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Research Article

Effects of chitosan nanoparticles on growth, development, and nutraceutical quality of tomato (*Solanum lycopersicum* L.)

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Abstract

The tomato (*Solanum lycopersicum* L.), considered one of the most desirable horticultural crops in the world for its nutritional and nutraceutical qualities, is also used as a model for many studies. The objective of this research was to compare different concentrations of chitosan nanoparticles (NPs) on the growth and development of tomato plants, as well as on fruit quality. Three concentrations of chitosan (1000, 2000 and 3000 mg L^{-1}) and a control were applied to tomato leaves in a randomized block design with three replicates and four treatments. Different growth variables (plant height, stem diameter, root length and fresh and dry biomass of different plant organs) as well as fruit yield and polar and equatorial diameter were evaluated. Fruit quality was evaluated by determining the contents of total soluble solids (TSS, %), vitamin C (mg g^{-1}), polyphenols (gallic acid mg g^{-1}), antioxidant capacity (trolox µmol g^{-1}), flavonoids (catechin mg. g^{-1}) and carotenoids (β-carotene per µg g^{-1}). The positive effect of foliar applications of chitosan nanoparticles was proven. The higher concentrations favoured yield and fruit quality, increasing yield and contents in the different quality variables, while the medium concentration stimulated growth in general.

Keywords: fresh and dry biomass; fruits; growth dynamics; postharvest quality

Introduction

Tomatoes are cultivated in warm climates and in sheds that provide adequate conditions. It has multiple benefits for human health, as it is rich in lycopene and antioxidants that reduce a human's predisposition to degenerative diseases. Because of this, tomatoes are a much sought-after fruit in markets. It has been used as a model for several investigations in agricultural sciences to study the effect of heavy metal accumulation and gene expression on tolerance and resistance to biotic and abiotic factors, amongst others (Stout *et al.*, 2018).

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However, few studies have confirmed the potential of nanoparticles (NPs) in the development and production of tomato plants. NPs can reduce production and labour costs and improve water and nutrient uptake efficiency, thereby improving the nutritional quality of the fruit (Amerany *et al.*, 2022). Gaining more knowledge about the role of nanoparticles in improving agricultural yields (i.e. of cucumber (*Cucumis sativus* L.) as an environmentally friendly technology will lead to safer food and ensure food safety (Sánchez *et al.*, 2024). In this regard, it has been asserted that the use of NPs in agriculture brings many benefits to food production. For example, increased production rates and superior plant health and control of plant diseases and pests (Sánchez *et al.*, 2024).

Since the 1980s, a breakthrough in chitosan research has been observed, particularly for its beneficial effect as a regulator of plant growth and development (Wang *et al.*, 2015). Nanoparticle use has emerged in modern agriculture to produce agro products, such as nanofertilisers, nanopesticides, nanoherbicides and nanosensors, which increase food yields in a sustainable way and reduce humanity's environmental impact (Lira *et al.*, 2018). In fact, NP use has increased thanks to the physical and chemical properties that NPs acquire on a nanometric scale compared to micro-sized material (Hojjat and Hojjat, 2015).

Chitosan is a natural biopolymer obtained by the partially or fully deacetylated form of chitin, which has randomly distributed N-acetylglucosamine and glucosamine units. Chitosan is the only polycationic biopolymer in nature (Aranaz *et al.*, 2021; Islam *et al.*, 2022; Riseh *et al.*, 2022) and is mainly obtained by chemical methods, enzymatic methods (Lopez *et al.*, 2019), fermentation or a combination of biological-chemical processes from marine debris, such as crab exoskeletons, shrimp waste and arthropods. It has also been isolated from algae and fungal cell walls (Yu *et al.*, 2021; Riseh *et al.*, 2022; Wan *et al.*, 2022). In agriculture, chitosan has been studied to enhance plant defence systems against plant pathogenic microorganisms by foliar or direct soil application as a fertiliser, as well as a carrier of bioactive compounds and encapsulation of agrochemicals for controlled release in crops (Riseh *et al.*, 2022).

Since chitosan nanoparticles possess properties and capacities that promote plant growth, they remain an underutilised resource that has the potential to reduce the pollution caused by industrial agriculture. Hence, the aim of the present work was to compare different concentrations of chitosan nanoparticles on the growth and development of tomato plants, as well as on fruit quality.

Materials and Methods

Area description and establishment of treatments

The study was carried out in a greenhouse belonging to "La María" Experimental Campus at the Faculty of Livestock and Biological Sciences at the State Technical University of Quevedo. The campus is located 7.5 km along the Quevedo-Empalme road in the Mocache canton, Los Ríos province at coordinates 01°06'24" South latitude and 79°29'70" West longitude, at an altitude of 75 m above sea level, average annual temperature is 24 °C, relative humidity is 84%, and average annual rainfall is 2,295 mm.

A randomised block design was used whith four replicates and four treatments based on different doses of chitosan NPs (1000, 2000 and 3000 mg L^{-1}) (Reyes-Perez *et al.,* 2022; 2023) and a control, in which water was applied. Each experimental unit consisted of 10 tomato plants (*Solanum lycopersicum* L.) of the 'Floradad' variety. Foliar applications were made on three occasions: at 15, 25 and 35 days after transplanting (DAT).

When the plants from the seedbed were the right size (9 ± 1 cm), they were transplanted into receptacles containing a substrate made up of soil and compost in a 3:1 ratio, duly homogenised.

Irrigation was carried out twice a week using a localised drip irrigation system, with a dripper placed in each container and a delivery of 1.5 L h^{-1}, so that the plants did not suffer from stress. The irrigation time varied according to the water needs of the plants.

Plant tutorship was established by placing a stake at each end of the rows, from which two lines of wire were extended 20 cm apart, to which the plants were carefully attached as they grew.

Variables evaluated

For growth performance, periodic (non-destructive) assessments of plant height (cm) and stem diameter (cm) were made at 35, 45 and 55 DAT on 10 plants per treatment. Then, at harvest, destructive assessments were made (Food Dehydrators brand Generic) on 5 plants per treatment to determine the accumulation of fresh and dry biomass (g) of different plant organs (root, stems and leaves) with a precision scale model H-9884, as well as root length (cm) using a digital caliper model H-7352. Yield was determined for 10 plants per treatment and expressed in g ha^{-1}.

Equatorial diameter and polar diameter (cm) were measured on 20 randomly selected fruits for each treatment, as well as different biochemical determinations of fruit quality, which are consisted of the content of total soluble solids (TSS, %), typically expressed as a percentage and measured using a refractometer, which determines the refractive index of the solution, which is related to the concentration of dissolved solids.

Vitamin C (mg·g^{-1}), polyphenols (gallic acid equivalents, mg·g^{-1}), antioxidant capacity (Trolox equivalents, µmol·g^{-1}), flavonoids (catechin equivalents, mg·g^{-1}), and carotenoids (β-carotene equivalents, µg·g^{-1}) were all analyzed using standardized techniques with high-performance liquid chromatography (HPLC) (Model: Agilent Technologies 1200 series). The system was equipped with a quaternary pump and a UV-Vis absorbance detector, using a reversed-phase 5 µm LiChrocart RP-18 column (4.0 × 250 mm) (Vasco *et al.*, 2008; Nadeem *et al.,* 2022).

The data were analysed using Analysis of Variance (ANOVA), and the means were compared using Tukey's test at a significance level of $p \leq 0.05$, performed with SPSS v.24. The results were subsequently visualized through graphical representations generated with SigmaPlot software.

Results

Plant height (cm) and stem diameter (cm)

The dynamics of plant height (Figure 1A) only showed significant differences in the evaluation carried out 35 DAT. Treatment 3, in which 3000 mg L^{-1} was applied, produced the greatest height, although it did not show significant differences with respect to Treatment 2. In the control treatment, the plants reached the lowest height value. In relation to stem diameter (Figure 1B), there were significant differences between treatments at the three points in time when the evaluation was carried out. In contrast to the results for plant height, the most striking result was that the lowest values were reached both in the control treatment and in the lowest concentration of chitosan NPs.

Fresh and dry biomass (g) in root, stems and leaves and, as well, root length (cm).

In Figure 2, the fresh biomass of 3 different plant organs - root, stems and leaves - only showed significant differences between treatments in the root. This is perhaps because the shoot system had already been losing physiological activity that requires a greater amount of water and is responsible for the plant gaining more weight; it must be considered that the evaluation was carried out at the end of the crop cycle (Reyes Pérez *et al.*, 2020). The values for fresh biomass were higher in the stem than in the leaves and roots.

The behaviour of the dry biomass in the plant organs showed significant differences between treatments. The highest dry biomass contents corresponded to the stem, followed by those of the leaves and roots. The behaviour of the significant differences between treatments in the root for the two variables was similar. The greatest amounts of dry biomass were found in the control and the lowest dose with significant differences with respect to the highest dose.

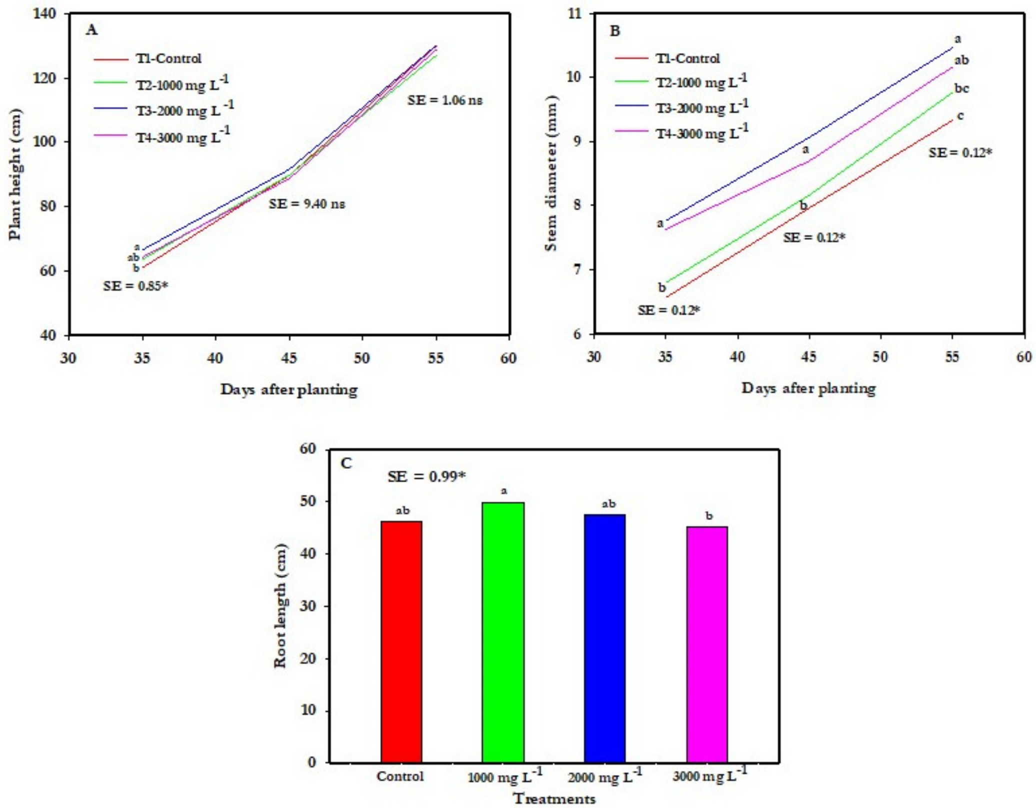

Figure 1. **(A)** Growth dynamics at 35, 45 and 55 days after transplanting of plant height, **(B)** stem diameter and **(C)** root length at the end of the crop cycle

Means with different letters means significant difference between treatments based on $p \leq 0.05$. SE: standard error

In the stem, the highest dry biomass content was observed in the 2,000 mg L^{-1} treatment and was significantly different from the rest of the treatments. This behaviour was similar in the leaves, although in this organ, for the two treatments with the lowest dose and the control, there were no significant differences, but there were with respect to the treatment with the highest concentration of chitosan NPs (Figure 2).

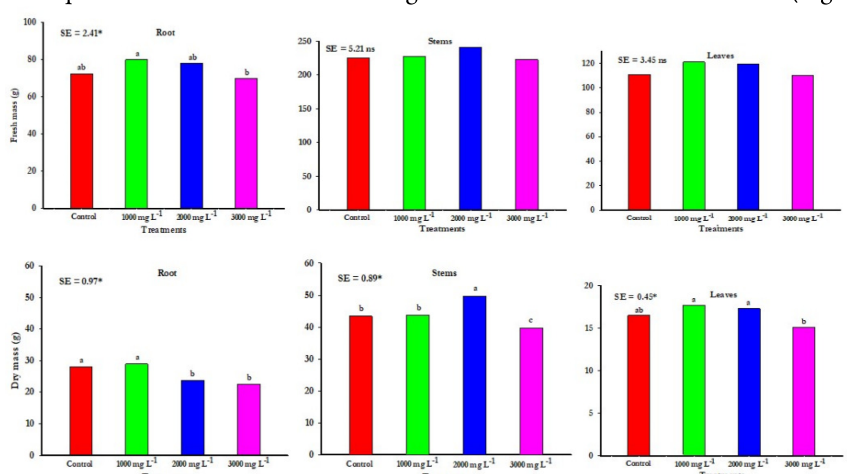

Figure 2. Fresh and dry biomass of root, stems and leaves at harvest time

Different letters above the bars represent significant differences between treatments based on $p \leq 0.05$. SE: standard error

In root length, evaluated at the end of the crop cycle, significant differences were found between treatments. But the highest values were found for Treatment 2 (1000 g L^{-1}) but without significant differences between this treatment and the control or Treatment 3.

Yield behaved differently from the variables analysed above, as the highest value was found using the highest concentration of chitosan NPs, with significant differences compared to the other treatments. In the control, the yield was lower and it should be noted that there were no significant differences between the lower concentrations ($p \leq 0.05$) (Figure 3).

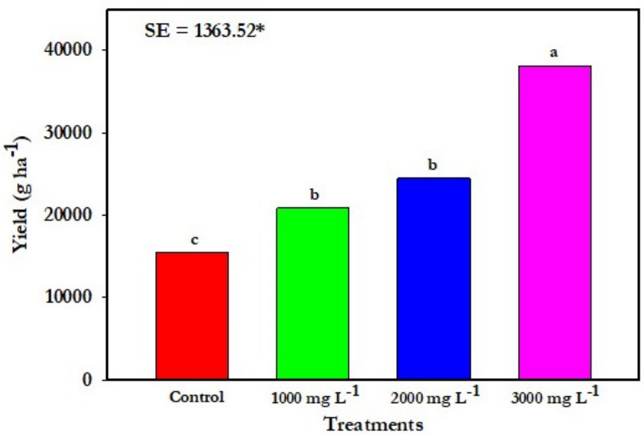

Figure 3. Effect of NPs of chitosan on yield in tomato plants (g ha^{-1})
Different letters above the bars represent significant differences between treatments based on $p \leq 0.05$. SE: standard error

Equatorial diameter and polar diameter (cm)

About fruit size (Figure 4), the result of the statistical analysis detected no significant differences between treatments for the polar diameter. However, for the equatorial diameter, the treatments that received chitosan NPs reached the largest sizes, with no significant differences between them. But these treatments did produce significantly different results from the control, which had the lowest value.

The polar diameter values were greater than those of the equatorial diameter, so a relationship between both variables in each treatment will always be > 1 in this type of fruit.

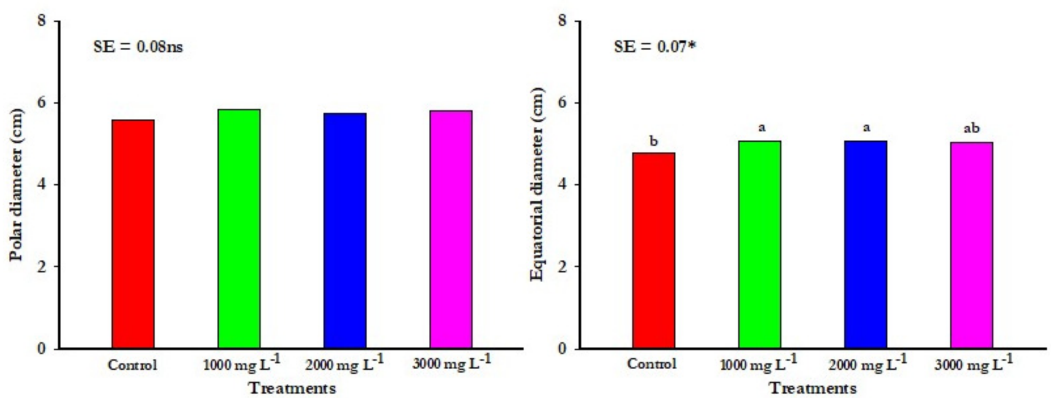

Figure 4. Size of tomatoes based on polar diameter and equatorial diameter
Different letters above the bars represent significant differences between treatments based on $p \leq 0.05$. SE: standard error

Biochemical determinations of fruit quality

When analysing some variables related to the nutraceutical quality of the fruits, it was found that for antioxidant capacity, vitamin C and carotenoid content (Figure 5), the trend was similar in terms of the influence of the treatments. There was an increase in values from the control treatment (lowest values) to the highest concentration of chitosan NPs, always with significant differences between treatments.

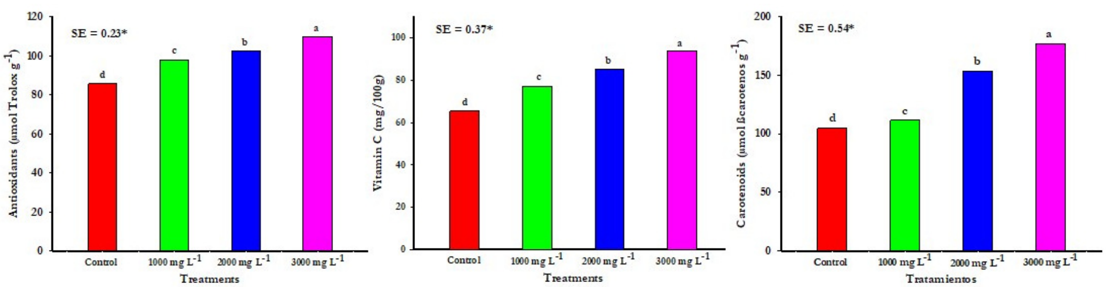

Figure 5. Antioxidant capacity, vitamin C and carotenoids in tomatoes
Different letters above the bars represent significant differences between treatments based on $p \leq 0.05$. SE: standard error

The rest of the variables analysed for the same purpose (flavonoid, polyphenol and TSS contents) showed similar behaviour to the previous ones. It was observed that the highest concentration of chitosan does not exceed 20 (catechin mg.g^{-1}), in polyphenols (7 mg galic acid/g) and total soluble solids (5%) (Figure 6).

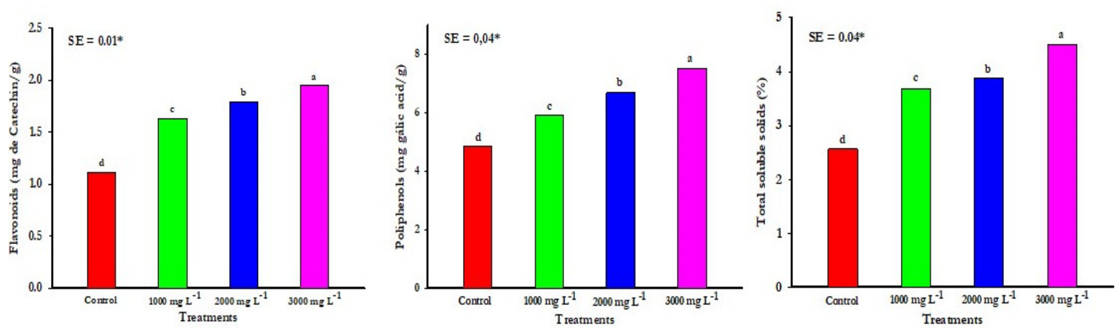

Figure 6. Contents of flavonoids, polyphenols and total soluble solids
Different letters above the bars represent significant differences between treatments based on $p \leq 0.05$. SE: standard error

Discussion

Chitosan nanoparticles can significantly improve the physiological functioning of tomato plants, including photosynthesis and nutrient uptake, resulting in better overall plant performance. These NPs are used to promote growth primarily because they are more efficient and have lower molecular weights, improved bioavailability, longer half-life and higher surface area to volume ratio in comparison to other nanoparticles (Ingle *et al.*, 2022).

Moreover, chitosan is known to promote growth in plant biomass (Behboudi *et al.*, 2018) and due chitosan nanoparticles has amino groups ($-NH_3^+$) that are positively charged and have affinity for cell membranes which causes higher reactivity in the plant system. In addition, chitosan has a nitrogen content of around 9-10%, which serves as a macronutrient for the plant (Agarwal *et al.*, 2015). Nitrogen is an important element for plants, given its incorporation into different metabolites that are necessary for plants to function properly.

There have been findings of chitosan increasing growth in plants' shoot systems (although in the present study, this effect was found only in the early stages of plant growth) and root growth (Lares *et al.*, 2019). Even though chitosan application decreases transpiration, it does not affect plant height, leaf area, root length or biomass variables according to Pandey *et al.* (2018). It is also of interest to keep in mind the growth stage at which the foliar application is made (Pongprayoon *et al.*, 2022), and it is for some of the reasons that can explain why the variables previously analysed in this study were not outstanding with the exception of the biochemical ones.

However, Yu *et al.* (2021) report how in some studies they have achieved increases of up to 25% in the polar diameter and 14% in the equatorial diameter when applying different doses of chitosan nanoparticles in their work. This contrasts with the present study, where no variations in the polar diameter or the equatorial diameter were observed; the increase was around 5%.

Besides, the results indicate a positive effect on plant growth, yet this has not always been the case. There are contradictions in the scientific literature, for instance cases in which the concentrations applied have had greater emphasis (Suwanchaikasem *et al.*, 2024). However, low concentrations have been found to be the most beneficial for growth, which is completely in line with the results of this work. On the other hand, Ramírez *et al.* (2021) evaluated the effect of chitosan NPs on the nutraceutical quality of sprouted triticale (x Triticosecale Wittmack), obtained similar results.

In terms of yield-production, this increased with higher doses of chitosan treatments compared to the control. This coincides with the results obtained by Pincay *et al.* (2021), who found a higher yield with the highest dose of chitosan in tomato plants. Their results also fully agree with the values for growth variables in the present work, in which the increase in root size is of essential importance. This is because larger-sized roots can explore a larger soil area, which mostly allows them a greater absorption of nutrients. Considering the small variation in the linear dimensions of the fruits (equatorial and polar diameters), a higher yield will be associated with a higher number of fruits per plant in the 3000 mg L^{-1} treatment, or with the biomass of the fruits, an aspect which is influenced by some variables related to the fruits' nutraceutical quality.

In general, it has been reported that chitosan has a significant effect on cell division, cell elongation, enzyme activation and protein synthesis, which results in faster growth rates of roots, stems and flowering and a higher number of flowers. These aspects can eventually lead to higher yields.

To another hand, it has been shown that the nutraceutical quality of fruits is also affected by chitosan NPs in different crops. Increased vitamin C content has an important impact on human health, as it is a water-soluble micronutrient with pleiotropic benefits due to its high antioxidant capacity (Carr *et al.*, 2017). Therefore, tomatoes with high amounts of this vitamin are of high quality and beneficial to human health.

The use of chitosan NPs can act as an inducing agent of metabolic processes, as they can increase the content of bioactive compounds highlighted in the development of the fruit and can stimulate the production of secondary metabolites (Xoca *et al.*, 2017; Xoca *et al.*, 2019; Montalvo *et al.*, 2020). What is more, an increase in the flavonoid content of sprouted triticale has been demonstrated by applying chitosan NPs to the leaves (Ramírez *et al.*, 2021). Significant increases in phenol content may be since the synthesis of this compound is also increased by the effect of chitosan application (Sanwam *et al.*, 2023).

A higher content of carotenoids will be associated with a more intense fruit colouration, which is verified at higher concentrations of chitosan NPs, although flavonoids are also important in this aspect and they also increased. Similar results were reported by Zheng *et al.* (2023). They found that high carotenoid contents are related to an increase in the genes involved in carotenoid synthesis, which increased noticeably in the fruits of plants treated with chitosan NPs compared to the untreated control. Carotenoids and chlorophyll degradation are largely responsible for the red colour of tomatoes (Wu *et al.*, 2022).

The increase of antioxidants in tomatoes after applying chitosan NPs favours the quality of the fruit and this contributes towards its beneficial effect on health. The high consumption of fresh and processed tomatoes makes this fruit a primary source of antioxidant molecules (ascorbic acid, vitamin E, carotenoids, flavonoids and phenolic acids) involved in the prevention of a wide range of diseases (Collins *et al.*, 2022). Higher amounts of antioxidants have been found in tomato varieties for industry use than in those produced for fresh consumption (Bianchi *et al.*, 2023). This denotes the varietal character of this variable in addition to the fact that the epidermis of the fruit is more abundant in antioxidants than other parts of the fruit, a characteristic that has also been found in other fruits (Rocha *et al.*, 2023).

Tomatoes' higher total soluble solids content could be attributed to the positive modulating effect of chitosan on the enzyme activity that catalyses the degradation of starch (the plants' reserve polysaccharide), cellulose (structural carbohydrate) and other cellular components of plant tissue (Alarcón *et al.*, 2020). It has also been argued (Falcón *et al.*, 2015) that chitosan oligosaccharides activate certain physiological and biochemical mechanisms, especially enzymatic and hormonal ones, which stimulate the degradation of certain reserve carbohydrates (sucrose and starch), so that they can be transformed into simpler sugars that increase the concentration of TSS in the fruit, preferably during ripening. However, it must be recognised that the values of TSS are also dependent on the varieties used (Pérez *et al.*, 2020).

It is evident that the field of nanotechnology applied to agriculture is very extensive and, therefore, opens a whole field of research and challenges. But one must be very precise with what one intends to identify, as Sánchez *et al.* (2024) pointed out.

Conclusions

The results obtained in this study once again demonstrate what is indicated in the scientific literature: high doses on plant growth or development variables (morphophenological) do not produce significant effects compared to controls. However, for the biochemical variables evaluated in fruit, the effects are positive. The results invite us to continue expanding our knowledge of chitosan nanoparticles and elucidating their physiological and biochemical effects, thus reducing the existing contradictions in the scientific literature and promoting how chitosan represents a key tool in modern agriculture, especially within the framework of organic and sustainable agriculture. Its dual function as a biofertilizer and biocontrol agent makes it a strategic input for improving crop productivity, reducing losses due to diseases, and minimizing the environmental impact of agricultural activity.

Authors' Contributions

Research, Conceptualization, Validation, Project Management and Fund Acquisition: JJRP. Writing: proofreading and editing: LTLLR. Research and methodology: EIJM. Writing: Original Draft Preparation and Writing: JJRP. Revision: Data curation, data analysis: BMA. Writing, revision, formal analysis: OFLO. Review and Editing: EORP.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest related to this article.

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