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Dose-Dependent Potential of Chitosan to Increase Yield or Bioactive Compound Content in Tomatoes

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Abstract: Chitosan is a natural polymer with multiple applications in agriculture due to its ability to stimulate plant growth and resistance to both biotic and abiotic stressors. The impact of chitosan application on fruit production and quality was studied under greenhouse conditions in a summer crop in a semi-arid climate. Treatments consisted of the spray application of this biostimulant to the aerial plant part at different doses (0, 0.1, and 1 g L^{-1}). Treatment with the lowest dose did not produce significant differences in yield (total production, number, and mean weight of the fruit), but increased the concentration of flavanones (trusses 2 and 7) and phloretin-*C*-diglucoside (truss 2) with regard to the control. On the contrary, the high-dose treatment increased the yield due to the rise in the number of fruits and produced a significant decrease in the concentration of vitamin C, lutein, *β*-carotene, and hydroxycinnamic acids (trusses 2 and 7); lycopene, phytoene, and phytofluene in truss 2; and flavanols and phloretin-*C*-diglucoside in truss 7. These results show the ability of chitosan to improve tomato yield or to enhance the accumulation of bioactive compounds (phenolic compounds) in fruit, depending on the dose. Results are explained on the basis of the ability of chitosan to activate yield and secondary metabolite production, the dilution effect due to an increased fruit load, and the interaction of chitosan with changing environmental factors throughout the crop cycle.

Keywords: biostimulants; production; bioactive; nutritional quality; pre-harvest

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1. Introduction

Chitosan is a bio-polysaccharide produced from the deacetylation of chitin, the structural element of cell walls in fungi, insect exoskeletons, and crustacean shells [1,2]. In the last decades, chitosan has been widely studied for different pharmaceutical and biomedical applications based on its antibacterial, antifungal, anti-HIV-1, antitumor, and antioxidant activity [3]. In agriculture, chitosan is used as a natural fungicide due to its capacity to inhibit the growth of many fungi that are pathogenic and mycoparasitic for plants by means of the permeabilization of fungi plasma membranes [4]. More recently, the application of chitosan as a plant biostimulant has received increasing attention [2,5]. At a cellular level, chitosan binds to the plant cell membrane and induces the generation of H_2O_2 and NO in the plant defense system via the octadecanoid and nitric oxide pathways, respectively. These two molecules act as signals that induce several plant responses to both biotic and abiotic stresses. Changes induced by chitosan at a plant level include stomata closure, increased chlorophyll content and net photosynthesis rate, enhanced plant growth, and activation of secondary metabolite production [6]. All these physiological changes have interesting agronomic implications since they could directly affect the yield and the quality of horticultural crops. Thus, a treatment of chitosan (28 kDa), at 0.5% dissolved in 0.5% lactic acid, increased the total weight (12.9%), germination rate (16%), and isoflavone content (11.8%) of sunflower sprouts [7], whereas a treatment in soybean sprouts with 0.05% chitosan (493 kDa) in 0.05% acetic acid solution increased the total weight (26%) and vitamin C content (14%) when compared with the control treatment [8].

Potential agronomic responses to chitosan and other biostimulants are especially relevant when plants grow under stress conditions. Among the abiotic factors that negatively affect crop yield and quality in semi-arid areas is high temperature [9]. The complex response of the plant to high-temperature stress is controlled by multiple genes and affects both the vegetative growth and the reproduction of plants [10]. Different air temperatures are reported to be optimal for tomato development depending on growth stage, air humidity, and environmental localization; however, generally speaking, the optimal range of temperatures for tomato cultivation falls between 18 and 32 °C during the entire growing season [11]. The optimal upper limit is usually exceeded under climate conditions of the Mediterranean summer months, with the subsequent detrimental effects on fruit yield and quality [12,13]. This problem is exacerbated in greenhouse crops, where temperature increase during summer months is more pronounced. Tomato yield reduction under high temperatures is mainly attributed to a photosynthetic and pollen viability decline [14,15]. This leads to a reduced fruit set and the subsequent decrease in fruit number and size. In addition, increased temperatures can modify tomato quality by altering the physical properties (size, color, etc.) of the fruit, as well as its sensorial and nutritional quality [13,16]. The effect of abiotic stress on tomato composition is of special interest because of its value as a functional food due to a high content of bioactive compounds; in particular, vitamins, carotenoids, and phenolic compounds [17,18].

In the current climate change scenario, the use of biostimulants in agriculture has become an option to improve plant resilience to adverse environmental conditions [19]. There is a wide range of substances with biostimulant properties on plants, but information on the peculiarities of each compound, its suitability for specific crops, and the most appropriate mode of application remains scarce and unclear. With regard to chitosan, several authors claim that exogenous applications of this compound improve the response of the plant to adverse conditions and, as a result, of defense response, as it can increase the concentration of bioactive compounds of interest in various crops [1]. This evidence shows the potential of chitosan to stimulate agronomic responses in horticultural crops under limiting conditions; however, due to the complexity of the response of the plant to biostimulation, it is necessary to obtain more information and perform specific studies for each crop and set of environmental conditions. In this study, the agronomic response and bioactive compounds of tomato arising as a result of application of chitosan were investigated under greenhouse cultivation in a semi-arid environment characterized by high-temperature conditions.

2. Materials and Methods

During the months of January to July, tomato plants (*Solanum lycopersicum* L. cv. Boludo) were grown in a chapel polyethylene greenhouse (300 m^2) in IMIDA's "Torreblanca" experimental farm, located in Torre Pacheco, Murcia, Spain (37°46′26.43″ N and 0°53′26.43″ W), with a Mediterranean climate and a soil classified as clay loam. Plants were irrigated by drip irrigation at a rate based on the FAO methodology [20] partially modified by [21]. The planting frame had 0.44 m between plants and 1 m between rows. Treatments with biostimulants consisted of spray application of chitosan (deacetylated chitin, medium molecular weight) (Sigma-Aldrich, St. Louis, MO, USA) at 0 (control), 0.1, and 1 g L^{-1} to leaves and fruits, directly on the area between trusses 2 and 4 (first application) and on the area between trusses 5 and 7 (second application). Control plants were treated with the same wetting agent that was used in the treatments (acetic acid 1%). In addition, a group of plants was sprayed only with water in order to evaluate the possible effect of acetic acid 1% on the production of the plants. Each treatment was applied three consecutive times to the aerial plant part, 15, 7, and 4 days before the harvest of the 2nd truss (99, 107, and 111 days after transplant, DAT) and again before the harvest of the 7th truss (136, 143, and 147 DAT). The experimental design consisted of randomized blocks, with two rows (blocks) per treatment, each row containing forty-two plants. Border rows along the edges of the test area and between treated rows were established to avoid a

"border effect". During the harvest period (May to July), the maximum temperature in the greenhouse reached values of 42 °C (Supplementary Figure S1), a value well-above the maximum temperature considered suitable for tomato cultivation (17–30 °C) [11]. More precisely, mean day/night temperatures during the development of the 2nd (May) and 7th (June) trusses were 26/15 °C and 30/19 °C, and the maximum temperatures inside the greenhouse were 31 and 35 °C, respectively.

For the evaluation of the total yield, number, and mean fruit weight, all the fruits from 12 plants per row (24 plants per treatment) were collected and weighed individually. For the analysis of fruit color and composition, three replicates per row (six per treatment) were established, each replicate consisting of ten fruits from two plants. For color determination, ten fruits from each replicate were used and measured using a Minolta CR-200 (Ramsey, NJ, USA) colorimeter through direct reading in three different areas of the surface of the fruit. In addition, quality parameters were evaluated on fruits from trusses 2 and 7 when completely red and ripe, discarding those that did not have a homogeneous color or had a defect. Ripeness of fruits was supervised daily to prevent over-ripening. For metabolite analysis, fruits belonging to the same replicate were cut into small pieces and mixed to conform a sample. They were then frozen in liquid N_2 and kept at −80 °C for further analysis following the methodologies described by [22] for tomato. After homogenization, soluble sugars were analyzed in an Agilent 1100 liquid chromatograph (HPLC) (Waldbronn, Germany) equipped with a refraction index detector and a 300 mm × 7.8 mm i.d., CARBOSep CHO-682 LEAD column using deionized water at a flow of 0.4 mL min^{-1} as mobile phase. Carotenoids were determined using a Hewlett-Packard mod. 1200 HPLC system (Santa Clara, CA, USA) with a photodiode array detector and a 250 mm × 4.6 mm i.d., 3 µm Prontosil C_{30} column (Bischoff, Leonberg, Germany) using a gradient of methanol (solvent A) and methyl terbutyl ether (solvent B) at a flow of 1.3 mL min^{-1} as follows: (1) initial conditions 15% solvent B and 85% solvent A, (2) a 10-min linear gradient maintaining 15% solvent B, (3) a 20-min linear gradient to 90% solvent B. Vitamin C and phenolic compounds were analyzed by HPLC-MS/MS (Agilent Series 1200, Agilent Technologies, Santa Clara, CA, USA) with an ESI interface operating in negative ion mode. For vitamin C, separation was carried out using a Prontosil C_{18} column of 250 mm × 3 mm and 3 µm particle size (Bischoff, Leonberg, Germany) and 0.2% formic acid at a flow of 0.4 mL min^{-1}. Separation of phenolic compounds was achieved in a Lichrosphere C_{18} analytical column of 250 mm × 4 mm and 5 µm particle size (Agilent Technologies, Waldbronn, Germany) with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 1 mL·min^{-1} in a gradient run that began with 5% B, reaching 10% B in 9 min, 30% B in 50 min, increased to 100% in 2 min and held at 100% B for an additional 3 min, returning to initial conditions in 1 min and remaining isocratic for 6 min. Full scan, neutral-loss scan, and precursor-ion scan experiments were carried out to confirm the identity of some compounds when the standard was not available. Selective reaction monitoring (SRM) transitions were optimized using several fragmentor voltages (F), from 20 to 200 V, and collision energies (CE), from 2 to 50 V.

Results were statistically analyzed by analysis of variance (ANOVA) using the statistical program IBM SPSS Statistics 25. Values were compared using Tukey's range test.

3. Results and Discussion

3.1. Fruit Yield

No significant differences were found in total production, mean weight, or number of fruits between plants sprayed with water and those sprayed with acetic acid 1% (control plants). Chitosan applied at the lowest dose (0.1 g L^{-1}) had no effect on total production, as it did not significantly affect either the number or mean fruit weight with regards to the control. However, treatment with the most concentrated dose (1 g L^{-1}) increased tomato production with regards to the control (27%) because a greater number of fruits was obtained (Table 1). The effect of chitosan as a crop biostimulant has shown to be highly dependent on the crop, the molecule structure, and the timing and rate of application [6].

Several studies have indicated that preharvest application of chitosan solutions increased plant growth and fruit yield at harvest in horticultural crops such as beans [23], strawberries [24], and bell peppers [25]. In tomatoes, foliar application of chitosan 0.06 g L^{-1} was shown to be an effective treatment to increase total yield under non-stressed conditions [26]. In addition, Mondal et al. [27] reported that foliar application of chitosan 0.75 g L^{-1} at the vegetative and early flowering stages enhanced plant growth and increased fruit yield in summer tomato cultivation under sub-tropical conditions. However, Parvin et al. [5] found that, although foliar application of chitosan increased tomato quality (biochemical parameters), treatments with a concentration between 0.06 and 0.12 g L^{-1} had no significant effect on tomato yield. The beneficial effect of chitosan application on plant development under non-stressed conditions is mainly attributed to the enhanced uptake of nitrogen and nutrients and its use as a carbon source for plant biosynthetic processes [28]. In many other cases, the increase in yield of horticultural crops was reported to be a consequence of disease reduction from chitosan since, in the absence of biotic stress, similar results were obtained for treated and untreated plants [29,30]. It has been indicated that chitosan reduced the electrolyte leakage from the cell membrane [31], which is important under heat conditions. Moreover, chitosan could alleviate heat stress by inducing ABA activity, which is related to stomatal closure [32], and inducing defense ABA-related genes [1]. Hidangmayum et al. [33] indicated that the mode, rate, and timing of application of chitosan are important in order to activate the various processes conducting to the increase in plant production under heat stress. In our trial, an increase in cumulative fruit yield between the chitosan-treated and untreated plants was only observed for the highest dose (1 g L^{-1}) (Figure 1). This yield enhancement started to be significant ($p < 0.05$) from 140 DAT onwards, and differences increased as the crop cycle progressed, coinciding with the second cycle of chitosan treatments and with the beginning of the period of higher environmental day and night temperatures and a higher crop load (increased demand and abiotic stress).

Table 1. Yield (kg $plant^{-1}$), number, and mean weight (g) of tomato fruits grown under different doses of chitosan (0, 0.1 y 1 g L^{-1}).

Chitosan Dose (g L^{-1})	Total Yield (kg $plant^{-1}$)	Fruit Number	Fruit Mean Weight (g)
Water	3.98 ^{a}	44.7 ^{a}	99.1 ^{b}
0 (control)	4.05 ^{a}	47.8 ^{a}	86.5 ^{ab}
0.1	4.13 ^{a}	52.8 ^{ab}	80.2 ^{a}
1	5.15 ^{b}	56.0 ^{b}	95.5 ^{b}
	**	**	**

** Significant differences between means at a 1% level of probability, respectively. Different letters in the same column indicate the presence of significant differences between means according to Tukey's test at the 5% level.

3.2. Fruit Color

Color is one of the most important quality attributes of tomatoes and is mainly due to carotenoid pigments, the concentration and profile of which are genetically determined and affected by the environment [34]. Lycopene is the major carotenoid responsible for its characteristic red color [35], and it increases during ripening. Fruit color was assessed in terms of hue (tone) and chroma (color purity or saturation), which are perceptual attributes of human color vision. Differences in color between fruits of various trusses were observed. Fruits of the seventh truss showed a more intense color (higher chroma values) than those of the second truss, probably due to the increase in radiation and temperature throughout the growing cycle. At the low dose (0.1 g L^{-1}), chitosan did not significantly affect color attributes. However, fruits treated with chitosan at a high dose (1 g L^{-1}) were redder (higher hue) but showed a less intense color (lower chroma) than those of the control treatment (Table 2). Therefore, treatment with chitosan at a high concentration could present an advantage when compared to fruits untreated or treated with a lower concentration of elicitor, taking into account that consumers have a preference for redder fruits. On the contrary, the decrease in chroma value (less intense color) could be a disadvantage, as variations in chroma are related to consumer acceptance when tomato

fruits are fully ripe [36]. Most of the studies on the effect of chitosan on fruit color focus on the post-harvest use of this compound. Some reports showed that tomatoes coated with chitosan became red more slowly than those in the control arms due to its ability to reduce the ripening processes [37]. However, others did not observe any effect on lycopene in tomatoes treated with chitosan during storage [38]. In a previous study by [39], pre-harvest applications of chitosan at higher concentrations than those used in this study (6 g L^{-1}) resulted in an increase in red color and carotenoid content in tomatoes. This increase was accompanied by a decrease in fruit yield, which may explain the differences found between our results and those of the previous study.

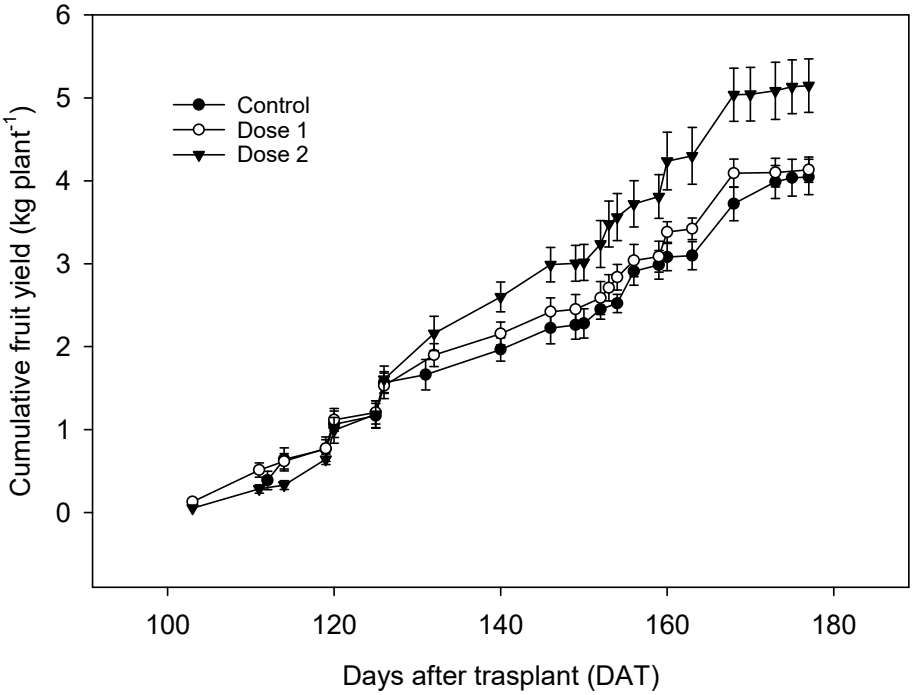

Figure 1. Cumulative fruit yield of tomato grown under control (0 g L^{-1}), dose 1 (0.1 g L^{-1}), and dose 2 (1 g L^{-1}) chitosan treatments.

Table 2. Soluble sugars (mg g^{-1} FW), vitamin C (mg g^{-1} FW), and color parameter (hue and chroma) in tomato fruits under different chitosan doses (0, 0.1, and 1 g L^{-1}).

		Chroma	Hue	Glucose (mg g^{-1})	Fructose (mg g^{-1})	Vitamin C (mg g^{-1})
Doses	0	25.4 ^{b}	43.7 ^{a}	17.4	16.4	25.1 ^{b}
	0.1	25.0 ^{b}	45.3 ^{ab}	17.6	15.4	24.1 ^{b}
	1	23.9 ^{a}	46.5 ^{b}	15.9	14.4	19.2 ^{a}
		***	**	n.s.	n.s.	***
Truss	2	24.2	45.3	16.2	14.9	22.5
	7	25.2	45.1	17.8	15.9	23.2
		***	n.s.	n.s.	n.s.	n.s.
INTERACTION						
Truss	Dose					
2	0	25.1	43.5	17.8	16.7	24.0 ^{c}
	0.1	24.7	45.4	16.1	14.6	22.7 ^{bc}
	1	23.0	47	14.6	13.3	20.7 ^{ab}
7	0	25.6	43.8	16.9	16.1	26.3 ^{c}
	0.1	25.2	45.1	19.0	16.2	25.6 ^{c}
	1	24.8	46.4	17.3	15.4	17.7 ^{a}
		n.s.	n.s.	n.s.	n.s.	***

, * Significant differences between means at a 1, or 0.1% level of probability, respectively; n.s., non-significant at p = 5%. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level.

3.3. Fruit Composition

Under our environmental conditions, chitosan treatments had no significant effect on the concentration of soluble sugars (Table 2). Sugars are key metabolites that have an influence on tomato quality and customer preferences [40]. In agreement with our results, El Amerany et al. [41] showed that pre-harvest foliar spray with chitosan did not affect soluble sugar concentrations of tomato fruits when compared to control. However, different results can be found in literature. Thus, treatment with a mixture of chitosan, jasmonic acid, and hydrogen peroxide in sweet bell pepper plants increased sugar content in fruit, mainly when the application was done at an advanced stage of maturity [42]. Similarly, total soluble solids (TSS) increased in tomato plants when they were treated with salicylic acid plus chitosan [43], and the same happened in cucumber plants treated with chitosan [44]. On the contrary, the application of chitosan foliar spray to tomato plants decreased TSS in tomato fruit [26]. The inconsistent results found in literature are attributable to the interaction of the chitosan treatment with other factors, such as dose, timing of application, and environmental conditions.

The health benefits of tomato fruits in the human diet are related to their bioactive components and synergistic effects, which confer cardiovascular, anti-cancer, and skin-health properties [45]. Most research focuses on the biological properties of lycopene. However, tomatoes are also a major source of other antioxidant and health-promoting compounds, such as β-carotene, phenolic compounds, and vitamin C [22]. The application of chitosan at the lowest dose did not affect the content of vitamin C of the fruit. However, chitosan applied at the highest dose caused a significant decrease in vitamin C concentration in the two trusses that were analyzed. This decrease was more pronounced in truss 7 (33% when compared to the control) than in truss 2 (14% when compared to the control), coinciding with a significant increase in production observed in plants under this treatment during the harvesting dates of the fruit corresponding to the seventh truss (from 151 to 159 DAT) (Supplementary Figure S1). Literature regarding the effect of chitosan on vitamin C is controversial. Increases with chitosan treatment during plant growth have been reported in broccoli [46] and tomato plants [5]. According to Khan et al. [47], the increase in vitamin C with chitosan could be explained by an increase in photosynthesis, which had a correlation with the synthesis of sugars, polysaccharides, and vitamins. The discrepancies found between these results and ours, with 1 mg L^{-1} chitosan decreasing the content of vitamin C, can be attributed to the fact that, in contrast to our study, these authors do not describe an increase in production and, therefore, a dilution effect is not likely to occur.

Lycopene and β-carotene accounted for 51% and 22% of the total carotenoids detected in fruit under control conditions (without chitosan), respectively (Table 3). Other major carotenoids detected in fruit were lutein and violaxanthin, in addition to the carotenoid precursors phytoene and phytofluene. A general decrease in lutein, β-carotene, and carotenoid precursors was observed in fruit from truss 7 when compared to truss 2. However, this effect was not observed for lycopene and violaxanthin. The trend of each of the individual compounds can be explained by a dilution effect, which is a consequence of a higher fruit load as the cycle progresses [48], or by the increase in temperature in the more advanced stages of the crop, which can lead to the accumulation of lycopene to the detriment of other products of metabolic channeling in the isoprenoid pathway [16]. At the lowest dose, chitosan did not significantly affect the concentration of carotenoids in fruit with regards to the control. However, treatment with chitosan at the highest dose (1 g L^{-1}) caused a general decrease in the concentration of the carotenoids that were analyzed, although this decrease was not statistically significant in the case of violaxanthin and, in the case of phytoene, phytofluene, and lycopene, was only observed in fruits from truss 2. This was probably due to the fact that, in fruits from truss 7, the effect of the treatments could be masked by the effect of the crop phenology (dilution effect as a consequence of a higher fruit load). The effect of the chitosan 1 g L^{-1} treatment on the carotenoid content of the fruit did not only affect their concentration, but also the profile of individual carotenoids. While the percentage of lycopene in fruits was 49% of the detected carotenoids in the control treatment,

in the treatment with chitosan at the highest dose, this number raised to 54%. These results were in accordance with the higher hue value (redder fruits due to a higher proportion of lycopene) and the lower chroma value (less intense color related to a lower total concentration of carotenoids) found in fruits from this treatment when compared to the control. Results obtained for lycopene may be a consequence of the impact of chitosan on fruit production depending on the dose and mode of application of chitosan. Parvin et al. [5] studied different chitosan application methods on tomato quality, indicating that, whereas chitosan treatments based on foliar application alone decreased lycopene concentration in the fruit, combined foliar and soil application of chitosan increased this value when compared to control fruits. Furthermore, in this earlier study, foliar application of chitosan at increasing concentrations was correlated with a decrease in lycopene concentration.

Table 3. Concentration of violaxanthin (Viol), lutein, phytoene (Phyto), phytofluene (Phytof), all trans-β-carotene (β-carot), and total lycopene (Lycop) (μg g^{-1} FW) in tomato fruits under different chitosan doses (0, 0.1, and 1 g L^{-1}).

		Viol	Lutein	Phyto	Phytof	β-Carot	Lycop
Dose	0	0.96	2.2 ^{b}	5.6 ^{b}	4.5	9.7 ^{b}	22.0
(g L^{-1})	0.1	0.92	2.1 ^{ab}	4.5 ^{ab}	3.5	8.9 ^{ab}	20.3
	1	0.89	1.8 ^{a}	3.5 ^{a}	2.8	7.8 ^{a}	19.6
		n.s.	*	***	n.s.	*	n.s.
Truss	2	0.97	2.2	5.7	4.6	9.7	20.2
	7	0.87	1.9	3.3	2.6	8.0	21.1
		n.s.	**	***	***	**	n.s.
	INTERACTION SECCION						
Truss	Dose						
2	0	1.07	2.5	7.5 ^{b}	6.0 ^{c}	11.0	22.6 ^{b}
	0.1	1.01	2.2	5.9 ^{b}	4.5 ^{bc}	9.8	19.8 ^{ab}
	1	0.85	1.9	3.9 ^{a}	3.1 ^{ab}	8.2	18.4 ^{a}
7	0	0.85	2.0	3.7 ^{a}	3.0 ^{a}	8.5	21.3 ^{ab}
	0.1	0.98	1.9	3.1 ^{a}	2.4 ^{a}	8.0	21.2 ^{ab}
	1	0.84	1.8	3.0 ^{a}	2.4 ^{a}	7.5	20.9 ^{ab}
		n.s.	n.s.	**	*	n.s.	*

*, **, *** Significant differences between means at a 5, 1, or 0.1% level of probability, respectively; n.s., non-significant at p = 5%. Different letters in the same column indicate the presence of significant differences between means according to Duncan's test at the 5% level.

The main phenolic compounds detected in tomato were flavanones (calculated as the sum of naringenin and naringenin-*O*-hexoside), hydroxycinnamic acids (chlorogenic, caffeic, ferulic-*O*-hexoside, caffeic-*O*-hexoside, like-chlorogenic, cryptochlorogenic and dicaffeilquinic acids), flavonols (rutin-*O*-hexoside, rutin-*O*-pentoside, rutin, kaempferol-3-*O*-rutinoside and quercetin) and phloretin-*C*-diglycoside (Table 4 and Supplementary Table S1). Unlike what was observed for carotenoids, an increase in the concentration of the main families of phenolic compounds was observed in the fruits of truss 7 with regards to those of truss 2 (Table 4). Phenolic compounds play an important role at a physiological level, increasing the stress tolerance of plants due to its antioxidant properties and ability to scavenge free radicals, protecting plant cells from the detrimental effects of oxidative stress [49]. Previous studies showed that the duration and intensity of light irradiance plays a predominant role in the regulation of phenolic compounds in plants with a longer period of light exposure and higher light intensities, showing higher concentrations of several families of phenolic compounds [50]. Thus, the increase in photoperiod and light intensity as the growing cycle progressed could be the responsible for the higher concentration of phenolic compounds found in the fruits of truss 7 when compared to those of truss 2. The response of phenolic compounds to chitosan application depended both on the family of compounds studied and on the concentration of the biostimulant. Chitosan at the lowest dose (0.1 g L^{-1}) increased flavanones in trusses 2 and 7, mainly due to the increase in naringenin, and phloretin-*C*-diglycoside in truss 2. However, chitosan at 1 g L^{-1} decreased hydroxycinnamic acids in both trusses, and flavonols and phloretin-*C*-diglycoside only in truss 7. Several authors have reported effects of chitosan on phenolic compounds; however, most of them refer to the total phenolic content and do not analyse

each individual phenolic compound. Liu et al. [51], studying the effect of chitosan on the control of postharvest diseases of tomato fruits, indicated that the activities of PPO and POD and the level of total phenolic compounds in chitosan-treated fruit increased. These results are in agreement with those reported by Coqueiro et al. [52], who suggested that the accumulation of trans-cinnamic acid derivatives in tomato was a consequence of the activation of the phenylpropanoid pathway by chitosan. Interestingly, different doses of chitosan showed different effects on tomato and aubergine. Thus, total phenolic content increased with the lowest tested dose of chitosan (60 ppm) in aubergine, but decreased in tomato, while no effect of 100 ppm chitosan was observed on phenolic content in either fruit [26]. As observed for other metabolites, the effect of chitosan on the accumulation of phenolic compounds in fruit is highly dependent on the concentration applied and its impact on fruit yield. Moreover, results may be affected by timing, as the interaction of biostimulant application with other factors such as temperature or fruit load varies throughout the crop cycle.

Table 4. Concentration of flavanones, hydroxycinnamic acids (Hydroxi.), flavonols, and phloretin-*C*-diglucoside (Phloretin) (μg g^{-1} FW) in tomato fruits under different chitosan doses (0, 0.1, and 1 g L^{-1}).

			Flavanones	Hydroxi.	Flavonols	Phloretin
Dose		0	36.5 ^{a}	29.8 ^{b}	19.0 ^{b}	3.3 ^{b}
		0.1	51.3 ^{b}	26.6 ^{b}	17.8 ^{b}	3.4 ^{b}
		1	35.1 ^{a}	19.9 ^{a}	12.5 ^{a}	2.6 ^{a}
			***	***	***	*
Truss		2	30.7	24.1	11.1	2.2
		7	51.3	28.2	22.5	4.0
			***	*	***	***
	INTERACTION					
Truss		Dose				
2		0	23.9	25.4	11.6 ^{ab}	1.9 ^{a}
		0.1	44.2	25.4	12.8 ^{ab}	2.6 ^{b}
		1	23.9	20.2	9.4 ^{a}	2.0 ^{ab}
7		0	49.1	34.2	25.9 ^{c}	4.7 ^{d}
		0.1	58.5	27.9	23.6 ^{c}	4.2 ^{cd}
		1	46.2	19.6	16.2 ^{b}	3.1 ^{bc}
			n.s.	n.s.	*	*

*, *** Significant differences between means at a 5 or 0.1% level of probability, respectively; n.s., non-significant at p = 5%. Different letters in the same column indicate the presence of significant differences between means according to Tukey's test at the 5% level.

4. Conclusions

Chitosan effect on tomato yield depended on the application dose. Foliar application of chitosan at 0.1 g L^{-1} did not have a significant effect on fruit production, whereas the highest dose (1 g L^{-1}) increased fruit number and consequently fruit yield. The effect of treatments on fruit composition was only observed for secondary metabolites (vitamin C, phenolic compounds, and carotenoids) and related color parameters. Conversely to yield, the highest dose (1 g L^{-1}) had a negative effect on the concentration of several secondary metabolites, while the lowest concentration (0.1 g L^{-1}) increased the concentration of some bioactive compounds. To sum up, treatment with the low dose increased the concentration of flavanones in both trusses and of phloretin-*C*-diglucoside in truss 2. However, the application of the high dose resulted in a significant decrease in the concentration of vitamin C, lutein, β-carotene, and hydroxycinnamic acids in both trusses; of lycopene, phytoene, and phytofluene in truss 2; and of flavanols and phloretin-*C*-diglucoside in truss 7. The results obtained from the fruit composition are explained as a combination of several factors: the role of chitosan on the activation of the production of secondary metabolites, a dilution effect because of chitosan treatment on the fruit load, and the interaction of chitosan with changing environmental factors throughout the crop cycle (mainly temperature and light).

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae8121152/s1, Figure S1: Evolution of the maximum and minimum temperatures during the growing season. Table S1. Concentration of individual phenolic compound (μg g^{-1} FW) in tomato fruits under different chitosan doses (0, 0.1, and 1 g L^{-1}).

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