

# Depolymerised Sodium Alginate as a Eco-Friendly Biostimulant for Improving Herb Yield and Nutrient Accumulation in Hyssop

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## ABSTRACT

Sodium alginate and its derivatives present a promising tool for improving the quality of crops. Hyssop is one of the most important herb used both in foods and traditional medicines. The aim of this study was to investigate the effect of depolymerised sodium alginate (DSA) on yield and mineral status of hyssop plants under greenhouse conditions. The outcomes demonstrated that foliar application of DSA with a molecular mass of 64,000 g·mol<sup>-1</sup> at 50 and 100 mg·dm<sup>-3</sup> significantly improved the leaf chlorophyll index (by 22.9 and 30.3%, respectively), above-ground plant both fresh (by 24.1% and 28.2%, respectively) and dry weights (by 30.1% and 32.8%, respectively) relative to the control. The DSA at 50 and 100 mg·dm<sup>-3</sup> stimulated nitrogen, calcium, copper, manganese and zinc concentration in above-ground hyssop tissues by 18.9–31.1%, 32.9–77.2%, 44.7–43.2%, 69.3–41.8 and 40.6–33.3%, respectively. Moreover, application of DSA at 100 mg·dm<sup>-3</sup> increased phosphorus and potassium concentration by 38.9% and 24.3%, respectively. The magnesium, boron and iron contents were unaffected by biostimulant treatment. The use of DSA has shown commercial potential to increase herb yield and some mineral nutrients in potted hyssop.

**Keywords:** biostimulant; natural polysaccharides, food crops; mineral elements.

## INTRODUCTION

The population is estimated to exceed 11 billion by the end of the 21st century. With this marked population growth and in an era of climate change, there is a global problem with ensuring so-called food security (Laurance et al., 2014; UNICEF, 2021). It is estimated that some 2 billion people worldwide suffer from malnutrition caused by insufficient food (acute hunger) or a deficiency of specific vitamins and needed minerals in the diet (latent hunger). (Gödecke et al., 2018; Praharaj et al., 2021). It is predicted that more than 60% of people are deficient in iron, more than 30% in zinc, 30% in iodine, and 15% in selenium. Dietary calcium, magnesium, and copper deficiencies are common in many developed and developing countries (White and Broadley, 2009). Plants are essential to the human diet due to their high nutritional value and bioactive content (Lea

et al., 2006; Crozier et al., 2006; Salachna et al., 2021). Vegetables and herbs are essential micronutrient sources and are healthier than cereals or meat (Liu, 2013; Ceccanti et al., 2021). A practical way to increase the macro- and micronutrient content of the edible parts of vegetables and herbs is to use fertilizers and biostimulants (Aftab et al., 2014; Zhang et al., 2020). There is a growing demand for new effective biostimulants that increase the quality of plant yields while not posing a threat to the environment (Salachna et al., 2019; Ahmad et al., 2020).

Hyssop *Hyssopus officinalis* L. (Lamiaceae) is a widespread spice and medicinal as well as ornamental plant (Fatemeh and Sanaz, 2011). This species is a perennial herb native to southern and south-eastern Europe (Stancheva et al., 2019). Hyssop leaves have a pleasant, balmy, sweet-camphoraceous scent and a tart spicy taste (Tahir et al., 2018). Hyssop has a peculiar aroma and

flavor and is therefore used in its natural state or processed as a food additive (Vlase et al., 2014; Zawisłak, 2016). Fresh and dried hyssop herb is used as a flavouring for soups, potatoes, cheeses, pates, meats, salads. Hyssop is also used to make teas, bitter liqueurs and vermouths (Baj et al., 2018). Hyssop has been a valued medicinal plant since ancient times. Hyssop herb exhibits diastolic, expectorant, diuretic, windmill, and stimulant effects, accelerate wound healing, and is used for excessive sweating. The major constituents of hyssop herb are essential oils, glycosides, tannins, flavonoids, resins, and organic acids (Vlase et al., 2014; Golubkina et al., 2020). Hyssop is also a source of valuable minerals for human health (Gonçalves et al., 2013; Saebi et al., 2021), while there is a lack of broader data on the content of nutrients in the herb of this plant species.

Currently, the crop cultivation technology is constantly augmented with new solutions improving biological value of the yield. Natural polysaccharides are compounds exhibiting multi-directional action in plants; they are also eco-friendly, biocompatible, reactively bioactive and cheap (Mukarram et al., 2021). Particular attention is paid to depolymerised polysaccharides, which are considered by higher biological efficiency compared to the products from which they were obtained (Salachna et al., 2019). Sodium alginate is an example of a naturally polysaccharide with biostimulatory properties (Aftab et al., 2013; Zhang et al., 2020). Alginates are naturally

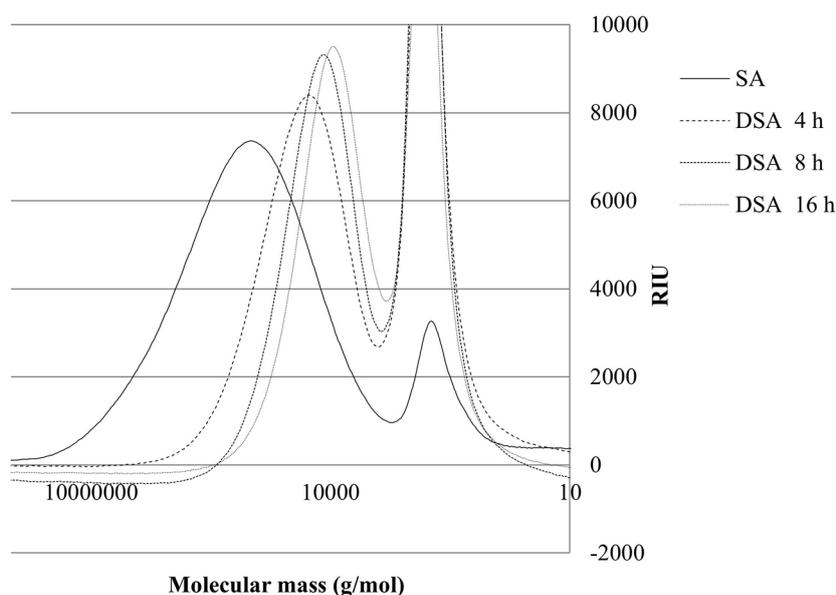
occurring polysaccharide copolymers produced by brown algae and some bacterial species. It has been proven that sodium alginate, especially in a depolymerized form with low molecular mass, positively affects the growth and quality of the crop yield (Aftab et al., 2014; Hossain et al., 2021; Khan et al., 2022).

There are few studies showing positive effects on growth and yield of hyssop such biostimulants as L-phenylalanine (Aghaei et al., 2019), seaweed fertilizer (Pirani et al., 2020), kaolin, chitosan and glycine amino acid (Khajeh Hosseini et al., 2021) and citrulline (Ahmadi et al., 2020). However, these referred research does not address the effect of biostimulants on mineral status of hyssop. The aim of this a greenhouse study was thus to investigate the effect of depolymerised sodium alginate with low molecular mass on the herbage yield and nutrient content of hyssop.

## MATERIALS AND METHODS

### Preparation of depolymerised sodium alginate

Depolymerised sodium alginate (DSA) was obtained from sodium alginate (Sigma-Aldrich, Poznań, Poland) as a result of acid hydrolysis. 20 g of starting sodium alginate was dissolved in 800 cm<sup>3</sup> of deionized water at 70 °C. Concentrated HCl was added to obtain final concentration of



**Figure 1.** The molecular mass distribution of sodium alginate and depolymerised sodium alginate (DSA) after 4, 8 and 16 hours of hydrolysis

0.2 M. Then the solution was incubated for 4, 8 or 16 hours while stirring. Following hydrolysis samples were cooled to room temperature, neutralized with NaOH to obtain pH 7 and partially evaporated using vacuum evaporator RVO 200A (INGOS, Czech Republic). DSA was precipitated using acetone, filtered, several times washed with acetone and dried at 50 °C for several hours. The evaluation of the physicochemical properties of the studied compounds was based on high performance size exclusion chromatography (HPSEC) and Fourier transform infrared spectroscopy (FTIR) methods (Salachna et al., 2018). Starting alginate had molecular mass of 325,000 g·mol<sup>-1</sup>, which decreased during 4, 8 and 16 hours of hydrolysis to 64,000 g·mol<sup>-1</sup>, 42,000 g·mol<sup>-1</sup> and 32,000 g·mol<sup>-1</sup>, respectively (Figure 1).

### Plant material and experimental design

The seeds of *Hyssopus officinalis* were purchased from W. Legutko company (Jutrosin, Poland) and sown on 5 March into a mixture of sand and peat (1:1). Seed trays kept in a climate-controlled greenhouse (with a set-point air temperatures of 22 °C/18 °C; day/night) of West Pomeranian University of Technology in Szczecin located on the Plant Growth Facility (53° 25' N, 14° 32' E, 25 m asl., sub-zone 7a USDA). After four weeks uniform seedlings were individually transferred into 1.2 dm<sup>3</sup> pots (one plant per pot) filled with a peat substrate with pH 6.1 and EC 0.9 mS·cm<sup>-1</sup>, supplemented with a fertilizer Hydrocomplex (Yara International ASA, Norway) consisting of 5% N-NO<sub>3</sub>, 7% N-NH<sub>4</sub>, 11% P<sub>2</sub>O<sub>5</sub>, 18% K<sub>2</sub>O, 2.7% MgO, 8% S, 0.015% B, 0.2% Fe, 0.02% Mn, and 0.02% Zn at 2 g·dm<sup>-3</sup>. All plants were grown in a greenhouse under natural light during the experimental period.

Four weeks after transplanting, the plants were divided into three groups: (i) plants treated by foliar spraying with tap water – control; (ii) plants treated by foliar spraying with DSA at 50 mg·dm<sup>-3</sup> and (iii) plants treated by foliar spraying with DSA at 100 mg·dm<sup>-3</sup>. The plants were treated with biostimulant or tap water a total of seven times, every five days, and each time on average 15–20 cm<sup>3</sup> per plant of the solution or tap water was applied. Based on previous preliminary studies, the study used DSA with a molecular mass of 64,000 g·mol<sup>-1</sup>. Doses of 50 and 100 mg·dm<sup>-3</sup> were adopted based on previous studies.

The experimental design of the pot experiment was a completely randomized complete block design with 3 treatments and 4 replicates (40 plants in total for each treatment). In plants, the SPAD (Soil and Plant Analysis Development) leaf greenness index was analyzed using the SPAD-502 optical apparatus (Minolta, Japan). The SPAD value was determined using the formula  $SPAD = (940 \text{ nm} - 650 \text{ nm}) / (650 \text{ nm} - 940 \text{ nm})$ . One week after the last treatment with the biostimulant, the above-ground part of individual hyssop plants was cut and its fresh weight was determined using an electronic scale. Then the plant samples were dried at 65 °C and ground in a laboratory mill.

### Plant material analyses

To determine above-ground hyssop tissues content of N, P, K, Ca and Mg, samples were mineralized for 1 h in 17 cm<sup>3</sup> of 96% H<sub>2</sub>SO<sub>4</sub>. Samples for B, Cu, Fe, Mn and Zn assessment were mineralized for 8 h in 30 cm<sup>3</sup> a mixture (1:4) of HNO<sub>3</sub> and HClO<sub>4</sub>. The content of total N was determined by titration (Kjeldahl method), K and Ca by flame photometry, P and B by spectrophotometry using a Spectronic GENESYS 6 UV-Visible Spectrophotometer (Thermo Electron Corporation, Cambridge, UK), and Mg, Cu, Fe, Zn and Mn by atomic absorption spectrophotometry (Zawadzińska et al., 2021). Each analysis was performed in triplicate.

### Statistical analyses

Data were analyzed using analysis of variance (the one-way ANOVA) by TIBCO Statistica® 13.3 software (StatSoft Poland). Treatments means were compared using Tukey's multiple range test.

## RESULTS AND DISCUSSION

Biostimulants such as derivatives of polysaccharides with low molecular mass can influence the growth of crops and their yield quality (Zhang et al., 2020). In the present study, plants of hyssopus were exposed or not to the foliar DSA treatments. This study found that treating plants with DSA at both concentrations significantly increased the leaf greening index (Table 1). Relative to the control, plants sprayed with DSA

at concentrations of 50 and 100 mg·dm<sup>-3</sup> had an increased the leaf greenness index, by 22.9 and 30.3%, respectively. The leaf greenness index correlates with the nitrogen and chlorophyll contents in leaves (Salachna and Zawadzińska, 2017). Increased concentrations of chlorophylls and carotenoids in leaves as a result of treatment with sodium alginate fractions with low molecular mass were also observed in *Eucomis autumnalis* (Salachna et al., 2018). Increased pigment content in plant leaves after DSA application may have influenced more intense photosynthesis, as observed in earlier studies in *Artemisia annua* (Aftab et al. 2013) and *Cymbopogon flexuosus* (Khan et al., 2022). The increase in the SPAD index observed in the study under the influence of DSA may result from a better supply of nitrogen and chlorophyll to the leaves, which may have led to an intensification of plant weight gain. The obtained results indicated the stimulating effect of both the tested concentrations of DSA on biomass of hyssop (Table 1). It was shown that plants treated with DSA at 50 and 100 mg dm<sup>-3</sup> were characterized by greater fresh mass of aerial parts after harvest in relation to control plants (by 24.1% and 28.2%, respectively). Also, the dry weight of the above-ground parts of plants was significantly increased as a result of the application of DSA at 50 and 100 mg dm<sup>-3</sup> (by 30.1%

and 32.8%, respectively) in comparison with untreated plants. Under the influence of DSA at 100 mg·dm<sup>-3</sup>, the plants were characterized by a higher fresh and dry weights of biomass versus to DSA 50 mg dm<sup>-3</sup>. The increase in fresh weight as a result of sodium alginate with low molecular mass application was also reported in *Cymbopogon flexuosus* (Khan et al., 2022), *Camellia sinensis* (Hossain et al., 2021) and *Eucomis autumnalis* (Salachna et al., 2018). The increased biomass of the plants treated with DSA may be due to enhanced uptake of water and minerals by roots, which in turn is associated with the intensification of plant growth.

The present study evaluated whether sodium alginate can change in hyssop mineral status. The use of depolymerised sodium alginate in the greenhouse cultivation of hyssop increased both macro- and micronutrients except for Mg, B and Fe (Table 2, Table 3). The effects were dose dependent. Derivatives of sodium alginate at 50 mg dm<sup>-3</sup> stimulated higher N, Ca, Cu, Mn and Zn accumulation in hyssop plants tissues by 18.9%, 32.9%, 44.7%, 69.3% and 40.6%, respectively, compared to control. However, plants treated with DSA at 100 mg·dm<sup>-3</sup> showed more content of N, P, K, Ca, Cu, Mn and Zn by 31.1%, 38.9%, 24.3%, 77.2%, 43.2%, 41.8% and 33.3%, respectively.

**Table 1.** Effect of foliar application of DSA solutions on the SPAD leaf greenness index and biomass of hyssop. Each value in the table is presented as a mean ± standard deviation for n = 3

Treatment	Leaf greenness index (SPAD)	Fresh weight (g)	Dry weight (g)
Control (water)	35.0 ± 1.89 b	33.6 ± 2.02 b	8.18 ± 0.77 b
50 mg dm <sup>-3</sup> DSA	43.0 ± 2.41 a	41.7 ± 0.25 a	10.6 ± 0.99 a
100 mg dm <sup>-3</sup> DSA	45.6 ± 2.92 a	43.1 ± 1.65 a	10.8 ± 0.55 a
<i>F</i>	6.08	4.17	7.52
<i>p</i>	<0.001	<0.001	0.011

**Note:** means values followed by different letter within the same column are significantly different ( $p \leq 0.05$ ) according to Tukey's multiple range test.

**Table 2.** Effect of foliar application of DSA solutions on macronutrients (% dry weight) accumulation in above-ground hyssop tissues. Each value in the table is presented as a mean ± standard deviation for n = 3

Treatment	N	P	K	Ca	Mg
Control (water)	1.32 ± 0.11 b	0.18 ± 0.01 b	1.40 ± 0.10 b	0.79 ± 0.11 c	0.27 ± 0.02 a
50 mg dm <sup>-3</sup> DSA	1.57 ± 0.04 a	0.19 ± 0.01 b	1.47 ± 0.06 b	1.05 ± 0.05 b	0.28 ± 0.02 a
100 mg dm <sup>-3</sup> DSA	1.73 ± 0.06 a	0.25 ± 0.02 a	1.74 ± 0.07 a	1.40 ± 0.04 a	0.28 ± 0.03 a
<i>F</i>	21.67	30.55	15.57	53.47	0.317
<i>p</i>	0.002	<0.001	0.004	<0.001	0.740

**Note:** means values followed by different letter within the same column are significantly different ( $p \leq 0.05$ ) according to Tukey's multiple range test.

**Table 3.** Effect of foliar application of DSA solutions on micronutrients (mg kg<sup>-1</sup> dry weight) accumulation in above-ground hyssop tissues. Each value in the table is presented as a mean ± standard deviation for n = 3

Treatment	B	Cu	Fe	Mn	Zn
Control (water)	20.3 ± 2.09 a	4.03 ± 0.42 b	87.6 ± 8.96 a	15.3 ± 4.21 b	41.1 ± 4.27 b
50 mg dm <sup>-3</sup> DSA	17.9 ± 2.01 a	5.83 ± 0.41 a	86.3 ± 12.6 a	25.9 ± 1.24 a	57.8 ± 1.96 a
100 mg dm <sup>-3</sup> DSA	19.2 ± 1.61 a	5.77 ± 0.21 a	89.2 ± 14.1 a	21.7 ± 1.76 a	54.8 ± 3.47 a
<i>F</i>	1.120	24.03	0.016	25.36	20.84
<i>p</i>	0.386	<0.001	0.984	<0.001	0.002

**Note:** means values followed by different letter within the same column are significantly different ( $p \leq 0.05$ ) according to Tukey's multiple range test.

Few studies have indicated that sodium alginate derivatives can induce changes at the biological and physiological levels by affecting the synthesis of assimilatory pigments and metabolites and the degree of plant nutrition. In *Artemisia annua*, irradiated sodium alginate at 80 mg·dm<sup>-3</sup> combined with nitrogen fertilization (80 kg N·ha<sup>-1</sup>) significantly increased herb yield and artemisinin content (Aftab et al., 2013). In another study, the application to *A. annua* crops of oligomers obtained from the depolymerization of sodium alginate in combination with phosphorus fertilization significantly improved nitrogen and phosphorus concentrations in the leaves of *A. annua* plants (Aftab et al., 2014).

The elemental content of herbs can compensate for their lack in the human body and affect the use of medicinal properties. Herbal plants containing even trace amounts of micronutrients contribute to the proper function of body enzymes. The human body can assimilate micronutrients contained in herbal raw materials (Hedges and Lister, 2007). The chemical composition of herbs is very labile and depends on the genotype, plant development stage, plant age, and environmental factors (Patel et al., 2016; Li, 2020). Here, irrespective of treatment, above-ground hyssop tissues contained the following levels of macronutrients (in % dry weight): K 1.40–1.74 > N 1.32–1.73 > Ca 0.79–1.40 > Mg 0.27–0.28 > P 0.18–0.25 (Table 2). According to results, K is one of the most abundant macronutrient and Fe is one of the most rich micronutrient in hyssop. Similar results were observed by Saebi et al. (2021), who reported that the K content in hyssop biomass was 1.54–1.65 % dry weight. Micronutrient content in tissues was as follows values (mg kg<sup>-1</sup> dry weight): Fe 86.3–87.6 > Zn 41.1–54.8 > Mn 15.3–30.7 > Cu 17.9–20.3 (Table 3). Golubkina et al. (2020) found 106–155 mg Fe per kg of hyssop dry weight. Nutrients are compounds essential for the proper functioning of the human

organism (Welch, 2002). They are constituents of bones, teeth, skin and hair, part of complexes that are essential for the body's metabolism and components or activators of enzymes (Soetan et al., 2010). Moreover, nutrients affect water-electrolyte and acid-base balance and neuromuscular excitability (Martínez-Ballesta et al., 2010). In the light of these considerations, it is necessary to look for new environmentally friendly ways to improve the content in herbs and spices of valuable compounds for health (Grusak 2002).

## CONCLUSIONS

It can be concluded based on the results obtained in this study that DSA used in the form of foliar spraying during cultivation, can improve plant biomass and content of some nutrients in hyssop. The DSA application at 50 and 100 mg·dm<sup>-3</sup> increased the leaf chlorophyll index, above-ground plant both fresh and dry weights as well as enhanced nitrogen, calcium, copper, manganese and zinc. However, treatment with 100 mg·dm<sup>-3</sup> of DSA resulted in a increase in leaf phosphorus and potassium contents. The DSA can be recommended in sustainable cultivation programs, where particular emphasis is placed on reducing the use of mineral fertilizers that have a negative impact on the environment.

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