# FOLIAR APPLICATION OF DEPOLYMERISED CHITOSAN ENHANCES THE GROWTH AND CONTENT OF POLYPHENOLS AND L-ASCORBIC ACID IN *Eucomis autumnalis*, AN ORNAMENTAL AND MEDICINAL PLANT

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## Abstract

*Chitosan and its derivatives are promising plant biostimulants with potentially broad applications in agriculture and horticulture. In this study, we determined the effect of the application method and dose of depolymerised chitosan on morphological characteristics and metabolite levels in Eucomis autumnalis, a medicinal and ornamental plant. Depolymerised chitosan (molecular weight ~154,500 g/mol, number average molecular weight ~22,800 g/mol and degree of deacetylation ~85%) was used at 50 and 100 mg/l by drenching plants or spraying. In general, depolymerised chitosan increased the length of plant leaves and roots, especially at the dose of 100 mg/l, and also increased the fresh weight of leaves, bulbs and roots, with a more potent effect found when depolymerised chitosan was applied as a foliar spray. In addition, spraying the plants with chitosan solution at 50 and 100 mg/l caused a significant increase in the total chlorophyll content, by 55.4% and 42.0%, respectively, and the total polyphenol content, by 77.7% and 59.0%, respectively, compared with the control. On the other hand, the biosynthesis of carotenoids was most favourably affected by the application of 100 mg/l depolymerised chitosan by drenching and 50 mg/l depolymerised chitosan by spraying. Foliar application of depolymerised chitosan led to an increase in leaf L-ascorbic acid content by an average of 36.4%, regardless of the dose. The results suggest that depolymerised chitosan applied as a foliar spray promoted the growth of E. autumnalis and contributed to increased biosynthesis of polyphenols, which may be a future means of obtaining metabolites in this species.*

**Keywords:** *polysaccharides, chitosan oligomers, elicitors, phenolic, pineapple lily*

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# 1. Introduction

Environmentally friendly biostimulants are increasingly being introduced into conventional crop production following the principles of sustainable development [1]. Chitosan is one of the better-known biostimulants in agriculture and horticulture, and its effects have been widely documented [2]. Chitosan can improve plant growth and yield, modify physiological and metabolic processes, and promote nutrient uptake [3, 4]. Chitosan-based biostimulants have limitations in application, as native high-molecular-weight chitosan does not dissolve in water at pH 7 and has a high viscosity [5]. Due to protonated amino groups, chitosan is susceptible to modification. The degradation of chitosan can produce lower-molecular-weight oligosaccharide derivatives that are soluble in water and often have high biological activity [6, 7]. Depolymerised chitosan can modify plant growth and induce resistance to adverse environmental conditions by enhancing primary and secondary metabolism [8, 9]. The effectiveness of chitosan and its derivatives as a plant biostimulant may depend on the genotype, the physical and chemical properties of the polysaccharide, the solution concentration or the application form [10–12]. Unfortunately, many studies lack basic information about the physicochemical properties of the applied chitosan; hence, it is often difficult to compare the results and conclusions regarding the effect of the biopolymer on plants.

The *Eucomis* genus includes more than a dozen bulbous plant species in South Africa. For a long time, the species *Eucomis autumnalis* has been among the most important medicinal plants in South Africa [13]. Extracts from *E. autumnalis* are used in natural medicine to treat various diseases. The compounds of *E. autumnalis* exhibit antioxidant, anticancer, anti-inflammatory, antibacterial and antifungal properties [14, 15]. In addition to its medicinal properties, *Eucomis* species and varieties are cultivated worldwide as desirable ornamental plants. Due to the excessive harvesting of bulbs from natural sites by local people, endemic *Eucomis* species are threatened with extinction [13]. Therefore, strategies are being sought to propagate and produce *Eucomis* plants easily by using environmentally friendly biostimulants [16]. To date, the few studies using chitosan for *Eucomis* cultivation have focused on using this biostimulant for bulb dressing before planting and propagation by leaf cuttings [17, 18]. So far, the response of *Eucomis* to chitosan treatment during plant growth is unknown.

This study aimed to determine the effect of the dose and method of application of depolymerised chitosan on the morphological parameters, biomass accumulation, pigment content and metabolites of *E. autumnalis* plants obtained from seeds. This study tested the hypothesis that depolymerised chitosan stimulates the growth of *E. autumnalis* and modifies the phytochemical composition of plants.

# 2. Materials and Methods

## 2.1. Preparation of Depolymerised Chitosan

Depolymerised chitosan obtained at the Center for Bioimmobilization and Innovative Packaging Materials at West Pomeranian University of Technology in Szczecin was subjected to controlled radical degradation [19] with the continuous addition of hydrogen peroxide at a final concentration of 6.2 mmol to a 2.5% chitosan solution. Depolymerised chitosan was characterised by a molecular weight of ~154,500 g/mol, an number average molecular weight of ~22,800 g/mol and a degree of deacetylation of ~85%.

## 2.2. Plant Material and Experiment Design

Ten-week-old *E. autumnalis* seedlings obtained from seeds sown in spring under controlled conditions (greenhouse) were repotted one at a time into pots (diameter = 8 cm) filled with a commercial peat substrate (pH 5.5). Seven days after repotting, the plants were treated with depolymerised chitosan solutions (50 or 100 mg/l) every 7 days for 8 weeks. The solutions were applied by drenching the plants (20 ml of solution per pot) or spraying (5 ml of solution per pot). Control plants were not treated with the biostimulant. The experiment was set up in a randomised block design in four replicates, with five plants per replicate. After 10 weeks of cultivation in the greenhouse (opening the vents at 22°C during the day and 18°C at night), the plants were removed from their pots, washed in water and, after drying, the length and width of the longest leaf and the length of the longest roots were measured, and the fresh weight of all the leaves, the bulb and roots was determined using a PS 200/2000/C/2 balance (RADWAG, Poland) with a reading accuracy of 0.001 g.

### 2.3. Determination of the Photosynthetic Pigment, Total Polyphenol and L-Ascorbic Acid Contents

Samples (2 g) were taken from fresh leaves for biochemical analyses. The content of assimilation pigments was determined based on absorbance at 441, 646, 652 and 663 nm using a Helios Gamma Spectrophotometer (Thermo Spectronic, UK). The content of each pigment was calculated based on formulas proposed by Lichtenthaler and Buschmann [20] and expressed as mg/g fresh weight. The total polyphenol content in the extracts was determined using the Folin–Ciocalteu reagent by measuring absorbance at 760 nm, and the result is presented in terms of gallic acid equivalents [11]. The content of vitamin C as L-ascorbic acid was determined using the Tillman method based on the reduction of 2,6-dichlorophenolindophenol by L-ascorbic acid [18]. All analyses of plant material were performed triplicate.

### 2.4. Statistical Analysis

The results were submitted to one-way analysis of variance followed by Tukey's honestly significant difference post hoc using the STATISTICA statistical software (StatSoft, Poland). A $p$ value $\leq 0.05$ indicated a statistically significant difference.

## 3. Results and Discussion

As a result of the application of chitosan and its breakdown products, changes can occur at the morphological and metabolic levels in plants, resulting in increased growth and development [21, 22]. In this study, the dose and the method of application of depolymerised chitosan significantly affected the length and width of leaves and the root length of *E. autumnalis* (Table 1, Figure 1). Both drenching and spraying increased the length and width of the leaves, with spraying showing greater efficacy. Plants sprayed with 100 mg/l depolymerised chitosan had the most extended and widest leaves. The control plants that were not treated with chitosan had the shortest leaves. Treatment with depolymerised chitosan stimulated development of the root system, with the most pronounced effect from spraying with 100 mg/l depolymerised chitosan on the root length. The results confirm the positive effect of foliar treatment of plants with chitosan on morphological traits, consistent with previous studies. Chen et al. [23] showed that spraying *Pinellia ternata* plants with a water-soluble chitosan solution (degree of deacetylation ≥ 90% significantly increased the leaf area and plant height. El-Serafy [24] demonstrated that chitosan oligosaccharides applied as a foliar spray at a dose of 50 mg/l effectively increased the root growth rate of potted *Cordyline terminalis* plants. The beneficial effect of foliar-applied chitosan on plant growth is most likely due to its easy absorption by the leaves of water-soluble chitosan [3, 4]. Due to the presence of amino groups, chitosan can penetrate the vascular system of plants and activate a number of metabolic and physiological pathways, causing strong changes in their growth and development [25].

**Table 1.** Influence of the depolymerised chitosan concentration and application method on the morphological characteristics of *Eucomis autumnalis*.

| Treatment | Application method | Leaf length [cm] | Leaf width [cm] | Root length [cm] |
|---|---|---|---|---|
| **Control** | - | $5.00 \pm 0.20^{c}$ | $1.80 \pm 0.07^{b}$ | $4.63 \pm 0.29^{c}$ |
| **Chitosan 50 mg/l** | Soil drench | $5.82 \pm 0.41^{b}$ | $2.17 \pm 0.29^{ab}$ | $7.87 \pm 0.29^{b}$ |
| **Chitosan 100 mg/l** | Soil drench | $5.93 \pm 0.51^{b}$ | $2.17 \pm 0.29^{ab}$ | $7.5 \pm 1.04^{b}$ |
| **Chitosan 50 mg/l** | Foliar spray | $9.17 \pm 0.67^{a}$ | $2.80 \pm 0.09^{a}$ | $7.90 \pm 0.46^{b}$ |
| **Chitosan 100 mg/l** | Foliar spray | $9.47 \pm 0.20^{a}$ | $2.77 \pm 0.22^{a}$ | $9.97 \pm 0.80^{a}$ |

*Note.* The data are presented as the mean ± standard error of the mean. In each column, means with the same superscript letter are not significantly different based on Tukey's test (α = 0.05).

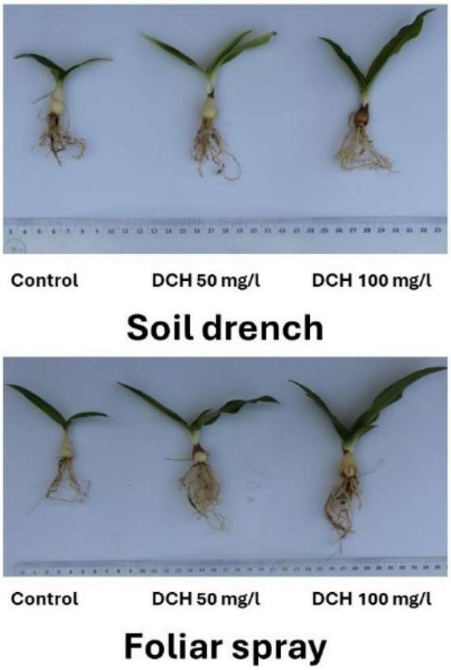


**Figure 1.** The effects of the depolymerised chitosan (DCH) concentration and application method on *Eucomis autumnalis* growth.

**Table 2.** The influence of the depolymerised chitosan concentration and application method on the fresh weight of *Eucomis autumnalis*.

| Treatment | Application method | Leaf fresh weight [g] | Bulb fresh weight [g] | Root fresh weight [g] |
|---|---|---|---|---|
| **Control** | - | $0.77 \pm 0.13^{c}$ | $0.91 \pm 0.01^{b}$ | $0.55 \pm 0.05^{c}$ |
| **Chitosan 50 mg/l** | Soil drench | $1.08 \pm 0.09^{b}$ | $0.96 \pm 0.05^{b}$ | $0.70 \pm 0.08^{b}$ |
| **Chitosan 100 mg/l** | Soil drench | $1.26 \pm 0.04^{b}$ | $1.09 \pm 0.03^{b}$ | $0.77 \pm 0.14^{b}$ |
| **Chitosan 50 mg/l** | Foliar spray | $3.18 \pm 0.33^{a}$ | $2.70 \pm 0.09^{a}$ | $1.47 \pm 0.08^{a}$ |
| **Chitosan 100 mg/l** | Foliar spray | $3.24 \pm 0.24^{a}$ | $2.87 \pm 0.14^{a}$ | $1.65 \pm 0.08^{a}$ |

*Note.* The data are presented as the mean ± standard error of the mean. In each column, means with the same superscript letter are not significantly different based on Tukey's test ($\alpha = 0.05$).

Table 2 shows the effect of depolymerised chitosan on plant biomass. The application of depolymerised chitosan significantly increased the fresh weight of leaves, bulbs and roots, with foliar spraying stimulating biomass growth more strongly than application by drenching. For plants treated with depolymerised chitosan at 50 or 100 mg/l via foliar spraying, the average increase in the fresh weight of leaves, bulbs, and roots was 3.2, 2.1 and 1.8 times higher, respectively, compared with the control. There were no significant differences in the leaf, bulb and root biomass depending on the biostimulant dose. In a previous study, researchers reported similar stimulating effects of 50 and 100 mg/l low-molecular-weight chitosan (~5,000 g/mol) applied via drenching on the fresh weight of leaves and roots of *Perilla frutescens* plants [11]. On the other hand, basil (*Ocimum basilicum*) showed an increase in fresh shoot weight when plants were sprayed with a 500 mg/l chitosan lactate solution [26]. The positive effect of chitosan and its derivatives on biomass growth may be related to more efficient uptake and utilisation by plants of macro- and micronutrients, mainly nitrogen, more intense fluorescence of photosystem II in the thylakoid membrane, and an increased rate of plant photosynthesis, which together ultimately lead to increased biomass production [25]. It is also worth noting that low-molecular-weight chitosan contains more nitrogen than non-degraded chitosan and can be a source of this yield-forming macronutrient for plants [3, 27].

The assimilation pigment contents in leaves are important biomarkers that determine the physiological status of plants. There were significant differences in the photosynthetic pigment contents depending on the application method and dose of depolymerised chitosan (Table 3). Both drenching and spraying leaves with the depolymerised chitosan solution stimulated total chlorophyll accumulation in *E. autumnalis*, with spraying exerted a more substantial effect. Relative to the control, foliar application of depolymerised chitosan at 50 and 100 mg/l increased the total chlorophyll content by 55.4% and 42.0%, respectively. Applying 100 mg/l chitosan via drenching and 50 mg/l chitosan via spraying had the most favourable effect on the carotenoid content, with a 21.3% and 22.1% increase compared with the control plants. In a previous study, there were similar results for sage (*Salvia abrotanoides*): after foliar application of chitosan, there was a marked 63% increase in the total chlorophyll content and a 68% increase in the carotenoid content compared with the control plants [28]. In another study, there was a significant increase in leaf accumulation of chlorophyll and carotenoids in potato (*Solanum tuberosum*) plants sprayed with an oligo-chitosan solution (molecular weight ~82.20 kDa) [9]. Chitosan-induced changes in the content of assimilation pigments are most likely related to the ability of chitosan to stimulate the production of endogenous cytokines and the expression of genes involved in chlorophyll synthesis. Increased synthesis of assimilation pigments may also be related to nitrogen in chitosan, a crucial component of the tetrapyrrole ring of chlorophyll [29, 30].

**Table 3.** The influence of the depolymerised chitosan concentration and application method on photosynthetic pigments in *Eucomis autumnalis*.

| Treatment | Application method | Total chlorophyll content [mg/g fresh weight] | Carotenoid content [mg/g fresh weight] |
|---|---|---|---|
| **Control** | - | $0.45 \pm 0.00^{c}$ | $0.17 \pm 0.01^{c}$ |
| **Chitosan 50 mg/l** | Soil drench | $0.57 \pm 0.01^{b}$ | $0.18 \pm 0.01^{bc}$ |
| **Chitosan 100 mg/l** | Soil drench | $0.58 \pm 0.01^{b}$ | $0.20 \pm 0.00^{a}$ |
| **Chitosan 50 mg/l** | Foliar spray | $0.70 \pm 0.03^{a}$ | $0.20 \pm 0.01^{a}$ |
| **Chitosan 100 mg/l** | Foliar spray | $0.64 \pm 0.08^{a}$ | $0.19 \pm 0.01^{ab}$ |

*Note.* The data are presented as the mean ± standard error of the mean. In each column, means with the same superscript letter are not significantly different based on Tukey's test ($\alpha = 0.05$).

Chitosan is believed to activate the biosynthesis of phytohormones, enzymes and secondary metabolites that help plants fight stresses and pathogens by inducing their defence responses [2, 31]. Table 4 presents the effects of depolymerised chitosan on the metabolite contents of *E. autumnalis* leaves. There was an apparent increase in the total polyphenol content after foliar application of 50 and 100 mg/l depolymerised chitosan – by 77.7% and 59.0%, respectively, compared with the control plants. The total polyphenol content increased significantly by 17% when plants were drenched with 100 mg/l depolymerised chitosan. Moreover, regardless of the dose, foliar treatment of plants with depolymerised chitosan significantly increased the L-ascorbic acid content in leaves by an average of 36.4% compared with the control plants. Application of 100 mg/l depolymerised chitosan by drenching increased the L-ascorbic acid content by 19.6% compared with the control plants, while the dose of 50 mg/l did not affect the L-ascorbic acid content. Chitosan has been shown to increase polyphenol levels in several plants, including Greek oregano (*Origanum vulgare* ssp. *hirtum*) treated with a foliar spray of 50 and 200 mg/l chitosan oligosaccharides (degree of polymerisation = 2–10 and degree of deacetylation > 95%) [8]; medicinal plant *Dracocephalum kotschyi* sprayed with 100 and 400 mg/l chitosan (degree of deacetylation = 85%) [12]; and in sage (*Salvia officinalis*) after foliar application of 0.25 and 0.5 g/l chitosan [21]. Conversely, in tomatoes (*Lycopersicon esculentum*), foliar treatment with chitosan (molecular weight ~ 50–150 kDa) dissolved in different organic acids increased polyphenols by 20%–70% and L-ascorbic acid by 5%–110% [32]. El Amerany et al. [33] also reported the stimulating effect of spraying 1 mg/ml chitosan (molecular weight ~ 318.53 kDa, degree of deacetylation = 4%–17%) on the L-ascorbic acid content in tomatoes.


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**Table 4.** The influence of the depolymerised chitosan concentration and application method on the total polyphenol and L-ascorbic acid contents of *Eucomis autumnalis*.

| Treatment | Application method | Total polyphenol content [mg gallic acid equivalents/100 g fresh weight] | L-ascorbic acid content [mg/100 g fresh weight] |
|---|---|---|---|
| **Control** | - | 28.02 ± 0.45[c] | 32.26 ± 0.89[c] |
| **Chitosan 50 mg/l** | Soil drench | 30.35 ± 0.66b[c] | 29.38 ± 0.82[c] |
| **Chitosan 100 mg/l** | Soil drench | 32.77 ± 0.57[b] | 38.59± 0.27[b] |
| **Chitosan 50 mg/l** | Foliar spray | 49.78 ± 0.91[a] | 43.64 ± 0.90[a] |
| **Chitosan 100 mg/l** | Foliar spray | 44.56 ± 1.92[ab] | 44.35 ± 0.84[a] |

*Note.* The data are presented as the mean ± standard error of the mean. In each column, means with the same superscript letter are not significantly different based on Tukey's test ($\alpha = 0.05$).

Chitosan increases the biosynthesis of specific secondary metabolites in plants by acting as a signalling molecule and modulating the expression of genes related to the synthesis of secondary metabolites, including polyphenols, responsible for inducing the plant's defence responses [3, 6, 12]. The results show that foliar treatment with chitosan of immature *E. autumnalis* plants can be an effective method to increase the production of secondary metabolites.

## 4. Conclusion

In conclusion, foliar application of depolymerised chitosan can stimulate growth and biomass gain and effectively increase the accumulation of chlorophylls, carotenoids, total polyphenols and L-ascorbic acid in *E. autumnalis* leaves. Foliar spraying of 100 g/l depolymerised chitosan provided the most potent biostimulatory effects. In the long term, research is needed to determine how depolymerised chitosan elicits the production of specific substances found in *E. autumnalis* that exhibit biological activity and have potential in the medical, pharmaceutical and food industries.

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