznie czynnych. W całym okresie trwania eksperymentu, największy plon surowca uzyskiwano po zastosowaniu następującej dawki nawożenia mineralnego: N – 60,0 $kg \cdot ha^{-1}$, P – 35,2 $kg \cdot ha^{-1}$, K – 83,0 $kg \cdot ha^{-1}$, bez obornika. Wprowadzenie obornika powodowało spadek przeciętnej masy kłączy z korzeniami o ponad 20%. Zastosowanie nawożenia mineralnego zwiększało średni plon surowca o około 30–40% i pozwalało uzyskać najwyższą masę organów podziemnych już w czwartym roku uprawy. Przeprowadzone badania wskazują ponadto, że nawożenie mineralne nie wpływa znacząco na poziom poszczególnych grup związków czynnych w surowcu, natomiast wprowadzenie obornika może istotnie obniżyć zawartość fenylopropanoidów. Na poziom związków czynnych ma również wpływ wiek uprawy. Rośliny dwuletnie wyróżniały się największą zawartością fenylopropanoidów oraz najmniejszą – fenyloetanoidów i kwasów fenolowych. W kolejnych latach uprawy nie obserwowano istotnych statystycznie zmian poziomu omawianych grup związków.

Słowa kluczowe: różeniec górski, nawożenie organiczne i mineralne, plon, jakość surowca zielarskiego

Accepted for print: 24.02.2015

For citation: Buchwald, W., Mordalski, R., Kucharski, W.A., Gryszczyńska, A., Adamczak A. (2015). Effect of fertilization on roseroot (*Rhodiola rosea* L.) yield and content of active compounds. Acta Sci. Pol. Hortorum Cultus, 14(2), 109–121.