The leaves of sea-buckthorn (Hippophae rhamnoides L.) can be a rich source of nutrients and biologically active substances. Their levels depend on growing conditions, agricultural technology and climate. The studies however, mainly focus on nutritious value of fruit and seeds and there is shortage of the information regarding to buckthorn leaves. The aim of the experiment was to determine the effect of symbiotic mycorrhizal fungi on the chemical composition and antioxidant activity of sea-buckthorn leaves. The study was conducted in 2014 and 2015 at the Experimental Station in Lipnik, Poland. Mycorrhization improved the nutritional value of leaves of sea-buckthorn by increasing levels of total protein (3%), N free extract (1%), Ca (9%), Na (16%), Fe (18%), Cr (34%), and partially elevating antioxidant activity by increasing the concentration of polyphenols (7%). Leaves of ‘Habego’ had a higher nutritional value, containing more total protein (4%), crude fat (7%), crude fiber (30%), and all fractions of dietary fiber. ‘Hergo’ had more beneficial levels of minerals (P-27%, K-67%, Mg-4%, Na-30%, Zn-8%, and Cr-21%), polyphenols (51%), total flavonoids (35%), carotenoids (10%), and L-ascorbic acid (8%), as well as higher antioxidant activity (40%). The results of our study partly confirmed the earlier scientific reports on the impact of mycorrhiza on the chemical composition of plants. However, it is not possible to compare our results with data on berry plants, including sea-buckthorn, due to the lack of information in the literature.

Key words: Antioxidants, chemical composition, fiber fractions, mycorrhiza, vitamins.

INTRODUCTION

Natural products, either as extracts or as pure compounds, give limitless possibilities for the discovery of new drugs and improving food quality, also thanks to their wide availability. Sea-buckthorn (Hippophae rhamnoides L.) contains a series of compounds including, among others, carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, and tannins. These compounds are of interest not only from the chemical point of view, but also because many of them exhibit biological and therapeutic activities, including antioxidant, antitumor, hepatoprotective, and immunomodulatory properties (Saikia and Handique, 2013). Evaluation of the nutritional value of sea-buckthorn usually concerns its fruit and seeds, while the leaves are rather neglected. However, some studies confirm that also its leaves are a rich source of compounds with anti-inflammatory (Padwad et al., 2006) and antibacterial properties (Upadhyay et al., 2011). They contain large amounts of crude protein, relatively high crude fat, and are a good source of macroelements (Jaroszewska et al., 2016). Therefore, sea-buckthorn leaves may be an excellent source of biologically active phytochemicals in both medicine and human nutrition.

The arbuscular mycorrhizal symbiosis is recognized for its multiple positive effects on plant growth and for its important contribution to the maintenance of soil quality. In spite of these benefits to agriculture, the realization of the full potential of this symbiosis has not yet been reached (Karagiannidis et al., 2012). During the establishment of the arbuscular mycorrhizal symbiosis, a range of chemical and biological parameters is affected in plants, including the pattern of secondary compounds (Karagiannidis et al., 2012). Previous studies confirm that arbuscular mycorrhizal fungi (AMF) affects the content of phenols (Hazzoumi et al., 2015). Higher content of flavonoids has been demonstrated in mycorrhizal roots of alfalfa (Medicago sativa L.) (Larose et al., 2002). The AMF root colonization factor leads to significantly increased concentrations of most of the phenolics in purple coneflower (Araim et al., 2009) and in strawberry fruits (Castellanos-Morales et al., 2010).

Currently, there is little information on the chemical composition and antioxidant properties of sea-buckthorn (Hippophae rhamnoides L.) leaves. There is also a lack of data concerning the potential impact of symbiotic mycorrhizal fungi on the quality of berry plants, including sea-buckthorn. Therefore, in this study we conducted a field experiment to evaluate the impact of symbiotic mycorrhizal fungi on the chemical composition of sea-buckthorn leaves.
and antioxidant activity of leaves of two varieties of sea-buckthorn.

**MATERIALS AND METHODS**

The study was conducted in 2014 and 2015 at the Experimental Station in Lipnik (53°20'35" N, 14°58'10" E, 7 m a.s.l.), Poland. The soil on which the experiment was carried out belonged to typical rusty soils (Polish Soil Classification, 2011), classified as Haplic Cambisol (Soil Classification, 2011), classified as Haplic Cambisol according to IUSS Working Group WRB (2015). At the Ap level, the soil has a slightly acidic loamy sand. The level of humus is formed from clay sands. The analysis of soil minerals showed high P levels and moderate Mg and K levels. The experiment was designed according to a completely randomized method in five replicates (one shrub = one repeat). Shrubs were planted in 4 × 3 m spacing. The size of single plot was 12 m². The total number of plots in the experiment was 20. The subject of the study included 2 and 3 yr old shrubs, female varieties Habego and Hergo. Mycorrhization was conducted with ectomycorrhizal mycelium, which is symbiotic for plants of the olive family. The isolate was obtained from the natural ecosystems in Croatia. It contains symbiotic mycorrhizal fungi (Glomus spp., G. gaspory sp., Pochonia spp., Lecanicillium spp.), and the root bacteria (Bacillus spp.) A dose of 15 mL was applied in two places the root zone of plants in the first year of experiment. The mycelium contained an addition of hydrogel (ensuring humidity essential for the initial fungi development). Leaves for the analysis were collected during harvest from shoots without fruiting. Leaves were collected from the outside of the bush, at half of their height. The leaves were taken from the 1 yr old shoots without any signs of aging or mechanical damage.

The experiment factors were mycorrhiza (half of the bushes from each variety were subject to mycorrhization, and half were used as a control group) and variety (Habego and Hergo).

All determinations were expressed on a dry weight (DW) basis. Dry matter, crude protein, ether extract, crude fiber, crude ash and N free extract (NFE) were determined by AOAC (2012). Dry matter was evaluated 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 Labortecnik AG, Flawil, Switzerland); crude fiber was determined with a fiber analyzer ANKOM 220 (ANKOM Technology, Macedon, New York, USA); total carbohydrates were calculated as: N free extract (NFE) (%) = 100 – % (moisture + crude protein + crude fat + crude ash + crude fiber).

The fiber components were determined using the detergent method according to Van Soest et al. (1991) performed with a fiber analyzer ANKOM 220. Determination of neutral detergent fiber (NDF) was conducted on an ash-free basis and included sodium dodecyl sulfate (NaCl2H2SO4) (822050, Merck, Kenilworth, Nueva Jersey, USA). Determination of acid detergent fiber (ADF) included hexadecyltrimethylammonium bromide (C16H33N(CH3)3Br (Merck 102342), while acid detergent lignin (ADL) was determined by hydrolysis of ADF sample in 72% sulfuric acid (H2SO4). Hemicellulose (HCEL) was calculated as the difference between NDF and ADF, while cellulose (CEL) as the difference between ADF and ADL.

The material for the macro-components concentration analyses was subjected to mineralization in concentrated sulfuric acid (H2SO4) and perchloric acid (HClO4), whilst the material for micro-components concentration analyses was digested in a mixture of nitric acid (HNO3) and perchloric acid (HClO4). The concentration of P was determined by the Egner-Riehm colorimetric method, with ammonium molybdate, at wavelength 660 nm, by using a Specol 221 apparatus (866287, Carl Zeiss Jena, Germany). An atomic absorption spectrometer (ASA) (iCE 3000 Series, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to determine K, Na, and Ca – by means of emulsion flame spectroscopy, and Mg, Zn, Fe, Pb, Cr and Cu – by means of absorption flame spectroscopy. The nitrate content was determined by potentiometry. Prior to assay Ca content, K and Mg trials were appropriately diluted. Other mineral compounds were determined in concentrated samples.

For total polyphenols, total flavonoids, and antioxidant activity determination, methanol extracts were prepared according to Kumaran and Karunakaran (2007). The total phenolic content of plant extracts was determined using Folin-Ciocalteu reagent (Yu et al., 2002); 0.1 cm³ plant extract was mixed with 0.5 cm³ Folin-Ciocalteu reagent and 1.5 cm³ 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 cm³ using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined. These data were used to estimate phenolic content using a standard curve obtained from various concentrations of gallic acid. Total polyphenol content in samples was calculated as the amount of gallic acid equivalent (GAE) in mg kg⁻¹ sample DW.

The total flavonoids content was determined by the method of Kumanan and Karunakaran (2007) using quercetin as a reference compound; 1 cm³ plant extract in methanol was mixed with 1 cm³ aluminum trichloride in methanol and a drop of acetic acid, and then diluted with ethanol to 25 cm³. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 cm³ plant extract and a drop of acetic acid, and then diluted to 25 cm³ with methanol.

The antioxidant capacity was assayed by Trolox Equivalent Antioxidant Capacity (TEAC) method (Re et al., 1999). This method is based on the generation of a stable colored free radical in aqueous solution and the measurement of antioxidant capacity, or free radical scavenging, as the decrease in the absorbance.
of the colored solution in a UV-VIS spectrometer following addition of the antioxidant. The radical used is 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) with an absorption maximum of 734 nm. ABTS was dissolved in deionized water to give a stock solution with a concentration of 7 mM. The ABTS radical cation (ABTS⁺) was produced by reacting 9 cm³ ABTS stock solution with 1 cm³ 24.5 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature overnight before use. The ABTS⁺ solution was diluted with deionized water (approximately 50-fold) to give an absorbance close to 0.70 at 734 nm. A 3 cm³ aliquot of ABTS⁺ solution was put into a cuvette in the spectrophotometer and absorbance measured. An aliquot of 30 mm³ of methanol extract was then added and a second absorbance reading taken after 6 min, by which time the discoloration was effectively complete.

Carotenoids were extracted with 80% solution of acetone and determined according to Lichtenhaler and Wellburn (1983). These compounds were determined in wavelength 440 nm, after having subtracted the concentration of chlorophyll A and B, using wavelengths 663 and 645 nm, respectively, and corresponding absorption coefficients at which carotenoids do not absorb.

Tocopherols were extracted from plants samples with hexane and were determined by Prieto et al. (1999) method. A sample volume of 0.5 cm³ hexane extract was mixed in a test tube with 5 cm³ reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 37 °C for 90 min with vigorous shaking. Absorbance of the aqueous phase at 695 nm was measured against the appropriate blank. A typical blank contained 5 cm³ reagent solution and 0.5 cm³ pure hexane, and it was incubated under the same conditions as the samples. An UV-VIS spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) was used.

Solution for extraction of water-soluble vitamins was made by mixing 50 cm³ acetonitrile with 10 cm³ glacial acetic acid and the volume was finally made up to 1000 cm³ with double distilled water. A sample of 10 g was transferred into conical flasks and 25 cm³ of 30 mm³ of methanol extract was then added and a spectrophotometer and absorbance measured. An aliquot made by mixing 50 cm³ acetonitrile with 10 cm³ glacial acetic acid and the volume was finally made up to 1000 cm³ with double distilled water. A sample of 10 g was added, water bath was kept shaking at 70 °C for 40 min. Thereafter, the sample was cooled down, filtered, and finally the volume was made up to 50 mL with extraction solution (Aslam et al., 2013).

The contents of vitamins thiamine (B1), riboflavin (B2), ascorbic acid (C), and niacin (PP) in extracts were measured using a high-performance liquid chromatographic (HPLC) method described by Klódka et al. (2008); using an HPLC system (Series 200, PerkinElmer, Shelton, Connecticut, USA) equipped with Hypersil ODS column (150 mm x 4.6 mm, 5 µm particle). The mobile phase was 50 mM KH₂PO₄ (A) and methanol (B) in gradient from 0% to 30% A in 8 min, then this ratio was maintained for 7 min. Wavelengths was λ = 245 nm, flow rate 1 cm³ min⁻¹, and injection of 20 mm³. Retention times and recoveries for C, B1, PP, and B2 were 1.45, 5.50, 7.20, 9.80 min, and 96.20, 93.51, 89.53, and 92.41 respectively.

Two-factor ANOVA was carried out on the experimental results using the mycorrhization and genotype as independent variables, after assessing normality and homogeneity of variance. The significance of differences between means was compared by Tukey’s multiple range tests (admissible error for determinations of chemical components was 5%). Results were presented as mean ± standard deviation of three independent determinations. Statistical significance was considered at p ≤ 0.05. The results from the experiment were analyzed using the Statistica version 12.0 software (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

We found nonsignificant effect of mycorrhization on the content of dry weight, crude ash, and crude fat in sea-buckthorn leaves (Table 1). Moisture content of fresh leaves used in the experiment was 68 ± 7% of fresh weight (FW). It was lower than the range of 82% to 96% FW in most vegetables (lettuce, green beans, asparagus, green peppers, and spinach) reported by Granado et al. (1992), but comparable with that (67% FW) of tea leaf (Li et al., 2012). Morgenstern et al. (2014) found the highest DW content in leaves of sea-buckthorn collected at the end of July (32%). Our analysis did not distinguish a variety of sea-buckthorn that differs significantly in DW content in the dried leaves (p ≤ 0.05). The average dry weight content was 95.3%, with a standard deviation of 0.9.

Mycorrhization resulted in an increase in protein content and NFE in sea-buckthorn leaves (by 2.63% and 1.21%, respectively, relative to the control) and a reduction in crude fiber content (by ~3.24%). In the present study variety had

| Item                  | Control       | Mycorrhiza    | P-value | Genotype     | Habego | Hergo | P-value |
|-----------------------|---------------|---------------|---------|--------------|--------|-------|---------|
| Moisture (g kg⁻¹ FW)  | 680.9 ± 70.3a | 625.9 ± 0.27a | 0.223   | 638.2 ± 14.2a | 667.2 ± 78.1a | 0.476 |
| Dry matter            | 953.4 ± 0.9a  | 953.1 ± 19.6a | 0.540   | 953.3 ± 1.1a  | 952.2 ± 0.8a  | 0.900 |
| Crude protein         | 140.9 ± 0.9b  | 144.7 ± 25.5a | 0.006   | 145.4 ± 27.4a | 140.3 ± 20.4b | 0.002 |
| Crude ash             | 40.9 ± 1.0a   | 39.1 ± 5.6a   | 0.081   | 37.2 ± 3.4b   | 42.9 ± 1.3a   | 0.000 |
| Crude fat             | 54.4 ± 4.6a   | 55.4 ± 0.7a   | 0.376   | 56.7 ± 1.9a   | 53.1 ± 3.2b   | 0.021 |
| Crude fiber           | 104.8 ± 10.6a | 101.4 ± 20.2b | 0.031   | 116.4 ± 6.9a  | 89.7 ± 3.1b   | 0.000 |
| NFE                   | 655.0 ± 14.8b | 663.0 ± 49.2a | 0.900   | 644.2 ± 36.7b | 673.9 ± 27.3a | 0.000 |

Mean values with the same letter in each line are not significantly different at p ≤ 0.05 according to the Tukey test, DW: Dry weight, FW: fresh weight, NFE: nitrogen free extract.
an impact on the level of crude ash, crude protein, crude fat, crude fiber, and NFE.

The leaves of the studied varieties of sea-buckthorn showed a considerable diversity in mineral content measured by crude ash. Its average level was 4% DW and ranged from 3.72% to 4.29% DW, with a standard deviation of 0.59. ‘Habego’ contained 13.3% more ash than ‘Hergo’. This study confirmed sea-buckthorn leaves as a rich source of protein. ‘Habego’ leaves had 145.4 g protein kg\(^{-1}\) DW, that is more than the level reported by Kashif and Ullah (2013) (120.35 g kg\(^{-1}\) DW). The difference is probably due to the genotype and the impact of environmental conditions (Zheng et al., 2012). It is worth noting that the protein content in sea-buckthorn fruit (more frequently used as food) stands at a more than 2.5 times lower level of 47 g kg\(^{-1}\) DW (Selvamuthukumaran and Farhath, 2014).

The leaves of the studied varieties of sea-buckthorn differed in crude fat content. Similar to protein, the highest crude fat levels were found in ‘Habego’ (56.7 g kg\(^{-1}\) DW). This confirms the results of other studies, which show that its leaves are a good source of lipids. Fulkerson et al. (2008) report that leaves of fodder radish, rape, chicory, and plantain contain on average 55.6% less crude fat than sea-buckthorn.

The properties of dietary fiber and its value depend on the source and the mutual proportions of the respective fractions. Dietary fiber contains many structures with diverse physical and chemical properties, and is capable of inducing physiological effects on the human body (Mann and Cummings, 2009). The leaves of ‘Habego’ contained ~ 23% more crude fiber than ‘Hergo’. Mycorrhization significantly reduced levels of crude fiber (Table 1) and neutral detergent fiber (NDF) (Figure 1). We also observed some downward trends in the levels of acid detergent fraction (ADF), hemicellulose (HCEL), and cellulose (CEL) in the mycorrhized leaves of sea-buckthorn, although these were nonsignificant. No such trend was observed for acid detergent lignin (ADL). The leaves of ‘Habego’ contained significantly more crude fiber (23%, Table 1) and all evaluated dietary fiber fractions (Figure 1). In the literature there are no data on the composition of the dietary fiber fractions obtained by the detergent method. In this study, the average content of NDF ranged from 219.3 to 278.7 g kg\(^{-1}\) DW, and again, it is impossible to compare this data to literature due to the lack of reports relating directly to sea-buckthorn. Fulkerson et al. (2008) examined NDF in fodder radish (Raphanus sativus L. var. oleiformis Pers.), rape (Brassica napus L.), chicory (Cichorium intybus L.) and plantain (Plantago lanceolata L.), which ranged from 156 to 489 g kg\(^{-1}\) DW.

The determined average ADF, consisting of cellulose and lignin, ranged from 167.9 to 205.1 g kg\(^{-1}\) DW. ‘Habego’ leaves contained ~ 18% of this fraction than those of ‘Hergo’. ADF level, just like NDF, was similar to fodder radish, rape, chicory, and plantain in the study by Fulkerson et al. (2008).

Acid detergent lignin (ADL), which to some extent influences the hardness of sea-buckthorn leaves, differed between ‘Habego’ (71.8 g) and ‘Hergo’ (65.9 g) by as much as 8%.

Hemicellulose content was in the range of 73.6 to 51.4 g kg\(^{-1}\) DW. A common significant source of HCEL are cereal grains, herbs, and vegetables (Biel and Jacyno, 2014; Černiauskiene et al., 2014). Schädel et al. (2010) examined four herb species (Geum urbanum L., Leontodon hispidus L., Salvia pratensis L., Silene flos-cuculi (L.) Greuter & Burdet) and reported HCEL content between the range of 60-220 g kg\(^{-1}\) DW. These authors found higher concentrations in silene, while the lowest HCEL concentrations were measured in leaves of geum, leontodon, and salvia, at less than 10% DW.

In this study, the average CEL content was 117.6 g kg\(^{-1}\) DW and varied from 102 to 133.2 g kg\(^{-1}\) DW. Fraser and Rowarth (1996) found a similar CEL level in the herbs of chicory and plantain. In the present study ‘Habego’ leaves contained significantly more CEL (~ 23%) than ‘Hergo’.

Sea-buckthorn leaves are a rich source of minerals (Jaroszewska et al., 2016), but their levels depend on many factors, including genetic characteristics, climate, soil conditions, maturity of the plant, and the time of harvesting, which was confirmed in our study (Table 2). Compared to the levels found in berries of sea-buckthorn (Ercisli et al., 2007) the average mineral content of the leaves was similar, with the exception of K (116% greater), Mg (31%), Ca (94%), and Fe (574%).

Mycorrhization significantly differentiated macro- and microelements in leaves of sea-buckthorn. Compared to control, leaves of the mycorrhized sea-buckthorn had significantly lower concentrations of P (by 0.5 g kg\(^{-1}\) DW,

**Figure 1.** Fiber components (n = 3) of sea-buckthorn leaves.

NDF: Neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, HCEL: hemicelluloses, CEL: cellulose, DW: dry weight. Mean values with the same letter are not significantly different at p ≤ 0.05 according to the Tukey test.
Table 2. Macro- and microelements (n = 3) and nitrates in sea buckthorn leaves.

| Item      | Control       | Mycorrhiza     | P-value | Habego       | Hergo        | P-value |
|-----------|---------------|----------------|---------|--------------|--------------|---------|
| Macroelements (g kg⁻¹ DW) |
| P         | 4.75 ± 0.4a   | 4.25 ± 0.9b    | 0.001   | 3.96 ± 0.5b  | 5.04 ± 0.1a  | 0.000   |
| K         | 14.9 ± 3.8a   | 12.2 ± 4.1b    | 0.000   | 10.2 ± 1.8b  | 17.0 ± 1.4a  | 0.000   |
| Mg        | 2.35 ± 0.1a   | 1.57 ± 0.0b    | 0.000   | 1.92 ± 0.4b  | 1.99 ± 0.5a  | 0.000   |
| Ca        | 3.45 ± 0.3b   | 3.77 ± 0.2a    | 0.001   | 3.69 ± 0.1a  | 3.57 ± 0.4b  | 0.026   |
| Na        | 84.2 ± 16.9b  | 98.1 ± 10.8a   | 0.000   | 79.1 ± 11.0b | 103.2 ± 5.0a | 0.000   |
| NO₃, mg kg⁻¹ DW |
| Fe        | 49.5 ± 3.9b   | 58.3 ± 1.1a    | 0.000   | 56.0 ± 3.6a  | 51.7 ± 6.5b  | 0.000   |
| Zn        | 33.7 ± 4.5a   | 30.1 ± 1.7b    | 0.000   | 30.6 ± 1.0b  | 33.1 ± 5.2a  | 0.000   |
| Cr        | 0.94 ± 0.0b   | 1.26 ± 0.2a    | 0.000   | 1.00 ± 0.1b  | 1.21 ± 0.3a  | 0.000   |
| Cu        | 3.82 ± 0.2a   | 3.66 ± 0.3a    | 0.294   | 3.82 ± 0.1a  | 3.66 ± 0.3a  | 0.293   |
| Pb        | nf            | nf             |         | nf           | nf           |         |

Mean values with the same letter in each line are not significantly different at p ≤ 0.05 according to the Tukey test. GAE: Gallic acid equivalent, DW: dry weight.

Table 3. Content of antioxidants (n = 3) in sea buckthorn leaves.

| Item              | Control       | Mycorrhiza     | P-value | Habego       | Hergo        | P-value |
|-------------------|---------------|----------------|---------|--------------|--------------|---------|
| Polyphenols, mg GAE kg⁻¹ DW | 8584.7 ± 2191.9b | 9174.6 ± 1834.9a | 0.023   | 7067 ± 570.3b | 10691.7 ± 417.9a | 0.000   |
| Total flavonoids, mg QE kg⁻¹ DW | 2546.2 ± 386.3a | 2284.9 ± 560.8a | 0.171   | 2054.1 ± 243.5b | 2777.1 ± 362.9a | 0.003   |
| Carotenoids, mg kg⁻¹ DW      | 1004.3 ± 8.3a  | 946.1 ± 92.9b   | 0.000   | 929.9 ± 74.9b | 1020.5 ± 14.2a | 0.000   |

Mean values with the same letter in each line are not significantly different at p ≤ 0.05 according to the Tukey test. GAE: Gallic acid equivalent, DW: dry weight. QE: quercetin equivalent.
The studied ‘Hergo’ had clearly superior antioxidant properties, with significantly more polyphenols (3624.7 mg GAE kg\(^{-1}\) DW, i.e. 51%), total flavonoids (723 mg QE kg\(^{-1}\) DW, i.e. 35%), and carotenoids (90.6 mg kg\(^{-1}\) DW, i.e. 10%). The leaves of this variety were also distinguished by higher antioxidant activities (by ~188.3 μmol Trolox\(^{-1}\) DW, 40%) (Figure 2). Morgenstern et al. (2015) showed that sea buckthorn leaves are very rich in beneficial phenolic compounds. The significant differences in antioxidant levels between the two varieties of sea-buckthorn were probably due to the varietal characteristics, as the plants were grown under the same conditions of climate and soil. The results as well as other analysis (Alfaro et al., 2013) highlight the importance of genotype as a crucial factor affecting the content of antioxidants in berry plants.

The harmful effects of free radicals are counteracted by antioxidants, which include, among others, vitamins A, C, and E, abundant in fruits and vegetables. In the studied sea-buckthorn leaves, mycorrhization and variety significantly differentiated only the level of ascorbic acid (Table 4). Depending on the ecotype, sea-buckthorn berries are reported to contain L-ascorbic acid at levels ranging from 360 to 2500 mg 100 g\(^{-1}\) (Bal et al., 2011). The examined leaves contained an average of 2139.6 mg kg\(^{-1}\) DW, compared to 155.3 mg kg\(^{-1}\) DW (7%) in control. Baslam et al. (2011) demonstrated a significant increase in L-ascorbic acid in fresh weight in mycorrhized lettuce. The leaves of ‘Hergo’ sea-buckthorn had a 164.1 mg kg\(^{-1}\) DW (8%) higher concentration of L-ascorbic acid than leaves of ‘Habego’. Although we did not find significant differences in the concentrations of the remaining vitamins (tocopherols, thiamine, riboflavin, and niacin) in sea-buckthorn leaves, there was a tendency towards their higher levels in the leaves of non-mycorrhized sea-buckthorn and ‘Hergo’ leaves.

CONCLUSION

The leaves of mycorrhized sea-buckthorn had a higher content of total protein, N free extract, Ca, Na, Fe, Cr, and polyphenols, which means that the cultivation of mycorrhized sea-buckthorn may improve the intake of these compounds with diet without the need to increase their consumption. Leaves collected from mycorrhized sea-buckthorn also showed an increased antioxidant activity.

The concentration of test compounds in the leaves of different varieties was clearly dependent on the varietal characteristics. The Habego variety was characterized by a higher nutritional value, containing more total protein, crude fat, crude fiber, and all tested fractions of dietary fiber. On the other hand, leaves of ‘Hergo’ sea-buckthorn had more minerals (P, K, Mg, Na, Zn, and Cr), polyphenols, total flavonoids, carotenoids, and L-ascorbic acid. The leaves of ‘Hergo’ also showed higher antioxidant activity.

Summing up the results of the experiment is difficult to assess the impact of arbuscular mycorrhizal fungi (AMF) on the chemical composition of sea buckthorn leaves and their antioxidant activity. The results of our study partly confirmed the earlier scientific reports on the impact of mycorrhiza on the chemical composition of plants (fruits, leaves). However, it is not possible to compare our results with data on berry plants, including sea-buckthorn, due to the lack of information in the literature. Therefore, further research in this area seems necessary.

| Item            | Mycorrhizal effect | Genotype effect | P-value | P-value |
|-----------------|--------------------|-----------------|---------|---------|
|                 | Control            | Mycorrhiza      |         |         |
| Tocopherols     | 40.98 ± 4.9a       | 38.19 ± 2.7a    | 0.094   |         |
|                 | mg kg\(^{-1}\) DW  |                  |         |         |
| L-ascorbic acid | 2217.3 ± 188a      | 2062.0 ± 16.0b  | 0.007   | 0.006   |
|                 | mg kg\(^{-1}\) DW  |                  |         |         |
| Thiamine        | 1.40 ± 0.1a        | 1.36 ± 0.2a     | 0.609   | 0.418   |
| Riboflavin      | 5.86 ± 0.3a        | 5.68 ± 0.2a     | 0.143   | 0.113   |
| Niacin          | 4.86 ± 0.4a        | 4.37 ± 0.2a     | 0.187   | 0.583   |

Mean values with the same letter in each line are not significantly different at p ≤ 0.05 according to the Tukey test.
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