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Stimulation effect of alginite on *Rhodiola rosea* L. *in vitro* growth

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Stimulating the growth of plants, increasing their productivity, and resistance to stress factors are important tasks facing scientists and producers of agricultural products. Different compounds of natural origin can be used as biostimulants. Such chemicals as humic, fulvic, and salicylic acids, mineral elements, amino acids, chitosan, vitamins, poly-, and oligosaccharides are the typical components of biostimulants. They affected not only plant growth. Biostimulants also improve biosynthetic activity, including chlorophyll synthesis, and accumulation of plant metabolites. Alginite, founded in Slovakia in Maar near Pinciná village northeast of Lučenec, was studied as a plant growth stimulator. The application of biostimulants to medicinal plants allows increasing in their biomass and productivity. The effect of alginite on *in vitro* growth of *Rhodiola rosea* L. plants and their antioxidant activity has been analyzed in this study. Adding 1 or 10 mL.L^{-1} of MS medium alginite extract solution diluted to the concentration of 1% resulted in intensive plant growth. Shoots weight increased 4.48–6.64-fold compared to the control. Adding alginite extract solution also stimulated root growth (3.00–5.58-fold). Despite the decrease in the specific content of flavonoids in the plants, grown on media with alginite, the total content of flavonoids in these plants was higher than in the control ones due to a significant increase in biomass. The antioxidant activity of the samples grown on the alginite medium was higher than that of the control plants. Thus, alginite, a compound of natural origin, can stimulate the growth of *R. rosea* and increase the bioactivity of these plants.

Keywords: *Rhodiola rosea*, alginite, growth stimulation, flavonoids, antioxidant activity

Introduction

Producers of food products face the problem of increasing the productivity of crops by stimulating plant growth. Such stimulation is possible due to the use of inorganic and organic fertilizers, as well as due to specific plant growth regulators. Chemical compounds used in agriculture can pose environmental risks by contaminating soils and water. They can also negatively affect the soil microflora, changing its component composition. Besides traditional fertilizers, different natural biostimulants can promote plant growth. These compounds do not supply nutrients directly to the plants. At the same time, biostimulants facilitate plant metabolic processes improving nutrient availability

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(Drobek et al., 2019). Regulation (EU) 2019/1009 of the European Parliament and of the Council laid down rules on the making available on the market of EU fertilizing products.

Natural compounds and various extracts containing bioactive components can be regarded as biostimulants. Humic, fulvic and salicylic acids, mineral elements, amino acids, chitosan, vitamins, poly-, and oligosaccharides are the typical components of biostimulants of different natures (Bulgari et al., 2015; EL Arroussi et al., 2018). Some organisms (bacteria and fungi) in plants and soil can be considered biostimulants because they can induce changes in plant growth and biosynthetic activity (Bashan et al., 2014; Egamberdieva et al.; 2017; Park et al., 2017). Biostimulants can be applied via the soil or leaves by irrigation systems (Drobek et al., 2019).

Biostimulants affected not only plant growth. They improve biosynthetic activity, including synthesis of chlorophyll, stress resistance, and accumulation of metabolites (for instance, flavonoids well-known as powerful antioxidants) (De Pascale et al., 2017; Van Oosten et al., 2017; Yakhin et al., 2017; Fleming et al., 2019; Paul et al., 2019; Amjad Bashir, 2021). This is partially due to their effect on phytohormones synthesis and gene expression (De Pascale et al., 2017; Rouphael et al., 2020;).

In particular, chitosan based biostimulants positively influenced the quality of strawberry fruits and increased the concentration of phenolic compounds (Soppelsa et al., 2019). Seaweed extract altered the nutraceutical and antioxidative potential and improved the growth and yield of *Glycine max* L. (Kocira et al., 2019). Arbuscular mycorrhizal fungi increased the polyphenol content in *Crocus sativus* L. (Caser et al., 2019). Humates and lignosulfonates increase root growth, enhance photosynthesis and stimulate the Nitrogen metabolism of *Zea mays* L. (Ertani et al., 2019). Micronized and concentrated vermicompost, diatomaceous earth, and soy flour enhanced seedling growth and increased the integrity and compressive strength of seeds of *Trifolium pratense* L. and *Lolium perenne* L. (Qiu et al., 2020). Seaweed extract, legume-derived protein hydrolysate, and tropical plant extract increased leafy vegetable productivity in low-fertility soils, the physiological and biochemical status of *Lactuca sativa* L. plants (Mola et al., 2019). *Moringa oleifera* Lam. leaf extract, used as foliar spray or seed soaking improves the growth and yields of *Pisum sativum* L. plants by alleviating the inhibitory effects of soil salinity stress (Desoky et al., 2016).

More and more attention is paid to the design of preparations that stimulate plant growth and are natural and safe for the environment. Great attention should be paid to environmental sustainability in the case of biostimulant use (Le Mire et al., 2016). Alginite as a complex of compounds of natural origin can be named among other biostimulants. Alginite is an organic-bituminous rock with different organic and inorganic components that were sedimented together with the clays in post-volcanic outbursts during geological periods appropriate for algae occurrence (Kulich et al., 2001). Alginite was found in Slovakia in Maar near Pinciná village northeast of the town of Lučenec (Vass et al., 1997). A study of alginite and its effect on plant growth was conducted by several research groups (Barančíková et al., 2003; Gömöryová et al., 2009; Brindza et al., 2021a; 2021b; Kropp et al., 2021).

In this work, we studied the effect of alginite extract on the growth and bioactivity of *Rhodiola rosea* L. plants in *in vitro* culture. *R. rosea*, or Golden root, is well known medicinal plant. Traditionally these plants are used as adaptogens, antidepressants, and anti-inflammatory remedies (Kelly, 2001; Bawa and Khanum, 2009; Panossian et al., 2010). They are rich in polyphenols with antioxidant activity (Chen et al., 2012). Numerous bioactive compounds were studied in *R. rosea* (Chiang et al., 2015). Such chemicals as gossypetin-7-O-L-rhamnopyranoside, rhodioflavonoside, gallic acid, *trans-p*-hydroxycinnamic acid, and *p*-tyrosol were identified. The compounds were evaluated for their antibacterial and cancer cell activities (Ming et al., 2005).

The cultivation of golden roots under sterile conditions is a necessary element of the biotechnology process. In particular, such plants can be used to study the peculiarities of the synthesis of biologically active compounds that are characteristic of *R. rosea*. In addition, *in vitro* grown plants are necessary for the development of technologies for the genetic transformation technic. The use of the *in vitro* system when testing the effects of various compounds allows for completely standardizing the conditions (medium composition, temperature regime, humidification, etc) and avoiding possible additional and side effects caused by microorganisms in the soil.

Material and methodology

Plant cultivation and treatment

Alginite product ALGEXr-2 from natural alginite from the Pincina region (Figure 1a) was created by a research team from the Institute of Agronomic Sciences, Faculty of Agrobiology and Food Resources at the Slovak University of Agriculture in Nitra. The solution was prepared by the extraction using a mixture of sodium pyrophosphate decahydrate and sodium hydroxide (5 : 1) (Figure 1b). The resulting extract was diluted to obtain 1% concentration and sterilized through a filter with a pore diameter of 0.2 µm, Sartorius, Minisart (test solution).

Rhodiola rosea L. plants from the *in vitro* collection of the Laboratory of Adaptational Biotechnology, Institute of Cell Biology and Genetic Engineering, NAS of Ukraine, were used in the experiment. The apical parts of the shoots were separated and transferred to Petri dishes (100 mm) with the solidified half-strength Murashige and Skoog nutrient medium (Duchefa Biochemie, Netherlands) containing 2% sucrose. Sterile test solution in the concentrations of 1 mL.L^{-1} and 10 mL.L^{-1} was added to the medium. The plants grown on half-strength Murashige and Skoog medium were used as the control ones. In 30 days of cultivation at a temperature of +24 °C, the plants were removed from the medium and washed with distilled water. Morphometric and biosynthetic parameters of seedlings were determined (wet weight (WW) of the shoots and roots; total content of flavonoids; antioxidant activity).

Flavonoid content assay

Flavonoid content was studied by a modified method (Matvieieva et al., 2019). Before this study, the shoots and roots were homogenized in 70% ethanol. The extracts were centrifuged for 10 min at 14000 rpm (Eppendorf Centrifuge 5415C). Supernatants were used for flavonoid content assay. The absorbance of the samples was measured at 510 nm using the spectrophotometer Fluorat-02 Panorama. Specific flavonoid content was calculated by the calibration plot: $C_{(rutin)}$ = 1.7427D (R^2 = 0.9936) and expressed as milligrams per gram of wet weight in rutin equivalent (mg RE.g^{-1} WW). Total flavonoid content was calculated as a product of the specific flavonoid content and the weight of the "hairy" root sample and expressed as milligrams in rutin equivalent (mg RE).

Antioxidant activity assay

The plant extracts obtained for the total content of flavonoids study were used for antioxidant activity analysis using 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) by the method described in (Brand-Williams et al., 1995). The optical density of the mixture was determined at 515 nm on the Panorama Fluorate-2 spectrophotometer. The radical scavenging activity was determined by the formula: RSA = 100(A_0 – A_1)/A_0, where A_0 – absorbance of DPPH*; A_1 – absorbance of the sample in the reaction. Equivalent concentration (EC_{50}) was calculated as the corresponding weight of plant material required to obtain the extract with a 50% DPPH* inhibition level.

Figure 1 Natural alginite (a) and the extract used as the test solution (b)

Statistical analysis

All analyses were carried out in triplicate. Values were represented as mean and standard error (SE). The data were analyzed for statistical significance using ANOVA followed by the Tukey HSD test. P values less than 0.05 were considered significant. The linear regression method was applied to determine the antioxidant activities as EC_{50} by establishing the relationship between RSA and extract weight on linear intervals of curves (for the data where RSA <75%).

Results and discussion

The effect of alginite added to the culture medium on *R. rosea* was studied. Since the stimulating activity of alginite was determined by plants of various species (Brindza et al., 2021a, 2021b; Eftimová et al., 2021; Horčinová Sedláčková et al., 2021), it was possible to expect the presence of a similar effect when using golden root plants. We used an *in vitro* model, as this way of testing allows us to avoid any possible influence of alginite on the soil microbiome and, thus, on plant growth.

It was found that the addition of the test solution at a concentration of 1 $mL.L^{-1}$ significantly stimulated the growth of both shoots and roots (Figure 2).

In particular, the average weight of shoots was 6.64 times greater than the same parameter in the control plants, and the weight of roots was 5.58 times more. The addition of the test solution at a higher concentration (10 $mL.L^{-1}$) also increased the weight of plants (shoots by 4.48 times and roots by 3 times), although this parameter was lower than when the test solution was used at the lower concentration (Figure 3).

Cultivation of *Rhodiola rosea* plants on a medium to which the test solution was added in a lower concentration has led to a decrease in the specific content of flavonoids (Figure 4, bars 2). A similar effect was found when the concentration of the test solution in the medium increased (Figure 4, bars 3). This effect can be explained by the more active growth of plants in variants 2 and 3 compared to the control (variant 1), which requires more energy and synthetic resources and inhibits the process of accumulation of metabolites. A similar negative correlation between weight gain and the specific content of flavonoids was observed earlier (Matvieieva et al., 2019).

However, the total content of flavonoids synthesized in the shoots and roots of *Rhodiola rosea* plants in the experimental variants was significantly higher than in the control, due to the stimulation of growth in the presence of the test solution and the excess weight of the shoots and roots. As can be seen from Figure 5, the total content of flavonoids in the roots and shoots of variant No 2 was 1.48 and 3.35 times higher than the similar parameter of the control plants. The total content of flavonoids in the roots and shoots of the plants of variant No 3 also exceeded the parameters of the control plants and was 0.315 and 0.374 mg RE, respectively.

It is known that different biostimulants can affect not only plant growth but also secondary metabolism. For instance, the stimulator application increased the number of leaves, flowerheads, and dry root matter of *Calendula officinalis* plants and stimulated flavonoid synthesis in the plants (Oliveira Machado et al., 2014). These results correlate with the data obtained in our experiments.

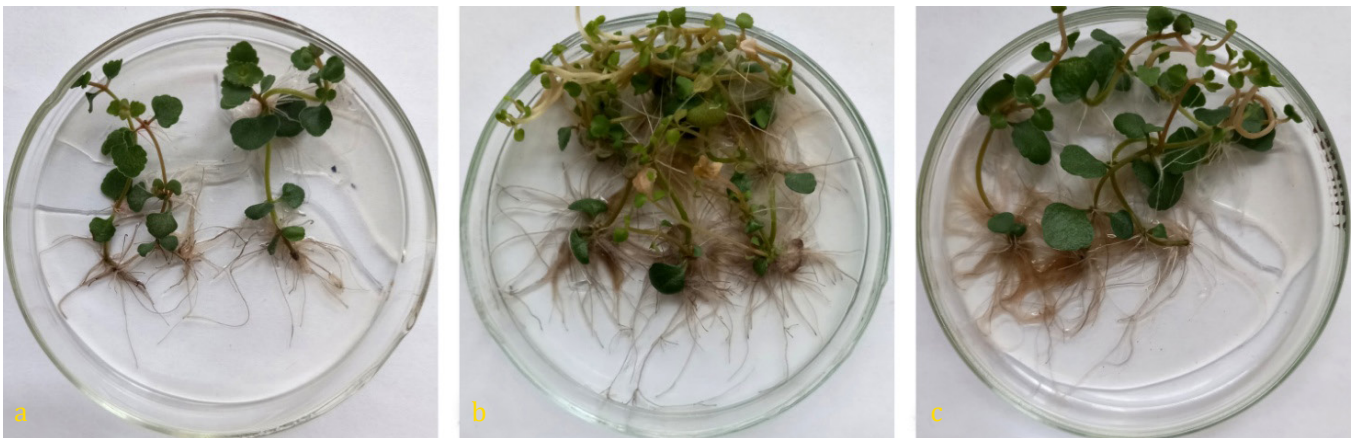

Figure 2 Growth of *Rhodiola rosea* L. plants on ½ MS medium
a – without test solution (the control); b – with the addition of the test solution, 1 $mL.L^{-1}$; c – with the addition of the test solution, 10 $mL.L^{-1}$

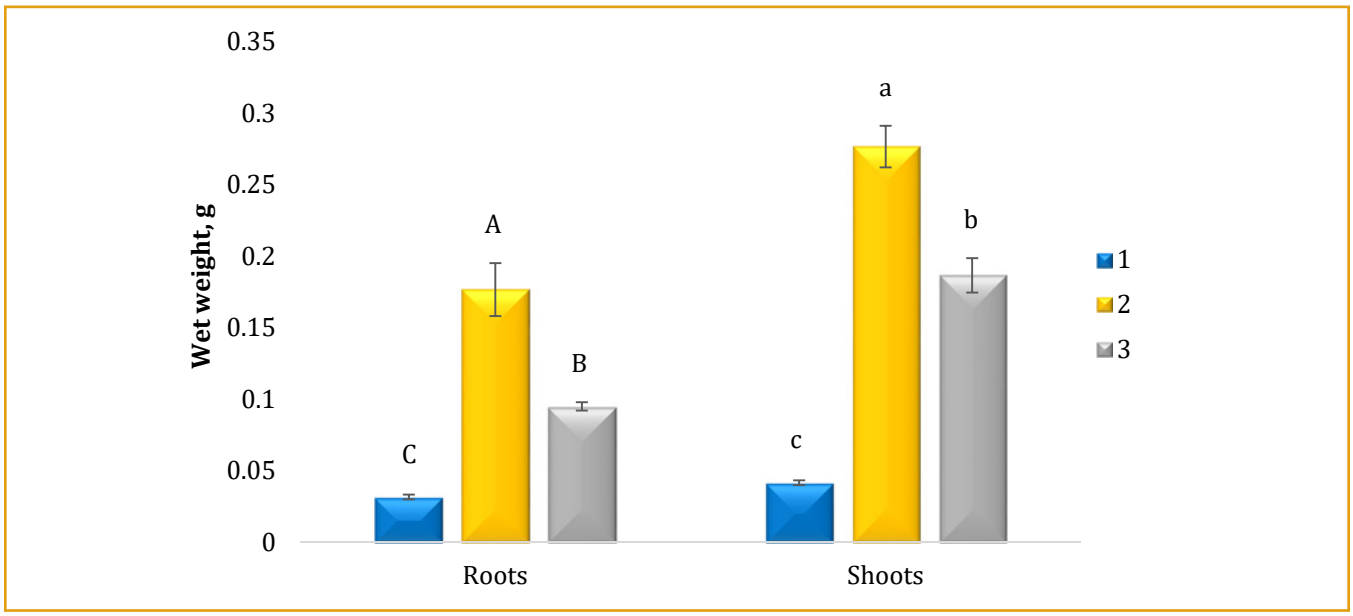

Figure 3 Weight of *Rhodiola rosea* L. plants (shoots and roots) grown on ½ MS medium
1 – the control; 2 – with the addition of the test solution, 1 mL.L^{-1}; 3 – with the addition of the test solution, 10 mL.L^{-1}. Upper and lower case letters belong to different comparisons

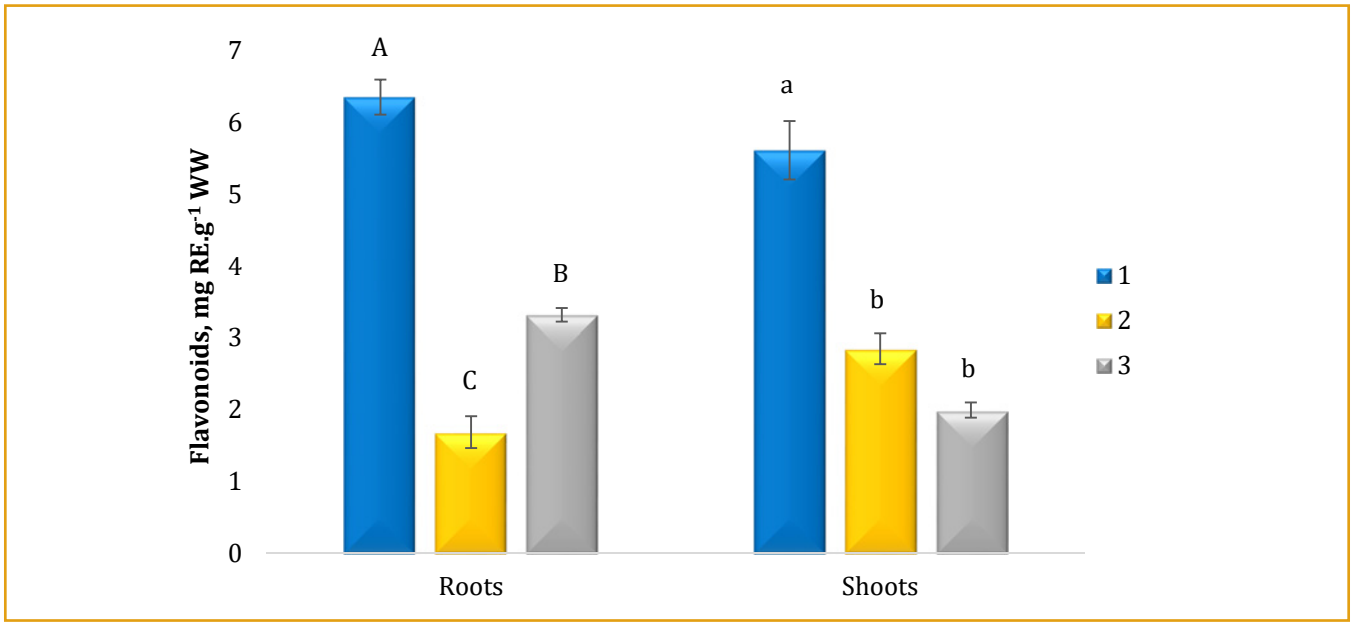

Figure 4 Specific content of flavonoids in shoots and roots of *Rhodiola rosea* L. plants on ½ MS medium
1 – control; 2 – with the addition of the test solution, 1 mL.L^{-1}; 3 – with the addition of the test solution, 10 mL.L^{-1}. Upper and lower case letters belong to different comparisons

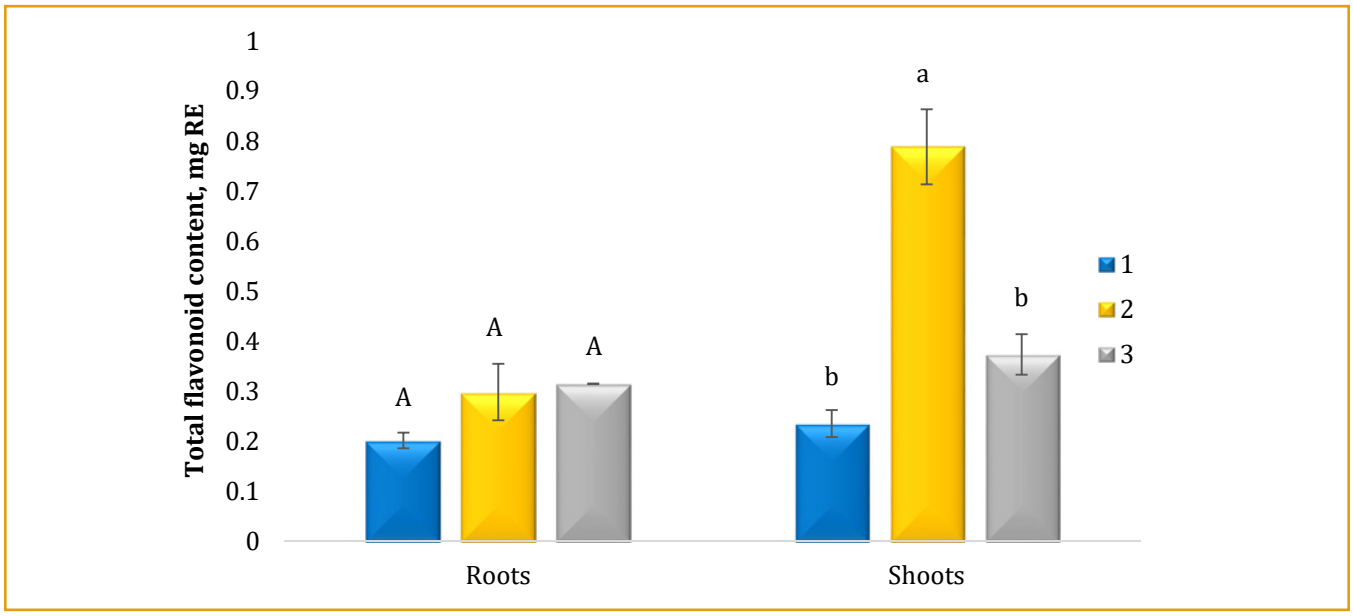

Figure 5 Total content of flavonoids in shoots and roots of *Rhodiola rosea* L. plants on ½ MS medium
1 – control; 2 – with the addition of the test solution, 1 mL.L^{-1}; 3 – with the addition of the test solution, 10 mL.L^{-1}. Upper and lower case letters belong to different comparisons

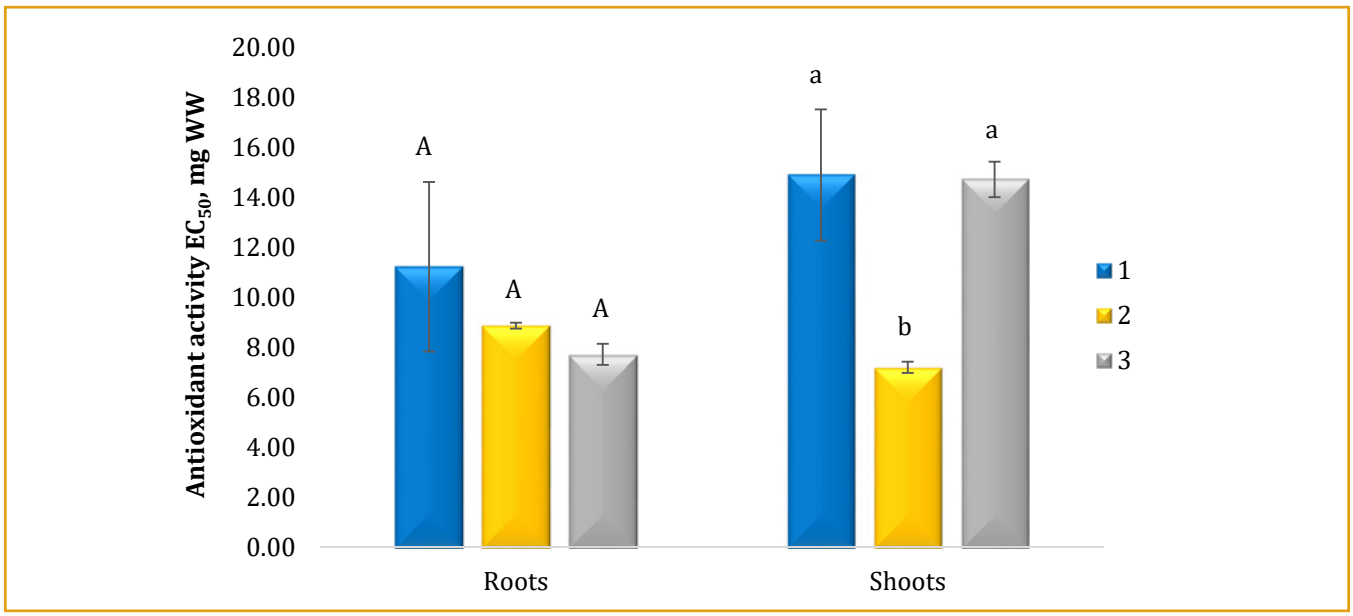

Figure 6 The antioxidant activity of root and shoot extracts of *Rhodiola rosea* L. plants
1 – control; 2 – with the addition of the test solution, 1 mL.L^{-1}; 3 – with the addition of the test solution, 10 mL.L^{-1}

The addition of alginite extract in a lower concentration has led to an increase in the level of antioxidant activity expressed in decreased equivalent concentration EC_{50} (Figure 6). When using the test solution in a lower concentration (1 mL.L^{-1}), a significant increase in the level of antioxidant activity in extracts from shoots was observed. However, using the test solution at a concentration of 10 mL.L^{-1}, the changes in antioxidant activity were within statistical error. The antioxidant activity of root extracts in the experimental variants also was not increased significantly.

The obtained results indicate significant stimulating activity of alginite extract. This effect was manifested both on roots and on shoots and was expressed in a 3.0–6.64-fold weight increase. The effect may be associated with humic acids in alginite (Barančíková et al., 2003), known as plant biostimulators. The stimulation effect

of these components was studied earlier (Chen et al., 2004). Authors have shown their positive influence on seed germination, root initiation, and total biomass growth of melon, ryegrass, and soybean plants. The humic acids improved Fe and possibly Zn nutrition in treated plants. Humic acids also promoted root growth (Jindo et al., 2020). It was studied that different biostimulants can affect root and shoot growth and increase plant biomass (Nardi et al., 2002; Jindo et al., 2012; Tužinský et al., 2015; Kim et al., 2019). They also affected the antioxidant and free radical-scavenging activities in treated plants (Zhu et al., 2006) and prevented plants from the negative effect of oxidative stress (Çimrin et al., 2010; Nephali et al., 2020; Omidbakhshfard et al., 2020). Alginite was also studied as a biostimulator that increases antioxidant activity in treated plants (Brindza et al., 2021a, 2021b; Eftimová et al., 2021; Horčinová Sedláčková et al., 2021).

Different aspects of alginite application as a possible biostimulant and its characteristics were studied in some institutions earlier (Beláček et al., 2002; Sarvašová, 2009; Nemcová et al., 2012–2015; Litavec and Barančíková, 2013; Barančíková and Litavec, 2016; Styková et al., 2016; Strompfová et al., 2018). Previously, according to the results obtained by scientists from the Slovak University of Agriculture in Nitra in 2021, the treatment of lawns (grass mixtures Liga, Midi, Renova, localization in Šajdíkove Humenice, Slovak Republic) with alginite extract containing humic acids led to increases in plant biomass. Such treatment also increased the seed and fruit weight of *Cucurbita pepo* L. var *oleifera* cultivated in Nitra in 2021. Leaves treatment increased the yield of *Triticum aestivum* L. var *aestivum* and *Avena sativa* L. (Vígľaš-Pstruša location) (Scientific report "Characteristics of treatment by alginite products in agriculture", Nitra, 2022, Slovak Agricultural University in Nitra).

Such results demonstrated the broad possibilities of using alginite extract to stimulate plant growth in field conditions. Our research showed that the *in vitro* addition of alginite extract solution also increased in biomass and accelerated the growth of the root system of *R. rosea* plants. This indicates that Alganite is a non-specific biostimulant of a vast spectrum of activity.

Conclusions

The addition of the test solution (1% alginite extract) at a concentration of 1 $mL.L^{-1}$ of the medium has led to a significant activation of the growth of both shoots and roots of Rhodiola plants in *in vitro* conditions. Such stimulation was accompanied by decreased specific flavonoid content in shoots and roots. However, due to the significantly greater weight of the plants, the total flavonoid content in the experimental variants was higher than in the control. An extract of alginite in a small concentration can be used both to stimulate the growth of *Rhodiola rosea* L. plants and to obtain an increased amount of flavonoids.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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