EFFECT OF CHITOSAN WITH DIFFERENT MOLECULAR WEIGHT ON THE GROWTH AND BULB YIELD OF ORIENTAL LILY ‘MONA LISA’

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Abstract

The potential of biodegradable and environmentally friendly substances that exhibit biostimulatory effects on plant growth and yield is now being exploited in horticultural production. This study evaluated the effect of chitosan on the growth, flowering, and yield of bulb of oriental lily ‘Mona Lisa’. Three types of chitosan differing in molecular weight (MW) were used: commercial chitosan (CCh, MW = 50,000–190,000 g/mol) and two forms of depolymerised chitosan (DCh 48, with a MW of ~48,000 g/mol, and DCh 154, with a MW of ~154,500 g/mol). The plants were watered twice with an aqueous solution of chitosan at a concentration of 2 g/l, with 100 ml of solution per pot. The control was plants watered with distilled water. Chitosan had a biostimulatory effect on the growth of lily plants. CCh increased the relative chlorophyll content of leaves (+11.3%), the fresh bulb weight (+11.0%), the bulb diameter (+12.3%) and the number of bulblets (+44.9%) compared with the control plants. DCh 154 significantly increased the fresh aboveground weight of plants (+31.3%), the fresh weight of leaves (+29.0%) and their number (+16.8%), the fresh weight of bulb (+12.3%) and their diameter (+11.0%), and the fresh weight of bulblet (+21.9%). DCh 48 increased the bulb diameter (+12.1%) and the number of bulblets (+45.4%), but decreased the length (-8.6%) and width of tepals (-6.8%) of the lily flowers. Finally, DCh 154 was the most effective in lily production. Using depolymerised chitosan as a biostimulant may contribute to developing new methods to produce high-quality lily plants.

Keywords: *oligochitosan, biostimulant, plant propagation, sustainable agriculture*

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1. Introduction

The production of ornamental plants is a rapidly growing and profitable segment in horticultural production [1]. Among the activities determining the continued development of the floriculture industry is the search for new strategies for growing and propagating plants [2]. Limitations in the use of agrochemicals, as well as economic factors, affect the yield and flower quality. Today, natural biostimulants are increasingly being used to improve the growth and increase the yield of plants [3]. This group includes many substances of natural origin and microorganisms with high biological activity [4]. One of the best-known is chitosan, a natural polysaccharide that consists of molecules of D-glucosamine and *N*-acetyl-D-glucosamine linked by β-1,4-glycosidic bonds [5]. Chitosan is formed by deacetylation of chitin contained in the chitinous carapaces of transformed crustaceans and the cell walls of fungi [6]. This compound is biodegradable and exhibits numerous biological effects [7]. Du Jardin [8] included chitosan and other polymers in one of seven categories of biostimulants. Studies on ornamental plants have proven that chitosan has a biostimulatory effects on plant growth, flowering and specific physiological processes [9, 10]. It has also been shown to stimulate plant defence mechanisms, inducing plant resistance to pathogenic fungi and bacteria. In addition, chitosan is effective in alleviating the effects of abiotic stresses [11, 12]. It can be applied alone as an aqueous solution for watering and spraying plants or as a component, such as hydrogels for coating seeds [13–15].

After tulips, lilies (*Lilium* spp.) are the most cultivated bulbous plants in the world; they are grown for cut flowers and as very decorative perennials for gardens and potted plants [16]. In addition, some species of lilies are offered as vegetables [17]. These plants are versatile in horticulture, so there is a great demand for their bulbs and improved production methods [18, 19]. Lilies from the group of oriental hybrids have very attractive flowers. They have a relatively long production time and are considered difficult to grow, mainly due to their high susceptibility to diseases, unfavourable growing conditions and low propagation rate [20]. On a production scale, lilies are propagated effectively and without great expense from scales, and growth regulators [21] and biostimulants [22] can be used to increase the yield of adventitious bulblets. Given the versatile properties of chitosan, this study evaluated the effects of chitosan with different molecular weights (MWs) on the growth, flowering and bulb yield of lilies.

2. Materials and Methods

The study was conducted at the West Pomeranian University of Technology in Szczecin (lat. 53°26′17″N, 14°32′32″E). The experiment used 'Mona Lisa' lily bulbs (average circumference 12–13 cm) of the oriental hybrid lily group recommended for growing in pots. The bulbs were imported from Dutch plantations. The bulbs were planted individually in pots (diameter = 16 cm, capacity = 2 dm^3) filled with TS1 medium (Klasmann-Delimann, Germany), with pH 6.3, electrical conductivity (EC) 0.63 mS/cm, and the following composition (mg/l): nitrate nitrogen ($N\text{-}NO_3$), 182; phosphorus (P), 131; potassium (K), 402; calcium (Ca), 1646; magnesium (Mg), 172; and chlorine (Cl), 18. Plants were grown for one growing season, from 19 March to 30 July 2019, under natural photoperiod conditions on growing tables in an unheated plastic tunnel covered with double polyethylene film. The average air temperature recorded in the tunnel during lily cultivation was: March, 10.1°C; April, 11.9°C; May, 18.7°C; June, 23.6°C; and July, 21.1°C.

Three types of chitosan were used: commercial chitosan (CCh) with an MW of 50,000–190,000 g/mol (Sigma-Aldrich, Iceland), depolymerised chitosan with a MW of ~48,000 g/mol (DCh 48) and depolymerised chitosan with a MW of ~154,500 g/mol (DCh 154). DCh 48 and DCh 154 with an average degree of deacetylation of ~85% were obtained by controlled free-radical degradation in the laboratory of the Center for Bioimmobilization and Innovative Packaging Materials at ZUT in Szczecin, Poland [23]. High-performance liquid chromatography (HPLC) was used to determine the DCh 48 and DCh 154 MWs. Chitosan was applied to watered plants as an aqueous solution (in distilled water) at a dose of 2.0 g/l. The dose was determined based on studies on bulbous plants [24]. Lilies were watered twice, after day 30 and 40 of cultivation, each time with 100 ml of chitosan solution per plant. Control plants were watered twice with 100 ml of distilled water. The experiment was set up in a randomised block design with three replicates, each with four plants.

When the first flower was developed, plant height [cm], tepal length [cm] and width [cm] were determined. At this stage, three plants were drawn from each replicate, in which the relative chlorophyll content as an indicator of leaf greenness was determined using the Soil and Plant Analysis Development (SPAD)-502 optical apparatus (Minolta, Japan). Measurements were taken on three fully expanded leaves of each plant. Flowers, buds and leaves were counted, and the fresh weight of the aboveground part of the plant [g] and the fresh weight of the leaves [g] were determined. The fresh weight was measured using an electronic scale (PS 200/2000/C/2, RADWAG, Poland) with an accuracy of 0.001 g.

After flowering, the bulbs removed from the pots were weighed [g], and their diameters [mm] were measured. The bulbs were used to propagate lilies by scales. For this purpose, three bulbs from each variant taken randomly (control, CCh, DCh 48 and DCh 154) were separated into scales, with four replicates per bulb. Scales from individual bulbs were placed separately in plastic bags (capacity = 2 dm^3) filled with a moist mixture of acid peat and perlite (1:1, v/v). The scales were stored for 12 weeks without light at 20–22°C, then for 16 weeks at 3–5°C with a relative humidity of approximately 90%. After this period, the number of bulblets formed on scales derived from one bulb and the fresh weight of bulblets [g] were determined.

The experiment was set up in a single-factor, random sub-block design. The results were subjected to analysis of variance (ANOVA) for univariate experiments followed by Tukey's test for multiple comparisons using the Statistica™ Professional 13.3.0 software (TIBCO Statistica, USA). A p value ≤ 0.05 was considered to be statistically significant.

3. Results and Discussion

Watering with the DCh 154 solution produced the highest fresh weight of the aboveground part of the plants (Table 1). In this case, the fresh weight gain was increased significantly by 31.3% compared with the control plants. In the available literature, there are no reports on improving oriental lily production technology using chitosan under production conditions. On the other hand, there is information on the beneficial effect of chitosan on the growth of several lily species and their regenerative ability in *in vitro* cultures. Unfortunately, the characterisation of chitosan is generally lacking in these studies. Khalafi et al. [25] added 200 mg/l chitosan to the medium of *Lilium ledebourii* (Baker) Boiss. and noted significantly stimulated plant growth and increased their fresh weight. Pourbeyrami et al. [26] obtained similar results using the same dose of chitosan in *in vitro* cultures of *Lilium regale* E.H. Wilson. In contrast, Kanchanapoom et al. [27] found that the addition of 25 mg/l chitosan to the nutrient solution of *Lilium longiflorum* Thunb. significantly increased the number of multiplied shoots and acted similarly effectively to the hormone benzyladenine. Shafiee-Masouleh [28] applied chitosan as nano- and magnetic particles at a concentration of 10–15 mg/l and found that it increased the fresh and dry weight of shoots and roots of oriental lily 'Arabian Red'. Based on previous studies [29, 30], it is possible that chitosan acts pleiotropically in cells to activate plant responses through various signalling pathways, transmitters and transcription factors that trigger the stimulatory effect.

Table 1. The effect of chitosan on plant height and the fresh weight of aboveground parts of oriental lily 'Mona Lisa'.

Treatment	Plant height [cm]	Fresh weight of the aboveground parts [g]
Control	46.0 ± 2.00 [a]	44.47 ± 3.10 [b]
CCh	47.5 ± 0.50 [a]	45.23 ± 0.80 [b]
DCh 48	48.8 ± 1.76 [a]	47.50 ± 2.51 [b]
DCh 154	48.1 ± 0.85 [a]	58.37 ± 1.10 [a]
p	0.172	0.0001

Note. The data are presented as the mean ± standard error of the mean. In each column, means followed by the same letter are not significantly different based on Tukey's test ($p \leq 0.05$). Abbreviations: CCh, commercial chitosan; DCh, depolymerised chitosan.

DCh 154 significantly affected the number of leaves and the fresh weight of leaves (Table 2). Compared with the control, plants watered with the DCh 154 solution showed a 16.8% increase in the number of leaves and a 29.0% increase in the fresh weight of leaves. The leaves of plants watered with the CCh solution showed the highest chlorophyll content, with a significant 11.3% increase compared with the control plants. Chamnanmanoontham et al. [31] showed that the elevated chlorophyll content in the leaves of chitosan-treated plants may be the result of increased expression of proteins involved in carbon metabolism and the photosynthetic process, leading to an increase in nutrients, primary metabolites, phytohormones and photosynthetic pigments necessary to stimulate plant growth [29].

Table 2. The effect of chitosan on number and weight of leaves and relative chlorophyll content of oriental lily 'Mona Lisa'.

Treatment	Number of leaves per plant	Leaf fresh weight [g/plant]	Relative chlorophyll content (SPAD)
Control	19.7 ± 1.15 [b]	25.5 ± 1.15 [b]	58.6 ± 0.59 [b]
CCh	20.3 ± 1.53 [ab]	26.0 ± 0.89 [b]	65.2 ± 1.34 [a]
DCh 48	22.0 ± 1.00 [ab]	26.2 ± 0.93 [b]	51.9 ± 2.64 [c]
DCh 154	23.0 ± 1.00 [a]	32.9 ± 1.36 [a]	62.8 ± 1.98 [ab]
p	0.032	0.001	0.001

Note. The data are presented as the mean ± standard error of the mean. In each column, means followed by the same letter are not significantly different based on Tukey's test ($p \leq 0.05$). Abbreviations: CCh, commercial chitosan; DCh, depolymerised chitosan; SPAD, Soil and Plant Analysis Development.

Regardless of its source and MW, chitosan did not affect the number of flowers and buds, but it did affect the length and width of lily petals (Table 3, Figure 1). Watering the plants with the DCh 48 solution significantly reduced the length and width of perianth tepals by 8.6% and 6.8%, respectively, compared with the control plants. Treatment with the CCh or DCh 154 solution did not significantly affect tepal parameters.

Table 3. The effect of chitosan on the number of flowers and buds and the length and width of the tepals of oriental lily 'Mona Lisa'.

Treatment	Number of flowers per plant	Number of buds per plant	Tepal length [cm]	Tepal width [cm]
Control	1.83 ± 0.29 [a]	1.67 ± 0.29 [a]	12.8 ± 0.05 [a]	6.50 ± 0.10 [a]
CCh	1.83 ± 0.29 [a]	1.83 ± 0.19 [a]	12.2 ± 0.10 [ab]	6.17 ± 0.21 [ab]
DCh 48	1.16 ± 0.21 [a]	2.50 ± 0.50 [a]	11.7 ± 0.13 [b]	6.06 ± 0.06 [b]
DCh 154	2.00 ± 0.01 [a]	1.83 ± 0.29 [a]	12.7 ± 0.58 [a]	6.22 ± 0.22 [ab]
p	0.055	0.080	0.007	0.046

Note. The data are presented as the mean ± standard error of the mean. In each column, means followed by the same letter are not significantly different based on Tukey's test ($p \leq 0.05$). Abbreviations: CCh, commercial chitosan; DCh, depolymerised chitosan.

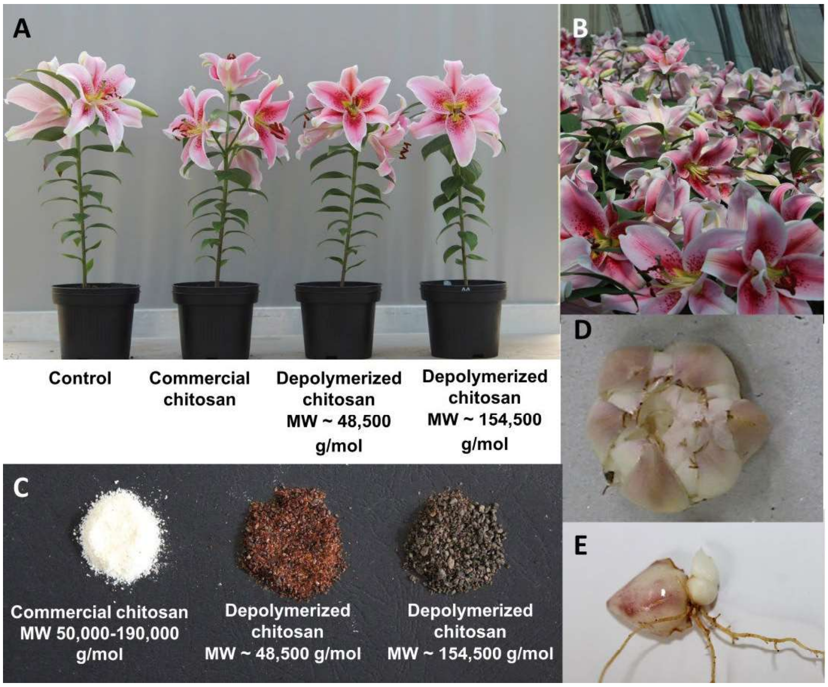

Figure 1. The effect of chitosan on flowering and the bulb yield of oriental lily 'Mona Lisa'. The photographs show (A) from left to right, an untreated control plant, and plants treated with commercial chitosan (CCh) with a molecular weight (MW) of 50,000–190,000 g/mol, depolymerised chitosan with a MW of ~48,000 g/mol (DCh 48) or depolymerised chitosan with a MW of ~154,500 (DCh 154); (B) flowering lilies in a foil tunnel; (C) chitosan used to the prepare aqueous solutions; (D) a lily bulb; (E) formation of bulblets on a bulb scale.

According to various authors, the response of plants to the type of chitosan used depends on its physical and chemical properties, such as the degree of deacetylation, MW and viscosity [29, 30, 32]. Table 4 shows the significant effect of watering plants with the chitosan solutions on the fresh weight of bulb and bulb diameter of the oriental lily 'Mona Lisa'. Compared with the control plants, bulbs watered with the CCh and DCh 154 solutions had a 11.0% and 12.3% higher fresh weight, respectively. Plants treated with CCh, DCh 48 and DCh 154 developed bulbs with 12.3%, 12.1% and 11.0% larger diameters, respectively, compared with the control plants. Khalafi et al. [25] demonstrated that chitosan significantly increased the yield of *L. ledebourii* bulbs. The authors reported an increased number of bulblets and plant roots after adding 200 mg/l chitosan to the nutrient solution.

Table 4. The effect of chitosan on the fresh weight and diameter of oriental lily 'Mona Lisa' bulb.

Treatment	Bulb fresh weight [g/plant]	Bulb diameter [mm]
Control	34.6 ± 1.83 [b]	47.2 ± 0.88 [b]
CCh	41.9 ± 2.41 [a]	53.0 ± 0.79 [a]
DCh 48	39.9 ± 2.01 [ab]	52.9 ± 1.01 [a]
DCh 154	42.5 ± 2.45 [a]	52.4 ± 0.58 [a]
p	0.008	0.001

Note. The data are presented as the mean ± standard error of the mean. In each column, means followed by the same letter are not significantly different based on Tukey's test ($p \leq 0.05$). Abbreviations: CCh, commercial chitosan; DCh, depolymerised chitosan.

Bulbs are organs of vegetative reproduction that show the ability to regenerate the entire plant. Churikoca and Barykina [33] and Feher [34] showed that the main reason for this regenerative ability is the totipotency of bulb cells. When bulbs are placed under conditions of high humidity and temperature without access to light, adventitious bulblets (Figure 1) are formed on the scales from buds in a dormant state. The rate of formation of adventitious bulblets on scales can be increased by using hormones and growth regulators, but with current pro-ecological trends and legal conditions, this approach is increasingly being abandoned [35]. In the present study, the effect of chitosan on bulblet yield – the fresh weight of bulbet and the number of bulblets – was evaluated on the scales of lily bulblets (Table 5). Bulblets formed on scales from bulbs treated with the DCh 154 solution had a 21.9% increase in fresh weight compared with the control. Scales from bulbs treated with the CCh and DCh 48 solutions yielded a significant 44.9% and 45.4% increase in the number of bulblets, respectively. The increased yield of bulblets may indicate a longer accumulation of the biostimulatory agent chitosan in the bulb and its secondary effect on the formation of bulblets on the scales. In addition, the resulting bulblets had no symptoms of disease, which could suggest that chitosan had a protective effect on them. Gong et al. [36] treated lanzhou lily (*Lilium davidii* var. *unicolor*) with chitosan in combination with natamycin to encapsulate the bulb slices. It was effective in maintaining their quality and preventing loss of weight, vitamin C, soluble sugars and proteins. Although there have been many studies, the mechanism of action of chitosan in plant organs is not yet completely clear [37, 38].

Table 5. Post harvest effects of chitosan on fresh weight of bulbet and number of bulblets of ‘Mona Lisa’ lily.

Treatment	Fresh weight of bulblet [g]	Number of bulblets
Control	0.32 ± 0.02 [b]	20.7 ± 8.39 [b]
CCh	0.34 ± 0.02 [b]	30.0 ± 4.58 [a]
DCh 48	0.31 ± 0.02 [b]	30.1 ± 6.56 [a]
DCh 154	0.39 ± 0.01 [a]	24.0 ± 6.93 [ab]
p	0.001	0.001

Note. The data are presented as the mean ± standard error of the mean. In each column, means followed by the same letter are not significantly different based on Tukey’s test ($p \leq 0.05$). Abbreviations: CCh, commercial chitosan; DCh, depolymerised chitosan.

4. Conclusions

The results presented in this study showed the usefulness of chitosan as a natural biostimulant in the production of oriental lilies. The DCh 154 solution had the strongest effect on growth and yield, with a significant increase in biomass, the number of leaves and leaf weight, of the bulb diameter and the fresh weight of bulb and bulblet. This treatment should be recommended for the production of high-quality lilies. Depolymerised chitosan is particularly important for the horticultural sector as an alternative to environmentally harmful chemicals. The need for integrated plant breeding and the growing interest in organic crops in horticulture may encourage the use of chitosan, also in the production of other bulb crops.

5. References

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