Available online at www.worldscientificnews.com

World Scientific News

WSN 62 (2017) 111-123 EISSN 2392-2192

Plant growth of *Verbena bonariensis* L. after chitosan, gellan gum or iota-carrageenan foliar applications

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ABSTRACT

Nowadays, the use of natural polysaccharides in the field of agriculture has increased in order to achieve sufficient yields and quality. Minimal research on effect of biopolymers on growth ornamental plants has been published. *Verbena bonariensis* L. is a valuable perennial species, recommended for cultivation in gardens and green areas, as well as cut and pot plant. The main objective of the investigation was to analyzed the effects of three polysaccharides on the growth and flowering of *V. bonariensis*. The plants were sprayed with aqueous solution of chitosan, gellan gum or iota-carrageenan at a concentration of 0.2%, five times. Control plants were treated with water. The results indicated that polysaccharides significantly enhanced the plant height, number of inflorescences and number of leaves of *V. bonariensis* plants. Application of chitosan and gellan gum significantly increased the stomatal conductance by 13.8 and 16.3 % and enhanced the number of shoots per plant by 29.4 and 37.5 % compared with that control, respectively. Among the three polysaccharides, gellan gum proved best and gave significantly maximum fresh weight of aboveground part and fresh weight of root by 34.3 and 114 % over the control one.

***Keywords*:** biostimulants; plant growth promoter; polysaccharides; purpletop vervain

1. INTRODUCTION

Natural polymers have widespread applications as a biostimulant to improve plant growth and have specific properties of being environmentally friendly and easily degradable [5,14,19]. Chitosan (poly-N-acetyl glucosamine), a natural non-toxic biopolymer, is a linear polycationic polysaccharide processed from the exoskeletons of crustaceans or cell wall of fungi [4,16]. Chitosan and its derivatives are produced by deacetylation of chitin (Fig. 1) and further derivatization [19]. Various biological and physiological activities of chitosan have been reported in plants. For example, chitosan promoted the seed germination, enhanced growth, flowering, photosynthesis, content of chlorophylls and mineral nutrient uptake [22,23]. Chitosan and its derivatives also stimulate defense against several pathogens in plants [4,16,25] and induce many enzymes and several genes in plants, such as glucanase and chitinase [19]. Effectiveness of chitosan depends on its physical-chemical properties, application methods, concentrations as well as the plant species and developmental stage [19,21-24].

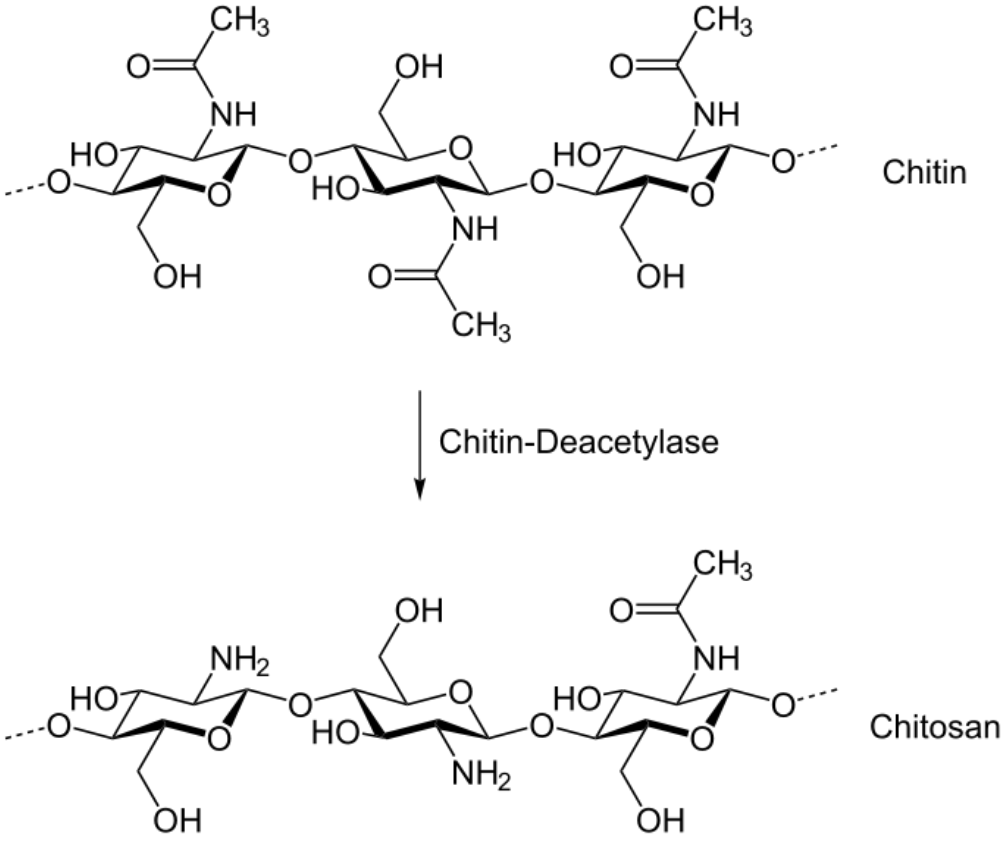

Figure 1. Synthesis of chitosan

Gellan gum is a water-soluble anionic polysaccharide produced by the bacterium *Sphingomonas elodea* (formerly *Pseudomonas elodea*) [27,31]. The polysaccharide is composed of tetrasaccharide repeating-units (Fig. 2) [12].

In micropropagation, gellan gum has been used as popular gelling agents [7-8,13,17,28]. The use of vermiculite-containing and gellan gum-solidified medium as the root-developing medium improved rooting of the microcuttings of *Castanea crenata* seedlings [24] and hybrid walnuts microcuttings [13,29] Limited attention has however been paid to the effects of these agents on plants growth *in vivo*.

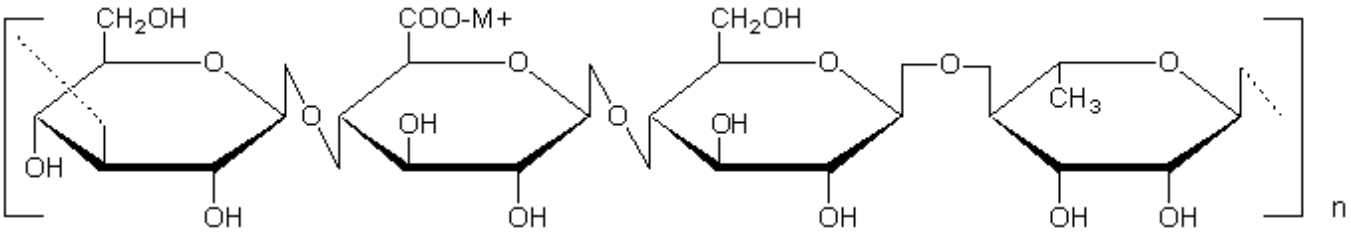

Figure 2. Structure of gellan gum

Carrageenans are natural polymers found in the cell walls of many red macroalgae (Rhodophyceae) with a repeating structure of alternating 1,3-linked β-D-galactophyranosyl arid 1,4-linked α-D-galactophyranosyl units [1]. There are three main varieties of carrageenans, which differ in their degree of sulphation (Fig. 3). Kappa-carrageenan has one sulphate group per disaccharide, iota-carrageenan has two, and lambda-carrageenan has three. Carrageenans may elicit various kinds of biological activities in plants [1-3,6,9,18,30]. Foliar application of oligo-carrageenans enhanced growth and productivity in tobacco, chickpea, maize, pechay, mint, pine and eucalyptus [1,5,10-11,26]. However, limited information is available for ornamental plants regarding effects of carrageenans or oligo-carrageenans.

Verbena bonariensis L., known as purpletop vervain, clustertop vervain, or tall verbena is a native plant to the tropical South America, from Colombia and Brazil to Argentina and Chile. It belongs to Verbenaceae with 211 species. Purpletop vervain is a rapid-growing, clump-forming tender perennial. Tall, upright branching stems hold clusters of magenta-purple flowers (Fig. 4) from early summer through late fall. The long lasting blooms of *V. bonariensis* attract clouds of bees and butterflies.

This perennial verbena has enjoyed a resurgence in popularity in recent years, associating beautifully with grasses for a tranquil planting scheme, or adding a touch of architectural style to the back of herbaceous borders. Purpletop vervain seems to be plant species tolerant to salinity and may be uses in urban landscape exposed to salt stress [20]. Furthermore, *V. bonariensis* is now becoming the promising both cut flower and container plant for outdoor space. However, the subject literature lacks data on *V. bonariensis* growth, which is a problem for the producers interested in wider use of this species. So far, no information has been published on the use of natural polysaccharides as plant growth promoter in *V. bonariensis* cultivation. In the present study, therefore, we analyzed the effects of three polysaccharides (chitosan, gellan gum and iota-carrageenan) on the growth and flowering of *V. bonariensis*.

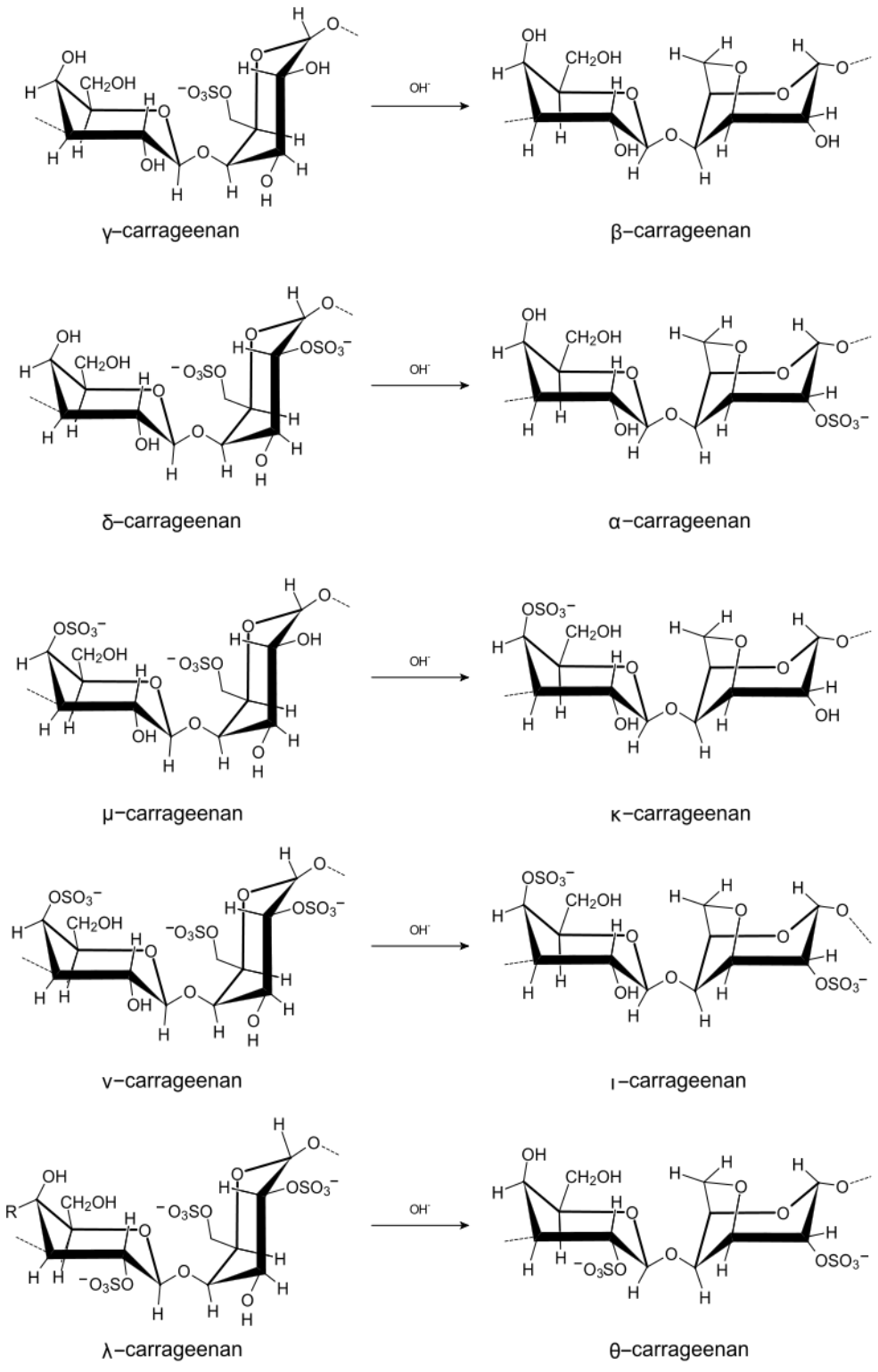

Figure 3. Structure of carrageenans

Figure 4. Cymes of the purpletop verbena (*Verbena bonariensis* L.)

2. MATERIALS AND METHODS

The study was conducted in a greenhouse and a plastic tunnel belonging to the West Pomeranian University of Technology in Szczecin (53°25' N, 14°32' E; 25 m a.s.l.). Sterilized seeds of *V. bonariensis* were sown on 12 March 2014 into boxes filled with substrates. Seeds were germinated under dark conditions in an environmentally controlled greenhouse with temperatures set 21 ºC day/ 17 ºC night and light intensity ranged from 314 to 890 µmol/s m^2. After germination, the seedlings were planted in the pots (0.4 L volume) containing a sphagnum peat (pH 6.5), supplemented with 1 g L^{-1} of a fertilizer Azofoska (8.1% N-NH_4, 5.5% N-NO_3, 6.4% P_2O_5, 19.1% K_2O, 4.5% MgO, 23.0% SO_3, 0.045% B, 0.18% Cu, 0.17% Fe, 0.27% Mn, 0.04% Mo, 0.045% Zn, and Cl >0.4%). On 18 May 2014 one plant was maintained in each pot (5 L volume). The pots were filed with a sphagnum peat (pH 6.5) with a mixture of fertilizer Azofoska (3 g·L^{-1}). The plants were grown under natural light in an unheated plastic tunnel and irrigated with tap water when substrate appeared dry. All temperature and relative humidity data were uploaded using the data logger Testo 175-H2 (Fig. 5-6). The plants were sprayed in the lower parts of the leaves with 10 ml of an aqueous solution of chitosan, gellan gum or iota-carrageenan at a concentration of 0.2%, five times, every seven days from 3 June 2014. Control plants were treated with water. Polysaccharides were purchased from Sigma-Aldrich. The beginning of anthesis was the day on which fully opened flowers in inflorescence were noticed in 5% of plants per individual variant. At this

stage, the stomatal conductance was measured with SC1 porometer (Decagon Devices Inc., Pullman, WA, USA). The measurements included six leaves and three readings were taken per each leaf (the abaxial side). Total plant height, number of shoots per plant, number of inflorescences per plant, number of leaves per plant, fresh weight of aboveground part and fresh weight of root were estimated on the last day (20 September 2014) of the cultivation.

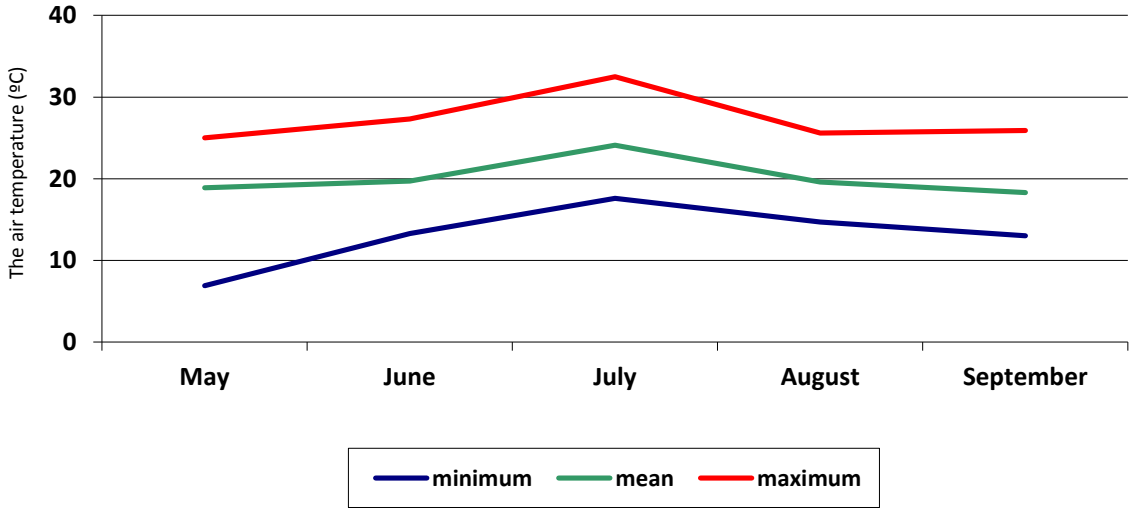

Figure 5. The air temperature (ºC) during the experiment

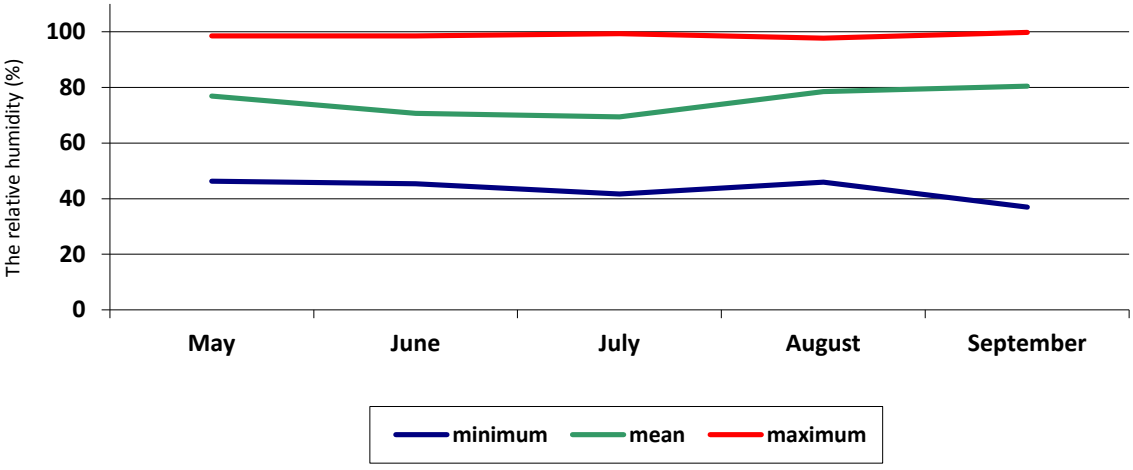

Figure 6. The relative humidity (%) during the experiment

Each treatment was replicated four times and each replicated included 8 plants. The pots were arranged in a complete randomized design. The mean values were calculated using the analysis of variance ANOVA using the Statistica 12.0 software (Statsoft, Poland).

3. RESULTS AND DISSCUSION

Statistical analysis of the date showed the different impact of chitosan, gellan and iota-carrageenan on stomatal conductance, plant height, number of shoots, inflorescences and leaves per plant, fresh weight of aboveground part and root of *V. bonariensis* (Fig. 7-13). In general, all polysaccharides had a significant positive effect on plant height, number of inflorescences and number of leaves per plant (Fig. 8, 10-11). Anthesis was not affected by polysaccharides treatments (date not shown).

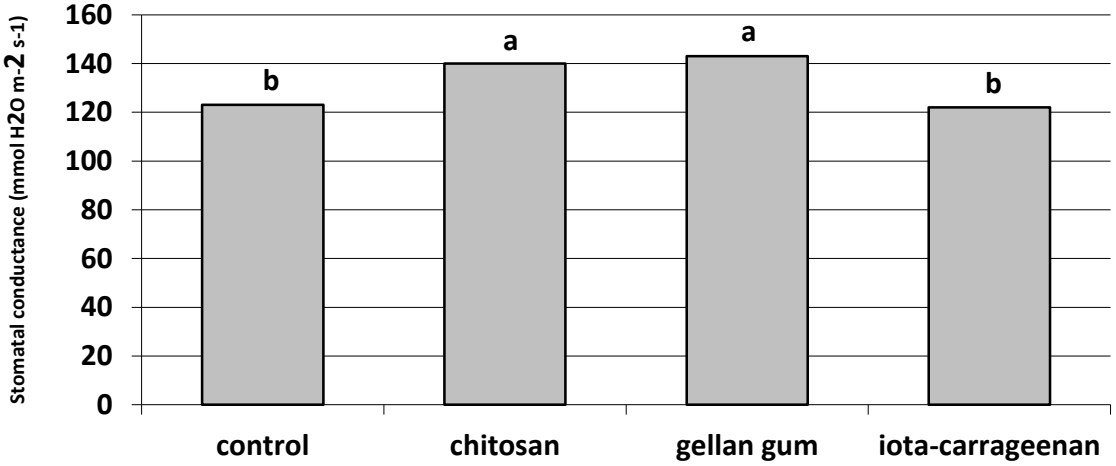

Figure 7. Effect of chitosan, gellan gum and iota-carrageenan on the stomatal conductance of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

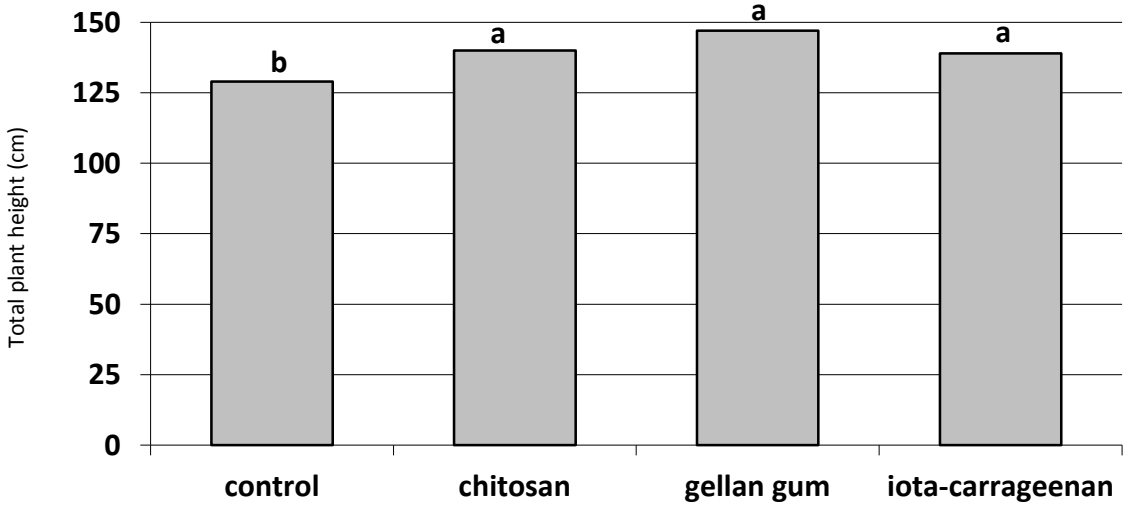

Figure 8. Effect of chitosan, gellan gum and iota-carrageenan on the total plant height of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

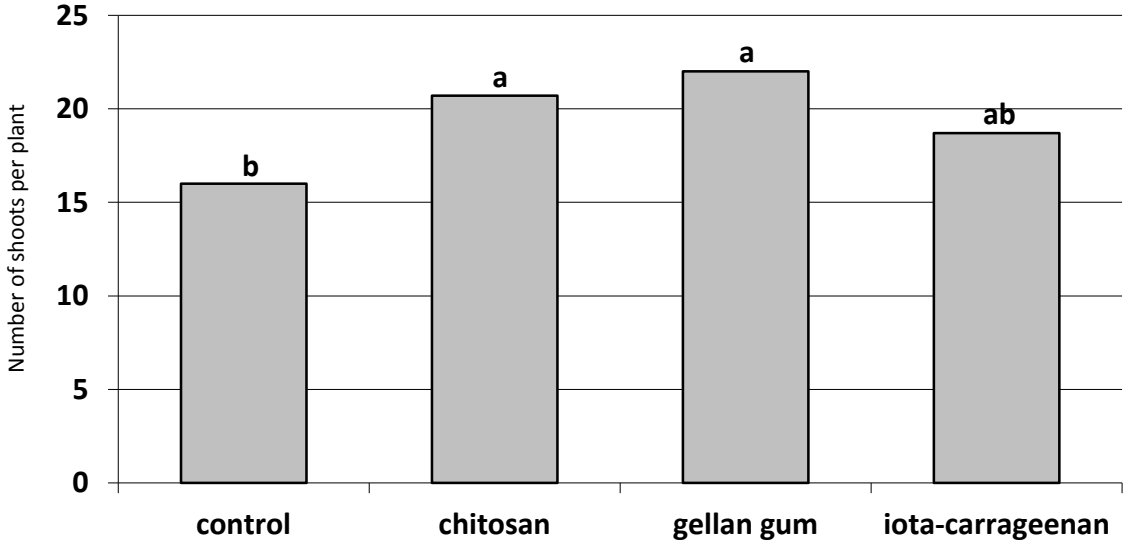

Figure 9. Effect of chitosan, gellan gum and iota-carrageenan on the number of shoots per plant of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

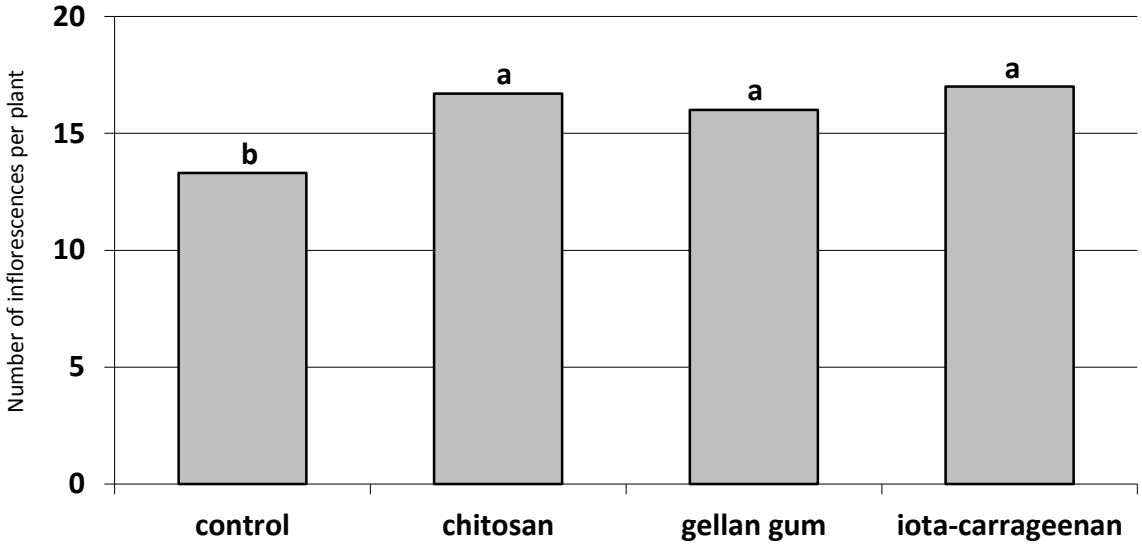

Figure 10. Effect of chitosan, gellan gum and iota-carrageenan on the number of inflorescences per plant of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

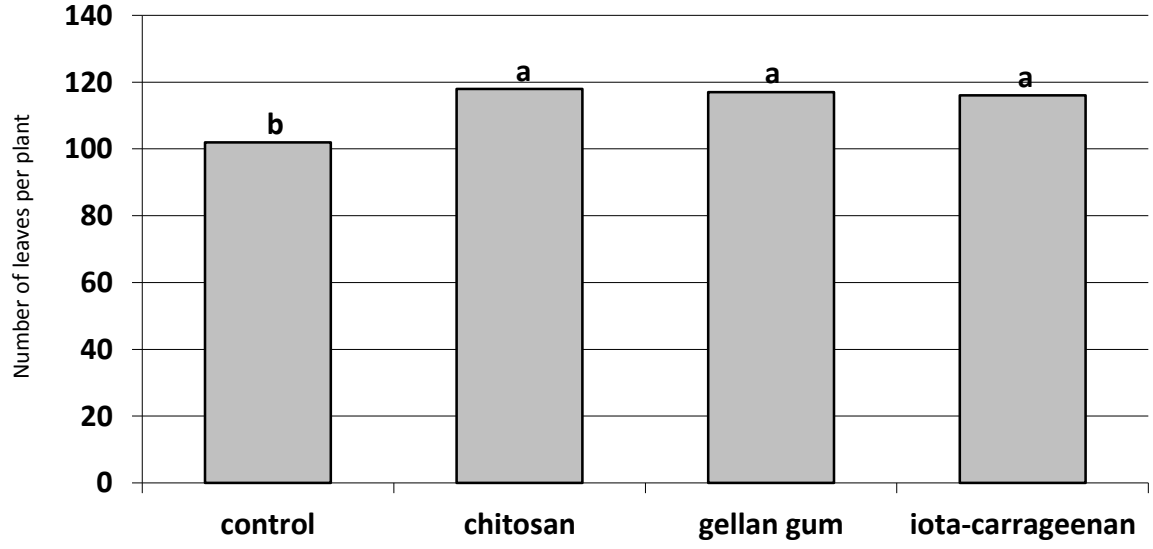

Figure 11. Effect of chitosan, gellan gum and iota-carrageenan on the number of leaves per plant of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

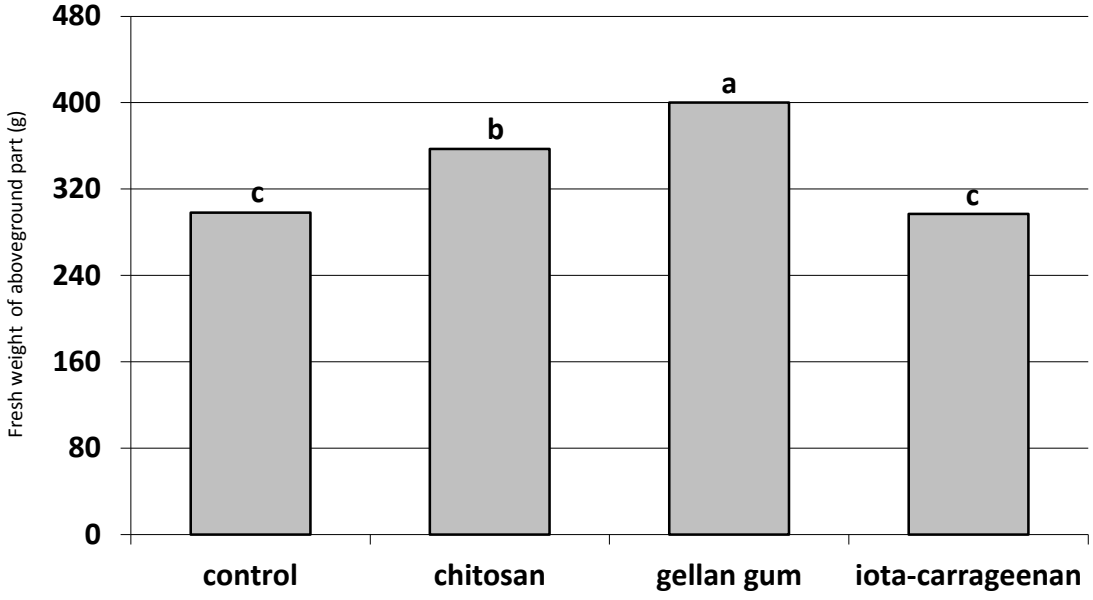

Figure 12. Effect of chitosan, gellan gum and iota-carrageenan on the fresh weight of aboveground part of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

Application of chitosan significantly increased the stomatal conductance by 13.8 % in comparison to the untreated plants (Fig. 7). The spray of chitosan also enhanced the plant height, number of shoots, number of leaves and fresh weight of aboveground part by 5.53, 29.4, 15.7 and 19.8 % compared with that control, respectively (Fig. 8-9, 11-12). Our results are in agreement with the previous reports on the effect of chitosan on other ornamental plants [19,22-23]. Many researchers reported that application of chitosan, or fractions such as chitosan oligomers increased the fresh biomass, shoot height, leaf number and root lenght [15-16,19,24]. Besides, chitosan also increased photosynthetic parameters such as net photosyntheis rate, stomatal conductance and internal CO_2 concentration [16,19]. The increase in stomatal conductance with chitosan treatments might be due to its positive effect on growth parameters of *V. bonariensis* plants.

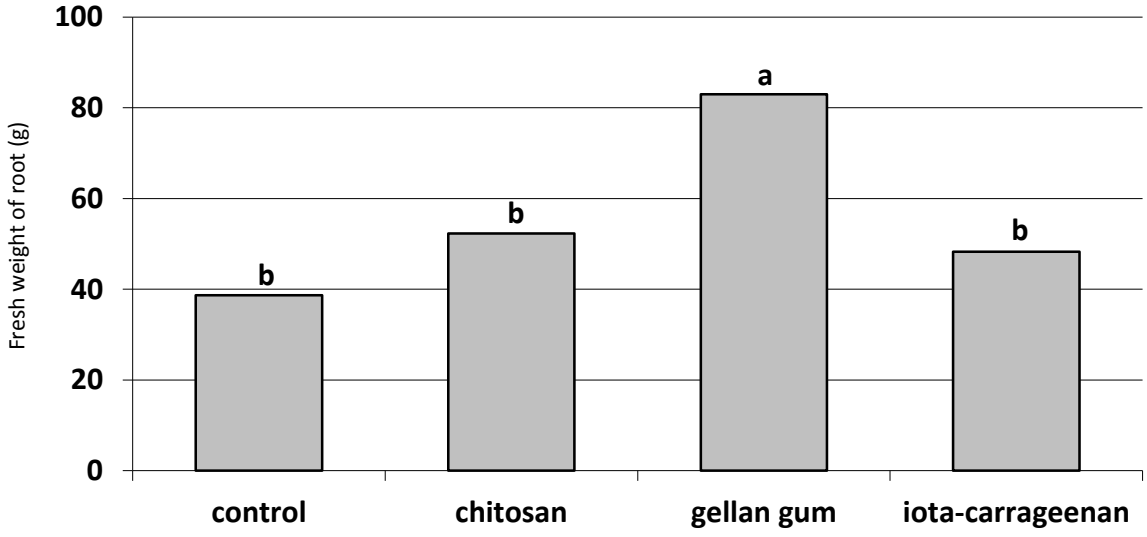

Figure 13. Effect of chitosan, gellan gum and iota-carrageenan on the fresh weight of root of purpletop vervain (*Verbena bonariensis* L.). Date are means of 4 replicates. Different letters on the columns indicate significant differences at $P < 0.05$ by Duncan's Multiple Range test.

Foliar spray of gellan gum has promotive effect on all the growth attributes studied (Fig. 7-13). As compared with the control, application of gellan gum significantly improved the stomatal conductance, plant height, number of shoots, number of inflorescences, number of leaves, fresh weight of aboveground part and fresh weight of root by 16.3, 13.9, 37.5, 20.3, 14.7, 34.2 and 114 %, respectively. Among different tratments, gellan gum proved the best and gave significantly maximum value for fresh weight of aboveground part and fresh weight of root. Recent results have shown that gellan gum, as compared to agar, improved the efficiency of *in vitro* culture giving more shoots with higher fresh and dry weight in giant reed (*Arundo donax* L.) [7].

Moreover, gellan gum-solidified media had higher number of total and bigger shoots of *Eucomis autumnalis* subspesies *autumnalis* when compared to agar treatment [17]. Coating of twin-scale cuttings of *Eucomis comosa* 'Sparkling Burgundy' and 'Twinkly Stars' in gellan

gum had a positive impact on the number and weight of the bulblets [24]. Coating *Ornithogalum saundersiae* cuttings in gellan gum significantly enhanced the adventitious root length [21]. Numerous gelling agents contain water-soluble root-stimulating substances such auxin or auxin-like compounds which could potentially affect the growth and development of plants.

V. bonariensis plants treated with iota-carrageenan showed an increase in plant height, number of inflorescences and number of leaves by 7.75, 27.8 and 13.7 % compared to control plants (Fig. 8, 10-11). Similarly, applications of carrageenans kappa obtained from *Hypnea musciformis* to the soli or to the leaves, promoted the growth and increased the height, number of pods, number of leaves, and induced an early anthesis of *Cicer arietinum* [5]. Previous study have shown that oligomeric forms of carrageenans stimulate plant growth by regulating a serial process of growth in plants, including cell division, metabolic pathways and photosynthesis [1-2,10-11,18]. Moreover, it has been identified that depolymerised carrageenans increase the content of essential oils and polyphenolic compounds with antipathogenic activities, and induce plant defense responses against insect and pathogens such as viruses, bacteria and fungi [3,9-11,18,30].

4. CONCLUSIONS

The overall results of the present work suggest that exogenous application of chitosan, gellan gum and iota-carrageenan improved the plant height, number of inflorescences and leaves of *V. bonariensis* plants. Moreover, treatment with gellan gum was the best to enhance both fresh weight of aboveground part and fresh weight of root. The resulting information in this report could be useful for *V. bonariensis* growers to produce high-quality plants. However, further investigations are required to comprehend the mechanism of chitosan, gellan gum and iota-carrageenan effect on plant growth, including physiological and biochemical responses at molecular level.

Acknowledgement

This study was supported by the Polish Ministry of Science and Higher Education, within the project UPB 518-07-014-3176-02/18 ZUT.

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(Received 12 December 2016; accepted 27 December 2016)