



Research Article



Effect of alginite in the form of ALGEX_r 6 preparation on the biomass formation and antioxidant activity of some medicinal plants

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The aim of the research is to determine the effect of application of the developed product (extract) called ALGEX_r 6 from natural mineral rock alginite in two different watering periods on the formation of root biomass and above-ground plant biomass of a selected group of medicinal plants (*Melissa officinalis* L., *Malva verticillata* L. and *Ocimum × citriodorum* Vis.) and determining the antioxidant activity in dried leaves and whole plants in aqueous and methyl alcohol extracts by DPPH method. The experiment was established in the Botanical Garden at the Slovak University of Agriculture in Nitra throughout 2020. ALGEX_r 6 was prepared by a research team at the Slovak University of Agriculture in Nitra in the form of an extract from the natural mineral rock alginite with an application of thermal and chemical treatment. In the experiment, ALGEX_r 6 was applied in the form of a watering in two variants with the same concentration of 3 % solution in 2 decilitres of water, but various application in terms of days in the pre-harvest stage of the above-ground plant biomass of 30 individual plants from each species. There are two diametrically opposite trends of ALGEX_r 6 application that are manifesting themselves in *M. officinalis* and *M. verticillata* by reducing the root and above-ground part biomass compared to the control variant. The percentage proportionality of root/above-ground part biomass in *M. officinalis* decreased from 62.48/30.31 % (control), to 45.57/18.85 % (variant 1) and to 36.07/17.27 % (variant 2), as well as in *M. verticillata* the root/above-ground part biomass decreased from 16.03/13.93 % (control), to 14.97/9.42 % (variant 1) and to 11.61/10.14 % (variant 2). In the species *Ocimum × citriodorum* Vis. the opposite trend manifested. The application of ALGEX_r 6 watering resulted in increasing the antioxidant activity on the tested plant parts, especially in aqueous extracts in *M. officinalis* (from 19.30/control to 33.61 %/variant 1, 2), also in *O. citriodorum* (from 26.56/control to 44.16 %/variant 1), while in methyl alcohol extracts, the antioxidant activity showed a slight increase in all tested species.

Keywords: ALGEX_r 6, *Melissa officinalis*, *Malva verticillata*, *Ocimum × citriodorum*, root, weight of above-ground part, antioxidant activity, aqueous extract, methyl alcohol extracta

Introduction

Medicinal herbs are used due to their health benefits, special aroma, taste and are considered one of the richest sources of bioactive compounds (Shanayda

and Korablova, 2015; Shymanska et al., 2018; Mňahončáková et al., 2019; Ivanišová et al., 2017, 2020). The commercial development of plants as sources of antioxidants to enhance health and food

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preservation is of current interest (Rice-Evans et al., 1997). Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases (Scalbert and Williamson, 2000; Martins et al., 2012). These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids, phenolic acids, lignan precursors, terpenes, mixed tocopherols, phospholipids, polyfunctional organic acids, other essential antioxidants such as β -carotene, vitamins C and E, selenium, zinc and others (Rice-Evans et al., 1996; Phippen, 1998, 2000; Svidenko et al., 2015a,b; Miraj et al., 2016). Thus, natural antioxidants have gained popularity in recent years. Antioxidants are primarily secondary metabolites contained in higher plants that eliminate free radicals that are harmful to the human body (Prugar, 2008). Antioxidants interfere with the process of oxidation by free radical reactions or reduction of hydroperoxides formed and the elimination of oxygen present. Natural antioxidants are obtained from plant material mainly as extracts, including medicinal and spicy plants such as *Salvia* spp. (Skybitska et al., 2015), *Origanum vulgare* L., *Melissa officinalis* L. (Rasmussen, 2011), *Ocimum basilicum* L., *Mentha piperita* L. (Svidenko et al., 2015a,b), *Rosmarinus officinalis* L. (Mňahončáková et al., 2019), *Allium ursinum* L. (Balaeva-Tichomirova and Leonovich, 2017), *Malva verticillata* L. (Mikhailova and Ebel, 2015), *Symphytum* spp. (Vergun et al., 2017) and other ones (Vergun et al., 2018, 2019, 2021; Shymanska et al., 2020).

Increasing or decreasing antioxidant activity in plants is conditioned by many factors. One of the factors is the growing environment. The amendment of organic or inorganic material to topsoil is considered a way to improve the physical properties of soil. Such materials affect the living space of soil microorganisms and thus also their activity (Dlapa et al., 2004; Fernandez et al., 2007; Ismail and Ozawa, 2007). The implementation of suitable natural materials that are non-toxic and their effect is almost in short time after application could be a chance for the future agriculture. One such material would be alginite. Alginite is a sedimentary laminated rock – an oil shale (Jámbor and Solti, 1975), which originated in basalt maar lakes. The name alginite originally belonged to petrographic constituent of coal residues consisting of algae (Solti, 1987). Dark laminae are rich in amorphous organic matter and well-preserved cells of green alga *Botryococcus braunii* (Ognjanova-Rumenova and Vass, 1998; Vass et al., 2003). The grey laminae are composed

of clay minerals derived from weathered basaltic tuff. In addition to organic matter, the rock contains considerable amounts of macronutrients such as P, K, Ca, Mg, as well as numerous trace elements. Alginite is quite rich in nutrients, except nitrogen. Release of phosphorus, potassium and microelements (Gregor and Bublinec, 1999) could significantly contribute to supplying the demands of the microbial population (Ognjanova-Rumenova and Vass, 1998; Motyleva et al., 2014). Moreover, both the clay minerals and organic matter contained in alginite have high cation-exchange capacities (in contrast to quartz sand), thus regulating cation concentration in the soil solution (Schachtschabel et al., 1984). The content of heavy metals lies below toxicity limits. A large specific surface area ranging from 300 to 650 m²/g results in a water retention capacity of approx. 110 % (Russell, 1990; Vass et al., 1997; Kulich et al., 2001). Tests of alginite from the deposits in Pula and Gerce (Hungary) showed that it can be used in agriculture and forestry to improve soil quality, soil water dynamics and nutrient content, to increase organic matter content, colloid content and to protect soil against acidification, desiccation and leakage of nutrients (Vass et al., 2003). No negative side effects for the environment have been observed (Kulich et al., 2001). In agriculture, alginite is also able to improve the water and nutrients regime and increasing of soil colloids (Beláček, 2006). Organic matter of alginite is a component of some types of kerogen with a predominance of type II alongside amorphous organic matter (Vass et al., 1997).

The aim of the work was to determine the effect of application of the developed product (extract) called ALGEX_r 6 from natural mineral rock alginite in two different watering periods on the formation of root and above-ground plant biomass of three species of medicinal plants with a determination of antioxidant activity in dried leaves and dried whole plants in aqueous and methyl alcohol extracts by DPPH.

Material and methodology

Environment of plants cultivation

From each tested species *Melissa officinalis* L. (MO), *Malva verticillate* L. (MV) and *Ocimum citriodorum* Vis. (OC), 30 individual plants were planted in containers with a diameter of 210 mm. Garden soil was used as a cultivation medium. The experiments were established in the Botanical Garden at the Slovak University of Agriculture in Nitra in 2020 at an altitude of 167 m a. s. level (Figure 1).



Figure 1 Demonstration of above-ground parts of medicinal plants: A – *Ocimum × citriodorum* Vis.; B – *Melissa officinalis* L.; C – *Malva verticillata* L. (Photo: Mňahončáková, 2020)

Application of ALGEX_r 6 preparation

ALGEX_r 6 was prepared by a research team at the Slovak University of Agriculture in Nitra in the form of an extract from a natural mineral rock with the application of thermal and chemical treatment. The product is not registered yet.

In the experiment, ALGEX_r 6 was applied in the form of a watering:

1. in variant 1 – only one application in the concentration of 3 % solution in 200 mL of water applied 10 days in the pre-harvest stage of the above-ground plant biomass,
2. in variant 2 – the first application in the concentration of 3 % solution in 200 mL of water applied 20 days in the pre-harvest stage of the above-ground plant biomass and the second application in the concentration of 3 % solution in 200 mL of water applied 10 days in the pre-harvest stage of the above-ground plant biomass,
3. control variant (marked C) – without application of ALGEX_r 6 was implemented in each plant species (Table 1).

At the end of our experiment, the plants were removed from the containers. The roots of the plants were washed from the soil under running water. After drying in an unheated greenhouse, the weight of the roots and above-ground plant biomass was determined individually for each plant.

Free radical scavenging activity

The antiradical activity of dried leaves and dried above-ground plant biomass of medicinal plants were determined in methanolic (ME) and aqueous extract (AE). The samples 1 g in 25 mL water/methyl alcohol were mixed for 12 hours and antiradical activity was determined after filtration of samples. In the frame of antiradical activity (ability to eliminate the free radicals) was tested the capacity of medicinal plants to remove DPPH• radicals (2,2-diphenyl-1-picrylhydrazyl) using methods of Brand-Williams et al. (1995). Absorbance at 515 nm has been registered in regular time intervals until the reaction equilibrium was reached – using the GENESYS 20 Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). First was measured the DPPH• (Sigma Aldrich, USA) absorbance without antioxidant substance (control). The inhibition of DPPH• radicals was calculated in percent of free DPPH• radicals in the samples using the method of Von Gadow et al. (1997):

$$\% \text{ Inh} = \frac{A_0 - A_1}{A_0} \cdot 100$$

where: A_0 is the absorbance of control in time $t = 0$ min (DPPH• solution), A_1 is the absorbance in the presence of antioxidant in time t (min), the result is in % of DPPH• radical inhibition

Statistical analysis

It was evaluated the variability of the test files in each character using descriptive statistics. For the characteristics of the files, it was used the basic

Table 1 Determination of antioxidant activity in plant parts in aqueous extracts (AE) and methyl alcohol extracts (ME) in tested plant species grown in variants

	Variants	<i>Melissa officinalis</i> L. (MO)	<i>Malva verticillata</i> L. (MV)	<i>Ocimum × citriodorum</i> Vis. (OC)
Dried leaves – DL	Control – C – AE	MOC-DLAE	MVC-DLAE	OCC-DLAE
	Variant 1 – AE	MO1-DLAE	MV1-DLAE	OC1-DLAE
	Variant 2 – AE	MO2-DLAE	*	*
	Control – C – ME	MOC-DLME	MVC-DLME	OCC-DLME
	Variant 1 – ME	MO1-DLME	MV1-DLME	OC1-DLME
	Variant 2 – ME	MO2-DLME	*	*
Dried herbs – DH	Control – C – AE	MOC-DHAE	MVC-DHAE	OCC-DHAE
	Variant 1 – AE	MO1-DHME	MV1-DHME	OC1-DHME
	Variant 2 – AE	MO2-DHME	*	*
	Control – C – ME	MOC-DHME	MVC-DHME	OCC-DHME
	Variant 1 – ME	MO1-DHME	MV1-DHME	OC1-DHME
	Variant 2 – ME	MO2-DHME	*	*

Note: * – untested variants; DL – dried leaves; DH – dried herbs; AE – aqueous extract; ME – methyl alcohol extract

descriptors of variability: average, minimum measured value, maximum measured value, the coefficient of variation (%). Data were analyzed with ANOVA test and differences between means compared through the Fisher test ($\alpha = 0.05$). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (Stehlíková, 1998).

Results and discussion

Melissa officinalis (MO)

In our experiment with the application of an innovated preparation of alginite (ALGEX_r 6) in the form of watering for selected medicinal herbs grown in containers in two different variants, we determined

the following effects on the evaluated parts of plants. The average weight of the root was determined in the range from 36.70 g (MO2) to 62.48 g (MOC), the average weight of the above-ground plant biomass in the range 17.27 g (MO2) – 30.31 g (MOC). The values of coefficients of variation indicate a medium to a high degree of variability (15.43 %/MOC – 34.44 %/MO2). After application of ALGEX_r 6, we recorded a higher proportion of roots (67.33 % in MOC) between the control variant (MOC) and the other two variants (MO1 and MO2) in comparison with above-ground plant biomass (32.67 %/MOC). After application of ALGEX_r 6 watering, we recorded approximately the same proportion of the weight of roots (70.73 %/MO1) and above-ground plant biomass (29.27 %/MO1) compared to the control variant (Figure 2). Analysis of variance confirmed the differences between the

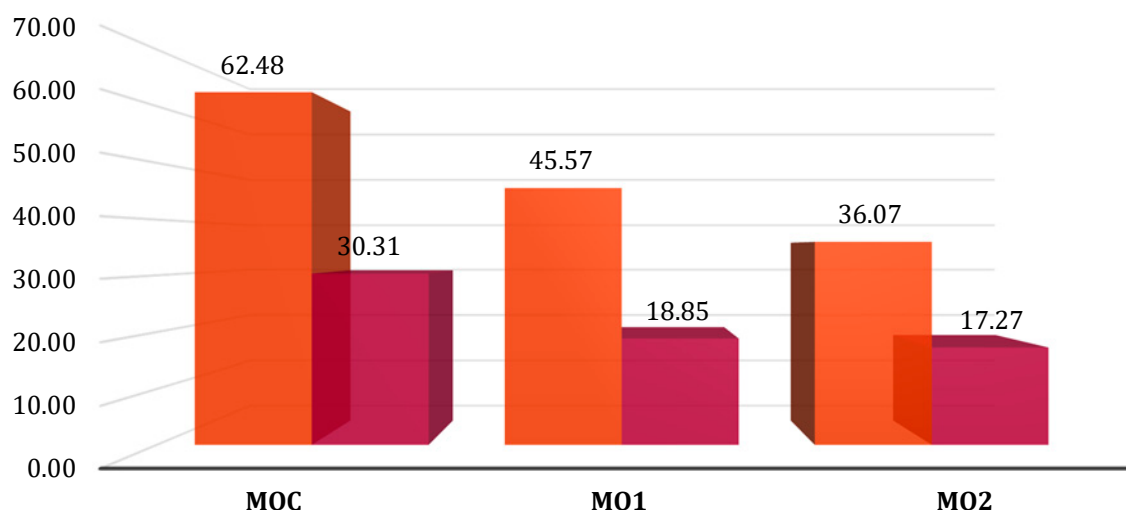


Figure 2 Comparison of the weight of *Melissa officinalis* L. root to the total weight of the above-ground plant biomass in the control variant and in the variants after application of ALGEX_r 6: MOC – control; MO1 – variant 1; MO2 – variant 2

Table 2 Analysis of variance to evaluate the effect of application of ALGEX_r 6 on the weight of roots and the weight of above-ground plant biomass of *Melissa officinalis* L. plants with statistical differences between the evaluated variants

Source of variation	SS	df	MS	F	p-value	F crit	Differences between variants				
							Weight of root (g)	Variants	\bar{x}	V %	MO2
Between variants	3579.19	2	1789.59	13.71	0.00	3.35	MOC	62.48	20.72	+++	++
Within variants	3522.66	27	130.46				MO1	45.57	23.41	-	
Total variability	7101.86	29					MO2	36.07	29.07		
Weight of above-ground plant biomass (g)											
Between variants	1013.50	2	506.75	11.63	0.00	3.35	MOC	30.31	29.22	+++	+++
Within variants	1175.92	27	43.55				MO1	18.85	21.75	-	
Total variability	2189.43	29					MO2	17.27	34.44		

Note: SS – sum of squares; df – degrees of freedom; MS – mean square; F – F statistic; p-value – probability ($\alpha = 0.05$); F crit – F-critical value; \bar{x} – arithmetic mean; V % – coefficient of variation (%); MO1 – variant 1; MO2 – variant 2

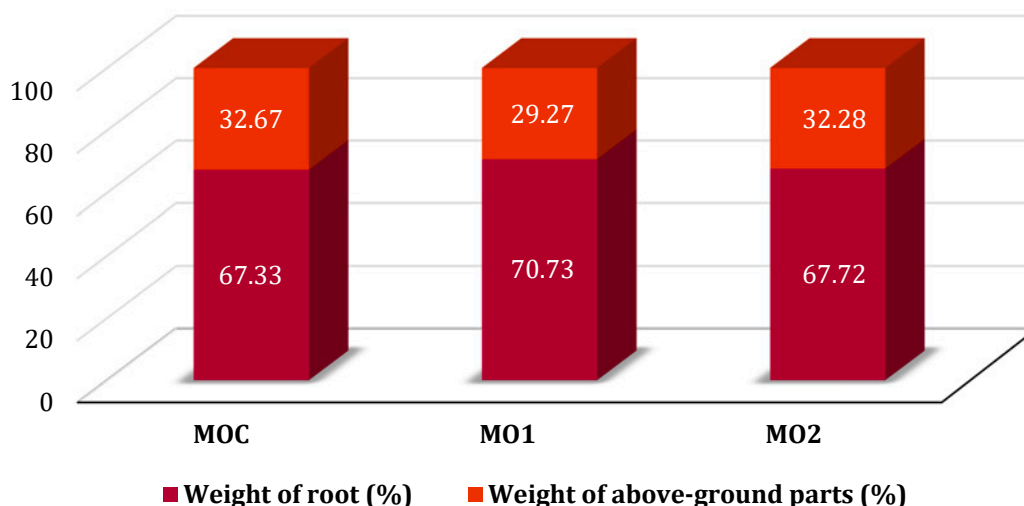


Figure 3 Comparison of the ratio of the weight of *Melissa officinalis* L. root (%) and the weight of above-ground plant biomass (%) of the total weight of the above-ground mass in the control variant and after application of ALGEX_r 6 in both variants: MOC – control; MO1 – variant 1; MO2 – variant 2

evaluated control variant and the two variants with different period ALGEX_r 6 watering. We determined a statistically highly significant effect on the reduction of root weight and weight of above-ground plant biomass in both MO1 and MO2 variants compared to the control variant (Table 2 and Figure 2). The results from the analysis of variance confirmed the statistically significant differences between the evaluated traits (Table 2).

In experiments, we applied ALGEX_r 6 two times (Variant 2 – MO2). In the aqueous extract, the smallest values of antioxidant activity were in the dried leaves of the control variant (19.30 %) and variant 1 (20.56 %). The highest values of antioxidant activity were achieved (MO2) dried leaves (77.74 %)

and samples of whole plants (MO2) in methyl alcohol extracts (76.27 %).

After the first application of ALGEX_r 6 the antioxidant activity increased especially in aqueous extracts in dried herbs (33.61 %). The effect on the increase of antioxidant activity in the evaluated traits was recorded after the second application of ALGEX_r 6 in both aqueous and methyl alcohol extracts (Figure 4). Results from the analysis of variance (ANOVA) of the evaluated traits (Table 3) confirm the statistically significant differences between aqueous and methyl alcohol extracts.

Table 4 and Figure 4 showed statistically significant differences between dried leaves and dried herbs in

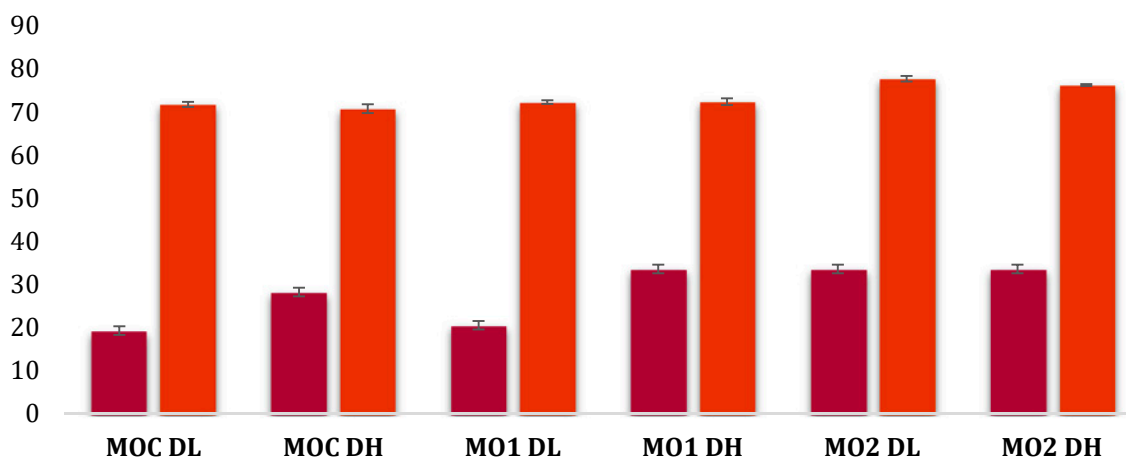


Figure 4 Comparison of antioxidant activity (%) in dried leaves (DL) and whole plants (DH) in aqueous and methanol extracts of *Melissa officinalis* L. grown in control variant (MOC) and after applications of ALGEX_r 6 in variants MO1 and MO2

Table 3 Analysis of variance to evaluate the antioxidant activity in aqueous and methanol extracts of *Melissa officinalis* L. grown in control variant (MOC) and after applications of ALGEX_r 6 in variants MO1 and MO2

Effect	SS	df	MS	F	p-value
AA – Aqueous Extract					
Absolute Member	10958.30	1	10958.30	454.10	0.00
Variation	508.62	5	101.72	4.22	0.02
Statistical Error	289.58	12	24.13		
AA – Methanol Extract					
Absolute Member	97368.54	1	97368.54	74412.82	0.00
Variation	115.91	5	23.18	17.72	0.00
Statistical Error	15.70	12	1.31		

Note: AA – antioxidant activity; SS – sum of squares; df – degrees of freedom; MS – mean square; F – F statistic; p-value – probability ($\alpha = 0.05$)

Table 4 Statistical differences in antioxidant activity between dried leaves (DL) and dried whole plants (DH) of *Melissa officinalis* (MO) determined in aqueous (AE) and methyl alcohol extracts (ME) by Fischer test (LSD)

Variants	x	V%	MOC-DLAE	MOC-DHAE	MO1-DLAE	MO1-DHAE	MO2-DLAE	MO2-DHAE	MOC-DLME	MOC-DHME	MO1-DLME	MO1-DHME	MO2-DLME
MOC-dried leaves aqueous extract	19.30	14.80											
MOC-dried herbs aqueous extract	28.24	1.10	+										
MO1-dried leaves aqueous extract	20.55	4.26	-	+									
MO1-dried herbs aqueous extract	33.61	31.47	+	-	+								
MO2-dried leaves aqueous extract	19.51	6.17	-	+	-	+							
MO2-dried herbs aqueous extract	26.80	17.66	+	+	+	+	+						
MOC-dried leaves methanol extract	71.77	1.41	+++	+++	+++	+++	+++	+++					
MOC-dried herbs methanol extract	70.78	2.46	+++	+++	+++	+++	+++	+++	-				
MO1-dried leaves methanol extract	72.30	1.02	+++	+++	+++	+++	+++	+++	-	-			
MO1-dried herbs methanol extract	72.41	1.87	+++	+++	+++	+++	+++	+++	-	-	-		
MO2-dried leaves methanol extract	77.74	1.42	+++	+++	+++	+++	+++	+++	-	-	-	-	
MO2-dried herbs methanol extract	76.27	0.53	+++	+++	+++	+++	+++	+++	-	-	-	-	-

Note: - - differences are disproven; + - differences are statistically significant; +++ - statistical significance with even smaller differences; arithmetic mean; V % – coefficient of variation (%)

methyl alcohol extracts in comparison of dried samples in aqueous extracts.

The concentration of an aqueous extract of *M. officinalis* capable to inhibit 50 % DPPH radical formation (IC_{50} value) was found to be 309 μ g dry leaves per extract mL, whereas for CAF 80 μ g/mL. Considering that a cup (250 mL) of a 2 % *M. officinalis* infusion or decoction contains according to findings ~1700–3300 mg dry extract it can be safely said that its consumption may effectively contribute to daily radical inhibitors intake (Papoti et al., 2019). Antioxidant extract yield from raw material *M. officinalis* leaves was 0.4 g extract/100 g plant material used rancimat method (Ribeiro et al.,

2001). *M. officinalis* ethanolic extracts showed a very good antioxidant activity in the DPPH test, correlated with the content in total phenols: higher in the case of *M. officinalis* leaves extract (32.76 mg GAE/g) and lower for *M. officinalis* stems extract (8.4 mg GAE/g) (Moacă et al., 2018).

Malva verticillata (MV)

The average weight of the root in *M. verticillata* was determined in the range from 11.60 (MV2) to 16.03 g (MVC), the average weight of the above-ground plant biomass in the range 9.42 (MV1) – 13.93 g (MVC). The values of the coefficients of variation indicate a medium

Table 5 Analysis of variance to evaluate the effect of application of ALGEX_r 6 on the weight of roots and the weight of above-ground plant biomass of *Malva verticillata* L. plants with statistical differences between the evaluated variants

Source of variation	SS	df	MS	F	p-value	F crit	Differences between variants				
							Weight of root (g)	Variants	\bar{x}	V %	MV2
Between variants	106.94	2	53.47	2.33	0.11	3.35	MVC	16.03	30.77	++	-
Within variants	617.37	27	22.86				MV1	14.97	39.00	-	
Total variability	724.32	29					MV2	11.61	27.47		
Weight of above-ground plant biomass (g)											
Between variants	117.35	2	58.67	4.00	0.02	3.35	MVC	13.93	35.20	++	+++
Within variants	395.27	27	14.63				MV1	9.42	26.73	-	
Total variability	512.62	29					MV2	10.14	36.25		

Note: SS – sum of squares; df – degrees of freedom; MS – mean square; F – F statistic; p-value – probability ($\alpha = 0.05$); F crit – F-critical value; \bar{x} – arithmetic mean; V % – coefficient of variation (%); MV1 – variant 1; MV2 – variant 2

to a high degree of variability (14.47 %/MV2) – 38.99 %/MV1). Analysis of variance determined the differences between the evaluated control variant and the two variants with different ALGEX_r 6 watering. We determined a statistically highly significant effect on the reduction of root weight in the MV2 variant compared to the control variant (Table 5 and Figure 5). The application of ALGEX_r 6 resulted in a statistically significant reduction in the formation of above-ground plant biomass in both variants MV1 and MV2 in comparison with the control variant (Table 6 and Figure 4). After application of ALGEX_r 6, we recorded the same proportion of roots (53 %) and above-ground plant biomass (46 %) between the control variant (MVC) and the MV2 variant. After application of ALGEX_r 6 watering, we recorded an increase in the proportion of roots (61 %) compared to the proportion of above-ground plant biomass (38 %/MV1). This is

documented in Figure 5. The results from the analysis of variance (Table 7) confirm the statistically significant differences between the evaluated traits.

DPPH provides a rapid, simple and sensitive method of evaluating the antioxidant activity of natural antioxidants. Application of ALGEX_r 6 in the experiment had the effect of increasing the antioxidant activity in aqueous and methyl alcohol extract. Mutual comparison of controls (MVC) and MV1 variants showed that in general we recorded higher values of antioxidant activity in methanol and aqueous extracts compared to control variants. It is evidenced that the effect of ALGEX_r 6 application determines the antioxidant activity (Figure 7).

Results from the analysis of variance (Table 6) confirmed the statistically significant differences in

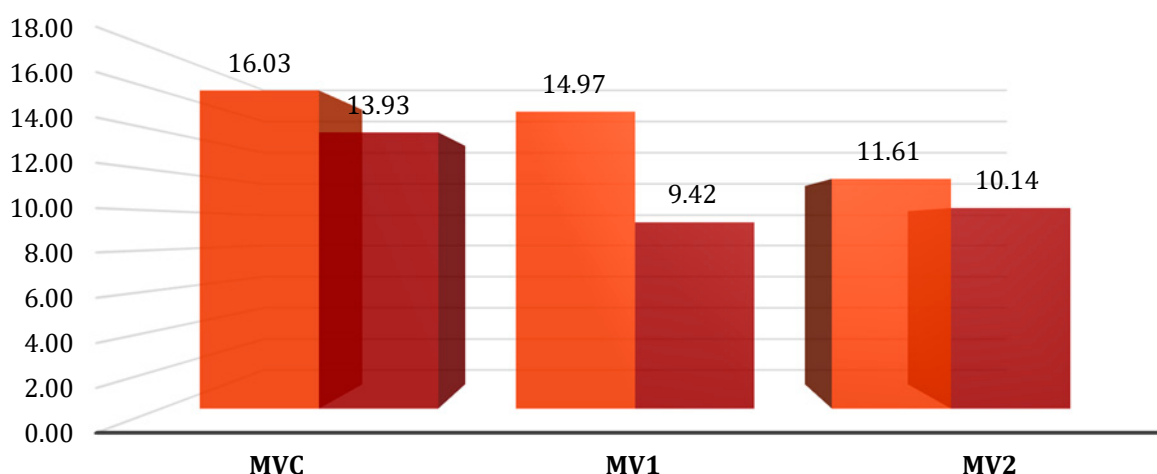


Figure 5 Comparison of the weight of *Malva verticillata* L. root to the total weight of the above-ground plant biomass in the control variant and in the variants after application of ALGEX_r 6: MVC – control; MV1 – variant 1; MV2 – variant 2

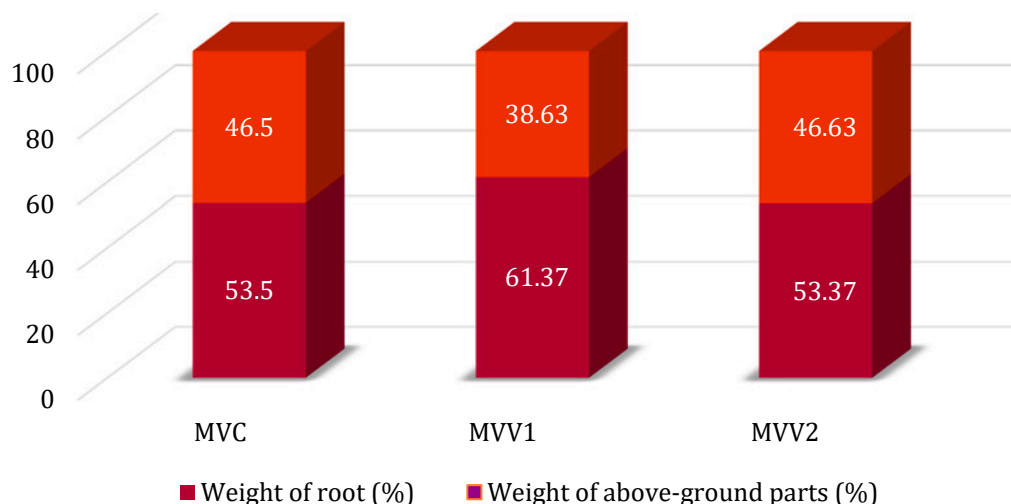


Figure 6 Comparison of the ratio of the weight of root (%) and the weight of above-ground plant biomass (%) of the total weight of the above-ground mass in the control variant and after application of ALGEX₆ in both variants: MVC – control; MV1 – variant 1; MV2 – variant 2

aqueous and methyl alcohol extracts after application of ALGEX₆.

Table 7 showed statistically significant differences between dried leaves and dried herbs after application of alginate preparation.

Bao et al. (2018) studied the antiradical scavenging activity of fresh leaves, stems and dried seeds of *M. verticillata* by three methods. The results showed that *M. verticillata* seeds had the highest ability to scavenge DPPH free radicals (22.14 ± 0.59 mg AAE/g extract), followed by the leaves (12.62 ± 0.41 mg AAE/g extract) and stems (5.15 ± 0.19 mg AAE/g extract), in that order. In addition, the seeds had higher levels of antioxidants than fatsia (19.08 ± 1.08 mg AAE/g extract), sesame seeds (11.09 ± 0.57 mg AAE/g extract),

bok choy (5.83 ± 0.44 mg AAE/g extract), and broccoli (3.96 ± 0.21 mg AAE/g extract) (Loizzo et al., 2016). The extracts of *M. verticillata* showed a strong ability to remove ABTS free radicals; the leaves had the strongest effect, at 363.83 ± 4.22 mg Trolox/g extract, followed by the seeds at 76.47 ± 5.37 mg Trolox/g extract and the stems, with the weakest effect of 46.72 ± 5.07 mg Trolox/g extract. The activity of each part of *M. verticillata* was stronger than those found in vegetables such as Chinese chives (30.63 ± 0.34 mg Trolox/g extract) and broccoli (45.17 ± 2.41 mg Trolox/g extract) (Loizzo et al., 2016). These results are different from the ability to scavenge DPPH free radicals. The leaves of *M. verticillata* exhibited the best ability to scavenge ABTS free radicals. The activity

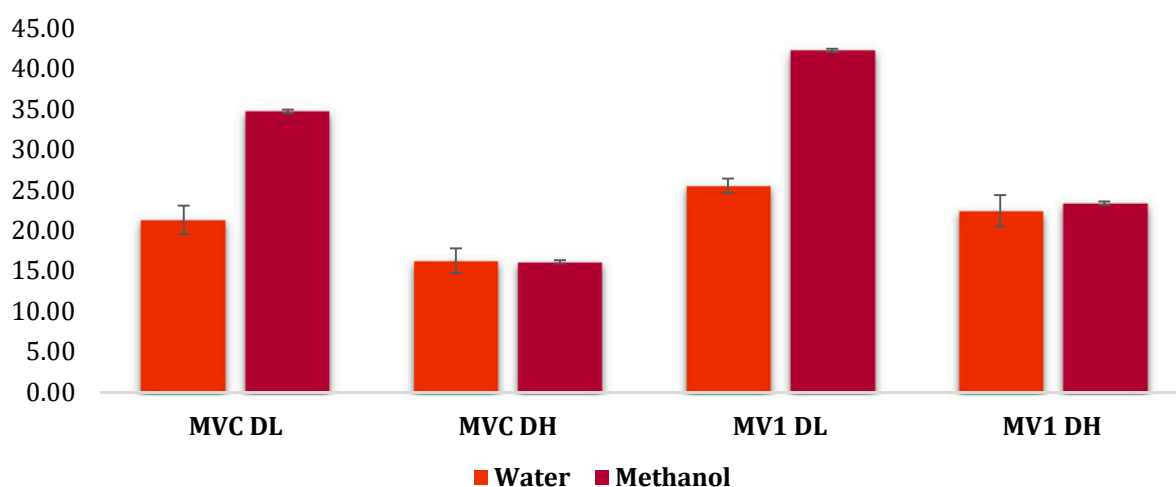


Figure 7 Comparison of antioxidant activity (%) in dried leaves (DL) and whole plants (DH) in aqueous and methanol extracts of *Malva verticillata* L. grown in control variant (MVC) and after applications of ALGEX₆ in variants MV1 and MV2

Table 6 Analysis of variance to evaluate the antioxidant activity in aqueous and methanol extracts of *Malva verticillata* L. grown in control variant (MVC) and after applications of ALGEX_r 6 in variants MV1 and MV2

Effect	SS	df	MS	F	p-value
AA – Aqueous Extract					
Absolute Member	5491.17	1	5491.17	735.65	0.00
Variant	133.40	3	44.47	5.9573	0.02
Statistical Error	59.72	8	7.46		
AA – Methanol Extract					
Absolute Member	10178.67	1	10178.67	23088.43	0.00
Variant	1217.33	3	405.78	920.43	0.00
Statistical Error	3.53	8	0.44		

Note: AA – antioxidant activity; SS – sum of squares; df – degrees of freedom; MS – mean square; F – F statistic; p-value – probability ($\alpha = 0.05$)

Table 7 Statistical differences in antioxidant activity between dried leaves (DL) and dried whole plants (DH) of *Malva verticillata* L. (MV) determined in aqueous (AE) and methyl alcohol extracts (ME) by Fischer test (LSD)

Variants	\bar{x}	V %	MVC-DLAE	MVC-DHAE	MV1-DLAE	MV1-DHAE	MVC-DLME	MVC-DHME	MV1-DLME	MV1-DHME
MVC-dried leaves aqueous extract	21.31	14.25								
MVC-dried herbs aqueous extract	16.27	16.27	+							
MV1-dried leaves aqueous extract	25.53	6.01	-	+						
MV1-dried herbs aqueous extract	22.42	14.94	-	+	-					
MVC-dried leaves methanol extract	37.73	2.38	+++	+++	+++	+++				
MVC-dried herbs methanol extract	16.12	2.67	+	-	+	+	+++			
MV1-dried leaves methanol extract	42.25	2.06	+++	+++	+++	+++	+	+++		
MV1-dried herbs methanol extract	23.38	1.51	-	+	-	-	+++	+	+++	

Note: - – differences are disproven; + – differences are statistically significant; +++ – statistical significance with even smaller differences; arithmetic mean; V % – coefficient of variation (%)

of *M. verticillata* leaves was stronger than that of the seeds.

Ocimum × *citriodorum* (OC)

We determined the average weight of the root in the range from 3.08 g (OCC) to 8.20 g (OC1), the average weight of the above-ground plant biomass in the range 8.61 g (OC2) – 9.95 g (OCC). The values of the coefficients of variation indicate a low to the high degree of variability (7.70 %/OCC) – 43.53 %/OC2). The differences between the evaluated control variant and the two variants with different watering of ALGEX_r 6 indicated a statistically highly significant effect on the increased root weight in the OC1 variant compared to the control variant and the demonstrable difference between OC1 and OC2 variant (Table 8 and Figure 8). After application of ALGEX_r 6, we recorded a different proportion of roots and above-ground plant biomass between the control variant (OCC) and other

variants (OC1 and OC2) where ALGEX_r 6 was applied 10 or 20 days in the pre-harvest stage of the above-ground plant biomass. We recorded a decrease in the proportion of above-ground plant biomass (51.56 %/OC1 and 62.60 %/OC2) compared to the proportion of above-ground plant biomass of the control variant (76.36 %). This is documented in Figure 9. The results from the analysis of variance (Table 8) confirm the statistically significant differences only for root weights.

The basil flowers are irrelevant in terms of their usage. It is scientifically proven (Majdi et al., 2020) that *O. × citriodorum* is used as a natural source of bioactive substances when consumed in the form of food or extract. In general, the dried leaves and whole herbs showed higher antioxidant activity especially in the methyl alcohol extracts (Figure 10). In dried leaves and dried whole herbs, we determined significantly increased antioxidant activity in aqueous extracts after

Table 8 Analysis of variance to evaluate the effect of application of ALGEX₆ on the weight of roots and the weight of above-ground plant biomass of *Ocimum × citriodorum* Vis. plants with statistical differences between the evaluated variants.

Source of variation	SS	df	MS	F	p-value	F crit	Differences between variants				
							Weight of root (g)	Variants	\bar{x}	V %	OBV2
Between variants	132.68	2	66.34	12.58	0.00	3.35	OCC	3.08	27.88	-	+++
Within variants	142.29	27	5.27				OC1	8.20	38.73	++	
Total variability	274.97	29					OC2	5.14	43.53		
Weight of above-ground plant biomass (g)											
Between variants	11.12	2	5.56	0.68	0.51	3.35	OCC	9.95	24.27	-	-
Within variants	218.50	27	8.09				OC1	8.73	35.11	-	
Total variability	229.62	29					OC2	8.61	34.96		

Note: SS – sum of squares; df – degrees of freedom; MS – mean square; F – F statistic; P-value – probability ($\alpha = 0.05$); F crit – F-critical value; \bar{x} – arithmetic mean; V % – coefficient of variation (%); OC1 – variant 1; OC2 – variant 2

alginate application, which results in increasing some biologically active substances soluble in water.

Results from the analysis of variance (Table 9) confirm the statistically significant differences between aqueous and methyl alcohol extracts.

Table 10 showed statistically significant differences between dried leaves and dried herbs in methyl alcohol extracts and aqueous extracts.

Juliani and Simon (2002) researched the antioxidant potential of dried leaves of five green and four purple basil cultivars and breeding lines by ABTS and FRAP assay showed that essential oils were a very low antioxidant activity varying from 0.05 % ‘Purples Ruffles’ > 0.7 % *O. sanctum* > > 2.0 *O. basilicum* to 5.9 % in ‘Sweet’ basil (FRAP) and from 0.1 % in ‘Purples

Ruffles’ > 0.2 % *O. citriodorum* > 0.4 % *O. sanctum* > 1.2 % *O. basilicum* to 4.1 % in ‘Sweet’ basil (ABTS).

Hakkim et al. (2008) studied the radical scavenging ability of antioxidants of eight *Ocimum* species in dried form. From the percentage scavenging values the *O. gratissimum* extract was the most potent scavenger (81.1 ± 2.1 %) followed by *O. americanum* (77.4 ± 1.4 %) > *O. minimum* (70.1 ± 2.2 %) > *O. citriodorum* (60.6 ± 2.5 %) > *O. kilimandscharicum* (56.2 ± 2.1 %) > *O. grandiflorum* (51.3 ± 2.3 %) > *O. lamiifolium* (46.2 ± 2.2 %) > *O. selloi* (42.4 ± 2.4 %).

Kovár et al. (2021) studied the influence of alginite (powder, crushed alginite) and extracts from it (sodium solution, potassium solution) on parameters as germination dynamics, average germination, germination rate and mean germination time of Kentucky bluegrass (*Poa pratensis* L.). The positive

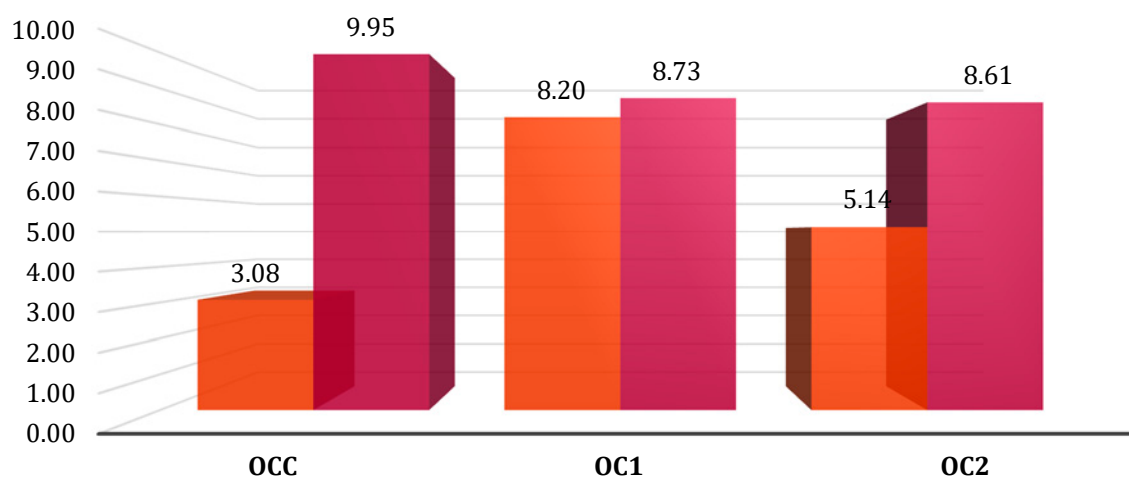


Figure 8 Comparison of the weight of *Ocimum × citriodorum* Vis. root to the total weight of the above-ground plant biomass in the control variant and in the variants after application of ALGEX₆: OCC – control; OC1 – variant 1; OC2 – variant 2

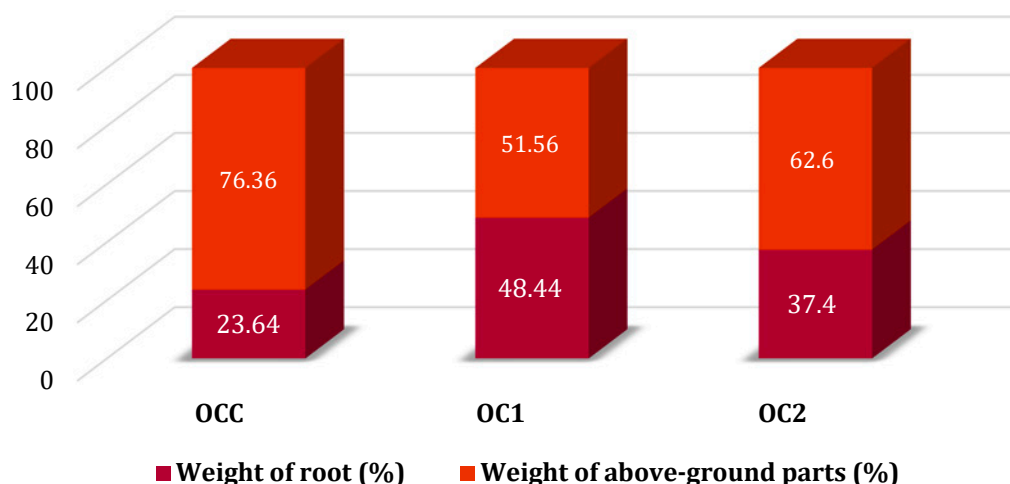


Figure 9 Comparison of the ratio of the weight of root (%) and the weight of above-ground plant biomass (%) of the total weight of the above-ground mass in the control variant and after application of ALGEX_r 6 in both variants: OCC – control; OC1 – variant 1; OC2 – variant 2

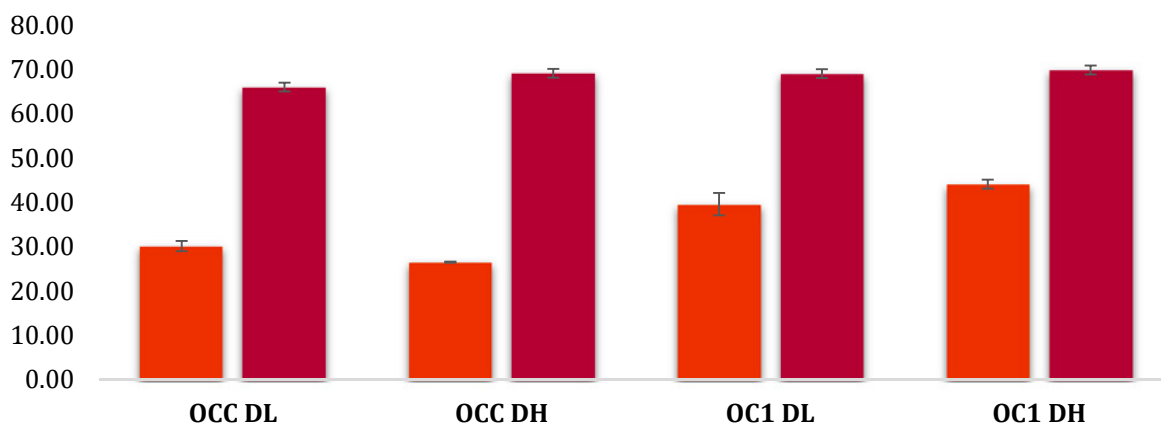


Figure 10 Comparison of antioxidant activity (%) in dried leaves (DL) and whole plants (DH) in aqueous and methanol extracts of *Ocimum × citriodorum* Vis. grown in control variant (OCC) and after applications of ALGEX_r 6 in variants OC1 and OC2

Table 9 Analysis of variance to evaluate the antioxidant activity in aqueous and methanol extracts of *Ocimum × citriodorum* (Vis.) grown in control variant (OCC) and after applications of ALGEX_r 6 in variants OC1 and OC2

Effect	SS	df	MS	F	p-value
AA – Aqueous Extract					
Absolute Member	14811.51	1	14811.51	2253.82	0.00
Variant	599.79	3	199.93	30.422	0.00
Statistical Error	52.57	8	6.57		
AA – Methanol Extracts					
Absolute Member	56435.06	1	56435.06	13218.96	0.00
Variant	26.15	3	8.72	2.04	0.19
Statistical Error	34.15	8	4.27		

Note: AA – antioxidant activity; SS – sum of squares; df – degrees of freedom; MS – mean square; F – F statistic; p-value – probability ($\alpha = 0.05$)

Table 10 Statistical differences in antioxidant activity between dried leaves (DL) and dried whole plants (DH) of *Ocimum × citriodorum* (OC) determined in aqueous (AE) and methyl alcohol extracts (ME) by Fischer test (LSD).

Variants	x	V %	OCC-DLAE	OCC-DHAE	OC1-DLAE	OC1-DHAE	OCC-DLME	OCC-DHME	OC1-DLME	OC1-DHME
OCC-dried leaves aqueous extract	30.16	6.66								
OCC-dried herbs aqueous extract	26.55	0.83	-							
OC1-dried leaves aqueous extract	39.65	11.02	+	+						
OC1-dried herbs aqueous extract	44.14	3.96	+++	+++	+					
OCC-dried leaves methanol extract	66.08	4.94	+++	+++	+++	+++				
OCC-dried herbs methanol extract	69.18	1.14	+++	+++	+++	+++	-			
OC1-dried leaves methanol extract	69.11	1.82	+++	+++	+++	+++	-	-		
OC1-dried herbs methanol extract	69.92	2.92	+++	+++	+++	+++	-	-	-	

Note: - - differences are disproven; + - differences are statistically significant; +++ - statistical significance with even smaller differences; arithmetic mean; V % - coefficient of variation (%)

and at the same time significant effect of alginite (crushed from) and its extracts was manifested especially in increasing of germination by 340.00 % (alginite extract), increasing average germination by 201.70 % (crushed alginite) and by 334.20 % (alginite extract), values of the mean germination time showed a shortening with using by 4.95 days (alginite extract), by 3 days (crushed alginite), by 2.82 days (powder application) compared to the controls.

Conclusions

Alginite as a bituminous rock is specific to the components of some types of kerogen with predominating kerogen type II. In addition to organic matter, the rock contains a spectrum of micronutrients – macroelements, mainly P, K, Ca and Mg, as well as a large number of microelements. Many plant species responded to the application of alginite with various effects during germination, growth, development, production of seeds, fruits, but also by increasing many biologically active substances, which also contribute to the quality of plant parts. We did not evaluate the content of their specific biologically active substances in the tested medicinal plant species. Reducing the weight of roots and above-ground parts of plants generally has a positive effect on the increase of biologically active substances in medicinal plant species. This trend was reflected by increasing antioxidant activity in the evaluated species.

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