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## EFFECTS OF DIFFERENT PLANT EXTRACTS ON THE MINERAL CONTENT OF BLACKBERRY LEAF (*R. fruticosus*) IN ORGANIC PRODUCTION

### SUMMARY

The aims of this study were to investigate the chemical content of plant extracts of nettle (*Urtica dioica*), alfalfa (*Medicago sativa*) and dandelion (*Taraxacum officinale*) and to determine their effect on the chemical content of blackberry leaves grown in organic production. Field and laboratory analysis were conducted over a two-year period. The plant extracts were fermented for 14 days before analysis. The field trial adopted a randomised block design with four repetitions. Each repetition included five blackberry bushes. The following properties were analysed: the chemical composition of plant extracts, macroelement concentration (%), microelement concentration ( $\text{mg kg}^{-1}$ ) and the fresh and dry weights of blackberry leaves (g). Analysis of the macronutrient content revealed the highest N content in the alfalfa plant extract, the highest P concentration in nettle plant extract, while the highest concentration of K was found in dandelion plant extract. The N and P concentrations were higher in all treatments than in the controls. The Fe concentration in blackberry leaves ranged from  $70.63 \text{ mg/kg}^{-1}$  (treatment with alfalfa plant extract) to  $76.68 \text{ mg kg}^{-1}$  (treatment with nettle plant extract). The difference in blackberry leaf Mn concentration between the dandelion treatment and the control were highly significant. The nettle and dandelion plant extracts influenced the Cu content of blackberry leaves. The Zn concentration in blackberry leaves treated with plant extracts was significantly higher than that in the controls. The highest dry mass content was found in blackberry leaves treated with nettle plant extract.

**Keywords:** plant extract, nettle, dandelion, alfalfa, blackberry leaves, micro and macroelements

### INTRODUCTION

Historically, humankind has known the effects of plant extracts for hundreds of years and has successfully used them in traditional agriculture (Lawless, 1995). A large number of plants grow freely in nature can serve to

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make quality foliar fertilizer. The prepared extracts from these plants are a source of readily available nutrients for cultivated plants.

In addition to providing the plant with sufficient plant nutrients, plant extracts may in part have insecticidal and fungicidal effects due to the bioactive chemicals present in the solution (Kim *et al.*, 2005; Daoubi *et al.*, 2005). Plant extracts used as foliar fertilizers could be a significant source of readily available elements; however, this may depend on the type and the quality of the soil on which these plants were grown (Popescu *et al.*, 2010; Markoski *et al.*, 2018). Nettle used to make plant extracts is rich in Mg, Ca, and K and also contains significant amounts of the trace elements Zn, Cu, Fe, Cr, Mn, Mo, Si, and V (Gohari *et al.*, 2018).

The use of plant extracts from different plant species gives fruit producers the opportunity to count on quality yield with better marketing possibilities. The application of cultivated plants can be done every 10 to 15 days without the fear of contamination or overyielding. These plant extracts are similar in purpose to those obtained from herbal teas and have been used for a long time on small farms in developing countries (Roy *et al.*, 2005).

The increase in yield of cultivated plants, which have been subjected to foliar treatments, is the result of the nutrient uptake from solution, which results in the intensification of photosynthesis and the accumulation of minerals in the edible part of the plant (Guievence and Badem, 2000; Batra *et al.*, 2002).

The mineral substances from the plant extracts are absorbed by the leaf cuticle, epidermis cells, stoma closure cells and through the hairs on the leaf. The diffusion of nutrients from the leaf surface to the free space within the cell wall is dependent on the difference in the nutrient concentration between the external environment and the concentration within the apoplasts. Nutrient uptake is higher with older leaves, as it has a larger total uptake area compared to younger leaves (Mengel, 2002). Aging of the leaves can affect the permeability of the leaf surface in such a way as to reduce the stoma opening process due to potassium retranslocation during senescence (Jordan and Brodribb, 2007). Young, partially-developed leaves are considered to have easier and faster nutrient uptake than older, fully-developed leaves. However, the influence of leaf age on foliar nutrient uptake has not been fully explained (Fernandez and Eichert, 2009).

The aim of the study was to investigate the chemical composition of plant extracts of nettle, alfalfa and dandelion and to determine their effect on the concentration of minerals in the blackberry leaves grown in organic production.

## MATERIAL AND METHODS

Field trials were conducted in the village of Brekinja near Kozarska Dubica in organic blackberry orchard. Laboratory tests of plant extracts were carried out in the Agricultural Institute of Republika Srpska in Banja Luka. Their health status was examined at the Veterinary Institute "Dr. Vaso Butozan" in Banja Luka. Laboratory analyzes of the mineral content of blackberry leaves as well as soil analysis were done in the Laboratory for agrochemistry of the Faculty of

Agriculture in Novi Sad. Field trials and the laboratory tests were conducted in the period of two years.

Plant extracts of nettle, alfalfa and dandelion were fermented for 14 days before the application. The plants used to make these extracts were young, finely chopped and mixed with rainwater. The fermentation took place in the shade with occasional stirring of the solution. One kilo of freshly chopped plant material was used for the nettle extract, while two kilos of chopped plant material was used for alfalfa and dandelion plant extract. After the fermentation, the mixtures were filtered and thereafter diluted with distilled water in 1:10 ratio.

The trial was set up as random block system with four repetitions. Each repetition consisted of five blackberry bushes (Čačanska bestrna variety) planted 3 m x 1.5 m apart.

Treatments were performed with a Villager DM-25 motor sprayer with a capacity of 15 l and operating pressure of 15-30 bars that was being regulated by the throttle. During the application the sprayer operated at almost full throttle. The first treatment started in the middle of May and the following were performed every 10 to 15 days until the first ripe blackberry fruits appeared.

During this study the following properties were analyzed: the chemical composition of plant extracts, macroelement concentration (%), microelement concentration (mg kg<sup>-1</sup>) and the fresh and dry weight of blackberry leaves (g). Examination of the health status of the plant extracts included testing for the presence of: Salmonella, Staphylococcus, Clostridia, Proteus species and Escherichia coli.

Mineral content of plant extracts were performed in the in the laboratories of the Department of Agroecology at the Agricultural Institute of Republika Srpska in Banja Luka.

The following macronutrients were tested in the plant extracts:

- a) Kjeldahl nitrogen - wet combustion (conc. H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> + 450°C - Kjeltec system I, Tecator), distillation.
- b) Phosphorus - wet burning with a mixture of acids (HNO<sub>3</sub> + HClO<sub>4</sub> + H<sub>2</sub>SO<sub>4</sub>); from solution, determination of phosphorus by staining method (dyeing in yellow) with optical density reading on a spectrophotometer.
- c) Potassium - wet burning with a mixture of acids (HNO<sub>3</sub> + HClO<sub>4</sub> + H<sub>2</sub>SO<sub>4</sub>); from solution, potassium readings by flame photometry.

Leaf sampling started after the last treatment of blackberry bushes. Sampling was performed in a matter that 30 leaves was collected from each repetition, resulting in 120 leaves in each treatment. The youngest, fully developed leaves were sampled in mid-July. The samples were weighed, than washed in distilled water, air-dried, and then dried in the oven at 70°C for 48 hours. Afterwards, dried leaves were finely grounded and weighed.

The concentration of macro and microelements in the leaf dry matter was determined in the Laboratory for Agrochemistry of the Faculty of Agriculture in Novi Sad.

The obtained results were analysed by the analysis of variance, and the least significance difference (LSD) test.

*Agro-ecological conditions*

Soil samples were analyzed at the Laboratory for Pedology at the Faculty of Agriculture in Novi Sad prior to field trial set up. During the course of the trial, meteorological parameters were gathered at the Hydrometeorological Station located in the Mlječanica Spa near Kozarska Dubica. Latitude and longitude of the field trial are 45°08'N and 16°43'E, respectively, and the climate is moderately continental. The elevation is 157 m.

*Soil conditions*

The trial was performed on peat soil suitable for growing fruit crops. The soil structure is crumbly, with a favorable water-air regime. The results of chemical analyzes of the soil samples taken before the start of the study are shown in Table 1.

Table 1. Results of chemical analyzes of the soil samples before the treatments

Depth (cm)	pH in KCl	pH in H <sub>2</sub> O	CaCO <sub>3</sub> (%)	Humus (%)	Total N (%)	Al-P <sub>2</sub> O <sub>5</sub> mg/100g	Al-K <sub>2</sub> O mg/100g
0 – 40	6,95	7,67	20,37	3,21	0,21	9,72	20,68

Based on the results of chemical analyzes of soil samples taken at a depth of 0-40 cm, the soil is poorly alkaline (pH 7.67 in H<sub>2</sub>O), and it is medium humic in terms of humus content (3.21%). The Ca content is high and thus the soil is classified as calcareous. The availability of readily available phosphorus is low (9.72 mg 100g<sup>-1</sup>), while the potassium content in soil is favorable for fruit production and amounts to 20.38 mg 100g<sup>-1</sup> K<sub>2</sub>O. The levels of Pb and Cd in the samples indicate that the soil is suitable for organic production (Tab. 2)

Table 2. Total cadmium (Cd) and lead (Pb) content in the soil

Depth (cm)	Pb (mg kg <sup>-1</sup> )	Cd (mg kg <sup>-1</sup> )
0 – 40	15,5	1,78

The concentration of Pb (15.5 mg kg<sup>-1</sup>) and Cd (1.78 mg kg<sup>-1</sup>) at 0-40 cm soil depth is lower than the amounts allowed in the soil intended for organic production.

Weather conditions. The average rainfall during the study was 600 mm, which was higher than the perennial average (442.7 mm). During the vegetation period (IV-VIII), the average air temperature varied compared to perennial average (18.9°C).

## RESULTS AND DISCUSSION

### *Chemical content of plant extracts*

Chemical analysis was performed to determine the content of macronutrients in plant extracts of nettle, dandelion and alfalfa (Table 3). Chemical analysis of plant extracts showed that the dandelion and alfalfa extracts were acidic, while the nettle extract had slightly acidic reaction. The pH values measured ranged from 5.2 (dandelion extract) to 6.4 (nettle extract). The highest N concentration was found in alfalfa extract (105.6 mg l<sup>-1</sup>). Alfalfa extract had twice as much N as the dandelion extract (41.0 mg l<sup>-1</sup>). The highest P content was measured with nettle extract (56.3 mg l<sup>-1</sup>). Potassium concentration in dandelion extract was 1205,3 mg l<sup>-1</sup>, which was over 40% higher than in nettle and alfalfa extracts.

Table 3. Chemical composition of plant extracts with a fermentation period of 14 days

No.	Extract	pH	N (mg l <sup>-1</sup> )	P (mg l <sup>-1</sup> )	K (mg l <sup>-1</sup> )
1.	Nettle	6,4	56,8	56,3	700,2
2.	Dandelion	5,2	41,0	42,5	1205,3
3.	Alfalfa	5,4	105,6	40,2	670,4

### *Mineral content of the blackberry leaves*

Mineral concentration of blackberry leaves is conditioned by the root activity and the ability of leaves to absorb ions from the applied plant extracts. The intensity of ion uptake depend on the leaf age, position as well as weather conditions during the study.

Concentration of macro and microelements in the blackberry leaves after the treatment with plant extracts are shown in Table 4.

Table 4. Concentration of macroand microelements (mg kg<sup>-1</sup>) in blackberry leaves before and after treatment with nettle, dandelion and alfalfa plant extracts

Treatment		Macroelements (%)			Microelements (mg kg <sup>-1</sup> )			
		N	P	K	Fe	Mn	Cu	Zn
Control		1,95	0,20	0,65	75,36	7,49	4,12	15,14
Plant extract	Nettle	1,99	0,23	0,63	76,68	8,17	4,57*	16,50*
	Dandelion	1,98	0,26	0,66	72,00	12,06**	4,08*	16,28*
	Alfalfa	1,98	0,26	0,65	70,63	10,86**	3,63	16,27*
LSD	0,05	0,24	0,11	0,14	7,25	1,35	0,42	1,06
	0,01	0,33	0,15	0,19	10,17	1,89	0,59	1,48

Blackberry leaves treated with plant extracts of nettle, dandelion and alfalfa had a higher concentration of nitrogen than the control, but the differences

found were not statistically significant. The highest concentration of N was in blackberry leaves treated with nettle extract (1.99%).

The highest average P concentration (0.26%) was found in blackberry leaves treated with dandelion and alfalfa extracts, and the lowest in the control (0.2%). All samples treated with nettle, dandelion and alfalfa extracts had higher P content compared to the control, however, the differences were not statistically significant. Blackberry leaves treated with nettle extract were found to have lower average P concentration of 0.03% compared to leaves treated with alfalfa and dandelion extracts.

The highest concentration of K was found in leaves treated with dandelion extract (0.66%). No statistically significant differences in K concentration were found between the control and the treatments with plant extracts. However, blackberry leaves treated with nettle extract had a 0.03% lower K concentration than the control. Potassium concentration was the same in leaves treated with alfalfa extract and the control (0.65%).

Different localities and soil types significantly affect the mineral composition of blackberry leaves (Licina and Oparnica, 2000a). The unfavorable agrochemical and physical properties of the soil significantly condition the mineral composition of the fruit leaves, and this is especially true for the N, K, Mn and Fe content (Licina and Oparnica, 2000 b).

Iron content in blackberry leaves after the treatments varied from 70,63 mg kg<sup>-1</sup> (alfalfa extract) to 76,68 mg kg<sup>-1</sup> (nettle extract). Blackberry leaves in the control treatment had a higher Fe content than the leaves treated with dandelion and alfalfa extracts. The differences in Fe content between the control and treatment with alfalfa extract were 4.73 mg kg<sup>-1</sup> and were not statistically significant.

The optimal concentration of Fe in the leaves of strawberries and berry fruits is within the narrow range of 70 - 80 µg/g (70-80 mg kg<sup>-1</sup>) of dry matter (Bergmann, 1983). The Fe concentration in healthy blackberry leaves was 142 µg/g (142 mg kg<sup>-1</sup>) in dry matter, while in diseased leaves it reached significantly higher values of 351 µg/g (351 mg kg<sup>-1</sup>) in dry matter (Stevanovic *et al.*, 1999). In this study Fe concentration found in blackberry leaves was within the optimal range.

The concentration of Mn in the leaves treated with nettle, dandelion and alfalfa extracts was higher than the control. The highest average concentration of Mn was found in blackberry leaves treated with dandelion extract (11.88 mg kg<sup>-1</sup>). A high concentration of Mn was also found in blackberry leaves treated with alfalfa extract. The differences in Mn concentration between treatments with dandelion and alfalfa extracts compared to controls were statistically significant. Treatment with nettle extract increased the Mn content of the blackberry leaves, but the differences compared to control were not statistically significant.

Optimal concentration of Mn in the blackberry leaf ranges from 40-150 µg/g (40-150 mg kg<sup>-1</sup>) of dry matter. Symptoms of the Mn deficiency occur at values lower than 10 µg/g (10 mg kg<sup>-1</sup>) of dry matter (Bergmann, 1983).

Manganese concentration in the leaves in the control treatment and the treatment with nettle extract was below the limit of  $10 \text{ mg kg}^{-1}$ .

During this study, nettle and dandelion extracts were found to statistically significant increase the concentration of copper in the blackberry leaves. At the same time, the concentration of copper in the blackberry leaves treated with alfalfa extract was lower than the control.

Copper is considered to be an essential microelements for higher plants, and its role in the transport of matter is predominantly catalytic. Plant deficiency symptoms are observed at concentrations less than  $4 \text{ }\mu\text{g/g}$  ( $4 \text{ mg kg}^{-1}$ ) of dry matter, and signs of excess at concentrations greater than  $30 \text{ }\mu\text{g/g}$  ( $30 \text{ mg kg}^{-1}$ ) of dry matter (Ubavić et al., 2001). During this study lower concentration of  $4 \text{ mg kg}^{-1}$  of dry matter was found in blackberry leaves treated with alfalfa extract ( $3.63 \text{ mg kg}^{-1}$ ), indicating its deficiency.

All treatments with plant extracts resulted in increased Zn concentration in the blackberry leaves relative to the control. The differences in Zn concentration between the control and the treatments were statistically significant. The highest concentration of Zn was found in blackberry leaves treated with blackberry extract ( $16.50 \text{ mg kg}^{-1}$ ).

Concentrations of Zn in blackberry leaves during the study were within the optimal range between  $20$  and  $70 \text{ }\mu\text{g/g}$  ( $20\text{-}70 \text{ mg kg}^{-1}$ ) of dry matter (Bergmann, 1983). The concentration of Zn in the blackberry leaves in all treatments was below  $20 \text{ mg kg}^{-1}$  of dry matter. During the two-year trials, the average concentration of Zn in the blackberry leaves was  $34 \text{ mg kg}^{-1}$  of dry matter (Glišić et al., 2006), which is higher than the results obtained during this study.

#### *Fresh and dry blackberry leaf weight*

The effect of plant extracts on the weight of fresh and dried blackberry leaves was observed as well as as the proportion of dry weight per fresh weight-dry matter content (Tab. 5).

Table 5. Fresh and dry blackberry leaf weight

Treatment	Fresh leaf weight (g)	Dry leaf weight(g)	Dry matter content (%)
Control	43,80	18,35	42,06
Nettle	49,23	20,74	42,25
Dandelion	44,25	17,93	40,27
Alfalfa	48,57	20,42	42,14
Average	46,46	19,15	41,68
LSD 0,05	18,84	8,86	2,10
0,01	26,41	12,42	2,94

The mass of fresh blackberry leaves treated with nettle, dandelion and alfalfa extracts was higher than the control, but the differences found were not

statistically significant. The average fresh leaf weight ranged from 43.80 g (control) to 49.23 g (nettle extract).

The highest leaf dry weight was obtained by treating fresh leaves with nettle extract (20.74 g). At the same time, the leaves that were treated with the dandelion extract had the lowest dry weight (17.93 g). Comparing treatments with the control indicates no statistically significant differences in the weight of dried blackberry leaves treated with different plant extracts.

The average dry matter content was 41.68%. The highest proportion of dry matter was found on the samples treated with nettle extract (42.25%) and the lowest in the treatment with dandelion extract (40.27%). The differences in the dry matter content between the control and the treatments were not statistically significant. Fresh blackberry leaves harvested in the spring can be used for chewing, brewing and teas. Freshly squeezed blackberry leaf juice is used in folk medicine for compresses with ulcers and insect bites. After harvesting, and drying blackberry leaves can be used to make teas and tea blends that are used in alternative medicine (Gursky, 1978).

### CONCLUSIONS

The dandelion and alfalfa plant extracts were acidic and the nettle slightly acidic reaction. Chemical analysis of the macroelement showed that alfalfa plant extract had the highest nitrogen content (105.6 mg l<sup>-1</sup>), nettle had highest phosphorus content (56.3 mg l<sup>-1</sup>), while the dandelion extract had the highest potassium content (1205.3 mg l<sup>-1</sup>).

In blackberry leaves treated with nettle, dandelion and alfalfa plant extracts, nitrogen and phosphorus concentrations were higher than in control treatment, but the differences found were not statistically significant. Potassium concentration was the same or below the levels in the control treatment (nettle extract treatment).

The differences in manganese concentration between blackberry leaves in the control treatment and dandelion and alfalfa plant extract treatments were statistically highly significant.

A lower copper concentration than 4 mg kg<sup>-1</sup> of dry matter was found in blackberry leaves treated with alfalfa extract (3.63 mg kg<sup>-1</sup>), indicating its deficiency. The concentration of manganese in blackberry leaves in control and nettle extract treatments was below the 10 mg kg<sup>-1</sup> limit when symptoms of its deficiency appear.

A significantly higher concentration of copper was found in blackberry leaves treated with nettle and dandelion plant extract compared to the control.

The concentration of zinc in the leaves treated with the plant extracts was significantly higher compared to the control. The highest dry matter content was found in blackberry leaves treated with nettle extract.

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