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ORIGINAL PAPERS

EVALUATION OF THE INFLUENCE OF SYMBIOTIC MYCORRHIZAL FUNGI ON BASIC CHEMICAL COMPOUNDS AND MINERALS IN SEA BUCKTHORN LEAVES

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Abstract

The study included an assessment of the chemical composition of three varieties of sea buckthorn (Ascola, Habego and Leikora) cultivated with and without an application of mycorrhizal fungi. Micorrhization was conducted with ectomicorrhizal mycelium, which is symbiotic for plants of the olive family. The study was conducted in 2014, at the Experimental Station in Lipnik (53°20′35″N 14°58′10″E), Poland. The soil in which the plants were grown belongs to rusty soils and is classified as Haplic Cambisol. The experiment was conducted in a randomized block design with five replications (one shrub - one replicate). The content of nutrients and selected macroelements was determined in leaves of the plants. The mean crude protein content in the leaves reached 20.3% of dry mass. Mycorrhization decreased the protein concentration in the leaves of the tested varieties except the variety Leikora, which responded with a protein content increase. The crude fat content in the leaves of mycorrhized plants was higher by 4.7% than in the samples without mycorrhization. Application of mycorrhizae decreased the crude fibre content in the examined varieties. The least crude fibre was found in leaves of cv. Ascola (9.6%). Sea buckthorn leaves are a rich source of macroelements (N, P, K, Mg and Ca). The results of the study on mycorrhization of sea buckthorn do not demonstrate unambiguously the direction of changes in the content of basic nutrients and macroelements.

Keywords: nutrients, *Hippophae rhamnoides* L., macroelements, mycorrhization.

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INTRODUCTION

Many plant species have already been tested for their potential biological, therapeutic and pharmaceutical properties (HUSSAIN et al. 2008). Sea buckthorn (*Hippophae rhamnoides* L.), a unique and valuable plant, has recently gained worldwide attention, mainly because of its medicinal and nutritional potential. Sea buckthorn (SBT) is a thorny nitrogen-fixing deciduous shrub of cold arid regions, native to Europe and Asia. It is currently domesticated in several parts of the world owing to its nutritional and medicinal properties. The word Hippophae has been derived from a Latin word 'hippo' meaning 'a horse' and 'phaos' which means 'shine'. In Greece, SBT leaves and twigs were used to feed animals to promote weight gain and shiny coat, especially in horses. It has a rich history of being used in treatment of numerous medical conditions (SURYAKUMAR, GUPTA 2011). The health promoting properties of SBT fruit and seeds are commonly recognized as they are a source of biologically active compounds, such as tocopherols, caroteinoids, vitamins, phenols, which exhibit significant antioxidant potential (VARSHNEYA et al. 2012). Sea buckthorn leaves, on the other hand, are less used in the food and feed industries. Also, references concerning the chemical composition of SBT leaves and its health promoting properties are limited. Sea buckthorn leaves contain nutrients and bioactive substances, which include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols. Leaves are equally rich in important antioxidants, including *β*-carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid. They also contain significant quantities of calcium, magnesium and potassium. UPADHYAY et al. (2010) showed that SBT leaves are rich in total phenols and in flavonoids, which are good antioxidants, showing immuno-stimulating and anti-inflammatory properties, which suggests the clinical potential of SBT leaves. The minerals in SBT leaves, such as iron, phosphorus and magnesium, have a positive effect on the processes of absorption of vitamins and other nutrients, and therefore enhance tissue building and prevent anemia. TIAN et al. (2004), GUAN et al. (2005) and NEGI et al. (2005) showed that fresh SBT leaves are rich in carotenoids (26.3 mg 100 g^{-1}) and chlorophyll (98.8 mg 100 g^{-1}), which are considered quality indicators for green vegetables. SBT leaves also contain high quantities of protein (21%), of which 0.73% is lysine and 0.13% are methionine and cystine (BISWAS et al. 2010, PATIAL et al. 2013). This comprehensive evaluation of SBT leaves is aimed at searching opportunities for its wider use in production of feeds for monogastric animals and, more importantly, of its use as a source of biologically active phyto-compounds in human nutrition.

Cultivation of sea buckthorn has also economic justification as it is a low-demanding plant and every part of it can be used (fruit, seeds and leaves). Taking into consideration the current trends in use of agrichemicals, it is necessary to return to more natural and environmentally friendly methods. Mycorrhizae are a symbiotic association of plants and fungi whose appearance limits the amount of artificial fertilisers, most of which are leached into the groundwater and contaminate it. Eliminating the use of plant protection products, especially fungicides, contributes to a better protection of the environment (SCHÜTZENDÜBEL, POLLE 2002). Hence, the aim of this research was to evaluate the chemical composition of leaves of three varieties of sea buckthorn cultivated with and without an application of mycorrhizal fungi.

MATERIAL AND METHODS

The study was conducted in 2014, at the Experimental Station in Lipnik (53°20′35″N 14°58′10″E), Poland. The experiment was set up in a randomized block design with five replications (one shrub – one replicate). Bushes were planted in 4x3 m spacing. The size of a single plot was 12 m^2. The total number of plots in the experiment was 30. The soil on which the experiment was conducted belongs to rusty soils (CPS, 2011) and is classified as *Haplic Cambisol* according to FAO-WRB (2007). In the Ap level, it has the texture of clay sand with a slightly acidic pH, the humus content (1.3-1.5%) and silt and clay (11 - 13%). The humus level was formed from clay sands. The analysis of the soil minerals showed high levels of phosphorus and moderate levels of magnesium and potassium.

Plant material

Two-year-old shrubs (with no fruit), which represented female varieties Ascola, Habego, Leikora and a male variety, a pollinator Pollmix, were studied. The outline of the experiment was set according to the factors:

- mycorrhization of some of the plants (5 shrubs), in the liquid form applied into the root zone of one-year-old shrubs. Additionally, the biological agent Fungilitic® (Adiumentum) was used on one- and two-year-old mycorrhized plants during the growing season;
- five shrubs with no mycorrhization or Fungilitic® were used as control;
- different varieties (Ascola, Habego, Leikora).

Mycorhhization was conducted with ectomicorrhizal mycelium, which is symbiotic for plants of the olive family. The isolate, which was obtained from the natural ecosystems in Croatia, contained symbiotic mycorrhizal fungi (*Glomus* spp., *Gigaspora* spp., *Pochonia* spp., *Lecanicillum* spp.), and the root bacteria (*Bacillus* spp). A dose of 15 ml was applied twice in each plant's root zone. The mycelium contained an addition of hydrogel (ensuring humidity essential for the initial fungi development). The plant protection agent Fungilitic® contains *Paenibacillus polymyxa* DCF strain, which exhibits anti-fungal and anti-bacterial properties and kills soil nematodes.

Weather conditions during the measurements

The atmospheric conditions in the whole year of the study are presented in Table 1. During the growing season, the air temperature and precipitation

Table 1

Temperature (°C) and rainfall (mm) during the experiment as compared with multiyear averages (1961 - 2004)

Years	Apr	May	June	July	Aug	Sept	Apr-Sept
Rainfall (mm)							
2014	37.0	100.5	48.5	95.0	66.5	106.0	453.5
Multiyears 1961 - 2004	34.9	48.6	61.7	70.9	54.1	51.6	321.8
Temperature (°C)							
2014	11.1	14.0	16.9	21.8	18.2	13.9	16.0
Multiyears 1961 - 2004	8.9	13.2	16.2	18.1	18.1	13.6	14.7

were measured at the weather station in Lipnik, localized near the experimental plots. The mean monthly temperature and precipitation in the experimental year were slightly different from the multiyear averages, being by 1.3°C and 131.7 mm higher, respectively.

During the growing season (02.08.2014), samples of leaves were collected for the laboratory analysis in order to determine their chemical composition and nutritional value. The leaves chosen for the measurements were well-developed, taken from the middle part of one-year-old shoots on the outer part of shrubs, in the middle of their height. The analysis was conducted on fully developed leaves without any signs of ageing or mechanical damage.

Chemical analysis

The samples were dried at 105°C in an oven with a fan. The chemical composition of the samples was determined according to the Association of Official Analytical Chemists procedures (AOAC, 2006): dry mass, by drying at 105°C to constant weight; ether extract, by Soxhlet extraction with diethyl ether; crude ash, by incineration in a muffle furnace at 580°C for 8 h; crude protein (N × 6.25), by Kjeldahl method using a Büchi Distillation Unit B-324 (Büchi Labortechnik AG, Switzerland); crude fiber, with a fiber analyser – ANCOM 220 (ANCOM Technology, USA); Nitrogen-free extract (NFE) was calculated as: NFE (%) = 100 - % (moisture + crude protein + crude fat + ash + crude fiber). The material for the macro-component concentration analyses was subjected to mineralization in concentrated H_2SO_4 and $HClO_4$ acids. Phosphorus (P) was assessed by the colourimetric method on a Specol 221 apparatus. An atomic absorption spectrometer (iCE 3000 Series, Thermo Fisher Scientific) was used to determine K. Calcium was determined by means of emulsion flame spectroscopy, whereas the concentrations of Mg, Fe and Zn were detected by absorption flame spectroscopy.

Statistical analysis

The results of the chemical composition analysis of SBT leaves (*Hippophae rhamnoides* L.) were processed statistically by variance analysis for short-term experiments, and the significance of differences with LSD of 0.05 was evaluated with the Tukey's test. The statistical analysis was carried out using the ANALWAR - 5.1.FR programme.

RESULTS AND DISCUSSION

The chemical composition of the analysed SBT varieties showed significant variation depending on the factors (Tables 2-3). Research of using leaf

Table 2

Mycorrhizal impact on the chemical composition in leaves of sea buckthorn (g kg^{-1} d.m.)

Object	Trait					
	dry matter (g kg^{-1} fresh)	ash	crude protein	crude fat	crude fibre	NFE
Control	321.8	55.7	204.2	67.5	109.6	563.1
Mycorrhizae	325.9	56.4	201.3	70.7	101.4	570.3
Ascola	341.1	57.9	190.0	64.6	108.9	578.6
Habego	314.2	51.5	216.7	70.3	111.3	550.3
Leikora	316.2	58.7	201.5	72.5	96.2	571.2
Mean	323.8	56.0	202.7	69.1	105.5	566.7
LSD $_{0,05}$ for mycorrhizae	*n.s.	n.s.	n.s.	1.65	3.41	n.s.
LSD $_{0,05}$ for variety	10.7	3.83	7.33	2.53	5.23	15.1

* n.s. – non-significant difference

Table 3

Mycorrhizal impact on the content of macroelements in leaves of sea buckthorn (g kg^{-1} d.m.)

Object	Trait				
	N	P	K	Mg	Ca
Control	28.2	4.78	15.7	1.98	5.82
Mycorrhizae	25.7	5.40	15.0	2.23	6.10
Ascola	26.0	5.06	14.9	2.36	7.11
Habego	26.0	4.80	15.0	1.91	5.63
Leikora	28.9	5.42	16.1	2.00	5.14
Mean	27.0	5.09	16.4	2.09	5.96
LSD $_{0.05\ for}$ mycorrhizae	1.19	0.13	0.25	0.01	0.09
LSD $_{0.05\ for}$ variety	1.82	0.20	0.42	0.02	0.13

extracts in human and animal nutrition started in the 1960s, when Pirie (1966) suggested using leaf extracts of green leafy plants in combating undernourishment in developing countries. The suggestion provoked numerous studies, which focused on various leaves extracts (Shah et al. 1981). Leaves of the studied SBT varieties are relatively high in protein. Kahif and Ullah (2013) examined selected medicinal plants and also found the most protein in SBT leaves, whereas significantly less protein was found in leaves of neem (*Azadirachta indica*), pomegranate (*Punica granatum*) and tulsi (*Ocimum tenuiflorum*) (99.6, 36.9 and 51.0 g kg^{-1} d.m., respectively). In this study, the protein content reached 202.7 g kg^{-1} d.m. (Table 2), which confirms the results of other authors. Li and Wardle (2002) determined the protein content in SBT leaves to be 20.6% in 2000 and 21.1% in 2001. Biswas et al. (2010) obtained similar results, i.e. 20.7%. The highest protein content in the present research was found in cv. Habego, which had 14% more protein in leaves than the protein-poorest Ascola (Table 2). The only variety in which the protein content increased after mycorrhization was Leikora (18.8% more protein in comparison to the control). In the remaining varieties, mycorrhization decreased the protein concentration in leaves (Figure 1). It should be empha-

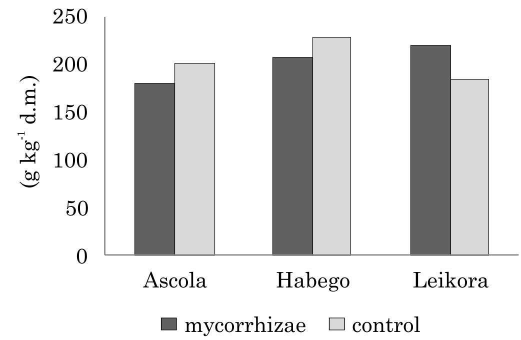

Fig. 1. The impact of mycorrhizae and variety on the content of crude protein in leaves of sea buckthorn, LSD for 0.05 = 10.4

sised that the protein content in SBT fruit (which has much wider application in human nutrition) is just about 5% (Selvamuthukumaran, Farhath 2014). Comparison of SBT leaves and fruit shows an over 20-fold higher protein concentration in leaves.

The fat content determines the energy contained in plant material to a wider extent than protein, total carbohydrates or fibre. It is a concentrated energy source for all tissues and cells, with the exception of the brain, kidneys and erythrocytes. In the studied SBT varieties, the mean fat content reached 69.1 g kg^{-1} d.m. (Table 2). The leaves of mycorrhized plants had 4.7% more fat than the control. The highest fat content was found in Leikora and the lowest one was in Ascola. Singh (1998) studied nutritional values of sea buckthorn and concluded that the fat content in *H. rhamnoides* ranged between 3.5 and 4.8% whilst in *H. salicifolia* it reached 4.6%. In this study, the values were higher, that is the mean fat content in the control reached 6.75% whilst in the mycorrhized sample it was 7.07%. The fat content in medicinal plants is lower even by 50% (Kashif, Ullah 2013).

Until the 1980s, the indigestible food ingredients of plant origin were considered to be a ballast in the human digestive tract (Nawirska, Kwaśniewska 2005). Currently, more attention is paid to such components because of their importance in the human and animal physiology. Crude fibre is a component of dietary fibre. In literature, there are few definitions of both dietary fibre and crude fibre. It is now known that dietary fibre has a complicated, heterogenous organisation. It contains a lot of structures of different physical and chemical properties that produce various physiological effects on the human organism (Cummings et al. 2004, Mann, Cummings 2009). Components of the crude fibre fraction are cellulose and lignin and partly hemicellulose. Crude fibre contains insoluble fractions, therefore its physiological effect is mainly to regulate intestinal peristalsis, prevent constipation, remove toxins and metabolites and, consequently, reduce the risk of cancer, especially the colon neoplasm (Thompson 2000). The present research shows that mycorrhization of SBT shrubs decreased the content of crude fibre in leaves by 7.5% (Table 2). The highest concentration of crude fibre and also protein was found in leaves of the Habego variety. In all the studied varieties, mycorrhization decreased the content of crude fibre (Figure 2), but the lowest levels were observed in leaves of Leikora (less by 15.2% than in the control).

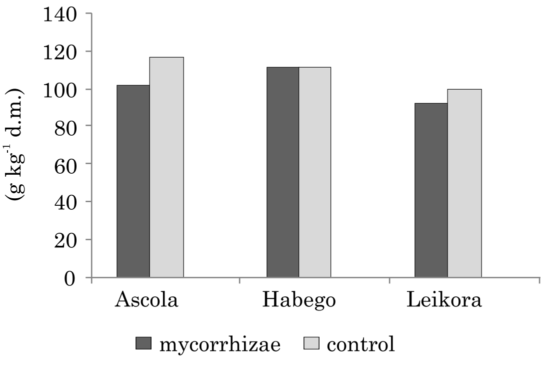

Fig. 2. The impact of mycorrhizae and variety on the content of crude fibre in leaves of sea buckthorn, LSD for 0.05 = 7.40

Sea buckthorn leaves are a good source of minerals, valuable for nutritional and processing purposes. The content of minerals, expressed as total ash, was higher by 11.7% in Ascola and Leikora than in Habego (Table 2). Mycorrhization increased the amount of total ash only in the Leikora variety (by 27.7%) – Figure 3. The values of total ash in SBT leaves (5.6%) were

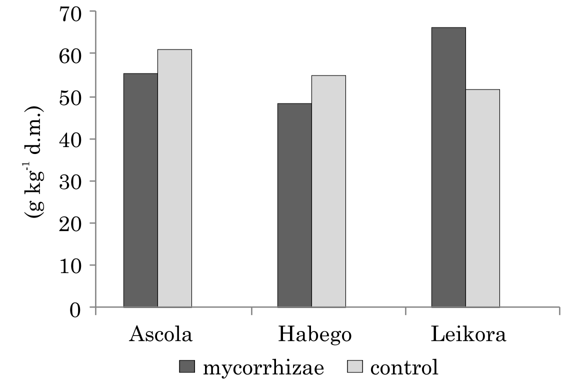

Fig. 3. The impact of mycorrhizae and variety
on the content of crude ash in leaves of sea buckthorn,
LSD for 0.05 = 5.41

similar to the levels reported by SINGH (1998) in leaves of *H. salicifolia* (5.1%). On the other hand, SELVAMUTHUKUMARAN and FARHATH (2014) revealed that the total ash content in fruit was lower than in leaves by 1.88%.

Total carbohydrates (NFE, nitrogen free extract) play metabolic and structural functions and are a storage of easily accessible energy. Through photosynthesis carbohydrates are the main component of dry mass in most of plant products. In the present study, NFE ranged from 550.3 to 578.6% g kg^{-1} d.m. The lowest NFE content was found in Habego (Table 2). Leaves of the mycorrhized Ascola and Habego varieties had respectively 7.6% and 4.4% more NFE than the control (Figure 4). SELVAMUTHUKUMARAN and FARHATH (2014) recorded 61.9% total carbohydrate content in SBT fruit.

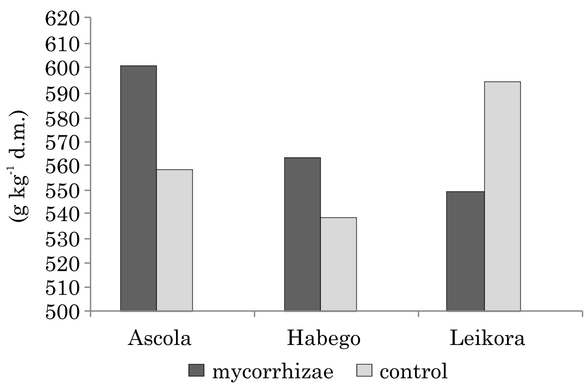

Fig. 4. The impact of mycorrhizae and variety
on the content of NFE in leaves of sea buckthorn,
LSD for 0.05 = 21.4

Dry matter of SBT leaves is the sum of components other than water, mostly protein, fat, carbohydrates, crude fibre and minerals The mean content of dry mass in all of the samples (converted to fresh weight) reached 323.8 g kg^{-1}. The higher dry matter content was recorded in the variety Ascola. In the cultivars Habego and Leikora, the dry mass content was lower by 7.9% and 7.3%, respectively, compared to Ascola (Table 2). Myccorhization increased the level of dry mass by 6.6% in Ascola and by 2% in Habego, while decreasing it by 4.8% in Leikora (Figure 5).

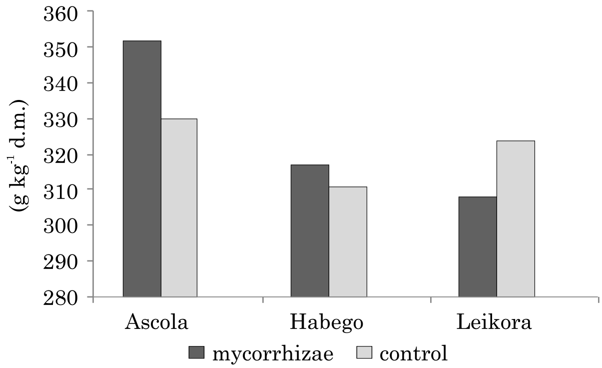

Fig. 5. The impact of mycorrhizae and variety on the content of dry matter in leaves of sea buckthorn, LSD for 0.05 = 15.2

The results of the research concerning mycorrhization do not indicate a clear direction of changes in the basic nutrients.

Herbs are a valuable source of many minerals and the form in which they occur is usually easily digestible to humans (Özcan 2004). In natural conditions, the content of micro- and macro-elements depends on a plant species and even on its variety. It is also affected by the soil type and properties as well as the agroclimate and pollution; moreover, it depends on the part of a plant and its stage of development (Smrkolj, Stibilj 2004, Ražic et al. 2005). Taking into consideration the importance of plants in medicine, it is clear that minerals are essential in any assessment of the nutritional value of sea buckthorn. The present study found a statistically significant influence of mycorrhization and a variety on the concentration of macro-elements (nitrogen, phosphorus, potassium, magnesium and calcium) in leaves of the three studied varieties (Table 3). Hyphae of the VA mycorrhizae fungi absorb from the soil such minerals as P, Ca, Zn, Cu, S, B, Cl and transfer them to the roots of symbiotic plants (Kothari et al. 1990). In leaves of the mycorrhized shrubs, there was less nitrogen and potassium and more phosphorus than in the control. Mycorrhizal fungi are capable of secreting metabolites that enhance the accessibility of hardly soluble phosphorus compounds, and the use of mycorrhizae increases the concentration of this element in the plant. Given the differences in the content of the analysed elements in the plant material, the macro-elements can be ranked in the following order: K > Ca > P > Mg. After the analysis of the macronutrient content in SBT leaves, it is worth mentioning that the variety Leikora contained the largest amounts of nitrogen, phosphorus and potassium in dry matter (Table 3). Nigerian medicinal plants (*Aspilia africana* and *Bryophyllum pinnatum*) are used in herbal medicine to cure diseases and heal injuries, although they contain significantly smaller amounts of the studies elements (Okwu, Josiah 2006) than the SBT leaves examined in the present study. More nitrogen (by 3.7%) was found in leaves collected from the mycorrhized Habego (Figure 6). More phosphorus was found in leaves collected from the mycorrhized Habergo and Leikora varieties (by 27.2% and 33.1%, respectively) – Figure 7. More

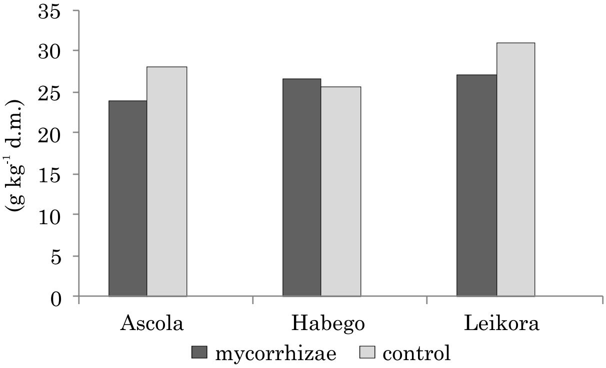

Fig. 6. The impact of mycorrhizae and variety on the content of N in leaves of sea buckthorn, LSD for 0.05 = 2.58

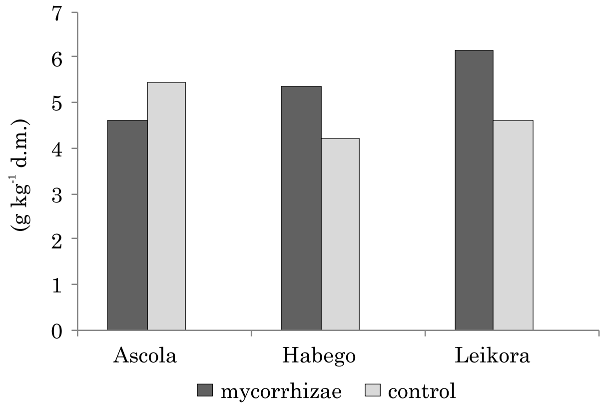

Fig. 7. The impact of mycorrhizae and variety on the content of P in leaves of sea buckthorn, LSD for 0.05 = 0.28

potassium was found in the mycorrhized Leikora variety (24% increase) – Figure 8. The analysis of the results indicates an increased uptake of magnesium (by 13.8%) and calcium (by 4.8%) by the mycorrhized plants. The highest content of both elements was found in the variety Ascola (Table 3).

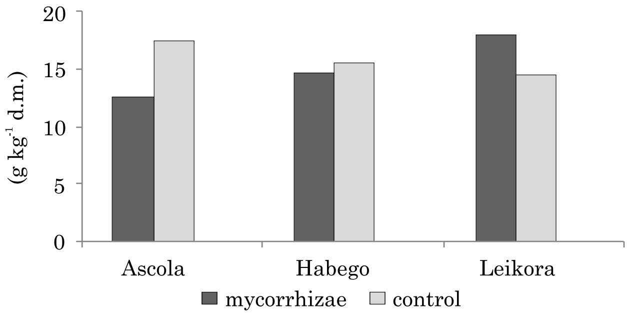

Fig. 8. The impact of mycorrhizae and variety on the content of K in leaves of sea buckthorn, LSD for 0.05 = 0.60

A significant increase in magnesium and calcium was recorded in leaves of the mycorhhized Ascola (more by 63.7% and 23%, respectively). A different response to mycorrhization was observed in the Habego variety, in which the content of these macronutrients decreased by 11.3% and 24.3%, respectively (Figures 9-10).

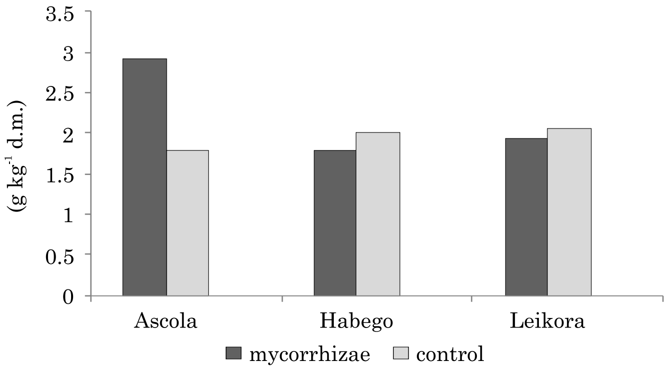

Fig. 9. The impact of mycorrhizae and variety on the content of Mg in leaves of sea buckthorn, LSD for 0.05 = 0.02

The leaves of sea buckthorn have the highest amounts of macronutrients among all the parts of this plant. SABIR et al. (2005) studied *Hippophae rhamnoides* ssp. *Turkestanica* fruit and reported the content of phosphorus at 0.11 - 0.13 g kg^{-1} d.m., potassium at 0.14 - 0.36 g kg^{-1} d.m, magnesium at 0.15 - 0.24 g kg^{-1} d.m, and calcium at 0.07 - 0.1 g kg^{-1} d.m. ZEB (2004) provided much lower values of the analysed macroelements both in juice and in dried SBT fruit. The research by POPESCU et al. (2010) demonstrated higher concentrations of sodium, manganese, iron and calcium in SBT leaves than in bilberry leaves and fruit, carrot roots, Jerusalem artichoke leaves and tubers, tomatoes, leek and hawthorn leaves and fruit.

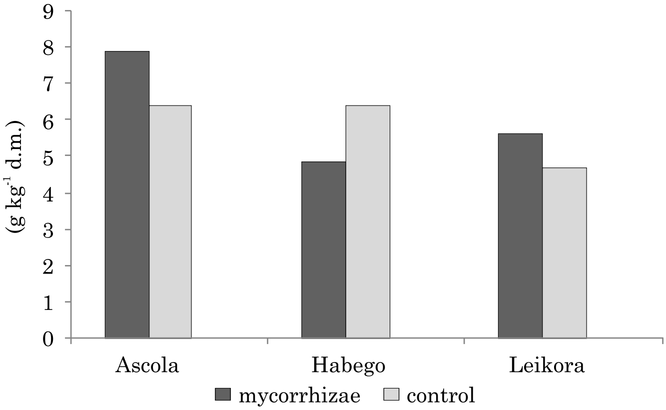

Fig. 10. The impact of mycorrhizae and variety on the content of Ca in leaves of sea buckthorn, LSD for 0.05 = 0.19

CONCLUSIONS

The results of the present study show the basic chemical composition and macroelements content in SBT leaves, thus filling in a large gap in literature concerning the nutritional value of sea buckthorn leaves. SBT leaves have a very high crude protein content, relatively high crude fat and are a good source of macroelements. The results of the research concerning mycorrhization do not indicate a clear direction of the changes in basic nutrients. The present study implicates a need to assess the nutritional value of the plant, which should be an integral part of the breeding work on this valuable species, especially the less appreciated leaves of sea buckthorn.

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