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ORIGINAL PAPER

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EFFECT OF FERTILIZATION AND PLANT SPACING ON YIELD AND CONTENT OF FLAVONOIDS IN FIREWEED HERB (*Epilobii herba*)

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ABSTRACT

Fireweed (*Epilobium angustifolium* L.) is a quite common species in our country. However, in order to obtain larger quantities of high quality *E. angustifolia* herb, it is necessary to introduce it into cultivation. The study covered four-year field experiment (2013–2016). The aim of the study was to investigate the fireweed reaction to the impact of various levels of mineral fertilization and spacing. It has been shown that the increased amount of fertilizer components at the level of 60 N, 70 P_2O_5 and 100 K_2O kg·ha^{-1} stimulated vegetative growth and had a positive effect on yielding. The highest biological efficiency of the plantation was found at the population density resulting from 45 × 30 cm spacing. Increasing the dose of mineral fertilization and increasing the plant spacing did not result in statistically proven impact on the quality of the raw material (flavonoid content).

Key words: *Epilobium angustifolium*, herb, yield, mineral fertilization, plant spacing, flavonoids

INTRODUCTION

Epilobium angustifolium L. syn. (*Chamaenerion angustifolium* (L.) Scop.) is a medicinal plant used in traditional medicine of many countries. Fireweed is herbaceous perennial plant growing in northern climates throughout the world [Fleenor 2016]. In Poland, it occurs throughout the country, in mountains to the dwarf pine level. It inhabits pioneer sites, particularly abundant in freshly transformed soils in coniferous or mixed forest ecosystems. Moreover, it is a common weed of roadsides, balks and on field borders [Szafer et al. 1986]. Plants are useful in forest restoring due to the ability to germinate and grow in reclamation areas [Pinno et al. 2014].

Species belonging to *Epilobium* genus are a rich source of polyphenolic compounds, including flavonoids, phenolic acids and tannins. They also contain lipophilic compounds, such as steroids, triterpenoids and fatty acids [Granica et al. 2014, Gryszczyńska et al. 2018, Jürgenson et al. 2012, Kaškonienė et al. 2015, Maruška et al. 2014]. The most important active ingredients are flavonoids and ellagitannins, including oenotein B. Fireweed has been used as an agent accelerating the healing of extensive wounds and as a disinfectant. In recent years, the species has gained increasing importance in the treatment of benign prostatic hyperplasia. Kosalec et al. [2013] compared antimicrobial activity of ethanol extracts from flowers and leaves of fireweed. The strains of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Candida albicans,*

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C. tropicalis, C. dubliniensis and *Saccharomyces cerevisiae* were susceptible to both extracts. Researchers suggest that because of high antimicrobial activity, the extracts can be used in the adjunctive therapy of benign prostatic hyperplasia. The effectiveness of fireweed extracts in the treatment of prostate disease was confirmed by Kiss et al. [2004, 2006]. They showed in biological tests that extracts of fireweed have an inhibitory effect on peptidases, which are responsible for the development of prostate diseases. The authors attribute this activity mainly to such compounds as flavonoids, phenolic acids and ellagitannins. Experimental studies have shown that extracts, as well as particular substances or groups of compounds obtained from herbs have a wide range of effects: antibacterial, stimulating epithelial tissue regeneration, antispasmodic, antioxidant and potentially anti-inflammatory [Granica et al. 2014, Jambor 2012, Kiss et al. 2004, 2006, Kosalec et al. 2013, Kujawski et al. 2010, Maruška et al. 2014, Schepetkin et al. 2016].

The previous research carried out with *E. angustifolium* concerned mainly the developmental biology and phytochemical assessment of the raw material. Therefore, there is a need to conduct the agricultural research on this species. The aim of the present study was to evaluate the reaction of fireweed to various levels of mineral fertilization (fertilizing experiment) and to determine the optimal spacing of plants (cultivation experiment).

MATERIAL AND METHODS

The research was conducted in the experimental field of the Department of Botany, Breeding and Agricultural Technology of Medicinal Plants INF&MP, Plewiska near Poznań, Poland, in 2013–2016. The climate is temperate there. During the vegetation period the highest mean of temperature was noticed in 2013, and the lowest in 2015 (Table 1). The highest sum of rainfalls was recorded in 2016, and the lowest in 2015. Plot experiments were established on lessivé soil developed from ground moraine with the granulometric composition of light loamy sands. In the years of research, the soil was characterized by following chemical properties: pH in H_2O: 6.6–7.0; $N\text{-}NO_3$ content: 17–21, P: 66–154, K: 110–150, Ca: 550–917, Mg: 42–50 mg · dm^{-3}; salinity: 0.12–0.21 g NaCl · dm^{-3}. Forecrop (mustard-phacelia mixture) was grown in the main crop for green manure and in summer was disked and ploughed. Immediately before planting fireweed, the soil was cultivate with a cultivation unit (string roller cultivator). The initial material were underground organs obtained from the natural sites (northwest region of Poznań). The cuttings obtained by dividing the thickened rhizomes were planted directly into the ground in autumn (October). The experiments were set up as unifactorial in a randomized block system with three replicates. Research was carried out by performing two types of experiments.

Fertilizing experiment

The variable factor were mineral fertilization doses, in variants:

a) 1 (NPK): N – 50, P_2O_5 – 60, K_2O – 80 kg·ha^{-1},

b) 2 (NPK): N – 60, P_2O_5 – 70, K_2O – 100 kg·ha^{-1}.

Commercial formulations of the following fertilizers were used: nitro-chalk (28% N), granular superphosphate (46% P_2O_5) and potassium salt (60% K_2O). The comparative object was control: "0" (without fertilization). The same planting spacing was used: 45 × 40 cm.

Cultivation experiment

The variable factor was the spacing of fireweed cuttings in the following variants:

a) 45 × 30 cm,

b) 45 × 40 cm,

c) 45 × 50 cm.

The same mineral fertilization was applied at the level: N – 50, P_2O_5 – 60, K_2O – 80 kg·ha^{-1}, using the same type of fertilizer as in the experiment.

For both experiments, the plot area to be planted was 12 m^2. The raw material (herb) was collected manually, in the first year of cultivation in the full flowering phase – once, while in the 2nd and 3rd – twice (in the full blooming and in the beginning of reflorescence). The types of basic agricultural measures as well as the dates of their performance are listed in table 2.

The scope of research included the assessment of fresh and dry herb yield by weight method (mass). The dry mass was determined after reducing the moisture content of the raw material to 12% in an GoBest UZ-108 electronic dryer, at a recommended tempera-

Table 1. Weather conditions during the vegetation period. Plewiska, 2013–2016

Month	2013		2014		2015		2016	
	mean of temp. (°C)	sum of rainfalls (mm)	mean of temp. (°C)	sum of rainfalls (mm)	mean of temp. (°C)	sum of rainfalls (mm)	mean of temp. (°C)	sum of rainfalls (mm)
April	11.6	17.1	10.9	29.5	8.7	20.3	8.8	0.3
May	18.1	63.4	13.7	113.8	13.4	39.6	15.6	33.0
June	21.1	98.6	16.5	26.2	15.9	93.7	18.4	102.6
July	23.6	35.2	21.7	60.2	19.3	87.4	19.1	118.1
August	22.5	30.6	17.2	79.0	22.1	16.8	17.7	36.3
September	15.9	65.5	15.3	51.6	14.6	26.2	16.8	11.2
November	13.7	15.0	10.8	31.2	7.9	33.5	8.4	77.7
Mean/∑	18.1	325.4	15.2	391.5	14.6	317.5	15.0	379.2

Table 2. Dates of agricultural measures and phenological stages of fireweed in field experiments. Plewiska, 2013–2016

	1st year of cultivation		2nd year of cultivation		3rd year of cultivation	
	2013	2014	2014	2015	2015	2016
Planting	12 X 2012	18 X 2013	–	–	–	–
Beginning of vegetation	29 IV	24 IV	18 IV	10 IV	10 IV	20 IV
Fertilization ($^1/_2$N, P, K)	30 IV	26 IV	23 IV	21 IV	21 IV	26 IV
Full of vegetation	6 V	9 V	29 IV	28 IV	28 IV	25 IV
Fertilization ($^1/_2$N)	6 VI	10 VI	30 V	18 V	18 V	1 VI
First harvesting of herb	3 VIII	28 VII	9 VI	1 VI	1 VI	24 V
Second harvesting of herb	–	–	8 VIII	4 VIII	4 VIII	17 VIII

ture of 50–55°C. The flavonoid content was determined spectrophotometrically after their extraction from the raw material and calculated as quercetin [Farmakopea Polska VI 2002] . The detection wavelength applied was 425 nm. In order to assess the significance of differences between experimental objects, an analysis of variance was carried out according to the model for the system of random blocks. Fisher-Snedecor's F test was used as the criterion of significance of differences, and LSD was calculated using the Student test t at a significance level of 0.05%. Voucher specimens were identified by the authors and deposited in the Institute Natural Fibres and Medicinal Plants (Poznań, Poland).

RESULTS

Fertilizing experiment. The yields of fresh and air-dried matter of fireweed herb are summarized in tables 3 and 4. Regardless of the plantation age, a significant effect of the mineral fertilization dose (nitrogen, phosphorus, potassium) on the yield level was found. Using dose 2 (NPK), the yield increased by 75% in first, by 25% in second and by 12% in third year of cultivation, in comparison to dose 1 (NPK). The increase of yield relative to the control ("0") was 95, 30 and 40%, respectively. Studies have shown that in the 2nd and 3rd year of cultivation two herb harvests are obtained, while the weight obtained from the first cut was 85–

Table 3. Effect of fertilization on the herb yield of fireweed. Fresh mass (q·ha^{-1}). Plewiska, 2013–2016

	1st year of cultivation 2013–2014	2nd year of cultivation 2014–2015			3rd year of cultivation 2015–2016			Σ 1st-3rd years of cultivation
		first harvesting	second harvesting	Σ	first harvesting	second harvesting	Σ	
1 NPK N – 50, P_2O_5 – 60, K_2O – 80	24.76	376.05	32.97	409.02	371.12	48.03	419.15	852.93
2 NPK N – 60, P_2O_5 – 70, K_2O – 100	40.00	468.15	37.28	505.43	403.49	69.65	473.14	1018.57
Control „0”	21.01	360.62	25.07	385.69	290.74	47.66	338.40	745.10
$LSD_{(0,05)}$	5.15	13.09	3.13	91.37	16.80	n.s.	122.42	85.08

n.s. – not significant difference

Table 4. Effect of fertilization on the herb yield of fireweed. Dry mass (q·ha^{-1}). Plewiska, 2013–2016

	1st year of cultivation 2013–2014	2nd year of cultivation 2014–2015			3rd year of cultivation 2015–2016			Σ 1st-3rd years of cultivation
		first harvesting	second harvesting	Σ	first harvesting	second harvesting	Σ	
1 NPK N – 50, P_2O_5 – 60, K_2O – 80	5.62	87.28	8.65	95.93	90.26	12.84	103.10	204.65
2 NPK N – 60, P_2O_5 – 70, K_2O – 100	9.78	109.02	9.63	118.65	96.55	18.43	114.98	243.41
Control „0”	4.95	84.57	6.68	91.25	67.91	12.47	80.38	176.58
$LSD_{(0,05)}$	1.17	22.76	0.94	22.33	6.54	n.s.	10.64	22.57

n.s. – not significant difference

90% of the total yield. As a result, on the 2–3-year-old plantations a multiple increase of yield was noted, as compared to 1-year (only one herb harvest). The average dry herb yield obtained using mineral fertilization was 7.70 in first, 107.29 in second and 109.04 q·ha^{-1} in third year of cultivation. During the study period, the highest yield amounting 118.65 q·ha^{-1} was obtained in the 2nd year of cultivation, using dose 2 (NPK). The flavonoid content in raw material calculated as quercetin is summarized in table 5. No statistically significant differences were found between the flavonoid content in the raw material and the fertilization level. Although based on the results obtained it can be concluded that the flavonoid accumulation increases in subsequent years of cultivation. In addition, the use of fertilization in the 2nd and 3rd year of cultivation resulted in a statistically insignificant, but noticeable decrease of flavonoid content in fireweed herb.

Cultivation experiment. The average yield of fresh and air-dry herb weight is summarized in tables 6 and 7. Based on the average results, with three years of research, it is possible to take into account the large variability of fireweed crop depending on the spacing used. Except for the first harvest in the third year of cultivation, regardless of the plantation age, the highest herb yield was obtained at the highest planting

Table 5. Effect of fertilization on the content of flavonoids in fireweed herb. Content of flavonoids calculated as quercetin (g·100 g^{-1}). Plewiska, 2013–2016

	1st year of cultivation 2013–2014	2nd year of cultivation 2014–2015			3rd year of cultivation 2015–2016			Weighted mean 1st-3rd years of cultivation
		first harvesting	second harvesting	weighted mean	first harvesting	second harvesting	weighted mean	
1 NPK N – 50, P_2O_5 – 60, K_2O – 80	0.41	0.41	0.42	0.41	0.60	0.35	0.57	0.46
2 NPK N – 60, P_2O_5 – 70, K_2O – 100	0.28	0.51	0.48	0.51	0.66	0.26	0.60	0.46
Control „0”	0.26	0.64	0.52	0.63	0.80	0.39	0.74	0.54
$LSD_{(0,05)}$	n.s.	n.s.	n.s.	n.s.	n.s	n.s	n.s.	n.s.

n.s. – not significant difference

Table 6. Effect of spacing on the herb yield of fireweed. Herb yield. Fresh mass (q·ha^{-1}). Plewiska, 2013–2016

	1st year of cultivation 2013–2014	2nd year of cultivation 2014–2015			3rd year of cultivation 2015–2016			Σ 1st-3rd years of cultivation
		first harvesting	second harvesting	Σ	first harvesting	second harvesting	Σ	
45 cm × 30 cm	31.83	402.10	43.71	445.81	351.48	77.91	429.39	907.03
45 cm × 40 cm	26.22	354.82	33.21	388.03	376.43	73.58	450.01	864.26
45 cm × 50 cm	17.66	355.07	35.35	390.42	327.02	66.18	393.20	801.28
$LSD_{(0,05)}$	8.15	n.s.	4,78	n.s.	21,10	n.s.	45.00	101.25

n.s. – not significant difference

Table 7. Effect of spacing on the herb yield of fireweed. Herb yield. Dry mass (q·ha^{-1}). Plewiska, 2013–2016

	1st year of cultivation 2013–2014	2nd year of cultivation 2014–2015			3rd year of cultivation 2015–2016			Σ 1st-3rd years of cultivation
		first harvesting	second harvesting	Σ	first harvesting	second harvesting	Σ	
45 cm × 30 cm	7.06	89.02	11.28	100.30	83.83	20.25	104.08	211.44
45 cm × 40 cm	5.97	83.46	8.65	92.11	90.50	19.14	109.64	207.72
45 cm × 50 cm	3.73	84.45	9.09	93.54	78.40	17.27	95.67	192.94
$LSD_{(0,05)}$	1.83	n.s.	0.78	n.s.	5.92	n.s.	n.s.	n.s.

n.s. – not significant difference

density tested, as a result of the 45 × 30 cm spacing. Increasing the spacing to 45 × 50 cm resulted in the first year of cultivation a significant reduction in the herb's dry matter yield by about 45%. In 2–3-year-old plantations the tested spacing did not significant differentiate the yield height statistically. The average dry herb yield was: 5.59 in the first, 95.32 in the second and 103.13 q·ha^{-1} of dry herb weight in the third year of cultivation. The highest total yield in the amount of 109.64 q·ha^{-1} was obtained in the third year of cultivation, with a spacing of 45 × 40 cm. The flavonoid content in the raw material calculated as quercetin is summarized in table 8. Statistical analysis showed no relationship between the flavonoid level in the raw material and the planting density, though increase of the content of these compounds in the following years of cultivation was observed.

Table 8. Effect of spacing on the content of flavonoids in fireweed herb. Content of flavonoids calculated as quercetin (g·100 g^{-1}). Plewiska, 2013–2016

	1st year of cultivation 2013–2014	2nd year of cultivation 2014–2015			3rd year of cultivation 2015–2016			Weighted mean 1st-3rd years of cultivation
		first harvesting	second harvesting	weighted mean	first harvesting	second harvesting	weighted mean	
45 cm × 30 cm	0.30	0.31	0.35	0.32	0.53	0.33	0.50	0.37
45 cm × 40 cm	0.30	0.32	0.36	0.33	0.52	0.36	0.49	0.37
45 cm × 50 cm	0.39	0.25	0.35	0.26	0.50	0.28	0.46	0.37
$LSD_{(0,05)}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. – not significant difference

DISCUSSION

The literature provides a comprehensive description of the habitat conditions, but the causal relationship remains to be clarified and how agroclimatic factors affect the dynamics of *E. angustifolium* development. Available publications show that factors that are important when studying the spatial distribution of this species in the field include primarily mineral nutrients and light requirements [Myerscough and Whitehead 1966, 1967]. The tendency to create dense stand and strong dependence of fireweed development on the soil's nitrogen content were described. Hence, the amount of mineral fertilization and plant density per unit area can be a potentially important element of the cultivation technology. The distance between plants determines the power of competition of plants for light, thus the planting density can limit or stimulate plant development.

Long-term plot experiments were conducted in Canada by Grainger and Turkington [2013]. They studied the impact of enrichment of natural sites with minerals (fertilizer applied once a year) on the growth and development of several plant species of boreal forest understory, including *E. angustifolium*. They used granulated fertilizer (N : P : K 35 : 10 : 5) in an amount of 17.5 N, 5 P and 2.5 K g·(m^2)$^{-1}$. Researchers hypothesized that fireweed adapts faster and better to a new nutrient-rich environment than other plant species tested (*Mertensia paniculata, Achillea millefolium, Festuca altaica*). Analysis of the results confirmed that in a situation where nutrients in the substrate become available, *E. angustifolium* closed stand and in conditions of limited amount of light, the plants grow taller, have a larger leaf area and produce more biomass. These conclusions are consistent with the results of own field research, in which the highest herb yield was obtained in combinations with the highest mineral fertilization level (2NPK) and at higher planting density. Confirmation of these results are experiments conducted in greenhouse conditions. Pinno et al. [2014] observed growth dynamics depending on nutrient uptake. Fireweed was cultivated on various types of reclamation soils, which differ significantly in terms of total nitrogen and phosphorus content. The best fireweed growth was found for nitrogen-phosphorus-potassium (NPK) fertilization. When fertilizing only with nitrogen or phosphorus and potassium, the response to growth depended on the type of soil, which indicates that specific fertilizer mixtures should be used at specific locations. In other studies, Myerscough and Whitehead [1967] cultivated *E. angustifolium* in a standard solution containing fertilizers and three consecutive tenfold dilutions. N, P and K levels in standard solutions were 558, 317 and 9.50 ppm, respectively. The degree of plant development and leaf surface showed large reduction. The intensification of red discoloration was also observed when the dilution of solution increased.

In the literature, the fireweed is often considered to be a nitrophilic plant, meanwhile, according to Myerscough and Whitehead [1966], its relationship with the habitat (spatial distribution) should also be properly related to conditions that prefer nitrification. In forests, especially on poorer soils, its occurrence is often associated with the presence of a large amount of decomposing wood, as well as with sites of recent fires, in which the substrate is usually rich in minerals. The same researchers also studied the germination biology of *E. angustifolium*. They observed that germination in conditions of reduced soil moisture and air humidity, as well as reduced supply of minerals, mainly nitrogen and potassium, was difficult and inefficient. In addition, they found that at low temperatures (10°C), the maximum percentage of germination occurs at higher levels of lighting, while the demand for light disappears as the temperature increases to 30°C [Myerscough and Whitehead 1966]. In turn, Van Andel and Vera [1977] studied another aspect of the developmental biology of *E. angustifolium* and competitive species characteristic of the forest thinnings (*Senecio sylvaticus*). Species were grown by sowing seeds into the soil at three levels of mineral nutrition. It was found that with the increase in soil nutrient levels, the ratio of flowering to non-flowering plants increases for fireweed.

According to literature data, taking into account spatial distribution of the species, the fireweeds prefers an exposure with a lot of light, moderately moist soils and sheltered areas where plants are not exposed to cold, drying winds in winter. In open sites, it does not tolerate hot and dry summers [Myerscough and Whitehead 1966]. Similar results were obtained in own research. In the case of high air temperatures, during summer, the leaf tips dried and the plant growth stopped. This situation occurred in 2013 (one-year plantation), with experiments carried out on light soils, in open and sunny sites. The changing weather conditions in the years of research strongly influenced on the level of yield. In 2015, due to the low rainfall in April to October (317.5 mm), the lowest herb yields were obtained during entire research period.

In the literature on *E. angustifolium* there are very few studies on field research. Myerscough and Whitehead [1967] conducted a series of plot experiments that complemented greenhouse research on the development of fireweed depending on the agricultural factors used. The studies have shown the large requirements of fireweed in terms of the content of basic nutrients in soil in relation to plant needs related to the growth of new shoots. They found that *E. angustifolium* easily spreads in the field by vegetative reproduction, but also has the ability to produce shoots in the same places in subsequent years. Therefore, the species is adapted to be survived in small, favourable locations. It also demonstrates a clear adaptation to growth in close stands due to compensation for the reduction in light intensity by increasing the leaf surface. These remarks confirm the usefulness of fireweed for establishing and running effective field plantations. The comparative studies of herb growth dynamics and phenolic compounds content of two-year-old plants of *Epilobium angustifolium* and *E. hirsutum* were carried out by Pelc and Węglarz [2006]. It was found that in the vegetative phase the mass of the *E. angustifolium* air-dry herb was greater and in the generative phase was lower than the mass of *E. hirsutum*.

In the fertilizer experiment, the highest flavonoid content ($0.80\ g \cdot 100\ g^{-1}$) was found on control plots (without fertilization) in the herb from the first harvest in the third year of cultivation. In the cultivation experiment, the highest level of flavonoids ($0.53\ g \cdot 100\ g^{-1}$) was recorded at a spacing of 45 × 30 cm in the herb from the first harvest in the third year of cultivation. These values are lower than those obtained in

E. angustifolium herb harvested in the massive blooming phase from different Lithuanian populations amounting 1.43–4.10 g·100 g^{-1} [Kaškonienė et al. 2015]. In turn, the levels of these compounds in the samples consisting of leaves and flowers, and obtained from 10 Finnish populations, were 0.58–1.66 g·100 g^{-1} [Baert et al. 2017]. Similar variability of flavonoids content, amounting 0.58–1.52 g·100 g^{-1}, was found from 30 populations of *E. angustifolium* in the eastern Poland [Pelc et al. 2007]. While the levels of flavonoids in *E. angustifolium* collected in Estonia were even lower, amounting 0.236 g·100 g^{-1} in leaves and 0.209 g·100 g^{-1} in flowers [Jürgenson et al. 2012]. Fireweed is characterized by significant inter-population phytochemical variability [Adamczak et al. 2019]. The variation of flavonoid contents increases when different plant parts are analysed, and the raw material is collected in various developmental phases. The highest contents was recorded in the massive flowering phase [Baert et al. 2017, Maruška et al. 2014, Jürgenson et al. 2012] or in the vegetative stage [Pelc and Węglarz 2016]. Leaves and flowers were the best plant material to obtain high amount of flavonoids. The obtained results suggest the dependence of the quality of the raw material on the plantation age and planting density. The highest flavonoid content calculated as quercetin in fireweed herb (especially from the first cut) was obtained in the 2nd and 3rd year of cultivation, i.e. in conditions of higher stand density. Similar regularities were found in the *Epilobium hirsutum*, *E. parviflorum* and *E. tetragonum* herbs, where the content of flavonoids in the full blooming phase was the highest in the second year of vegetation [Pelc et al. 2005].

CONCLUSIONS

E. angustifolium cultivated on light soils strongly reacted to the level of mineral fertilization and the spacing of rhizome cuttings. The reaction force varied depending on the age of plantation. Increased amount of fertilizer ingredients at the level of: 60 N, 70 P_2O_5 and 100 K_2O kg·ha^{-1} stimulated the vegetative growth of the aboveground parts of plants and had a positive effect on yielding. The highest efficiency in field cultivation occurred at plant density resulting from the 45 × 30 cm spacing. Increase of dose of mineral fertilization and plant density did not result in deterioration of the quality of the raw material (flavonoid content).

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