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CONTENT OF MACRO- AND MICRONUTRIENTS IN CHITOSAN TREATED FREESIA (*Freesia* Eckl. ex Klatt) LEAVES

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ABSTRACT

Freesia grown under cover to be marketed as cut flower is highly sensitive to substrate temperatures exceeding 15–18°C. However, freesia varieties of Beach group are especially attractive plants that may be easily cultivated under cover and do not require substrate cooling. Experiments were conducted in summer and fall of 2011 and 2012 in an unheated plastic tunnel. Planting material consisted of daughter bulbs of 'Summer Beach' freesia. The plants were treated with chitosan with a molecular weight of 8000 $g \cdot dm^{-3}$. Experimental variants involved methods of chitosan application (watering vs. spraying), its concentration (0.2 vs. 0.4%) and frequency of application (7 vs. 14 days). During the flowering period and at the end of vegetation, freesia leaves were collected to determine the content of following micro- and macronutrients: N, P, K, Ca, Mg, Zn, Cu, Mn, and Fe. The leaves collected at the end of vegetation season contained more P, K, Ca, Fe, Mn, and Zn than those collected during flowering. The content of Mg and Cu was similar in both cases, but N level was lower at the end of vegetation. Irrespective of experimental variant, leaves of all plants treated with chitosan accumulated more N, P, Ca, Cu, Fe and Mn and less Zn during the entire vegetation season than the control ones. At the end of the vegetation season, plants sprayed with chitosan revealed higher concentration of N, P, Ca, Mg, Fe, and Zn, and lower concentration of K, Cu, and Mn than those watered with the investigated compound. No clear patterns of micro- and macronutrient accumulation depending on chitosan concentration were observed. Plants treated with 0.2% chitosan contained more P, K, Mg, Mn, and Zn than those exposed to its two times higher concentration. A contrary response was observed for the leaf accumulation of N and Fe.

Key words: application method, concentration, frequency of application, 'Summer Beach'

INTRODUCTION

Chitosan improves a structure of sandy soils by increasing the cohesion between their particles [Górnik et al. 2008]. It also facilitates plant production on soils contaminated with large amounts of minerals [Bassi et al. 2000, Kamari et al. 2012]. According to Bennewitz and Hlusek [2006], this compound facilitates nutrient uptake from the soil, and may be also used for controlled release of agrochemicals [Pérez Quinones et al. 2011] and fertilizers [Khorrami et al. 2012] or as a fertilizer for plant cultivation [Sukwattanasinitt et al. 2001]. Research literature contains numerous reports on positive effects of chitosan on growth, development and decorative value of ornamental plants [Ohta et al. 2004, Żurawik et al. 2017]. However, the way it affects the mineral uptake from the substrate and chemical composition of leaves exposed to its presence is still unknown. Chemical properties of chitosan depend on its morphological

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structure – crystallinity [Mucha 2010], degree of deacetylation – degree of chitin into chitosan conversion [Kananont et al. 2010, Rodríguez et al. 2010] and degree of polymerization – i.e. molecular weight [Limpanavech et al. 2008]. The effects chitosan exerts on plants during cultivation depend on multiple factors, e.g. plant species [Kananont et al. 2010, Obsuwan et al. 2010], variety [Żurawik and Bartkowiak 2009], method of application [Algam et al. 2010], concentration [Mahdavi et al. 2011, Sheikha and AL-Malki 2011], and number of treatments [El-Tanahy et al. 2012, Żurawik et al. 2017].

Therefore, the aim of this study was to determine the effects of the application method, concentration and frequency of chitosan application on chemical composition of 'Summer Beach' freesia cultivated for cut flowers.

MATERIAL AND METHODS

The study was conducted in 2011–2012 in an unheated plastic tunnel (14°31'E and 53°26'N). The freesias were cultivated in spring and summer without substrate cooling. Daughter bulbs of 'Summer Beach' freesia weighing 5.63–6.48 g (2011) and 12.34–13.75 g (2012) were planted. The bulbs were put into 45.5 dm^3 boxes filled with substrate based of high peat and deacidified up to pH 6.2 based on a neutralization curve by adding 5 g of chalk (CaO 36.9%) and 5 g of dolomite (CaO 45.0, MgO 15.0%) per 1 dm^3. Nutrients were supplemented in a form of a multicomponent fertilizer Azofoska (N 13.6, P_2O_5 6.4, K_2O 19.1, MgO 4.5, B 0.045, Cu 0.180, Fe 0.17, Mn 0.27, Mo 0.040, Zn 0.045%), at the dose of 1.5 $g \cdot dm^{-3}$. During growth, the plants were fertilized every two weeks by watering with 0.2% solution of Peters Professional Floral Feed (N 27.0, P_2O_5 15.0, K_2O 12.0, MgO 0.1, B 0.02, Cu 0.063, Fe 0.12, Mn 0.06, Mo 0.001, Zn 0.06%), and then with Peters Professional Blossom Booster (N 10.0, P_2O_5 30.0, K_2O 20.0, MgO 2.0, B 0.20, Cu 0.015, Fe 0.12, Mn 0.06, Mo 0.01, Zn 0.015%). The last dose of the fertilizer was applied six weeks before the end of experiment.

Plants were treated with chitosan of molecular weight 8000 $g \cdot mol^{-1}$ at 0.2% or 0.4%. The compound was applied by watering or spraying. The first application was conducted at the stage of two leaves, and the treatments were repeated every 7 or 14 days. In total, there were 24 or 12 treatments, each using 10 ml of the solution per plant. Control plants were watered or sprayed with water. A solution of 1% chitosan was prepared as follows: 8 g of the compound were dissolved in 300 ml of distilled water and pH was adjusted to 7.7 with 12 ml of 1 M NaOH. Then the solution was diluted with distilled water to a weight of 500 g. Subsequently, it was mixed with 500 g of 0.2 M CH_3COOH. Solutions of lower concentrations were obtained by adding 0.1 M CH_3COOH as a solvent.

Plant material was harvested at the peak of flowering period and after completing the vegetation period. The analyzed leaves were fully developed, with no signs of disease or pest infestation. They were analyzed for the content of following micro- and macronutrients: N, P, K, Ca, Mg, Zn, Cu, Mn, and Fe. Chemical analyses were performed as described by Ostrowska et al. [1991]. Total nitrogen was determined according to Kjeldahl method (mineralization in concentrated sulfuric acid and adding selenium mixture), total phosphorus with colorimetric method according to Barton, potassium and total calcium by flame photometry, and magnesium, copper, iron, manganese and zinc by atomic absorption spectrometry (ASA) following sample mineralization in 1 : 1 mixture of nitric and chloric acids.

They study evaluated 12 experimental variants combining the method of application (2), concentration (3) and frequency of chitosan application (2). Each variant comprised 45 plants divided into three replicates of 15 individuals. The results were statistically verified by means of a fully randomized three-factor analysis of variance for individual years and also collectively for two years. Mean results were compared using Tukey's test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

As reported by Babel and Kurniwan [2003], Bautista-Baños et al. [2006] and El Hadrami et al. [2010], chitosan is capable of chelating and binding metal ions. Becker et al. [2000] claimed that it might be used in plant production as a fertilizer or a coating that retards nutrient release from fertilizers [Wu et al. 2008, Chen et al. 2013]. In our study, the use of chitosan at the peak of flowering improved accumulation of all analyzed macronutrients in the leaves, irrespective of other parameters (Tab. 1). According to Winkler et al. [2017], plants may be capable of degrading chitosan and releasing small fragments of chitin that may be used as a source of nutrients. In our study, plants sprayed with the compound contained more nitrogen than those watered with chitosan solution. A reverse observation was made for P, K and Ca. No significant effects of chitosan application method were found on leaf content of magnesium. Irrespective of chitosan concentration, freesia leaves treated every seven days contained more nitrogen than those treated every 14 days. Leaves of plants treated every two weeks accumulated more potassium and calcium. The study showed no significant effect of chitosan application on the plant content of phosphorus and magnesium. Żurawik [2013] reported that chitosan of molecular weight 10 000 $g{\cdot}mol^{-1}$ and at concentration of 0.4% increased phosphorus and potassium leaf content in 'Silver Beach' freesia. These outcomes were not confirmed in our study for 'Summer Beach' variety treated with chitosan of molecular weight 8000 $g{\cdot}mol^{-1}$. Leaves of plants treated with 0.2% chitosan contained twice as more these macronutrients as those treated with 0.4% chitosan. The differences are most probably due to variable plant response to the molecular weight of chitosan [Winkler et al. 2017]. Irrespective of chitosan application method and its concentration, the lowest content of K and Ca was detected in control leaves, while the highest in those treated with 0.2% solution. Nitrogen and magnesium were more abundant in plants treated with 0.2% and 0.4% chitosan than in the control samples. The freesias treated with chitosan at 0.2% concentration were found to have the highest content of phosphorus as compared with those treated with chitosan at 0.4% and control ones. Żurawik [2013] demonstrated 50% difference in leaf content of potassium in 'Silver Beach' freesia exposed to chitosan. In our study, the differences were considerably lower and ranged from 9.0% to 11.6%, which might be due to variable response of freesia from Beach variety to chitosan treatment [Żurawik and Bartkowiak 2009]. A significant correlation was found during flowering between chitosan concentration and its application method and N, P and Ca content in the leaves. However, variable relationships between chitosan concentration and frequency of its application were found for the accumulation of N, K and Ca. Analysis of variance for K and Ca content revealed significant correlation between chitosan application method and its use frequency.

Previous studies of Żurawik [2013] demonstrated a relationship between the content of micronutrients in freesia leaves and chitosan application method. This was confirmed in our study, in which chitosan with molecular weight of 8000 $g{\cdot}mol^{-1}$ was used during flowering (Tab. 2). More Cu, Fe and Mn was detected in the leaves of 'Summer Beach' freesia when the plants were sprayed than watered with chitosan. A reverse relationship was observed for zinc. Regardless of the application method and concentration, plants treated with chitosan solution every seven days accumulated more Cu and Fe than those treated every two weeks. Contrasting results were obtained for Mn and Zn. Exposure of 'Silver Beach' freesia to a solution of chitosan with molecular weight of 10 000 $g{\cdot}mol^{-1}$ did not significantly affect leaf content of Fe and Mn [Żurawik 2013]. These outcomes were not confirmed in our study for 'Summer Beach' freesia. It was probably due to different polymerization degree that affects the biological activity of the polymer [Zou et al. 2017]. Application of both 0.2% and 0.4% solutions of 8000 $g{\cdot}mol^{-1}$ chitosan resulted in higher content of these micronutrients in treated vs. non-treated plants. Considering Cu content, it was the highest in plants treated with 0.4% solution of the biopolymer and the lowest in those from the control variant. The highest content of Zn was determined for the leaves of plants exposed to 0.2% chitosan solution. Further increase of the biopolymer concentration to 0.4% resulted in lower Zn content as compared with control plants. For all determined micronutrients, significant correlation was found between chitosan concentration and its application method as well as between its concentration and frequency of application. In terms of Zn content, no clear relationship was determined for chitosan application method and frequency of its use.

Table 1. Content of macronutrients (g·kg^{-1} d.w.) in leaves of 'Summer Beach' freesia depending on chitosan concentration, application method and frequency of its application – flowering peak

Macronutrient (g·kg^{-1} d.w.)	Concentration (%) (C)	Application method (A)		Frequency of use (B)		Mean
		watering	spraying	I	II	
N	0.0	26.8	27.0	27.2	26.6	26.9
	0.2	28.5	29.7	29.0	29.2	29.1
	0.4	28.9	29.5	29.6	28.9	29.2
Mean		28.1	28.7	28.6	28.2	28.4
$LSD_{0.05}$		A – 0.26 B – 0.26 C – 0.39 A × C – 0.46 C × A – 0.55 A × B – n.s. B × C – 0.46 C × B – 0.55 A × B × C – n.s.				
P	0.0	5.65	5.33	5.45	5.53	5.49
	0.2	7.30	5.98	6.80	6.48	6.64
	0.4	6.00	5.30	5.80	5.50	5.65
Mean		6.32	5.54	6.02	5.84	5.93
$LSD_{0.05}$		A – 0.296 B – n.s. C – 0.425 A × C – 0.490 C × A – 0.654 A × B – n.s. B × C – n.s. A × B × C – n.s.				
K	0.0	27.2	26.1	26.2	27.1	26.7
	0.2	30.3	29.3	28.4	31.2	29.8
	0.4	29.4	28.7	28.8	29.4	29.1
Mean		29.0	28.0	27.8	29.2	28.5
$LSD_{0.05}$		A – 0.26 B – 0.26 C – 0.39 A × C – n.s. A × B – 0,37 B × A – 0.37 B × C – 0.45 C × B – 0.55 A × B × C – n.s.				
Ca	0.0	7.03	7.43	7.15	7.30	7.23
	0.2	9.30	8.90	9.05	9.15	9.10
	0.4	9.24	7.80	7.96	9.08	8.52
Mean		8.52	8.04	8.05	8.51	8.28
$LSD_{0.05}$		A – 0.263 B – 0.263 C – 0.396 A × C – 0.464 C × A – 0.553 A × B – 0.372 B × A – 0.372 B × C – 0.464 C × B – 0.553 A × B × C – n.s.				
Mg	0.0	1.88	1.95	1.98	1.85	1.92
	0.2	2.65	2.65	2.73	2.58	2.65
	0.4	2.45	2.30	2.18	2.58	2.38
Mean		2.33	2.30	2.30	2.34	2.32
$LSD_{0.05}$		A – n.s. B – n.s. C – 0.347 A × C – n.s. A × B – n.s. B × C – n.s. A × B × C – n.s.				

Explanations: I – every 7 days, II – every 14 days, n.s. – not significant difference

Table 2. Content of micronutrients (mg·kg^{-1} d.w.) in leaves of 'Summer Beach' freesia depending on chitosan concentration, application method and frequency of its application – flowering peak

Micronutrient (mg·kg^{-1} d.w.)	Concentration (%) (C)	Application method (A)		Frequency of use (B)		Mean
		watering	spraying	I	II	
Cu	0.0	4.29	4.28	4.25	4.31	4.28
	0.2	4.26	4.68	4.72	4.22	4.47
	0.4	4.49	5.12	5.10	4.51	4.81
Mean		4.35	4.70	4.69	4.35	4.52
$LSD_{0.05}$		A – 0.029 B – 0.029 C – 0.043 A × C – 0.051 C × A – 0.061 A × B – n.s. B × C – 0.051 C × B – 0.061 A × B × C – n.s.				
Fe	0.0	112.9	112.8	114.8	110.9	112.9
	0.2	124.0	148.0	134.2	137.8	136.0
	0.4	122.6	150.2	142.4	130.4	136.4
Mean		119.8	137.0	130.5	126.4	128.4
$LSD_{0.05}$		A – 0.45 B – 0.45 C – 0.66 A × C – 0.77 C × A – 0.94 A × B – n.s. B × C – 0.77 C × B – 0.94 A × B × C – n.s.				
Mn	0.0	108.3	108.0	106.9	109.4	108.2
	0.2	120.4	130.9	120.8	130.5	125.7
	0.4	122.5	129.5	128.7	123.3	126.0
Mean		117.1	122.8	118.8	121.1	120.0
$LSD_{0.05}$		A – 0.39 B – 0.39 C – 0.58 AxC – 0.68 C × A – 0.82 A × B – n.s. B × C – 0.68 C × B – 0.82 A × B × C – n.s.				
Zn	0.0	63.5	63.4	61.7	65.2	63.5
	0.2	74.7	66.1	72.1	68.7	70.4
	0.4	60.0	62.8	60.3	62.5	61.4
Mean		66.1	64.1	64.7	65.5	65.1
$LSD_{0.05}$		A – 0.36 B – 0.36 C – 0.54 A × C – 0.63 C × A – 0.76 A × B – 0.51 B × A – 0.51 B × C – 0.63 C × B – 0.76 A × B × C – n.s.				

Explanations: I – every 7 days, II – every 14 days, n.s. – not significant difference

Table 3. Content of macronutrients (g·kg^{-1} d.w.) in leaves of 'Summer Beach' freesia depending on chitosan concentration, application method and frequency of its application – end of vegetation period

Macronutrient (g·kg^{-1} d.w.)	Concentration (%) (C)	Application method (A)		Frequency of use (B)		Mean
		watering	spraying	I	II	
N	0.0	21.4	21.3	21.5	21.2	21.4
	0.2	22.2	24.7	24.3	22.6	23.5
	0.4	24.0	23.5	25.6	21.9	23.8
Mean		22.5	23.2	23.8	21.9	22.9
$LSD_{0.05}$		A – 0.34 B – 0.34 C – 0.50 A × C – 0.58 C × A – 0.71 A × B – 0.48 B × A – 0.48 B × C – 0.58 C × B – 0.71 A × B × C – n.s.				
P	0.0	79.0	82.5	80.5	81.0	80.8
	0.2	101.3	99.4	96.5	104.1	100.3
	0.4	94.6	100.0	87.1	107.5	97.3
Mean		91.6	94.0	88.0	97.5	92.8
$LSD_{0.05}$		A – 0.67 B – 0.67 C – 0.99 A × C – 1.15 C × A – 1.40 A × B – 0.94 B × A – 0.94 B × C – 1.15 C × B – 1.40 A × B × C – n.s.				
K	0.0	44.3	45.1	44.9	44.5	44.7
	0.2	48.9	43.5	46.4	46.0	46.2
	0.4	44.6	43.9	43.8	44.7	44.3
Mean		45.9	44.2	45.0	45.1	45.1
$LSD_{0.05}$		A – 0.38 B – n.s. C – 0.56 A × C – 0.66 C × A – 0.79 A × B – n.s. B × C – 0.66 C × B – 0.79 A × BxC – n.s.				
Ca	0.0	10.7	10.5	10.7	10.5	10.6
	0.2	10.4	11.7	11.2	10.9	11.1
	0.4	10.8	11.4	12.0	10.2	11.1
Mean		10.6	11.2	11.3	10.5	10.9
$LSD_{0.05}$		A – 0.31 B – 0.31 C – 0.47 A × C – 0.55 C × A – 0.66 A × B – n.s. B × C – 0.55 C × B – 0.66 A × B × C – n.s.				
Mg	0.0	2.15	2.10	2.20	2.05	2.13
	0.2	2.15	2.50	2.50	2.15	2.33
	0.4	1.98	2.18	2.15	2.00	2.08
Mean		2.09	2.26	2.28	2.07	2.18
$LSD_{0.05}$		A – 0.12 B – 0.12 C – 0.17 A × C – 0.20 C × A – 0.24 A × B – 0.16 B × A – 0.16 B × C – n.s. A × B × C – n.s.				

Explanations: I – every 7 days, II – every 14 days, n.s. – not significant difference

Table 4. Content of micronutrients (mg·kg^{-1} d.w.) in leaves of 'Summer Beach' freesia depending on chitosan concentration, application method and frequency of its application – end of vegetation period

Micronutrient (mg·kg^{-1} d.w.)	Concentration (%) (C)	Application method (A)		Frequency of use (B)		Mean
		watering	spraying	I	II	
Cu	0.0	5.06	4.83	4.83	5.06	4.95
	0.2	5.88	5.28	6.23	4.93	5.58
	0.4	5.65	5.38	5.56	5.47	5.52
Mean		5.53	5.16	5.54	5.15	5.35
$LSD_{0.05}$	A – 0.054 B – 0.054 C – 0.080 A × C – 0.093 C × A – 0.113 A × B – 0.076 B × A – 0.076 B × C – 0.093 C × B – 0.113 A × B × C – n.s.					
Fe	0.0	215.3	217.6	216.4	216.5	216.5
	0.2	213.5	243.5	228.5	228.5	228.5
	0.4	227.9	241.2	229.9	239.2	234.6
Mean		218.9	234.1	224.9	228.1	226.5
$LSD_{0.05}$	A – 1.17 B – 1.17 C – 1.73 A × C – 2.02 C × A – 2.44 A × B – 1.65 B × A – 1.65 B × C – 2.02 C × B – 2.44 A × B × C – n.s.					
Mn	0.0	140.7	143.6	142.7	141.6	142.2
	0.2	162.2	153.9	161.5	154.6	158.1
	0.4	158.6	150.4	155.8	153.2	154.5
Mean		153.8	149.3	153.3	149.8	151.6
$LSD_{0.05}$	A – 1.29 B – 1.29 C – 1.91 A × C – 2.23 C × A – 2.70 A × B – 1.82 B × A – 1.82 B × C – 2.23 C × B – 2.70 A × × B × C – n.s.					
Zn	0.0	84.3	87.9	86.8	85.4	86.1
	0.2	77.9	91.5	83.7	85.7	84.7
	0.4	82.9	81.0	82.6	81.3	82.0
Mean		81.7	86.8	84.4	84.1	84.3
$LSD_{0.05}$	A – 0.82 B – n.s. C – 1.21 A× C – 1.41 C × A – 1.71 A × B – 1.15 B × A – 1.15 B × C – 1.41 C × B – 1.71 A × B × C – n.s.					

Explanations: I – every 7 days, II – every 14 days, n.s. – not significant difference

Wojcieszczuk et al. [2000] claim that during the vegetation season, Ca content in freesia leaves grows and Mg content declines. In our study, at the end of the vegetation season, freesia leaves contained more P, K and Ca, and less N than during flowering, while Mg levels remained fairly unchanged (Tab. 3). Leaves of 'Summer Beach' freesia treated with chitosan solution accumulated more N, P, Ca, Cu, Fe and Mn than non-treated plants (Tabs. 3 and 4). As claimed by Bennewitz and Hlusek [2006], higher content of minerals in the leaves might result from the facilitation of chitosan-mediated nutrient uptake from the soil. Chitosan also improves the soil structure, both under cover and in the open field [Winkler et al. 2017]. In the view of Iriti et al. [2009], 1.0% chitosan added to the medium intensified rooting of *Arisaematernatipartitum* bulbs. Chitosan solution stimulated growth and development of roots in *Phaseoluscoccineus* (0.5%) [Sheikha and Al-Malki 2011] and *Freesia hybrida* 'Silver Beach' (0.2% and 0.4%) [Żurawik 2013], which could also improve the mineral uptake from the substrate and increase their accumulation in the leaves. Spraying the experimental plants with chitosan at the end of the vegetation period significantly enhanced the content of N, P, Ca and Mg as compared with watering variant. However, the report of Shehta et al. [2012], claiming that plants sprayed with chitosan absorbed more K from the substrate, was not confirmed. In our study, more K was accumulated in the leaves of plants watered vs. sprayed with chitosan. Leaves of 'Summer Beach' freesia had higher levels of N, Ca and Mg when chitosan was applied every seven days than when it was supplied every 14 days. A reverse observation was made for P level. Only potassium content was not affected by the frequency of chitosan application. The study revealed variable effects of chitosan concentrations on leaf content of macronutrients. More nitrogen and calcium was detected in the leaves treated with 0.2% or 0.4% solution than in the control ones. The highest levels of phosphorus were found in samples from plants treated with 0.2% chitosan, and the lowest in non-treated ones. The finding of Żurawik [2013], who claimed that treating 'Silver Beach' freesias with 10 000 $g \cdot mol^{-1}$ chitosan at 0.4% resulted in higher content of potassium in the leaves than when 0.2% solution was used, did not find any confirmation in present study. In this experiment, K was most abundantly detected in the leaves exposed to 0.2% chitosan solution, as compared with non-treated plants and those exposed to 0.4% chitosan. Similar relationship was observed for magnesium. At the end of the vegetation season, significant correlation was found between chitosan concentration and its application method and N, P, K and Ca content in the leaves. However, variable relationships between chitosan concentration and frequency of its use were observed for the accumulation of N, P, K and Ca. Variance analysis for N, P and Mg content in the examined plant material revealed significant correlation between chitosan application method and its use frequency.

Chemical composition of freesia leaves depends on its developmental stage and cultivar [Wojcieszczuk et al. 2000]. Samples collected at the end of the vegetation season contained considerably more Fe, Mn and Zn than those harvested during flowering, while Cu concentration was similar in both cases (Tab. 4). Watering plants with chitosan solution significantly improved the leaf content of Cu and Mn, as compared with spraying. A contrary observation was made for Fe and Zn. Irrespective of the application method and concentration, supplying chitosan every seven days significantly increased Cu and Mn content and reduced Fe content in freesia leaves. Changes in frequency of the biopolymer application had no effects on Zn content in the leaves of 'Summer Beach' freesia. Salachna et al. [2017] reported that coating the bulbs of *Ornithogalum saundersiae* with chitosan and gellan gum enhanced the leaf accumulation of Zn and Mn. In our study, leaves of plants treated with 0.2% or 0.4% chitosan contained more Cu, Fe and Mn, but less Zn than the control ones. Our findings confirmed those reported by Kamari et al. [2012] and Żurawik [2013] that chitosan limited the uptake of zinc from the soil. For all determined micronutrients, significant correlations were found between chitosan concentration and its application method, chitosan concentration and frequency of its application, and application method and application frequency.

CONCLUSIONS

1. The use of chitosan in the cultivation of 'Summer Beach' freesia facilitated the uptake of N, P, Ca, Cu, Fe and Mn and limited the absorption of Zn.

2. Chitosan effect on leaf chemical composition depended on the method of its application. Spraying with the biopolymer increased the content of N, P, Ca, Mg, Fe and Zn at the end of vegetation, and watering caused enhanced accumulation of K, Cu and Mn.

3. Application of chitosan 24 times during the vegetation season improved the levels of N, Ca, Mg, Cu and Mn in leaves, as compared with 12 treatments.

4. For both application methods and frequency of application over the entire cultivation period, increasing the chitosan solution concentration from 0.2% to 0.4% reduced the accumulation of P, K and Zn in freesia leaves.

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