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Effects of Depolymerized Gellan with Different Molecular Weights on the Growth of Four Bedding Plant Species

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Abstract: New solutions allowing for the shortening of the growing cycle and improvements in plant quality are constantly sought in order to improve the efficiency of bedding plant production under covers. Biodegradable polysaccharides and their derivatives have become increasingly popular in horticulture as plant growth promoters. A greenhouse pot experiment was conducted to evaluate the effects of depolymerized gellan of different molecular weights (M_W 56 kDa and 77 kDa) on the growth and physiological parameters of ornamental bedding plants *Rudbeckia hirta* L., *Salvia splendens* Sellow ex J.A. Schultes, *Scabiosa atropurpurea* L., and *Tithonia rotundifolia* (Mill.) S.F. Blake. The results showed that the application of depolymerized gellan accelerated flowering and stimulated the growth of all assessed species, regardless of M_W. The plants treated with depolymerized gellan grew higher and had greater fresh weight of their above-ground parts, higher leaf relative chlorophyll content (SPAD; soil and plant analysis development), and higher stomatal conductance (g_s). The use of 56 kDa gellan fraction resulted in the formation of inflorescences with the greatest fresh weight in *S. atropurpurea*. Leaves of *R. hirta* treated with this fraction showed the highest values of SPAD and g_s. This study demonstrated that gellan derivatives of low M_W may be used for the production of innovative plant biostimulants.

Keywords: biopolymers; polysaccharides; floriculture; plant growth regulators

1. Introduction

Production of bedding plants, including annual, biennial, and perennial species, is one of the most important branches of the horticultural market. The sector has enjoyed dynamic growth due to a high public demand for ornamental plants and the increasing use of bedding plants in urban green areas. Standard and well-known species constitute the base of the bedding plant inventory and are being supplemented with new cultivars. Moreover, less common species are constantly being introduced to the market, as they are gaining popularity and can be sold at higher prices [1–3]. The sustainable development of floriculture requires modern and environmentally friendly solutions that can maximize the display of plant potential [4,5]. To this end, growers increasingly often use biostimulants that help to intensify plant propagation, growth, and flowering and improve stress tolerance [6–8]. Studies on the use of natural biostimulants in the cultivation of ornamental plants are considerably fewer than in the case of vegetables, fruits, and other crops [9,10], and this gap should be urgently filled.

Biostimulants comprise a wide and diverse group of plant growth improving compounds, including natural carbohydrate polymers and their derivatives [11,12]. The best known and widely tested biostimulating polysaccharides involve chitosan, alginate, and carrageenan [13–15]. They positively affect plant growth and flowering, improve plant stress resistance, and may serve as carriers of biologically active substances that further enhance their applicability. A number of research

works indicated have that depolymerized forms of biostimulating polysaccharides exerted stronger effects on plant growth than their parent compounds [16–20]. This may be due to their low molecular weight (M_W), which determines the biological activity of modified biopolymers [21,22].

Gellan gum is a natural extracellular polysaccharide produced during microbiological fermentation conducted by selected strains of *Sphingomonas elodea*, and is commonly used in food, cosmetic, and pharmaceutical products [23]. Due to its gelling and stabilizing properties, it is also a popular component of plant tissue culture media [24]. Studies showed that high gellan gum concentrations improved in vitro germination frequencies in *Pinus strobus* L. embryos [25] and somatic embryo development in *Pseudotsuga menziesii* (Mirb.) Franco [26]. In vivo application of native gellan gum and its derivative enhanced biomass growth, photosynthesis intensity, and mineral content in *Eucomis bicolor* L'Hér. and *E. comosa* Hort. ex Wehrh., with particularly strong stimulating effects of the depolymerized form of gellan gum with low M_W [27]. Investigating plant response to gellan gum derivatives of different M_W seems to be an interesting research challenge.

In this work, two depolymerized fractions of gellan gum of low M_W were obtained and used as potential biostimulants in the cultivation of four species of bedding plants: *Rudbeckia hirta* L. (black-eyed Susan), *Salvia splendens* Sellow ex J.A. Schultes (scarlet sage), *Scabiosa atropurpurea* L. (sweet scabious), and *Tithonia rotundifolia* (Mill.) S.F. Blake (Mexican sunflower). *S. splendens* and *R. hirta* belong to the most popular ornamental plants used in flowerbeds and gardens, while *S. atropurpurea* and *T. rotundifolia*, being less known bedding plants, deserve greater attention due to their attractive inflorescences that remain decorative for a long time. The objective of this study was to check whether and how gellan derivatives of different M_W affect plant growth, flowering, leaf relative chlorophyll content, and stomatal conductance (g_s).

2. Materials and Methods

2.1. Preparation and Determination of Depolymerized Gellan

Gellan fractions with low M_W were prepared by acid hydrolysis. A solution of gellan gum (Sigma-Aldrich, Poznań, Poland) was prepared by dissolving 20 g in 800 mL of deionized water at 70 °C. Concentrated 36% HCl (Chempur, Piekary Śląskie, Poland) was added to reach a final concentration of 0.2 M. The solution was incubated at 70 °C for 4 or 16 h with stirring.

M_W of the starting gellan gum and depolymerized fractions was determined by HPSEC using a S1000 pump, a S2300 refractive index detector, and a 20 µL sample loop (Knauer, Berlin, Germany). Separation was carried out using SUPREMA 10,000 Å 10 µm column (PSS, Mainz, Germany) eluted at a flow rate of 1 mL/min at 60 °C with 0.02% NaN_3 and 10 mM of disodium edetate to avoid gellan aggregation. More information on obtaining gellan gum fractions and their characteristics is provided in an earlier work [27].

2.2. Plant Material and Experimental Design

Seeds of *R. hirta*, *S. splendens* cv. 'Luna', *S. atropurpurea*, and *T. rotundifolia* obtained from W. Legutko Breeding and Seed Company (Jutrosin, Poland) were sown on 15 March 2016 into plastic boxes filled with TS1 substrate (Kronen, Cerkwica, Poland). After four weeks, single seedlings matched for size were transplanted into round plastic pots (13 cm in diameter, 1.0 dm^3 volume). The substrate consisted of deacidified peat (Kronen, Cerkwica, Poland), pH 6.1, supplemented with a multicomponent Hydrocomplex fertilizer (Yara International ASA, Oslo, Norway) at a dose of 3 $g \cdot dm^{-3}$, and containing 12% N (5% N-NO_3 and 7% N-NH_4), 11% P_2O_5, 18% K_2O, 2.7% MgO, 8% S, 0.015% B, 0.2% Fe, 0.02% Mn, and 0.02% Zn. The plants were grown under natural photoperiod, on tables arranged in a heated glasshouse with ventilation (22/18 °C day/night), located at the premises of the West Pomeranian University of Technology in Szczecin (53°25'44'' N, 14°33'10'' E).

The treatment with depolymerized gellan of different M_W (56 kDa and 77 kDa) began a week after transplanting. The plants were drenched seven times every three days with aqueous solutions of

gellan derivatives at a concentration of 100 mg/L (determined based on a preliminary trial). Each plant in the pot was drenched with 50 mL of the solution. Control plants were drenched with tap water. Each treatment included four replicates, and each experimental unit consisted of ten plants. Pots were arranged in a randomized complete block.

Daily observations enabled the determination of the number of days from sowing to the beginning of flowering, which was marked by the appearance of the first flowers in the inflorescences. At this stage, relative chlorophyll content, as determined according to the leaf greenness index SPAD (soil and plant analysis development, was measured using a chlorophyll meter SPAD 502 (Minolta, Osaka, Japan), and g_s was measured with a SC1 porometer (Dekagon Devices, Pullman, WA, USA). The measurements involved two fully developed, representative leaves of five plants matched for size from each replicate. The measurements were repeated three times for each leaf, and the mean value was calculated. At full stage flowering, five plants matched for size from each replicate were cut off at the substrate line and compared for their height, fresh weight of the above-ground part, and fresh weight of fully open inflorescence.

2.3. Statistical Analysis of Experimental Data

The results were checked for normality using the Shapiro–Wilk test and analyzed individually for each species using one-way analysis of variance (ANOVA) and Statistica 13.3 package (TIBCO Software Inc. StatSoft, Cracow, Poland). The Tukey's HSD test was employed to assess the significance between means, assuming a significance level of $p \leq 0.05$.

3. Results

Acid hydrolysis yielded degraded fractions of gellan gum of low M_W. Figure 1 shows changes in the M_W of depolymerized gum after hydrolysis. The starting gellan had M_W of 1000 kDa, which dropped after 4 and 16 hours of hydrolysis to 77 kDa and 56 kDa, respectively.

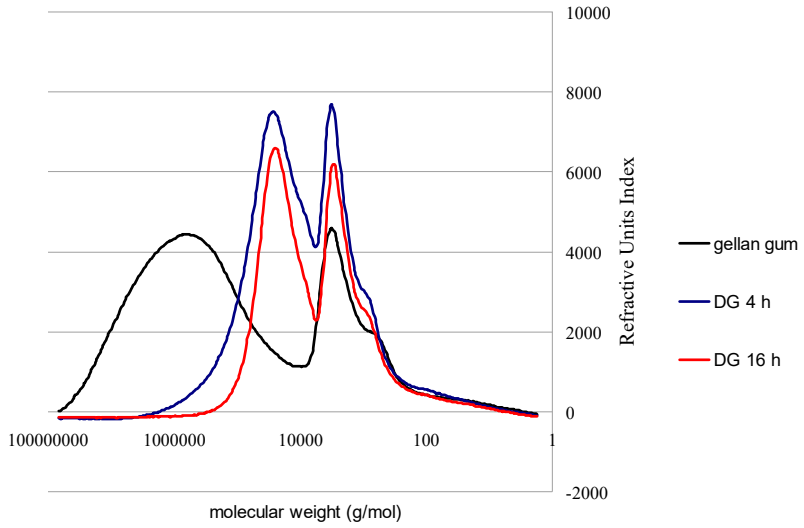


Figure 1. The molecular weight distribution of gellan gum and depolymerized gellan (DG) after 4 and 16 hours of hydrolysis.

The present data demonstrate that depolymerized gellan with low M_W stimulated the growth and flowering of all species tested (Figure 2, Figure 3a,b). The plants treated with depolymerized gellan of M_W 56 kDa and 77 kDa started flowering 4–5 days (*R. hirta*), 4–6 days (*S. splendens*), 6–7 days (*S. atropurpurea),* and 9–11 days (*T. rotundifolia*) earlier than the control plants. They were also 9.4–15.1% (*R. hirta*), 8.1–12.3% (*S. splendens*), 6.0–9.3% (*S. atropurpurea*), and 15.7–19.0% (*T. rotundifolia*) taller than non-treated plants. Gellan derivatives significantly increased the fresh weight of the

above-ground parts of the assessed plants (Figure 4a). Following depolymerized gellan application, plants grew by 29.0–33.2% (*R. hirta*), 18.8–26.6% (*S. splendens*), 13.8–21.9% (*S. atropurpurea*), and 47.0-72.4% (*T. rotundifolia*). The M_W of the derivatives had no effects on the flowering onset, height, and fresh weight of the above-ground parts.

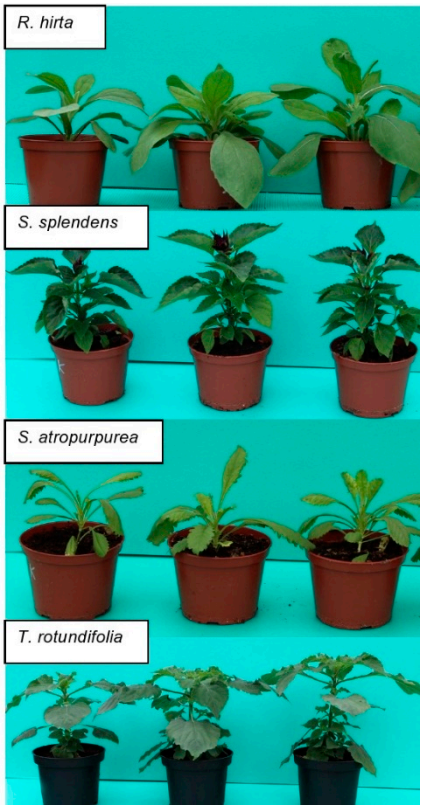
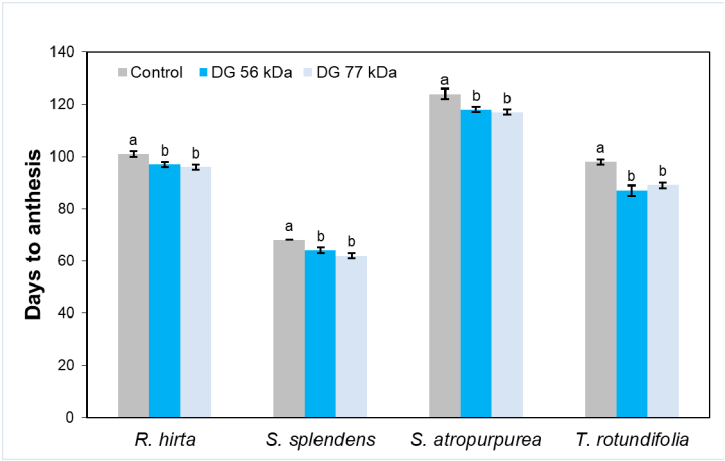
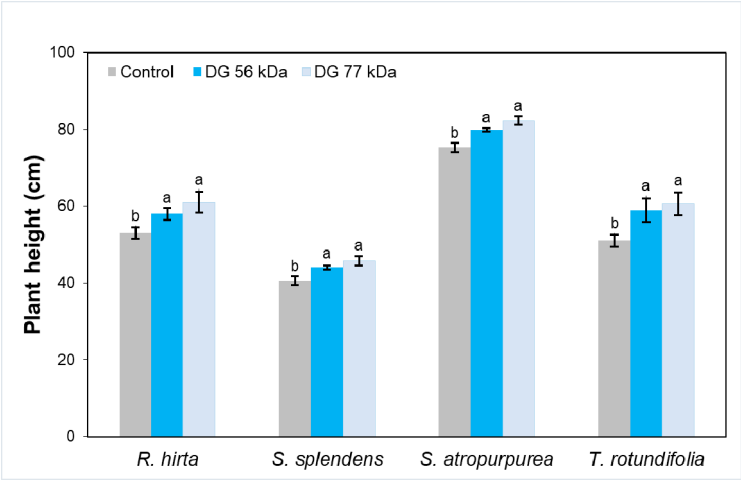


Figure 2. Physical appearance at week 10 from the sowing of four bedding plant species treated with depolymerized gellan (DG) with different molecular weights (M_W 56 kDa and 77 kDa); left to right: untreated control, DG 56 kDa, and DG 77 kDa.



(**a**)

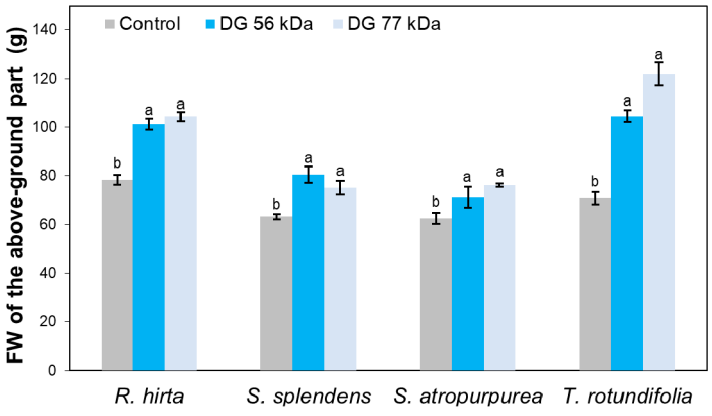
Figure 3. *Cont.*



(**b**)

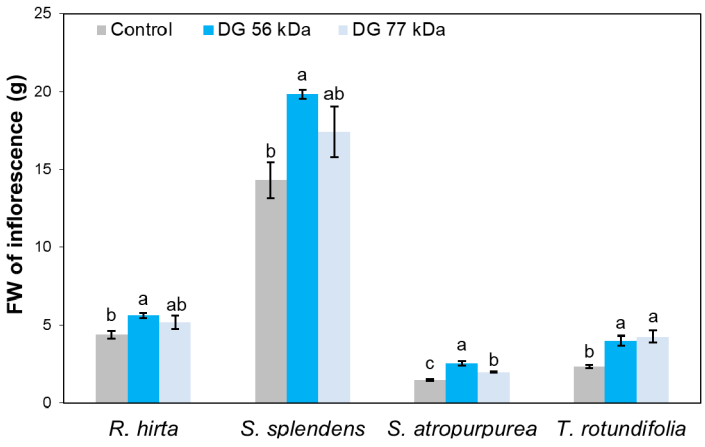
Figure 3. Days to anthesis (**a**) and plant height (**b**) of bedding plants treated with depolymerized gellan (DG) with different molecular weights (M_W 56 kDa and 77 kDa). Each treatment included four replicates and the values shown are the mean ± SEM. Columns labeled with the same letters are not significantly different at $p \leq 0.05$.

The results showed that depolymerized gellan positively affected inflorescence fresh weight and the effect depended on the species and M_W (Figure 4b). In *R. hirta* and *S. splendens*, drenching with depolymerized gellan of M_W 56 kDa enhanced the fresh weight of the inflorescences by 17.5–28.0% and 21.8–38.6%, respectively. In the remaining species, both derivatives (i.e., of M_W 56 kDa and 77 kDa) clearly stimulated plant growth by increasing inflorescence the fresh weight by 33.3–69.3% in *S. atropurpurea* and 71.7–83.3% in *T. rotundifolia*. In *S. atropurpurea*, the M_W of the depolymerized gellan significantly affected inflorescence weight, and the effect was more pronounced for M_W 56 kDa. Inflorescences of *T. rotundifolia* showed no significant differences in fresh weight depending on the depolymerized gellan M_W.



(**a**)

Figure 4. *Cont.*

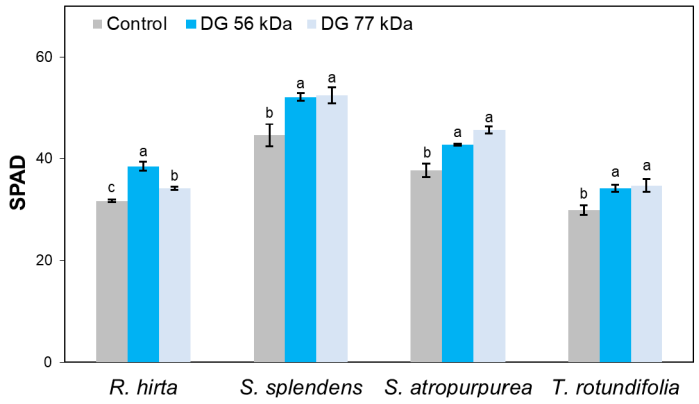


(**b**)

Figure 4. The effects of depolymerized gellan (DG) with different molecular weights (M_W 56 kDa and 77 kDa) on the fresh weight (FW) of the above-ground parts (**a**) and inflorescence (**b**) of greenhouse-grown bedding plants. Data represent the values at full stage flowering. Each treatment included four replicates and the values shown are the mean ± SEM. Columns labeled with the same letters are not significantly different at $p \leq 0.05$.

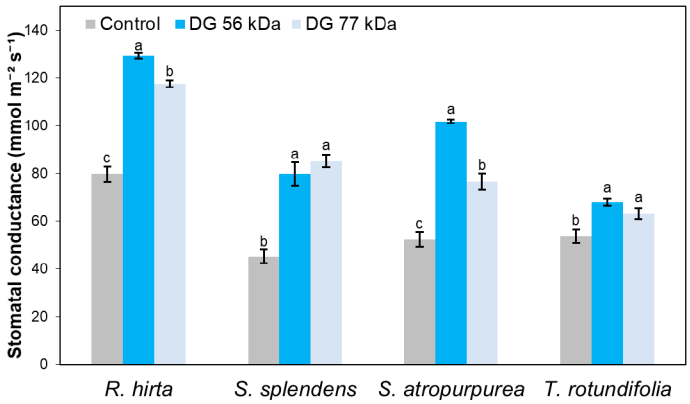
Treatments with both gellan fractions of different M_W enhanced relative leaf chlorophyll content (expressed as SPAD index) in all examined species (Figure 5a). SPAD in depolymerized gellan-treated plants was 7.9–21.5% (*R. hirta)*, 17.0–17.7% (*S. splendens*), 13.3–21.0% (*S. atropurpurea),* and by 14.2–16.2% (*T. rotundifolia)* higher than in the control plants. In *R. hirta*, the effect of the stimulant on SPAD depended significantly on its M_W. The depolymerized gellan of M_W 56 kDa most effectively enhanced SPAD vs. the non-treated plants.

The biostimulant also enhanced g_s by 47.4–62.1% in *R. hirta*, 46.5–94.6% in *S. atropurpurea*, 76.2–88.1% in *S. splendens,* and 17.8–26.7% *in T. rotundifolia* vs. the control plants (Figure 5b). In *R. hirta* and *S. atropurpurea*, g_s depended on the gellan M_W and was the highest in plants exposed to that of M_W 56 kDa.



(**a**)

Figure 5. *Cont*.



(**b**)

Figure 5. The effects of depolymerized gellan (DG) with different molecular weights (M_W 56 kDa and 77 kDa) on relative chlorophyll content-SPAD (soil and plant analysis development) index (**a**) and stomatal conductance (**b**) of greenhouse-grown bedding plants. Data represent the values at early stage flowering. Each treatment included four replicates and the values shown are the mean ± SEM. Columns labeled with the same letters are not significantly different at $p \leq 0.05$.

4. Discussion

This is the first study to compare the effects of depolymerized gellan of different M_W on growth, flowering, and selected physiological parameters of four ornamental plants. It was observed that drenching with depolymerized gellan solution accelerated flowering in all tested species, and thus shortened the growing cycle in the greenhouse. These results confirmed the results of a previous study that showed that gellan gum and its derivatives may affect the course of plant developmental stages and advance flowering [27]. This may be due to the fact that plants treated with depolymerized gellan were quicker to reach minimum parameters of, for example, weight and height that allow for flowering [28,29]. The transition from vegetative to generative stage is a highly complex process regulated and controlled by phytohormones and other growth regulators and chemical compounds, including oligosaccharides, which serve as signal molecules [30–32]. So far, the role of depolymerized gellan and other biostimulants based on polysaccharide derivatives in the induction of plant flowering is unknown.

The data indicated that the depolymerized fractions of gellan stimulated plant growth, as manifested by their increased height, fresh weight of the above-ground parts, and inflorescences. Degraded gellan gum exerted similarly beneficial effects on the biomass growth of *Perilla frutescens* (L.) Britt. var. *crispa* f. *purpurea* exposed to salt stress [33]. Stimulating activity of depolymerized gellan on plant growth may be associated with the enhanced activity of basic physiological processes, including photosynthesis, as shown in studies on geophytes *E. comosa* and *E. bicolor* [27]. Plant biomass increment largely depends on an internal photosynthesis related factor, that is, the content of assimilation pigments. Another crucial aspect is the condition of stomata directly affecting carbon dioxide assimilation and transpiration [34–36]. The present data demonstrated that the application of depolymerized gellan enhanced g_s and SPAD index (correlating with chlorophyll content), which might have eventually stimulated growth and intensified biomass accumulation.

Research studies [37,38] seem to agree that the biological activity of biopolymers depends on their structure and physical properties, particularly M_W. For example, even a small change in chitooligomer M_W may affect growth and physiological and biochemical processes in wheat seedlings [39]. This study showed no significant effects of M_W of gellan gum derivatives on flowering time, height, and the fresh weight of the above-ground parts of the analyzed plants. However, significant differences were found for inflorescence fresh weight in *S. atropurpurea* and for physiological parameters in *R. hirta*, indicating a stronger stimulating effect of the depolymerized gellan of lower M_W of 56 kDa. Other studies [21,40]

also reported intensified biomass production and photosynthesis following treatment with low M_W polysaccharide derivatives. Stronger biological activity of low M_W biopolymers may be due to their faster and easier penetration into plant tissues and cells, where they bind with cell membrane receptors and initiate metabolic changes [16,18,21].

Studies to date have indicated that carbohydrate polymers may stimulate metabolism in plant systems, including stress response metabolism [15,20,22,31]. This is because the natural bioactive polymers are similar to biotic elicitors [41]. At the cellular level, elicitor perception by pattern-recognition receptors activate plant innate immunity [42,43]. Derivatives of gellan gum seem to act as plant elicitors, as under salinity stress they stimulate the synthesis of secondary metabolites [33]. It is therefore possible that gellan derivatives induce anti-stress protective mechanisms in plants. Improved tolerance to unfavorable environmental conditions may translate into more intense plant growth and their increased physiological activity. The biostimulating effects of gellan derivatives may also be associated with beneficial bacteria of the *Sphingomonas* genus and their ability to produce sphingans, exopolysaccharides related to gellan gum [44]. It has been proven that *Sphingomonas* sp. Cra20 positively affects the growth of *Arabidopsis thaliana* (L.) Heynh. and increases plant tolerance to drought stress [45]. Similarly, enhanced growth and improved chlorophyll contents were observed in *Lycopersicon esculentum* Mill. inoculated with endophytic *Sphingomonas* sp. LK11 [46]. This study was an initial experiment, and the research will be continued in order to better understand the mechanism of gellan derivatives' biostimulatory action.

5. Conclusions

Plant growth and development may be positively affected by using natural biostimulants. This study assessed the effects of depolymerized gellan of different M_W (56 kDa and 77 kDa), yielded from gellan gum hydrolysis, on the growth and physiological parameters of four ornamental plants. Both gellan fractions positively affected growth, accelerated flowering, and increased the biomass of the above-ground parts. Depolymerized gellan triggered growth stimulation associated with enhanced relative content of chlorophyll and intensified g_s. Depending on the species, oligomer M_W differently affected inflorescence weight and physiological parameters. Gellan gum derivatives of low M_W may be used in floriculture to shorten the production time and enhance the bedding plant quality.

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Conflicts of Interest: The author declares no conflict of interest.

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