

Article

# Oligo-Alginate with Low Molecular Mass Improves Growth and Physiological Activity of *Eucomis autumnalis* under Salinity Stress

Piotr Salachna <sup>1,\*</sup> , Monika Grzeszczuk <sup>1</sup>, Edward Meller <sup>2</sup> and Marcin Soból <sup>3</sup>

<sup>1</sup> Department of Horticulture, West Pomeranian University of Technology, 3 Papieża Pawła VI Str., 71-459 Szczecin, Poland; monika.grzeszczuk@zut.edu.pl

<sup>2</sup> Department of Soil Science, Grassland Management and Environmental Chemistry, West Pomeranian University of Technology, Słowackiego 17 Str., 71-434 Szczecin, Poland; edward.meller@zut.edu.pl

<sup>3</sup> Center of Bioimmobilisation and Innovative Packaging Materials, West Pomeranian University of Technology, 35 Janickiego Str., 71-270 Szczecin, Poland; marcin.sobol@zut.edu.pl

\* Correspondence: piotr.salachna@zut.edu.pl; Tel.: +48-91-4496-359

Academic Editor: Marek M. Kowalczyk

Received: 10 March 2018; Accepted: 31 March 2018; Published: 2 April 2018



**Abstract:** Biopolymers have become increasingly popular as biostimulators of plant growth. One of them, oligo-alginate, is a molecule that regulates plant biological processes and may be used in horticultural practice as a plant growth regulator. Biostimulators are mainly used to improve plant tolerance to abiotic stresses, including salinity. The aim of the study was to assess the effects of salinity and oligo-alginate of various molecular masses on the growth and physiological activity of *Eucomis autumnalis*. The species is an ornamental and medicinal plant that has been used for a long time in the traditional medicine of South Africa. The bulbs of *E. autumnalis* were coated using depolymerized sodium alginate of molecular mass 32,000; 42,000, and 64,000 g mol<sup>-1</sup>. All of these oligo-alginates fractions stimulated plant growth, and the effect was the strongest for the fraction of 32,000 g mol<sup>-1</sup>. This fraction was then selected for the second stage of the study, when plants were exposed to salt stress evoked by the presence of 100 mM NaCl. We found that the oligo-alginate coating mitigated the negative effects of salinity. Plants treated with the oligomer and watered with NaCl showed smaller reduction in the weight of the above-ground parts and bulbs, pigment content and antioxidant activity as compared with those not treated with the oligo-alginate. The study demonstrated for the first time that low molecular mass oligo-alginate may be used as plant biostimulator that limits negative effects of salinity in *E. autumnalis*.

**Keywords:** oligosaccharides; degree of polymerization; NaCl; plant growth promoter

## 1. Introduction

Biostimulators are enjoying considerable attention as chemical or microbial agents that improve plant growth and yield quality [1,2]. The worldwide market for biostimulators is expected to be worth over two billion dollars in 2018 [3]. Algae-derived polysaccharides are popular biostimulators with multidirectional effects in plants [4]. They are biodegradable, biocompatible, non-toxic, biologically reactive, and cheap [5]. Of particular interest are alginates—natural polysaccharide copolymers composed of two types of monomers:  $\beta$ -D-mannaric acid and  $\alpha$ -L-guluronic acid [6]. Alginate is a well-known polysaccharide obtained from marine algae, mainly brown algae (Phaeophyceae) or

produced extracellularly by some bacteria [7]. The literature review shows that sodium alginate in depolymerized form stimulates seed germination [8], plant growth and development [9–11], affects photosynthesis [12], ion uptake and transport [13,14], and increases the content of assimilation pigments [15], proteins [16], and secondary metabolites such as artemisinin [12], morphine, codeine [17] or essential oils [18,19]. In addition, sodium alginate is effective in inhibiting certain plant diseases [20], enhances plant tolerance to drought [21] and alleviates toxic effects of heavy metals [22]. Sodium alginate has been used for many years in horticulture for producing biopolymeric seed coatings, sometimes supplemented with fertilizers and plant protection products [23]. It is also successfully used for coating fruits and vegetables to prolong their shelf life [24]. Sodium alginate is useful for encapsulation of in vitro somatic embryos and cultured shoot tips to develop synthetic seeds of many plant species [25,26].

Bioactive oligosaccharides of a specific structure and size act as signal molecules [27,28] that may control plants' growth [29] and basal metabolism [30] by regulating their gene expression [31]. Research shows that depolymerized sodium alginate is a more effective plant growth promoter than a non-polymerized form [12,13,32]. This might be due to its low molecular mass, which is one of the most important factors affecting biological activity of many chemically modified polysaccharides [33–35].

Biostimulators, including polysaccharides, are often applied in the form of spraying or watering solutions, which requires a lot of work and large amounts of water. An answer to this problem may be the use of hydrogel coatings containing the biostimulators [36]. Such coatings are the result of a polyelectrolyte complex formation by interaction of anionic functional groups of the polyelectrolyte with metal cations, or of a reaction at the interface of aqueous solutions of polyelectrolytes with functional groups of opposite charges [37]. Polysaccharides applied via coatings may positively affect plant growth and flowering [38] and stimulate root system development [39]. Specific effects depend on the components used [40].

*Eucomis autumnalis* (Mill.) Chitt (Asparagaceae formerly Hyacinthaceae) is an ornamental plant, highly popular due to its extremely original, decorative and durable inflorescences. It is grown as a pot plant and for cut flowers [41,42]. The plants may be grown in gardens and urban green areas, but their sensitivity to excess soil salinity should be taken into account [43]. *E. autumnalis* is the most important medical plant, used for a long time in traditional South African medicine [44]. Bulbs, leaves and roots of *E. autumnalis* show multidirectional pharmacological effects, including antioxidant, anti-inflammatory, bactericidal, fungicidal and cytostatic activity [45,46]. As the bulbs have been in very high demand for pharmacological purposes, the species is now threatened with extinction [47]. Therefore, the aim of many scientific studies is to develop new solutions aimed at providing the plants with the most favorable growth conditions [48,49].

Salinity is one of the crucial abiotic stresses limiting plant growth [50]. Exceeding the plant tolerance threshold for salt triggers numerous adverse changes in plant growth and development [51]. Negative effects of salt stress include mainly the accumulation and toxicity of Na<sup>+</sup> and Cl<sup>-</sup> ions, disturbance of ionic equilibrium, dysfunction of plasma membranes, changes in metabolism and enzymatic activity, and limitation of photosynthesis and respiration intensity [52]. Moreover, salinity limits growth and flowering, induces wilting, drying and falling of leaves, disrupts water management and generates oxidative stress [53]. To improve plant tolerance to unfavorable factors, breeders may use anti-stress substances, including e.g., polysaccharides [54] and their derivatives such as chitosan-PVA [55], sulfated chitoooligosaccharides [56] or gamma irradiated carrageenan [57]. So far, data are missing on the effects of sodium alginate derivatives on the growth of plants exposed to salt stress.

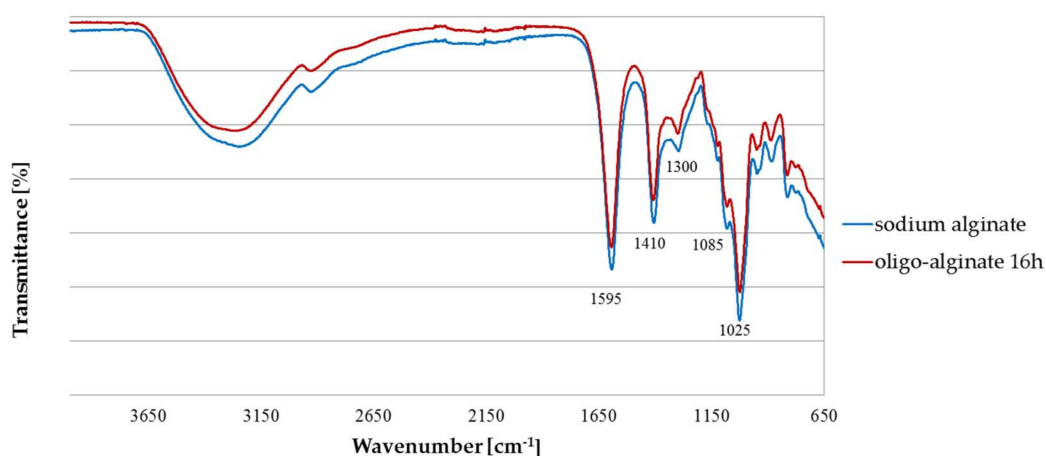
This study verified a research hypothesis that sodium alginate of various molecular masses, and therefore with different physical and chemical properties, applied in the form of bulb coating, affects the growth of *E. autumnalis* to varying degrees. We also made the first attempt at alleviating the

effects of salt stress in *E. autumnalis* by treating the plants with a selected fraction of sodium alginate with the strongest biostimulatory effect.

## 2. Results and Discussion

### 2.1. Fourier Transform Infra Red (FTIR)

Spectroscopic analyses were performed in order to confirm whether alginates undergo any chemical modification after the depolymerization process. Sodium alginate and oligo-alginate spectrum (Figure 1) showed bands at  $\sim 1595\text{ cm}^{-1}$  and  $\sim 1410\text{ cm}^{-1}$ , which is given by the antisymmetric and symmetric  $\text{COO}^-$  stretch, respectively. Band at  $\sim 1300\text{ cm}^{-1}$  is assign to the skeletal vibration [58]. Peak at  $\sim 1085\text{ cm}^{-1}$  is attributed to C–O–C stretching and at  $\sim 1025\text{ cm}^{-1}$  to the OH bending of guluronate [59].



**Figure 1.** Fourier transform infra red (FTIR) spectra of sodium alginate and oligo-alginate obtained after 16 h hydrolysis.

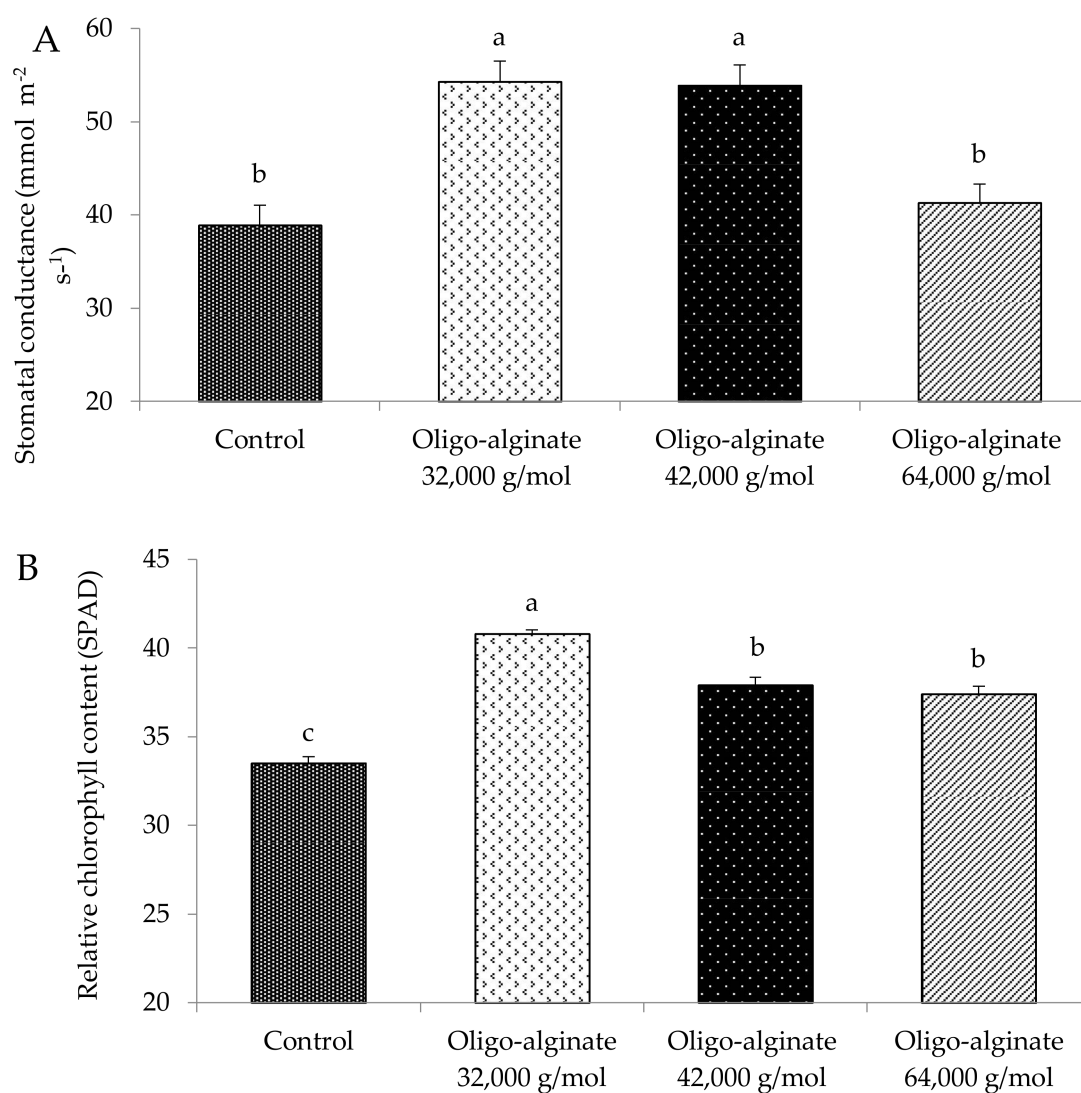
### 2.2. Effects of Oligo-Alginates with Different Molecular Mass on Growth Attributes of *Eucomis autumnalis*

In the first year, we assessed the effects of coating *E. autumnalis* bulbs in oligo-alginates of different molecular mass on plant morphological features and physiological parameters (Table 1, Figure 2). Compared with control plants, the plants treated with all fractions of oligo-alginates flowered earlier (on average by 5.5 days), and had longer leaves (33.2%) and inflorescences (33.6%). Molecular mass of the oligomers did not affect these variables. The oligo-alginate of molecular mass  $32,000\text{ g mol}^{-1}$  caused the highest increase in plant height (30.8%), plant width (28.6%), florets number per inflorescence (29.3%) and the relative chlorophyll content (21.8%) in comparison with control plants. Moreover, plants treated with 32,000 and 42,000  $\text{g mol}^{-1}$  oligo-alginates had greater than controls stomatal conductance, by 39.6% and 38.6%, respectively. The oligomers did not affect the number of produced leaves and inflorescences. These results corroborated those from other studies in which depolymerized sodium alginate positively influenced morphological features of plants depending on their degree of polymerization. The irradiated alginate with molecular mass below  $10^4$  considerably stimulated growth of rice and peanut through a hydroponic solution [9]. Media supplemented with irradiated alginate of molecular mass approximately  $1.43 \times 10^4\text{ Da}$  positively affected shoot height, root length and fresh weight of in vitro propagated ornamental plants *Chrysanthemum × grandiflorum*, *Eustoma grandiflorum* and *Limonium latifolium* [10]. Irradiated alginate of molecular mass within 1–3 kDa most effectively stimulated growth and development of barley and soybean [11]. Research showed that oligosaccharides in higher plants shorten the cell cycle, stimulate cell elongation and cell division, enhance mitotic indexes as well as decrease chromosomal aberration frequency [22,60]. Stronger effects

of low molecular mass oligosaccharides may result from faster and easier penetration of their molecules into plant tissues and individual cells [61].

**Table 1.** Effect of oligo-alginate with different molecular mass on growth and flowering of *Eucomis autumnalis*. Means ( $\pm$ standard errors) followed by the same small letter in the same row did not differ by Tukey test ( $p \leq 0.05$ ).

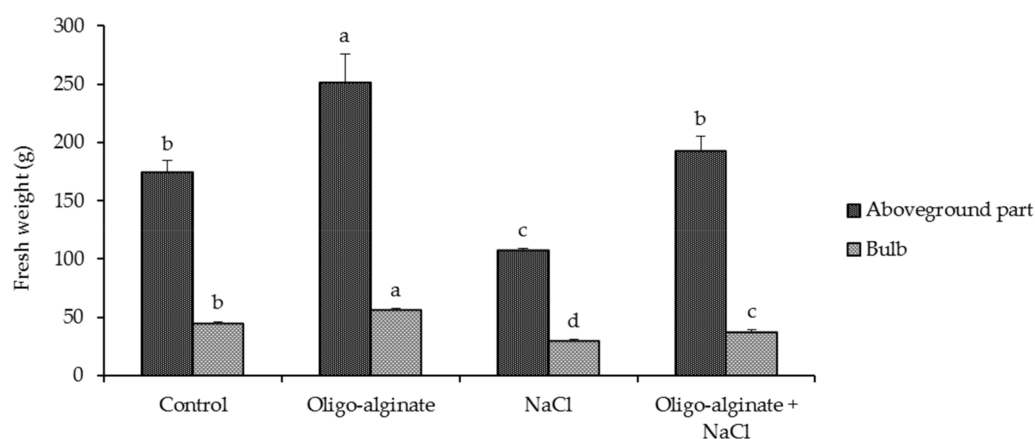
Parameters	Oligo-Alginate Molecular Mass ( $\text{g mol}^{-1}$ )			
	Control	32,000	42,000	64,000
Days to anthesis	81.6 $\pm$ 0.78 a	75.2 $\pm$ 0.61 b	76.0 $\pm$ 0.50 b	77.2 $\pm$ 0.52 b
Plant height (cm)	31.2 $\pm$ 1.59 c	40.8 $\pm$ 1.01 a	37.5 $\pm$ 1.04 a,b	34.3 $\pm$ 1.64 b,c
Plant width (cm)	28.3 $\pm$ 1.04 b	36.4 $\pm$ 1.68 a	32.0 $\pm$ 1.80 a,b	32.5 $\pm$ 0.76 a,b
Leaf length (cm)	23.7 $\pm$ 1.29 b	32.0 $\pm$ 1.99 a	30.3 $\pm$ 0.79 a	32.4 $\pm$ 1.84 a
Number of leaves	5.33 $\pm$ 0.17 a	5.50 $\pm$ 0.29 a	5.50 $\pm$ 0.29 a	5.50 $\pm$ 0.29 a
Number of inflorescences	1.10 $\pm$ 0.06 a	1.10 $\pm$ 0.03 a	1.08 $\pm$ 0.04 a	1.03 $\pm$ 0.58 a
Inflorescence length (cm)	15.3 $\pm$ 0.88 b	20.3 $\pm$ 0.33 a	19.8 $\pm$ 0.33 a	21.2 $\pm$ 1.36 a
Number of florets	68.6 $\pm$ 4.03 c	88.7 $\pm$ 2.23 a	81.4 $\pm$ 1.99 b	81.4 $\pm$ 2.45 b



**Figure 2.** Effect of oligo-alginate with different molecular mass on stomatal conductance (A) and the relative chlorophyll content (B) of *Eucomis autumnalis*. Data are presented as means ( $\pm$ standard errors) and bars with different letters in each graph are significantly different by Tukey test ( $p \leq 0.05$ ).

### 2.3. Effects of Oligo-Alginate and Salinity Stress on Growth and Physiological Attributes of *Eucomis autumnalis*

The next phase of the study focused on the response of plants treated with sodium-alginate to salt stress. The bulbs were coated in oligo-alginate of molecular mass  $32,000 \text{ g mol}^{-1}$  that most strongly stimulated plant height, number of florets per inflorescence and the leaf greenness index Soil and Plant Analysis Development SPAD (Table 1, Figure 2). The effects of the oligomer and salinity on plant growth were estimated based on fresh biomass increase, as this parameter is useful in determining the quality of ornamental plants (Figure 3). Plants treated with oligo-alginate had considerably greater fresh weight of the above-ground parts and bulbs by 46.5% and 26.0%, respectively, as compared with controls. The increased biomass of the plants treated with the degraded alginate may be due to enhanced uptake of water and minerals by roots, and thus higher levels of nutrients in plant tissues, as reported for *Eucalyptus citriodora* [13], *Artemisia annua* [14] and *Cymbopogon flexuosus* [16].

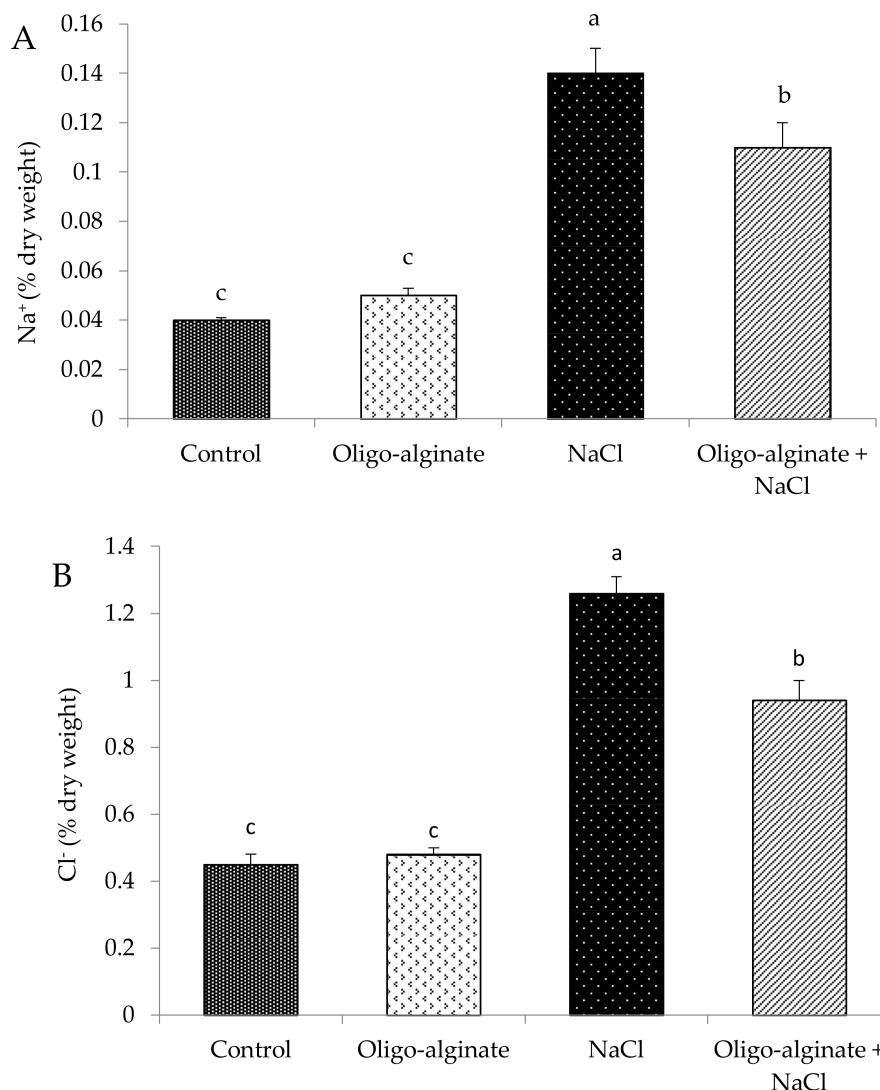


**Figure 3.** Effect of oligo-alginate and NaCl on fresh weight of above-ground part and bulb of *Eucomis autumnalis*. Data are presented as means ( $\pm$ standard errors) and bars with different letters in each graph are significantly different by Tukey Test ( $p \leq 0.05$ ).

The fresh weight of the above-ground parts and bulbs in salt-treated plants dropped markedly, by 37.8% and 34.5%, respectively, in comparison with non-treated plants (Figure 3). The adverse effect of salt stress on the biomass of leaves, inflorescences or bulbs was also observed in other geophytes [62,63]. Salt stress inhibits mitoses and elongation growth of cells and consequently reduces fresh and dry weight, especially of the above-ground parts [64].

In this study, oligo-alginate treatment effectively protected plants from biomass loss caused by exposure to salinity. Salt-induced reduction in fresh weight of the above-ground parts and bulbs was smaller in the plants treated with oligo-alginate vs. those treated with NaCl alone (Figure 3). Oligosaccharides may facilitate plant survival in unfavorable environments by interacting with cellular membrane receptors in a hormone-like manner and affecting gene regulation [65]. Oligosaccharides may increase plant stress tolerance by inducing the enzymes of the antioxidant system and stimulating synthesis of phenolic compounds [66,67]. Stress alleviating activity of the oligomers depends largely on their physical and chemical properties, including the degree of polymerization [34] and acetylation [68].

Leaves of the plants exposed to salinity accumulated significantly more sodium and chlorine than the control plants by 3.5- and 2.8-fold, respectively (Figure 4). The same trend was also reported for other bulbous plants [64,69]. Coating the bulbs in oligo-alginate curbed the accumulation of sodium and chlorine in salt exposed plants. The mechanisms of tolerating increased concentration of salt involve a synthesis of compatible metabolites that protect the structure of proteins and membranes (amino acids, amines, carbohydrates and polyols) [51,70].



**Figure 4.** Effect of oligo-alginate and NaCl on the Na<sup>+</sup> (A) and Cl<sup>-</sup> (B) ion concentration in leaves of *Eucomis autumnalis*. Data are presented as means ( $\pm$ standard errors) and bars with different letters in each graph are significantly different by Tukey Test ( $p \leq 0.05$ ).

The content of photosynthetic pigments provides a lot of information on how the biostimulators work under salt stress [71]. Quantitative proportions of chlorophyll *a*, *b* and carotenoids are also important for controlling photosynthesis intensity [72]. Our study confirmed positive effects of bulb coating with the oligo-alginate on leaf content of photosynthetic pigments (Table 2). The plants treated with the oligo-alginate contained significantly more chlorophyll *a* (by 13.8%) and chlorophyll *a* + *b* (by 13.8%) than the non-treated ones. Enhanced content of leaf pigments in oligo-alginate treated plants might improve photosynthetic efficiency, which is usually associated with improved uptake of minerals and finally leads to greater biomass production. Foliar application of oligo-alginate increased the content of photosynthetic pigments in the leaves of other medicinal plants [15,16]. This was probably due to a positive effect of the oligomer on the efficiency of the photosynthetic apparatus. Oligo-alginate has been reported to improve net photosynthetic rate, stomatal conductance, internal CO<sub>2</sub> concentration, as well as the activity of carbonic anhydrase—an enzyme transferring carbon dioxide to RuBisCO during photosynthesis [11–13].

**Table 2.** Effect of oligo-alginate and NaCl on photosynthetic pigment concentrations in leaves of *Eucomis autumnalis*. Means ( $\pm$ standard errors) followed by the same small letter in the same row did not differ by Tukey test ( $p \leq 0.05$ ).

Parameters	Treatment			
	Control	Oligo-Alginate	NaCl	Oligo-Alginate + NaCl
Chlorophyll <i>a</i> (mg kg <sup>-1</sup> FW)	434.2 $\pm$ 6.90 b	497.0 $\pm$ 5.50 a	342.3 $\pm$ 15.41 c	396.0 $\pm$ 4.51 b
Chlorophyll <i>b</i> (mg kg <sup>-1</sup> FW)	166.6 $\pm$ 3.91 a,b	186.8 $\pm$ 0.96 a	146.5 $\pm$ 8.29 b	160.3 $\pm$ 1.72 b
Chlorophyll <i>a/b</i> ratio	2.61 $\pm$ 0.06 a,b	2.66 $\pm$ 0.04 a	2.34 $\pm$ 0.03 c	2.47 $\pm$ 0.02 b,c
Chlorophyll <i>a + b</i> (mg kg <sup>-1</sup> FW)	600.8 $\pm$ 9.24 b	683.8 $\pm$ 5.12 a	488.8 $\pm$ 23.66 c	556.3 $\pm$ 6.02 b
Carotenoid (mg kg <sup>-1</sup> FW)	170.2 $\pm$ 5.68 a,b	187.8 $\pm$ 2.05 a	153.3 $\pm$ 9.22 b	176.1 $\pm$ 2.53 a,b
Chlorophyll/carotenoid ratio	3.54 $\pm$ 0.06 a	3.64 $\pm$ 0.01 a	3.19 $\pm$ 0.06 b	3.16 $\pm$ 0.03 b

Plants exposed in our study to salt stress had 21.1% lower content of chlorophyll *a*, 12.1% chlorophyll *b*, 18.6% chlorophyll *a + b* and 9.9% carotenoids than the control ones. They also had reduced chlorophyll *a* to chlorophyll *b* ratio and chlorophyll *a + b* to carotenoids ratio. Oligo-alginate treatment in salinity-exposed plants significantly limited the loss of chlorophyll *a* and chlorophyll *a + b*, and slightly reduced the decrease in carotenoids and chlorophyll *a* to chlorophyll *b* ratio vs. plants non-treated with the oligomer (Table 2). Excessive amounts of salt accumulated in chloroplasts exert a direct toxic effect on photosynthesis through destabilization of protein complexes and destruction of photosynthetic pigments [52,53]. A drop in the chlorophyll *a + b* to carotenoids ratio may be a sign of oxidative stress and also shows that a plant has activated the mechanisms of antioxidant defense by increasing the content of carotenoids responsible for protecting photosystems against reactive oxygen species [71–73].

Apart from yield increase, the positive effects of oligosaccharide application often include improvements in yield quality, including secondary metabolites [15,17,18]. Therefore, we decided to determine total polyphenol content (TPC) in the leaves. We also assessed content of L-ascorbic acid and plant antioxidant activity (DPPH) to better understand the response of the antioxidant system to oligo-alginate and NaCl treatment (Table 3).

**Table 3.** Effect of oligo-alginate and NaCl on content of total polyphenols, L-ascorbic acid and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical reduction) of *Eucomis autumnalis*. Means ( $\pm$ standard errors) followed by the same small letter in the same row did not differ by Tukey Test ( $p \leq 0.05$ ).

Parameters	Treatment			
	Control	Oligo-Alginate	NaCl	Oligo-Alginate + NaCl
Total polyphenols (mg GAE 100 g <sup>-1</sup> FW)	39.6 $\pm$ 1.56 c	57.6 $\pm$ 1.19 a	46.4 $\pm$ 2.29 b	49.1 $\pm$ 1.75 b
L-ascorbic acid (mg 100 g <sup>-1</sup> FW)	27.4 $\pm$ 1.49 c	42.6 $\pm$ 2.02 a	30.8 $\pm$ 2.27 b	36.0 $\pm$ 2.25 a,b
Antioxidant activity (% DPPH)	1.64 $\pm$ 0.11 d	2.94 $\pm$ 0.25 a	2.18 $\pm$ 0.09 c	2.41 $\pm$ 0.08 b

The highest TPC, L-ascorbic acid and antioxidant activities were observed in the plants grown from the bulbs coated with oligo-alginate. These variables were the lowest in the control plants. Salt stress induced a significant increase in TPC, L-ascorbic acid and a stimulate in antioxidant activity vs. plants non-treated with NaCl. Application of oligo-alginate in NaCl treated plants did not change their total polyphenol content and slightly enhanced content of L-ascorbic acid and antioxidant activity. The positive effect of oligo-alginate on limiting the drop in L-ascorbic acid and antioxidant activity under salt stress indicates protective properties of this agent. Sodium alginate derivatives change plant metabolism and affect biosynthesis of various substances, including active constituents [12,15,18], but the mechanism remains unclear.

The results discussed above demonstrate that sodium alginate derivatives favorably affect morphological features and physiological and biochemical parameters of *Eucomis autumnalis* and improve the medicinal value of the plant. The plants treated with oligo-alginate achieved markedly

higher biomass, pigment content, total polyphenol content and antioxidant activity. Polysaccharides and their derivatives stimulate or inhibit specific enzymes or enzymatic pathways involved in metabolic processes [27,31,74]. They may also act inside plant cells and control their hormonal activity [75]. Sodium alginate oligomers may stimulate plant growth by enhancing expression of selected genes. For example, alginate oligosaccharides induced expression of an auxin-related gene in rice, which changed auxin content in roots and promoted root formation and elongation [76]. Therefore, future studies evaluating the response of *Eucomis autumnalis* to oligo-alginate could include examination of genetic factors affecting plant growth and resistance to various microorganisms.

Our study demonstrated that oligo-alginate alleviates negative effects of salt stress in *Eucomis autumnalis*. We hypothesize that its mechanism of action consisted in limiting the accumulation of harmful  $\text{Cl}^-$  and  $\text{Na}^+$  ions in leaf tissues, which might finally result in reduced biomass loss and smaller decrease in the content of assimilation pigments. Interestingly, salt-exposed plants treated with oligo-alginate had higher leaf levels of L-ascorbic acid and higher antioxidant activity (DPPH), which may suggest activation of stress adaptation mechanisms. Plants experiencing salt stress remove reactive oxygen species (ROS) via non-enzymatic mechanisms, such as scavenging with ascorbic acid, and by increasing production of antioxidant enzymes [52,53]. Oligo-alginate may stimulate ROS detoxifying enzymes and enhance activity of isozymes responsible for stress protection. This hypothesis was confirmed by Zhang et al. [77], who reported that alginoligosaccharides induced the activity of phenylalanine ammonia lyase (PAL), peroxidase (POD) and catalase (CAT) in rice cells and protected plants against pathogens. Changes in the activity of antioxidant enzymes may most probably determine the anti-stress effect of oligo-alginate in plants, but this requires further research.

### 3. Materials and Methods

#### 3.1. Plant Materials and Growth Conditions

The study was conducted in the years 2014–2015 in a plastic tunnel located in the area of West Pomeranian University of Technology in Szczecin (53°25' N, 14°32' E; 25 m above mean sea level). Plant material included the bulbs of *E. autumnalis*, with a perimeter of 14–16 cm, purchased in the Netherlands. The bulbs were planted each year in mid-April into PVC pots (18 cm) filled with peat substrate enriched with 3 g dm<sup>-3</sup> of a multicomponent fertilizer Hydrocomplex (Yara International ASA, Oslo, Norway) containing (in % mass): N-NO<sub>3</sub> (5), N-NH<sub>4</sub> (7), P<sub>2</sub>O<sub>5</sub> (11), K<sub>2</sub>O (18), MgO (2.7), S (8), B (0.015), Fe (0.2), Mn (0.02), and Zn (0.02). The peat substrate of pH 5.7 contained 0.37 g NaCl dm<sup>-3</sup> and also (in mg dm<sup>-3</sup>): N-NO<sub>3</sub> (10), P (37), K (22), Ca (1980), Mg (122), and Cl (16). The plants were cultivated until mid-September under natural photoperiod and watered on average three times a week with tap water of the following composition (in mg dm<sup>-3</sup>): N-NO<sub>3</sub> (1.53), P (1.50), K (6.2), Ca (97.4), Mg (16.6), Na (24.0), Cl (24.0), Cu (0.62), Zn (0.42), Fe (1.3), HCO<sub>3</sub> (194), pH 6.4, and electrolytic conductivity 0.64 mS cm<sup>-1</sup>. Mean air temperature (°C) and relative humidity (%) in the plastic tunnel in 2014 were as follows: April 15.1/71.4, May 18.9/76.9, June 19.7/70.6, July 24.1/75.0, August 19.6/78.5, September 18.2/80.4, and in 2015: April 13.6/73.3, May 15.4/60.8, June 18.1/73.3, July 20.8/68.3, August 24.1/59.5, and September 17.4/70.6.

#### 3.2. Preparation and Determination the Molecular Mass of Oligo-Alginates

Oligo-alginates were prepared by acid hydrolysis. Twenty grams of starting sodium alginate from algae (Sigma-Aldrich, Poznań, Poland) was dissolved in 800 mL of deionized water at 70 °C. Concentrated HCl (Chempur, Piekary Śląskie, Poland) was added to obtain final concentration of 0.2 M. Then the solution was incubated for 4, 8 or 16 h while stirring. Starting sodium 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. Following hydrolysis samples were cooled to room temperature, neutralized with NaOH to obtain pH 7 and partially evaporated using vacuum evaporator (RVO 200A, INGOS, Praha, Czech Republic). High Performance Size Exclusion Chromatography



(HPSEC) was utilized to define molecular masses of starting and depolymerized sodium alginates. High-pressure dosing pump S 1000, refractive index detector S 2300 and a 20  $\mu\text{L}$  sample loop (Knauer, Berlin, Germany). Separation was performed using SUPREMA 10,000  $\text{\AA}$  10  $\mu\text{m}$  column (PSS, Mainz, Germany) eluted with 0.5 M NaCl at a flow rate of 1  $\text{mL min}^{-1}$  in room temperature. Relative molecular mass was determined using pullulans with narrow polydispersity (PSS, Mainz, Germany) as standards for calibration. Peak molecular mass of pullulan standards was as follow: 342; 1080; 6100; 9600; 21,100; 47,100; 107,000; 194,000; 344,000; and 708,000  $\text{g mol}^{-1}$ .

### 3.3. Fourier Transform Infra Red (FTIR) Analysis

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were recorded using Spectrum 100 spectrometer, equipped with a diamond ATR crystal (Perkin Elmer Spectrophotometer, Spectrum 100, Waltham, MA, USA), with a resolution of 4  $\text{cm}^{-1}$  in the 4000–650  $\text{cm}^{-1}$  wavenumber range.

### 3.4. Experiments

#### 3.4.1. Effects of Oligo-Alginates with Different Molecular Mass on Growth of *Eucomis autumnalis*

In the first year of the study *E. autumnalis* bulbs were coated with the oligo-alginates of different molecular weight (32,000; 42,000 and 64,000  $\text{g mol}^{-1}$ ). The controls were non-coated bulbs. The coating method was based on polyelectrolyte complexes [36]. The bulbs were soaked for 30 s in 1% aqueous solutions of oligo-alginates. The 40 bulbs were coated per treatment, ten per each repetition. After drying, the bulbs were planted individually into pots, which were then placed on tables in the plastic tunnel.

#### Determination of Growth Parameters

We determined the number of days from planting the bulbs to the beginning of flowering marked by the appearance of the first flowers. At this phase, 10 plants from each repetition were randomly selected to measure their height and width, the number of leaves and length of the longest leaf, and the number and length of inflorescences. Moreover, leaf greenness index SPAD (Soil and Plant Analysis Development) was measured with Chlorophyll Meter SPAD 502 (Minolta, Osaka, Japan) and leaf stomatal conductance was assessed with SC1 porometer (Dekagon Devices, Pullman, WA, USA). The measurements involved two fully developed leaves of five randomly selected plants from each repetition. They were repeated three times for each leaf and mean value was calculated. When the flowering was over, the number of flowers was assessed based on the number of inflorescence pedicels.

#### 3.4.2. Effect of Oligo-Alginate and Salt Stress on Plant Growth and Physiological Activity

In the second year of the study, the following experimental variants were tested: (I) control, non-treated plants; (II) bulbs coated with oligo-alginate before planting; (III) plants watered with 100 mM NaCl; (IV) bulbs coated with oligo-alginate before planting and plants watered with 100 mM NaCl. The concentration of NaCl solution was determined based on earlier studies that demonstrated considerable biomass decrease in the presence of 100 mM NaCl. Each variant involved 40 bulbs, ten per repetition. Considering the results from the first year, the oligo-alginate of molecular mass 32,000  $\text{g mol}^{-1}$  was selected for further bulb coating studies. The coated bulbs were planted individually into pots. After 30 days from planting, we started to water the plants with 100 mM NaCl (Chempur, Piekary Śląskie, Poland) solution. The plants were watered twice a week, for eight weeks, with 100 mL of the solution per plant per treatment. The plants in the other variants were treated with water. When the salt treatment ended, the plants were removed from the pots and fresh weight of their above-ground parts and bulbs was assessed. Fresh leaves were used for analyses.

### Determination of Photosynthetic Pigments

Leaf pigment content was determined using a spectrophotometer [78]. Fresh plant material was extracted with 80% acetone. Absorption of the extract was measured with spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK) at 441, 646, 652, and 663 nm. The following formulas were used in the calculations:

$$\text{chlorophyll } a \text{ (mg kg}^{-1} \text{ FW)} = (12.21 \times E_{663} - 2.81 \times E_{646}) \times (V/1000 \times m),$$

$$\text{chlorophyll } b \text{ (mg kg}^{-1} \text{ FW)} = (20.13 \times E_{646} - 5.03 \times E_{663}) \times (V / 1000 \times m),$$

$$\begin{aligned} \text{carotenoids (mg kg}^{-1} \text{ FW)} = & [(1000 \times E_{441}) - 3.27 \times (12.21 \times E_{663} - 2.81 \times E_{646}) \\ & - 104 \times (20.13 \times E_{646} - 5.03 \times E_{663})] \times [V/1000 \times (m \times 229)], \end{aligned}$$

where

E—extinction at specific wavelength,

V—volume of a volumetric flask [cm<sup>3</sup>],

m—sample weight in g.

### Determination of L-Ascorbic Acid

The content of vitamin C as L-ascorbic acid was determined by the Tillmans method, based on the color reaction of ascorbic acid with 2,6-dichloroindophenol solution [79].

### Determination of Total Polyphenols

Phenolic compounds were extracted from leaves as described before [80]. Total polyphenol content (TPC) was determined spectrophotometrically with Follin–Ciocalteu reagent and gallic acid as standard [81]. The results were read using spectrophotometer (Helios Gamma, Thermo Spectronic) at 760 nm. TPC was expressed as gallic acid equivalents (GAE) in mg 100 g<sup>-1</sup> FW.

### Determination of Antioxidant Activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Reduction

Leaf antioxidant activity was determined by a reduction of free radicals (DPPH), according to Yen and Chen [82], and DPPH inhibition percentage was calculated according to the formula provided by Rossi et al. [83]. Antioxidant potential of the investigated solutions was expressed by the percentage of DPPH inhibition using the following equation:

$$\% \text{ of DPPH inhibition} = 100 - [(A_t/A_r) \times 100], \quad (1)$$

where

A<sub>t</sub>—absorbance of the test solution

A<sub>r</sub>—absorbance of the reference solution

### Determination of Na and Cl

To determine leaf content of Na and Cl, plant material was dried (80 °C for 48 h), pulverized and wet mineralized with 17 cm<sup>3</sup> of concentrated H<sub>2</sub>SO<sub>4</sub> (d = 1.84) to obtain a 2.0 g air-dried sample. Sodium levels were assessed using a flame photometer AFP-100 (Biotech Engineering Management, Nicosia, Cyprus). Chlorine determination involved quantitative precipitation of chlorides in a neutralized solution by titration with AgNO<sub>3</sub> in the presence of K<sub>2</sub>CrO<sub>4</sub> as an indicator [84].

### Data Analysis

Both experiments were run as univariate ones in a complete randomization arrangement. All determinations were repeated four times. The measurements were statistically verified using

the analysis of variance and Statistica 13 software (Statsoft, Cracov, Poland). Confidence intervals were calculated with Tukey's test ( $p \leq 0.05$ ).

#### 4. Conclusions

Sodium alginate is a compound with versatile applications and is completely safe for the environment. In horticulture and agriculture, it is a popular polymer used for encapsulation of seeds and plant tissues in micropropagation. Sodium alginate derivatives exert a biostimulatory effect on plant growth and development and may cause plant resistance to pathogens and stress. Our study showed that low molecular mass oligo-alginate used for bulb coating may be a potential candidate as a bioregulator in the cultivation of *Eucomis autumnalis*. The biostimulating effect was the most pronounced for the oligo-alginate of molecular mass  $32,000 \text{ g mol}^{-1}$ . Plants treated with this oligomeric fraction were of markedly better quality, their leaves contained more pigments and total polyphenols and they exhibited slightly increased antioxidant activity. Also, our study was the first that showed that oligo-alginate may be used to improve plant tolerance to salt stress. The oligomer limited biomass loss, increased leaf content of photosynthetic pigments and curbed accumulation of sodium and chlorine in NaCl treated plants. These outcomes may help optimize the production technology of *Eucomis autumnalis* and possibly other bulbous plants. However, we need to remember that the effects of biostimulators may be different under controlled vs. field conditions, and the differences are due to the interaction of many factors, such as climatic conditions or soil properties. The mechanism of action of sodium alginate derivatives is still being researched. Further studies shall explore the relationships between the oligomer effectiveness and plant genotype, physical and chemical properties of the compound, its concentration or application method.

**Acknowledgments:** The study was supported by the National Science Centre, Poland (Project No. NN 310777840) and by the Polish Ministry of Science and Higher Education (Project UPB 518-07-014-3176-02/18 ZUT).

**Author Contributions:** Piotr Salachna conceived, designed the experiments, performed the experiments; analyzed the data, and wrote the paper. Monika Grzeszczuk performed the analysis of laboratory and analyzed the data; Edward Meller performed the analysis of laboratory; Marcin Soból performed the experiments and analyzed the data. All authors were responsible for processing information and manuscript writing. All authors read and approved the final manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

1. Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chem. Biol. Technol. Agric.* **2017**, *4*, 5. [[CrossRef](#)]
2. Yakhin, O.I.; Lubyantsev, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in plant science: A global perspective. *Front. Plant Sci.* **2017**, *7*, 2049. [[CrossRef](#)] [[PubMed](#)]
3. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [[CrossRef](#)]
4. Battacharyya, D.; Babgohari, M.Z.; Rathor, P.; Prithiviraj, B. Seaweed extracts as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 39–48. [[CrossRef](#)]
5. Khan, W.; Rayirath, U.P.; Subramanian, S.; Jithesh, M.N.; Rayorath, P.; Hodges, D.M.; Critchley, A.T.; Craigie, J.S.; Norrie, J.; Prithiviraj, B. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* **2009**, *28*, 386–399. [[CrossRef](#)]
6. Vera, J.; Castro, J.; González, A.; Moenne, A. Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. *Mar. Drugs* **2011**, *9*, 2514–2525. [[CrossRef](#)] [[PubMed](#)]
7. Hay, I.D.; Rehman, Z.U.; Moradali, M.F.; Wang, Y.; Rehm, B.H.A. Microbial alginate production, modification and its applications. *Microb. Biotechnol.* **2013**, *6*, 637–650. [[CrossRef](#)] [[PubMed](#)]
8. Hu, X.; Jiang, X.; Hwang, H.; Liu, S.; Guan, H. Promotive effects of alginate-derived oligosaccharide on maize seed germination. *J. Appl. Phycol.* **2004**, *16*, 73–76. [[CrossRef](#)]

9. Hien, N.Q.; Nagasawa, N.; Tham, L.X.; Yoshii, F.; Dang, V.H.; Mitomo, H.; Makuuchi, K.; Kume, T. Growth promotion of plants with depolymerized alginates by irradiation. *Radiat. Phys. Chem.* **2000**, *59*, 97–101. [[CrossRef](#)]
10. Luan, L.Q.; Hien, N.Q.; Nagasawa, N.; Kume, T.; Yoshii, F.; Nakanishi, T.M. Biological effect of radiation-degraded alginate on flower plants in tissue culture. *Biotechnol. Appl. Biochem.* **2003**, *38*, 283–288. [[CrossRef](#)] [[PubMed](#)]
11. Luan, L.Q.; Nagasawa, N.; Ha, V.T.T.; Hien, N.Q.; Nakanishi, T.M. Enhancement of plant growth stimulation activity of irradiated alginate by fractionation. *Radiat. Phys. Chem.* **2009**, *78*, 796–799. [[CrossRef](#)]
12. Aftab, T.; Khan, M.M.A.; Idrees, M.; Naeem, M.; Hashmi, N.; Varshney, L. Enhancing the growth, photosynthetic capacity and artemisinin content in *Artemisia annua* L. by irradiated sodium alginate. *Radiat. Phys. Chem.* **2011**, *80*, 833–836. [[CrossRef](#)]
13. Ali, A.; Khan, M.M.A.; Uddin, M.; Naeem, M.; Idrees, M.; Hashmi, N.; Dar, T.A.; Varshney, L. Radiolytically depolymerized sodium alginate improves physiological activities, yield attributes and composition of essential oil of *Eucalyptus citriodora* Hook. *Carbohydr. Polym.* **2014**, *112*, 134–144. [[CrossRef](#)] [[PubMed](#)]
14. Aftab, T.; Khan, M.M.A.; Naeem, M.; Idrees, M.; Siddiqi, T.O.; Varshney, L. Effect of irradiated sodium alginate and phosphorus on biomass and artemisinin production in *Artemisia annua*. *Carbohydr. Polym.* **2014**, *110*, 396–404. [[CrossRef](#)] [[PubMed](#)]
15. Idrees, M.; Naeem, M.; Alam, M.; Aftab, T.; Hashmi, N.; Khan, M.M.A.; Varshney, L. Utilizing the  $\gamma$ -irradiated sodium alginate as a plant growth promoter for enhancing the growth, physiological activities, and alkaloids production in *Catharanthus roseus* L. *Agric. Sci. China* **2011**, *10*, 1213–1221. [[CrossRef](#)]
16. Idrees, M.; Dar, T.A.; Naeem, M.; Aftab, T.; Khan, M.M.A.; Ali, A.; Uddin, M.; Varshney, L. Effects of gamma-irradiated sodium alginate on lemongrass: Field trials monitoring production of essential oil. *Ind. Crops Prod.* **2015**, *63*, 269–275. [[CrossRef](#)]
17. Khan, Z.H.; Khan, M.M.A.; Aftab, T.; Idrees, M.; Naeem, M. Influence of alginate oligosaccharides on growth, yield and alkaloid production of opium poppy (*Papaver somniferum* L.). *Front. Agric. China* **2011**, *5*, 122–127. [[CrossRef](#)]
18. Naeem, M.; Idrees, M.; Aftab, T.; Khan, M.M.A.; Varshney, L. Depolymerised carrageenan enhances physiological activities and menthol production in *Mentha arvensis* L. *Carbohydr. Polym.* **2012**, *87*, 1211–1218. [[CrossRef](#)]
19. Sarfaraz, A.; Naeem, M.; Nasir, S.; Idrees, M.; Aftab, T.; Hashmi, N.; Khan, M.A.A.; Varshney, M.; Varshney, L. An evaluation of the effects of irradiated sodium alginate on the growth, physiological activities and essential oil production of fennel (*Foeniculum vulgare* Mill.). *J. Med. Plants Res.* **2011**, *5*, 15–21.
20. Sano, Y. Antiviral activity of alginate against infection by tobacco mosaic virus. *Carbohydr. Polym.* **1999**, *38*, 183–186. [[CrossRef](#)]
21. Khan, N.; Mishra, A.; Chauhan, P.S.; Nautiyal, C.S. Induction of *Paenibacillus lentimorbus* biofilm by sodium alginate and CaCl<sub>2</sub> alleviates drought stress in chickpea. *Ann. Appl. Biol.* **2011**, *159*, 372–386. [[CrossRef](#)]
22. Ma, L.J.; Zhang, Y.; Bu, N.; Wang, S.H. Alleviation effect of alginate-derived oligosaccharides on *Vicia faba* root tip cells damaged by cadmium. *Bull. Environ. Contam. Toxicol.* **2010**, *84*, 161–164. [[CrossRef](#)] [[PubMed](#)]
23. Sarrocco, S.; Raeta, R.; Vannacci, G. Seeds encapsulation in calcium alginate pellets. *Seed Sci. Technol.* **2004**, *32*, 649–661. [[CrossRef](#)]
24. Espino-Díaz, M.; Molina-Corral, F.; Sepulveda, D.; González-Aguilar, G.; Olivas, G. Alginate coatings containing high levels of isoleucine improve aromatic and standard quality in fresh-cut apple. *Eur. J. Hortic. Sci.* **2016**, *81*, 175–184. [[CrossRef](#)]
25. Bach, A.; Pawłowska, B.; Malik, M. Plantlets from encapsulated meristems of *Gentiana pneumonanthe* L. *Acta Physiol. Plant.* **2004**, *26*, 53–57. [[CrossRef](#)]
26. Sakhanokho, H.F.; Pounders, C.T.; Blythe, E.K. Alginate encapsulation of *Begonia* microshoots for short-term storage and distribution. *Sci. World J.* **2013**. [[CrossRef](#)] [[PubMed](#)]
27. Darvill, A.; Augur, C.; Bergmann, C.; Carlson, R.W.; Cheong, J.J.; Eberhard, S.; Hahn, M.G.; Lo, V.M.; Marfa, V.; Meyer, B.; et al. Oligosaccharins—Oligosaccharides that regulate growth, development and defence responses in plants. *Glycobiology* **1992**, *2*, 181–198. [[CrossRef](#)] [[PubMed](#)]
28. Winkler, A.J.; Dominguez-Nuñez, J.A.; Aranaz, I.; Poza-Carrión, C.; Ramonell, K.; Somerville, S.; Berrocal-Lobo, M. Short-chain chitin oligomers: Promoters of plant growth. *Mar. Drugs* **2017**, *15*, 40. [[CrossRef](#)] [[PubMed](#)]

29. González, A.; Moenne, F.; Gómez, M.; Sáez, C.A.; Contreras, R.A.; Moenne, A. Oligo-carrageenan kappa increases NADPH, ascorbate and glutathione syntheses and TRR/TRX activities enhancing photosynthesis, basal metabolism, and growth in *Eucalyptus* trees. *Front. Plant Sci.* **2014**, *5*, 512. [[CrossRef](#)] [[PubMed](#)]
30. El-Mohdy, H.A. Radiation-induced degradation of sodium alginate and its plant growth promotion effect. *Arab. J. Chem.* **2017**, *10*, 431–438. [[CrossRef](#)]
31. Farmer, E.E.; Thomas, D.M.; Michael, J.S.; Clarence, A.R. Oligosaccharide signaling in plants. *J. Biol. Chem.* **1991**, *266*, 3140–3145. [[PubMed](#)]
32. Shabbir, A.; Khan, M.M.A.; Sadiq, Y.; Jaleel, H.; Ahmad, B.; Uddin, M. Regulation of functional activities and essential oil production in *Vetiveria zizanioides* L. Nash after  $\gamma$ -irradiated sodium alginate elicitation. *Turk. J. Biol.* **2017**, *41*, 661–672. [[CrossRef](#)]
33. Salachna, P.; Wilas, J.; Zawadzińska, A. The effect of chitosan coating of bulbs on the growth and flowering of *Ornithogalum saundersiae*. *Acta Hort.* **2015**, *1104*, 115–118. [[CrossRef](#)]
34. Zou, P.; Tian, X.; Dong, B.; Zhang, C. Size effects of chitoooligomers with certain degrees of polymerization on the chilling tolerance of wheat seedlings. *Carbohydr. Polym.* **2017**, *160*, 194–202. [[CrossRef](#)] [[PubMed](#)]
35. Salachna, P.; Mizielińska, M.; Soból, M. Exopolysaccharide gellan gum and derived oligo-gellan enhance growth and antimicrobial activity in *Eucomis* plants. *Polymers* **2018**, *10*, 242. [[CrossRef](#)]
36. Bartkowiak, A.; Startek, L.; Zurawik, P.; Salachna, P. Method of Hydrogel Coating Formation on the Surface of Plant Organs. Patent PL 197101, 29 February 2008.
37. Bartkowiak, A.; Hunkeler, D. New microcapsules based on oligoelectrolyte complexation. *Ann. N. Y. Acad. Sci.* **1999**, *875*, 36–45. [[CrossRef](#)] [[PubMed](#)]
38. Startek, L.; Bartkowiak, A.; Salachna, P.; Kaminska, M.; Mazurkiewicz-Zapalowicz, K. The influence of new methods of corm coating on freesia growth, development and health. *Acta Hort.* **2005**, *673*, 611–616. [[CrossRef](#)]
39. Salachna, P.; Zawadzińska, A.; Wilas, J. The use of natural polysaccharides in *Eucomis autumnalis* (Mill.) Chitt. propagation by twin-scale cuttings. *Acta Hort.* **2015**, *1104*, 225–227. [[CrossRef](#)]
40. Salachna, P.; Grzeszczuk, M.; Soból, M. Effects of chitoooligosaccharide coating combined with selected ionic polymers on the stimulation of *Ornithogalum saundersiae* growth. *Molecules* **2017**, *22*, 1903. [[CrossRef](#)] [[PubMed](#)]
41. Salachna, P.; Zawadzińska, A. Effect of daminozide and flurprimidol on growth, flowering and bulb yield of *Eucomis autumnalis* (Mill.) Chitt. *Folia Hort.* **2017**, *29*, 33–38. [[CrossRef](#)]
42. Masondo, N.A.; Finnie, J.F.; Van Staden, J. Pharmacological potential and conservation prospect of the genus *Eucomis* (Hyacinthaceae) endemic to southern Africa. *J. Ethnopharmacol.* **2014**, *151*, 44–53. [[CrossRef](#)] [[PubMed](#)]
43. Carlson, A.S.; Dole, J.M. *Eucomis* for specialty cut flower production. *Greenh. Prod. News* **2011**, *5*, 18–22.
44. Bisi-Johnson, M.A.; Obi, C.L.; Hattori, T.; Oshima, Y.; Li, S.; Kambizi, L.; Vasaikar, S.D. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. *BMC Complement. Altern. Med.* **2011**, *11*, 14. [[CrossRef](#)] [[PubMed](#)]
45. Koorbanally, C.; Crouch, N.R.; Mulholland, D.A. The phytochemistry and ethnobotany of the southern African genus *Eucomis* (Hyacinthaceae: Hyacinthoideae). In *Phytochemistry: Advances in Research*; Imperato, F., Ed.; Research Signpost: Trivandrum, India, 2006; pp. 69–85. ISBN 81-308-0034-9.
46. Mizielińska, M.; Salachna, P.; Ordon, M.; Łopusiewicz, Ł. Antimicrobial activity of water and acetone extracts of some *Eucomis* taxa. *Asian Pac. J. Trop. Med.* **2017**, *10*, 892–895. [[CrossRef](#)] [[PubMed](#)]
47. Taylor, J.L.S.; Rabe, T.; McGaw, L.J.; Jäger, A.K.; Van Staden, J. Towards the scientific validation of traditional medicinal plants. *Plant Growth Regul.* **2001**, *34*, 23–37. [[CrossRef](#)]
48. Masondo, N.A.; Aremu, A.O.; Rengasamy, K.R.R.; Amoo, S.O.; Gruz, J.; Šubrtová, M.; Doležal, K.; Van Staden, J. Growth and phytochemical response in *Eucomis autumnalis* (Mill.) Chitt. treated with phenolic biostimulants from brown alga, *Ecklonia maxima*. *S. Afr. J. Bot.* **2015**, *98*, 211. [[CrossRef](#)]
49. Salachna, P.; Zawadzińska, A.; Wierzbiński, Ł.; Senderek, W. Enhancing growth in *Eucomis autumnalis* (Mill.) Chitt. seedlings with exogenous application of nitric oxide. *J. Hort. Res.* **2016**, *24*, 13–17. [[CrossRef](#)]
50. Hura, T.; Szewczyk-Taranek, B.; Hura, K.; Nowak, K.; Pawłowska, B. Physiological responses of *Rosa rubiginosa* to saline environment. *Water Air Soil Pollut.* **2017**, *228*, 81. [[CrossRef](#)] [[PubMed](#)]
51. Munns, R.; Termaat, A. Whole-plant responses to salinity. *Funct. Plant Biol.* **1986**, *13*, 143–160. [[CrossRef](#)]

52. Negrão, S.; Schmöckel, S.M.; Tester, M. Evaluating physiological responses of plants to salinity stress. *Ann. Bot.* **2017**, *119*, 1–11. [[CrossRef](#)] [[PubMed](#)]
53. Parihar, P.; Singh, S.; Singh, R.; Singh, V.P.; Prasad, S.M. Effect of salinity stress on plants and its tolerance strategies: A review. *Environ. Sci. Pollut. R.* **2015**, *22*, 4056–4075. [[CrossRef](#)] [[PubMed](#)]
54. Sharma, H.S.; Fleming, C.; Selby, C.; Rao, J.R.; Martin, T. Plant biostimulants: A review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *J. Appl. Phycol.* **2014**, *26*, 465–490. [[CrossRef](#)]
55. Hernandez-Hernandez, H.; Gonzalez-Morales, S.; Benavides-Mendoza, A.; Ortega-Ortiz, H.; Cadenas-Pliego, G.; Juarez-Maldonado, A. Effects of chitosan–PVA and Cu nanoparticles on the growth and antioxidant capacity of tomato under saline stress. *Molecules* **2018**, *23*, 178. [[CrossRef](#)] [[PubMed](#)]
56. Zou, P.; Li, K.; Liu, S.; He, X.; Zhang, X.; Xing, R.; Li, P. Effect of sulfated chitoooligosaccharides on wheat seedlings (*Triticum aestivum* L.) under salt stress. *J. Agric. Food Chem.* **2016**, *64*, 2815–2821. [[CrossRef](#)] [[PubMed](#)]
57. Singh, M.; Khan, M.M.A.; Uddin, M.; Naeem, M.; Qureshi, M.I. Proliferating effect of radiolytically depolymerized carrageenan on physiological attributes, plant water relation parameters, essential oil production and active constituents of *Cymbopogon flexuosus* Steud. under drought stress. *PLoS ONE* **2017**, *12*, e0180129. [[CrossRef](#)] [[PubMed](#)]
58. Lawrie, G.; Keen, I.; Drew, B.; Chandler-Temple, A.; Rintoul, L.; Fredericks, P.; Grøndahl, L. Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS. *Biomacromolecules* **2007**, *8*, 2533–2541. [[CrossRef](#)] [[PubMed](#)]
59. Sakugawa, K.; Ikeda, A.; Takemura, A.; Ono, H. Simplified method for estimation of composition of alginates by FTIR. *J. Appl. Polym. Sci.* **2004**, *93*, 1372–1377. [[CrossRef](#)]
60. González-Pérez, L.; Vázquez-Glaría, A.; Perrotta, L.; Acosta, A.; Scriven, S.A.; Herbert, R.; Cabrera, J.C.; Francis, D.; Rogers, H.J. Oligosaccharins and Pectimor<sup>®</sup> stimulate root elongation and shorten the cell cycle in higher plants. *Plant Growth Regul.* **2012**, *68*, 211–221. [[CrossRef](#)]
61. Dzung, P.D.; Phu, D.V.; Du, B.D.; Ngoc, L.S.; Duy, N.N.; Hiet, H.D.; Hien, N.Q. Effect of foliar application of oligochitosan with different molecular weight on growth promotion and fruit yield enhancement of chili plant. *Plant Prod. Sci.* **2017**, *20*, 389–395. [[CrossRef](#)]
62. Shillo, R.; Ding, M.; Pasternak, D.; Zaccari, M. Cultivation of cut flower and bulb species with saline water. *Sci. Hort.* **2002**, *92*, 41–54. [[CrossRef](#)]
63. Salachna, P.; Zawadzińska, A.; Podsiadło, C. Response of *Ornithogalum saundersiae* Bak. to salinity stress. *Acta Sci. Pol.-Hortorum Cultus* **2016**, *15*, 123–134.
64. Cassaniti, C.; Romano, D.; Flowers, T.J. The response of ornamental plants to saline irrigation water. In *Irrigation-Water Management, Pollution and Alternative Strategies*; Garcia-Garizabal, I., Ed.; InTech Europe: Rijeka, Croatia, 2012; pp. 131–158.
65. Akimoto, C.; Aoyagi, H.; Tanaka, H. Endogenous elicitor-like effects of alginate on physiological activities of plant cells. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 429–436. [[CrossRef](#)]
66. Ma, L.J.; Li, X.M.; Bu, N.; Li, N. An alginate-derived oligosaccharide enhanced wheat tolerance to cadmium stress. *Plant Growth Regul.* **2010**, *62*, 71–76. [[CrossRef](#)]
67. Liu, H.; Zhang, Y.H.; Yin, H.; Wang, W.X.; Zhao, X.M.; Du, Y.G. Alginate oligosaccharides enhanced *Triticum aestivum* L. tolerance to drought stress. *Plant Physiol. Biochem.* **2013**, *62*, 33–40. [[CrossRef](#)] [[PubMed](#)]
68. Zou, P.; Li, K.; Liu, S.; Xing, R.; Qin, Y.; Yu, H.; Zhou, M.; Li, P. Effect of chitoooligosaccharides with different degrees of acetylation on wheat seedlings under salt stress. *Carbohydr. Polym.* **2015**, *126*, 62–69. [[CrossRef](#)] [[PubMed](#)]
69. Veatch-Blohm, M.E.; Sawch, D.; Elia, N.; Pinciotti, D. Salinity tolerance of three commonly planted *Narcissus* cultivars. *HortScience* **2014**, *49*, 1158–1164.
70. Carter, C.T.; Grieve, C.M. Salt tolerance of floriculture crops. In *Ecophysiology of High Salinity Tolerant Plants*; Khan, M.A., Weber, D.J., Eds.; Springer: Amsterdam, The Netherlands, 2006; pp. 279–287.
71. Chaves, M.M.; Flexas, J.; Pinheiro, C. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Ann. Bot.* **2009**, *103*, 551–556. [[CrossRef](#)] [[PubMed](#)]
72. Chen, Y.; Lin, F.; Yang, H.; Yue, L.; Hu, F.; Wang, J.; Luo, Y.; Cao, F. Effect of varying NaCl doses on flavonoid production in suspension cells of *Ginkgo biloba*: Relationship to chlorophyll fluorescence, ion homeostasis, antioxidant system and ultrastructure. *Acta Physiol. Plant.* **2014**, *36*, 3173–3187. [[CrossRef](#)]

73. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J.I. Plant salt-tolerance mechanisms. *Trends Plant Sci.* **2014**, *19*, 371–379. [[CrossRef](#)] [[PubMed](#)]
74. Karaki, N.; Aljawish, A.; Humeau, C.; Muniglia, L.; Jasniewski, J. Enzymatic modification of polysaccharides: Mechanisms, properties, and potential applications: A review. *Enzyme Microb. Technol.* **2016**, *90*, 1–18. [[CrossRef](#)] [[PubMed](#)]
75. Albersheim, P.; Darvill, A.G. Oligosaccharins. *Sci. Am.* **1985**, *253*, 58–65. [[CrossRef](#)]
76. Zhang, Y.; Yin, H.; Zhao, X.; Wang, W.; Du, Y.; He, A.; Sun, K. The promoting effects of alginate oligosaccharides on root development in *Oryza sativa* L. mediated by auxin signaling. *Carbohydr. Polym.* **2014**, *113*, 446–454. [[CrossRef](#)] [[PubMed](#)]
77. Zhang, S.; Tang, W.; Jiang, L.; Hou, Y.; Yang, F.; Chen, W.; Li, X. Elicitor activity of alginoligosaccharide and its potential application in protection of rice plant (*Oryza sativa* L.) against *Magnaporthe grisea*. *Biotechnol. Biotechnol. Equip.* **2015**, *29*, 646–652. [[CrossRef](#)]
78. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [[CrossRef](#)]
79. Association of Official Analytical Chemists. *AOAC Official Methods of Analysis of the Association of Official Analytical Chemists*; Association of Official Analytical Chemists: Washington, DC, USA, 1990.
80. Salachna, P.; Grzeszczuk, M.; Wilas, J. Total phenolic content, photosynthetic pigment concentration and antioxidant activity of leaves and bulbs of selected *Eucomis* L'Hér. taxa. *Fresenius Environ. Bull.* **2015**, *24*, 4220–4225.
81. Singleton, V.L.; Rossi, J.A., Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
82. Yen, G.C.; Chen, H.Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27–32. [[CrossRef](#)]
83. Rossi, M.; Giussani, E.; Morelli, R.; Scalzo, R.; Nani, R.C.; Torreggiani, D. Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Res. Int.* **2003**, *36*, 999–1005. [[CrossRef](#)]
84. Ostrowska, A.; Gawliński, S.; Szczubiałka, Z. *Methods for Analyzing and Assessing the Properties of Soil and Plants*; Instytut Ochrony Środowiska: Warsaw, Poland, 1991; pp. 1–333. (In Polish)

**Sample Availability:** Samples of the compounds are not available from the authors.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).