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Evaluation of Carrageenan, Xanthan Gum and Depolymerized Chitosan Based Coatings for Pineapple Lily Plant Production

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Abstract: Some natural polysaccharides and their derivatives are used in horticulture to stimulate plant growth. This study investigated the effects of coating bulbs with carrageenan-depolymerized chitosan (C-DCh) or xanthan-depolymerized chitosan (X-DCh) on growth, flowering, and bulb yield as well as physiological and biochemical attributes of pineapple lily (*Eucomis autumnalis*). The results showed that treatment with C-DCh or X-DCh significantly increased all growth parameters, bulb yield, greenness index, stomatal conductance, total N, total K, and total sugar content of bulbs and accelerated anthesis as compared with untreated bulbs. The positive impact of coatings on plant growth and physiological attributes depended on the type of biopolymer complexes. The X-DCh treatment exhibited the greatest plant height, fresh weight, daughter bulb number, greenness index, stomatal conductance, total N, K, and sugar content. However, this treatment induced a significant decrease in L-ascorbic acid, total polyphenol content and antioxidant activity. Overall, the results of this study indicated high suitability of C-DCh and X-DCh as bulb coatings for pineapple lily plant production.

Keywords: biostimulants; polysaccharides; bulb coating; plant enhancement; metabolites

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1. Introduction

Currently, biostimulants are used to improve growth and development of horticultural plants [1,2]. Biostimulants are a broad group of substances and microorganisms with high biological activity [3–5]. Of particular interest are biodegradable polysaccharides and their depolymerized derivatives exhibiting multi-directional actions in plants [6–10]. Chitosan is one of the best known natural polysaccharides with biostimulatory properties obtained in the process of chitin de-N-acetylation [11,12]. Chitosan and its oligomeric forms have stimulated plant growth and flowering, increased photosynthesis and nutrient uptake, and protected plants against stress [13–16]. Many studies have reported the benefits provided by chitosan on various ornamental plants, such as *Begonia* × *hiemalis* Fotsch [17], *Chrysanthemum morifolium* Ramat [18], *Eucomis bicolor* Baker [19], *Freesia* × *hybrida* [20], and *Petunia* × *atkinsiana* D. Don [21]. In practice, chitosan solution is applied as a spray or drench [22–24] as well as hydrogels for coating seeds, but hydrogels from "pure" chitosan have low stability and durability [25]. Moreover, the wider use of chitosan is limited due to its poor water solubility [26]. The application of depolymerized chitosan with low molecular weight and ionic biopolymers in the form of hydrogel coating formed on the surface of plant organs based on polyelectrolyte complexes may be the solution to the problem [27]. This type of coating formed by chitosan and ionic polymers can positively affect plant growth and flowering [28]; however, it is reasonable to conduct broader research, including evaluation of the effectiveness of various biopolymers as coating components [29]. Carrageenans are a family of anionic polymers extracted from red algae used as plant biostimulants [10,30,31]. Carrageenans and their breakdown products can stimulate plant productivity and root system development, and enhance net photosynthesis, basal, and secondary metabolisms [32–36]. Among natural biopolymers, xanthan gum, an

anionic, high-molecular-weight exo-polysaccharide secreted by the bacterium *Xanthomonas campestris* is also known [37]. Application of xanthan gum can influence plant growth and physiology, content of phenolic compounds, and antioxidant activity [38–40]. Xanthan gum used in micropropagation as an alternative to agar has a positive effect on the regenerative potential of some plants [41], which may indicate its biostimulative action. However, no information is available regarding the effect of xanthan gum as a biostimulant on plant growth and flowering.

Pineapple lily (*Eucomis autumnalis* (Mill.) Chitt. Asparagaceae) is a prospective bulbous ornamental plant grown in gardens, for cut flowers, and as a potted plant for indoor display [42–44]. The bulbs produce a rosette of smooth leaves and original decorative raceme-type inflorescences with a tuft of leaf-like bracts on top, composed of star-shaped white flowers with a pleasant scent. After flowering, the plants set decorative and durable green capsules. Besides its ornamental use, pineapple lily is one of the most popular plant species in traditional medicine in southern Africa [45]. The extracts of pineapple lily exert multidirectional effects, including antioxidant, anti-inflammatory, bactericidal, fungicidal and cytostatic effects [45,46]. The species is threatened with extinction in its natural habitat due to the excessive collection of bulbs for medicinal purposes as well as low vegetative propagation rate [45]. Thus, proper production methods of pineapple lily using various plant biostimulants is needed [47,48].

Previous work [49] reported that oligochitosan and sodium alginate can be successfully used for the preparation of hydrogel coatings for the bulbs of pineapple lily. However, systematic study of the effects of other biopolymers on growth, plant physiological status, and biochemical parameters of pineapple lily remains to be investigated. The current study was aimed to compare the effects of coatings containing hydrogels based on carrageenan or xanthan gum with depolymerized chitosan on the growth characteristics, flowering, bulb yield, physiological parameters, nutrients, L-ascorbic acid, and total polyphenol content, as well as antioxidant activity of pineapple lily. It was hypothesized that a coating treatment with polysaccharides would enhance the growth and bulb production of pineapple lily.

2. Materials and Methods

2.1. Plant Culture and Treatment

Bulbs of pineapple lily (*E. autumnalis*) with 12–14 cm circumference were imported from The Netherlands by Ogrodnictwo Wiśniewski Jacek Junior (Góraszka, Poland) and treated for 30 min in a suspension of 0.7% Topsin M 500 SC and 1% Captan 50 WP fungicides. Before planting, the uniform bulbs were coated according to the technology described by Startek et al. [29] in hydrogels based on 1% (*w/v*) carrageenan or 1% (*w/v*) xanthan gum in which bulbs were dipped for 30 s, and 0.2% (*w/v*) depolymerized chitosan in which the bulbs were soaked for 10 min. Control bulbs were soaked in distilled water. Depolymerized chitosan obtained by controlled free radical degradation [28] had a molecular weight of 154, 500 g mol^{-1}, the number-average molecular weight of 22,800 g mol^{-1}, and deacetylation degree of 85%. Carrageenan-depolymerized chitosan (C-DCh) and xanthan-depolymerized chitosan (X-DCh) were produced. Iota-carrageenan and xanthan gum were purchased from Sigma-Aldrich. Polysaccharides were prepared by solubilization using a magnetic stirrer. Each treatment was replicated four times and each replicate had 10 bulbs.

Coated bulbs were planted in a randomized block design on 15 April 2016 and 13 April 2017 into polyethylene boxes (60 × 40 × 19 cm) filled with peat substrates (pH 6.3) supplemented with a fertilizer Hydrocomplex (12% N, 4.5% P, and 15% K plus micronutrients; Yara International ASA, Oslo, Norway) at a dose of 3 g L^{-1}. Each box contained 10 bulbs. The boxes were transferred to a non-heated tunnel covered with a double layer of plastic located in the area of West Pomeranian University of Technology in Szczecin (53°25′ N, 14°32′ E; 25 m a.s.l.). Air temperature inside the tunnel was controlled with vents that were opened when the temperature exceeded 20 °C.

2.2. Measurement of Growth Parameters

The number of days to anthesis was recorded. When the first flowers opened in the raceme, plant height, diameter of the plant, and inflorescence length were recorded. At the end of the flowering period, the number of florets in the inflorescence were counted and fresh weight of the excised aboveground part was measured. On 3 October 2016 and 6 October 2017, the plants were removed from boxes, and fresh weight of bulbs per plant and the number of daughter bulbs were determined.

2.3. Measurement of Physiological Parameters

At the flowering stage, relative leaf chlorophyll content measurements were performed using a SPAD-502 Chlorophyll Meter (Minolta, Osaka, Japan) and stomatal conductance was assessed with a SC-1 Leaf Porometer (Dekagon Device, Pullwan, WA, USA). SPAD and stomatal conductance measurements were calculated based on four readings of four uniform leaves selected from five plants of each treatment.

2.4. Total N, P, K, and Total Sugar Content in Bulb Determination

At the end of the growing season, the bulb samples were collected, dried at 65 °C for 72 h and ground. Powdered samples (2.0 g) were digested in 17 mL concentrated 96–97% H_2SO_4. The total forms of N, P, and K were determined as outlined by Ostrowska [50]. Total N was determined according to the Kjeldahl method, P with colorimetric method according to Barton, and K by flame photometry [50]. The content of total sugar in samples of fresh bulbs was determined following the Luff-Schoorl method [51]. Nutrients and total sugar content were determined using three replicates per treatment.

2.5. L-Ascorbic Acid, Total Polyphenol Content, and Antioxidant Activity Determination

At the flowering stage, fully developed leaves were taken for biochemical analyses. Before homogenization, leaves were washed with water to remove soil, cut into slices, and dried in a circulating-air oven (35 °C ± 2 °C). Vitamin C was determined as L-ascorbic acid by the Tillman's titration method of the reduction of 2.6-dichlorophenolindophenol [52]. The preparation of plant extracts for the determination of the total polyphenol content and antioxidant activities was performed using the method of Wojdyło et al. [53] with some modifications. The sample of leaves was treated with 70% aqueous methanol (MeOH). Total polyphenol content was analyzed spectrophotometrically using the Folin–Ciocalteu colorimetric method as described by Wojdyło et al. [53]. The absorbance was measured at 760 nm. Antioxidant activity of leaves on DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined according to the procedure of Yen and Chen [54], and DPPH inhibition percentage was calculated according to the formula provided by Rossi et al. [55]. All determinations were carried out in three replicates.

2.6. Statistical Analysis

Data were normally distributed and passed Levene's test ($\alpha \leq 0.05$) for homogeneity of variance. Data were statistically analyzed by one-way ANOVA using Statistica™ Professional 13.3.0 software (TIBCO Statistica, Palo Alto, CA, USA). After checking the goodness of fit of the model, post hoc comparisons were done using the Duncan's Multiple Range Test (DMRT) at $\alpha \leq 0.05$. The results are presented as a mean from two years of the study.

3. Results

The effect of coating with carrageenan-depolymerized chitosan (C-DCh) or xanthan-depolymerized chitosan (X-DCh) on growth and flowering of pineapple lily is shown in Table 1. The C-DCh and X-DCh applications significantly increased plant height by 8% and 16%, respectively, plant width by 39% and 40%, respectively, fresh weight of the aboveground part by 71% and 95%, respectively, inflorescence length by 32% and 25%, respectively, and the number of florets by 8% and 9%, respectively, as well as accelerated flowering by 17 and 13 days, respectively. Statistically significant differences were observed

between X-DCh and C-DCh treatments. Bulbs coated in X-DCh were taller by 7% and had a greater fresh weight of the aboveground part by 16%, compared with bulbs coated in C-DCh.

Table 1. Growth and flowering parameters of pineapple lily treated with carrageenan-depolymerized chitosan (C-DCh) or xanthan-depolymerized chitosan (X-DCh).

Parameter	Type of Coatings		
	Control	C-DCh	X-DCh
Plant height (cm)	32.6 ± 0.96 c [z]	35.1 ± 0.59 b	37.7 ± 1.07 a
Plant diameter (cm)	25.7 ± 1.61 b	35.8 ± 1.86 a	36.0 ± 1.17 a
Fresh weight of the aboveground part (g)	107 ± 1.91 c	183 ± 4.10 b	209 ± 4.07 a
Length of inflorescence (cm)	16.5 ± 1.70 b	21.7 ± 1.19 a	20.7 ± 0.72 a
Number of florets	72.6 ± 3.39 b	78.6 ± 1.46 a	79.2 ± 1.15 a
Days to anthesis	161 ± 2.08 a	144 ± 3.21 b	148 ± 2.52 b

[z] Means (±SD) followed by the same small letter in the same row did not differ by Duncan's Multiple Range Test at $\alpha \leq 0.05$.

The fresh weight of bulbs, number of daughter bulbs, total N, K, and total sugar content in the pineapple lily bulbs were significantly affected by C-DCh or X-DCh complexes (Table 2). The coating of bulbs with C-DCh and X-DCh enhanced fresh weight of bulbs by 39% and 61%, respectively, and number of daughter bulbs by 24% and 48%, respectively. Moreover, the application of C-DCh and X-DCh increased levels of N by 49% and 54%, respectively, K by 46% and 57%, respectively, and total sugar content by 12% and 17%, respectively, in comparison with the control. The treatment with X-DCh resulted in the greatest fresh weight of bulbs, number of daughter bulbs, and total N, K, and sugar content. Bulb treatment with C-DCh and X-DCh did not affect total P content.

Table 2. Fresh weight of bulbs, number of daughter bulbs, total N, P, K, and total sugar content in bulb of pineapple lily treated with carrageenan-depolymerized chitosan (C-DCh) or xanthan-depolymerized chitosan (X-DCh).

Parameter	Type of Coatings		
	Control	C-DCh	X-DCh
Fresh weight of bulbs (g)	31.0 ± 1.87 c [z]	43.0 ± 2.61 b	50.0 ± 4.95 a
Number of daughter bulbs	0.75 ± 0.15 c	0.93 ± 0.07 b	1.11 ± 0.10 a
Total N content (% DW)	0.39 ± 0.03 c	0.58 ± 0.02 b	0.64 ± 0.02 a
Total P content (% DW)	0.05 ± 0.01 a	0.06 ± 0.01 a	0.05 ± 0.01 a
Total K content (% DW)	0.44 ± 0.02 c	0.64 ± 0.03 b	0.69 ± 0.02 a
Total sugar content (% FW)	6.66 ± 0.39 b	7.47 ± 0.15 a	7.76 ± 0.16 a

[z] Means (±SD) followed by the same small letter in the same row did not differ by Duncan's Multiple Range Test (DMRT) at $\alpha \leq 0.05$.

As shown in Figure 1, SPAD chlorophyll meter measurements and stomatal conductance were significantly increased due to C-DCh and X-DCh treatment in comparison to control. The C-DCh increased SPAD and stomatal conductance by 11% and 55%, and X-DCh by 7% and 31%, respectively. The SPAD and stomatal conductance of plants treated with X-DCh were 3% and 19%, respectively, greater than that of C-DCh treatment.

Figure 2 shows the effects of bulb coatings on the content of L-ascorbic acid and total polyphenols and the antioxidant activity. In comparison with the control the application of C-DCh or X-DCh significantly decreased total polyphenol content by 13% and 17%, respectively. Furthermore, the application of X-DCh significantly decreased L-ascorbic acid content by 33% and free DPPH radicals by 56%. The plant L-ascorbic acid content and antioxidant activity showed no statistically significant differences between control and C-DCh treatment.

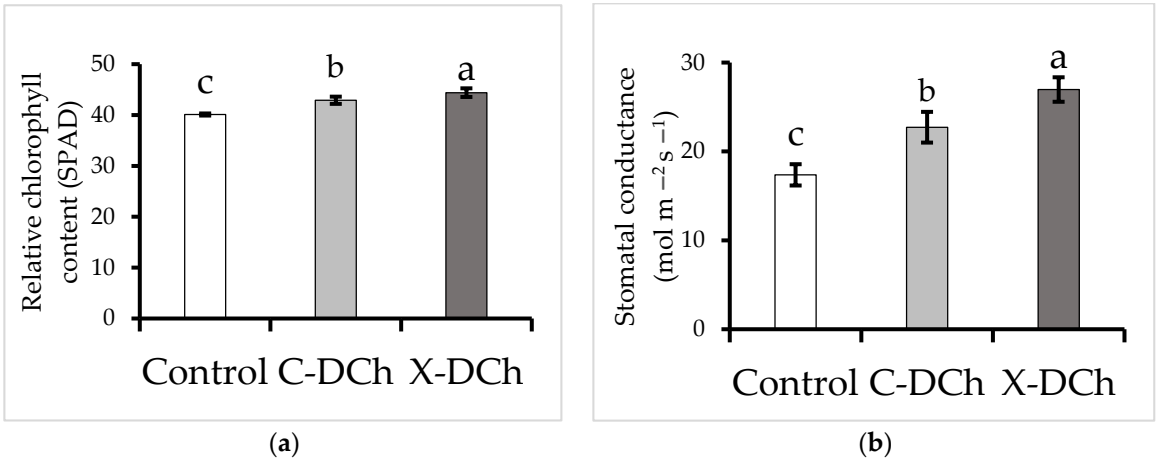

Figure 1. Relative leaf chlorophyll content (SPAD) (**a**) and stomatal conductance (**b**) of pineapple lily treated with carrageenan-depolymerized chitosan (C-DCh) and xanthan-depolymerized chitosan (X-DCh). Data are presented as means (±SD) and bars with different letters in each graph are significantly different by Duncan's Multiple Range Test (DMRT) at $\alpha \leq 0.05$.

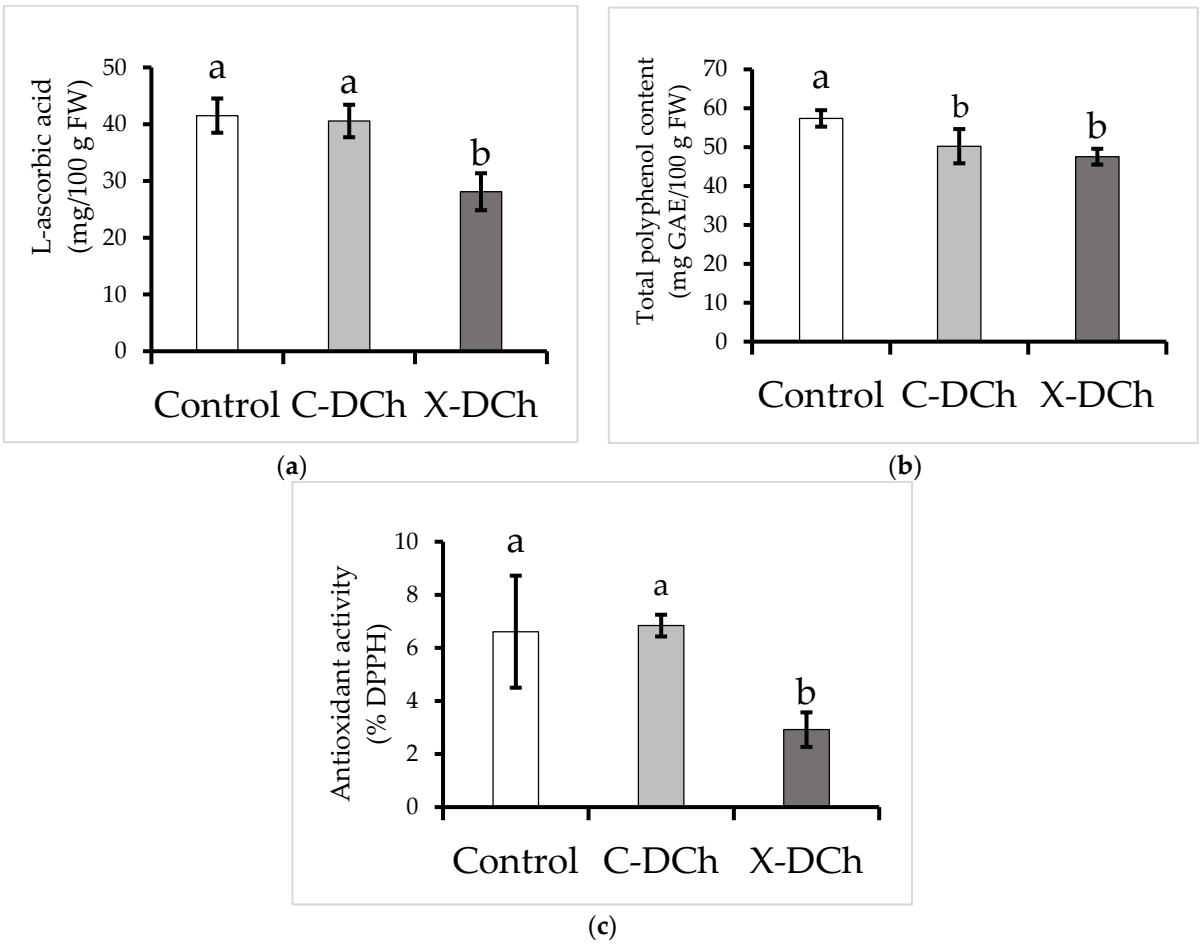

Figure 2. L-ascorbic acid (**a**) and total polyphenol content (**b**) and antioxidant activity (DPPH) (**c**) of pineapple lily treated with carrageenan-depolymerized chitosan (C-DCh) and xanthan-depolymerized chitosan (X-DCh). Data are presented as means (±SD) and bars with different letters in each graph are significantly different by Duncan's Multiple Range Test (DMRT) at $\alpha \leq 0.05$.

4. Discussion

Most studies on the application of biostimulant coatings focus on seeds, while data on coating other plant organs such as bulbs, tubers or rhizomes are far less available [56]. Our study is the first to use two biostimulant complexes, containing depolymerized chitosan and carrageenan (C-DCh) or xanthan (X-DCh), for coating pineapple lily bulbs. We found a stimulatory effect of both types of coatings on plant growth and development manifested in accelerated flowering and clearly higher yield of flowers and bulbs. In addition, plants grown out of biostimulant-coated bulbs featured more efficient gas exchange and better nutrient content. Some results of the current study are in agreement with our earlier work [29]. We reported a considerable improvement in plant growth, as assessed by morphological and physiological parameters and nutrient content in *E. autumnalis* when the bulbs were coated with oligochitosan and sodium alginate, and in *Ornithogalum saundersiae* Baker when the bulbs were coated with chitooligosaccharide and sodium alginate, carrageenan, gellan gum, or xanthan gum [28,49]. Stimulating effects of the coatings were probably due to the fact that their components enhanced plant tolerance to stresses from the beginning of their development, similar to that noted with seed coating [56]. Chitosan can improve growth and structure of a plant root system by limiting the presence of soil pathogens [11,12]. As a source of carbon for microorganisms, it also indirectly boosts soil microbial activity and thus improves absorption of minerals and water by plant roots [15]. In consequence, plants grow faster and stronger and produce better yield. Carrageenan may act as an elicitor that induces plant defense response against viroids, viruses, bacteria, or fungi, and it also may improve plant growth by controlling numerous metabolic processes including photosynthesis and assimilation of nitrogen and sulfur [10,30,35]. Xanthan gum is also capable of inducing local and systemic resistance against diseases and shows the same efficiency in plant protection against some phytopathogens as fungicides [38,57]. Soil application of xanthan gum may increase root biomass production and plant tolerance to drought and other environmental stresses [40].

The results presented in this paper demonstrated that the positive effects of coating pineapple lily bulbs on plant growth depended on the type of biopolymer complexes. The strongest plant growth stimulation was observed in plants obtained from bulbs treated with X-DCh complex. We assume that joint application of the biostimulants in X-DCh coatings may induce a stronger synergistic effect than C-DCh coating alone. It is commonly known that many biologically active substances change their properties when interacting with other substances [58,59]. Interestingly, the stimulating effect of X-DCh on the growth of the aboveground plant tissues and bulb biomass and the content of N, K, and total sugars in pineapple lily was accompanied by a clear drop in the levels of L-ascorbic acid and total polyphenols and by reduced antioxidant activity, responses not recorded in plants treated with C-DCh. The inhibitory effect of X-DCh treatment on secondary metabolite production may be a result of a trade-off between the production of plant biomass and secondary metabolism [60]. It is well known that defense and plant growth cannot usually be successfully executed at the same time [61,62]. Another possible interpretation, in line with previously cited research, is that xanthan gum induced a reduction in polyphenol content due to activation of some cellular biochemical mechanisms involved in plant resistance [57]. Still, further studies are necessary to validate either of these hypotheses.

5. Conclusions

The biostimulant complexes carrageenan-depolymerized chitosan (C-DCh) and xanthan-depolymerized chitosan (X-DCh) used for bulb coating improved plant productivity, allowing growers to speed up the production cycle in protected culture and to obtain higher quality flowers and bulbs of pineapple lily. Particularly strong biostimulant activity was shown for coatings containing derivatives of X-DCh. Bulb coatings in biostimulants seems a prospective, efficient, and environmentally friendly method of improving plant growth that can be recommended for sustainable production of ornamental plants.

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