Effects of Chitosan Application on the Yield and Quality of Lettuce

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Modern agricultural production has adopted the principle of maximizing yields in an environmental-friendly, inexpensive, and non-hazardous way. With these characteristics, chitosan has become an increasingly preferred biostimulant in sustainable agriculture. The aim of this study was to determine the effects of chitosan application on yield and quality in lettuce cultivation. For this purpose, Yedikule 5107 lettuce variety was used, and foliar chitosan applications were applied 3 times at 4 different doses of 0, 75, 150, and 300 ppm levels. The total yield, leaf number, leaf width, chlorophyll, total phenolic, and antiradical activity values ranged from 2970 to 3290 kg da^{-1}, from 35.8 to 37.7, from 11.27 to 12.33 cm, from 38.05 to 41.82, from 21.50 to 38.71 mg/100 g, and 46.0% to 54.9%, respectively, and the highest values were obtained in the 300 ppm chitosan application. The highest root collar and plant diameter values were determined at 75 ppm and 150 ppm chitosan doses, respectively. All chitosan applications increased leaf length. Furthermore, chitosan applications were effective in preventing nitrate accumulation. In conclusion, it was determined that the effect of pre-harvest chitosan application was positive, and especially 300 ppm chitosan dose was more effective than other doses.

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INTRODUCTION

Vegetables, which are among the basic nutritional elements, are plants that have an important place in our nutrition in terms of vitamins, minerals, anthocyanins, phenolic, and antioxidant compounds they contain. Raw vegetables are one of the most preferred plant groups for a balanced and healthy diet. They are becoming increasingly popular and consumed because of their health benefits, as well as their accessibility and ease of consumption (Balki 2021; Kaçar 2022). In addition to nutritional health, colour and flavour (taste and aroma) are important quality criteria for consumers (Bhuiyan *et al.* 2015). Total soluble solids contribute to flavour formation and are responsible for the sweet taste (Vargas-Arcila *et al.* 2017).

Lettuce is one of the easiest crops to cultivate together with other agricultural products. In addition, due to its high yield and nutrient content, there is an increase in the amount of production in parallel with the awareness of healthy nutrition. For these reasons, considering consumption habits and production values, the importance of practices that will benefit sustainable agriculture in lettuce cultivation is increasing daily (Kaçar 2022). Moreover, the majority of governments and other organizations recognize that the nutritional quality of food is strategically important. Therefore, it is extremely important to identify and develop sustainable, innovative, and inexpensive approaches to increase vegetable production and reduce nitrate concentration at the same time. Excessive nitrate accumulation in vegetables is a widespread problem that poses a potential threat to human health (Bian *et al*. 2020). This problem is more common in vegetable species, such as lettuce, which has a short vegetation period of 2 to 3 months and whose vegetative parts are consumed. Nitrite, which is formed by the reduction of nitrates because of digestion, causes poisoning, cancer, vitamin A deficiency, “methemoglobinemia”, and other diseases (Karademir 2019).

Regarding the growing global population and the necessity to increase crop production in a sustainable way, the agricultural sector needs innovative ways to improve productivity and resource efficiency. Accordingly, biostimulants have appeared as new, hopeful, and eco-friendly products to enhance the general sustainability of production systems. Among biostimulants, chitosan, which has proven potential to improve plant growth and increase crop quality and yield, has recently attracted considerable attention (Afonso *et al*. 2022).

Chitosan is a natural, inexpensive, and safe biopolymer that can be obtained in abundant quantities from natural sources. It is of great interest due to its non-toxic, biocompatible, and easily biodegradable properties. Chitosan is used in many industries such as agriculture, food, pharmacy, medicine, cosmetics, wastewater treatment, and textiles (Malerba and Cerena 2016). Along with having antibacterial, antiviral, and antifungal effects, it additionally acts by enhancing the plant's defensive mechanism to prevent and slow the spread of infections. Additionally, its use in agriculture has become important because of its many positive effects such as chelating metal ions in the environment (soil, water, *etc*.) and preventing the uptake of toxic metals in plants (Vasconcelos 2014). Chitosan’s effect on plants varies depending on the plant species, application conditions and time, chemical structure, concentration, and molecular weight (Kurtuluş and Vardar 2020). This is supported by a number of studies. For example, Monirul *et al*. (2018) reported that after applying different doses of chitosan (0, 25, 50, 75, and 100 ppm), the best results were obtained from the dose of 75 ppm in pepper and 50 ppm in tomato. In a study on strawberries, chitosan applications with different molecular weights (5, 12, 21, 50, 125, and 500 kDa) were compared. As a result, the highest yield values were obtained from chitosan with molecular weights of 12, 21 and 50 kDa (Ochmian *et al.* 2022). In another study, Sultana *et al.* (2015) reported that as a result of different doses of chitosan (0, 50, 75, 100 ppm) applied to spinach, the 100 ppm chitosan dose significantly increased yield by promoting growth. Elsharkawy and Ghoneim (2019) reported that as a result of applying chitosan to artichoke in different seasons (2014 and 2015) and doses (0, 150, and 300 ppm), the highest yield values were obtained from the 300 ppm chitosan dose in both seasons, but the yield values were lower in the second season than in the first season. This study aimed to determine the effects of different doses of chitosan applications on yield, quality, and nitrate accumulation in lettuce.

EXPERIMENTAL

Experimental Site

This study was conducted in Pamukkale district, Denizli province, Türkiye from October 2019 to February 2020 (37º55' N, 29º6' W). The soil texture of the study area was loamy, slightly alkaline (pH: 7.8), salt-free (0.0172%), high in lime (24.05%), low in organic matter (1.19%), high in potassium (K_2O - 48.17 kg/da), and very low in phosphorus (P_2O_5 - 2.75 kg/da). Table 1 presents some climatic parameters of the experimental site.

Table 1. Some Meteorological Data of the Experimental Site between 2019-2020

	October	November	December	January	February
Monthly Total Precipitation (mm)	11.2	18.0	103.2	29.8	52.6
Monthly Average Temperature (°C)	20.1	14.9	70.1	65.0	67.4
Monthly Average Relative Humidity (%)	50.6	59.6	70.1	65.0	67.4
10 cm Average Soil Temperature (°C)	20.1	13.2	6.8	3.6	6.6
10 cm Maximum Soil Temperature (°C)	30.2	22.1	12.8	9.5	14.2
10 cm Minimum Soil Temperature (°C)	12.0	7.0	1.3	-0.3	-0.2
20 cm Average Soil Temperature (°C)	20.4	13.7	7.4	4.1	6.7
20 cm Maximum Soil Temperature (°C)	26.6	19.0	12.7	7.7	11.5
20 cm Minimum Soil Temperature (°C)	14.2	9.1	3.4	0.9	1.1

Materials

Yedikule 5701 lettuce variety was used as plant material in the study. Seedlings were obtained from Ufuk Greenhouse Seedlings and Seeds Company (Denizli, Türkiye). Low molecular weight chitosan (Sigma–Aldrich, St. Louis, MO, USA) was used for the applications. Chitosan solutions were prepared in 0.15% acetic acid solution at 75 ppm, 150 ppm, and 300 ppm doses, and polyethylene glycol sorbitan monolaurate (Tween 20) (0.5%) was added as a spreader-adhesive. The doses in this study were determined by considering the doses of chitosan that gave the best results in studies conducted on some vegetable species. For example, 75 ppm in pepper and Malabar spinach (Mondal *et al.* 2011; Monirul *et al.* 2018), 100 and 125 ppm in okra (Mondal *et al.* 2012), 100 ppm in spinach (Sultana *et al.* 2015), and 300 ppm in artichoke (Elsharkawy and Ghoneim 2019).

Experimental Design and Cultural Practices

The experiment was configured in randomized plots, with three replicates, with 15 plants per replicate. Seedlings were planted in the field on 09.10.2019 at a distance of 40 × 25 cm^2 between rows and above rows. Four different doses (0, 75 ppm, 150 ppm, and 300 ppm) of chitosan as foliar spraying started 4 weeks after transplanting and were applied 3 times with 10-day intervals (November 9, November 19, and November 29). The fertilisation programme for lettuce was calculated and applied according to Güçdemir (2006), based on the data obtained from the soil analysis and taking into account the nutrients contained in the fertilisers to be applied. Therefore, a total of 20 kg mono ammonium phosphate, 10 kg ammonium sulfate, 5 kg potassium sulfate, and 2 kg zinc sulfate were applied twice during the vegetation period (on the 20^{th} and 40^{th} days after planting). Yield and quality analyses of lettuce harvested on February 6, 2020, were performed at Isparta University of Applied Sciences, Faculty of Agriculture, Horticulture Laboratory.

Variables Analyzed

Total yield (kg da^{-1}), plant height (cm), plant diameter (cm), root collar diameter (mm) number of leaves, leaf length and width (cm) were analysed in the study.

Chlorophyll and color values

Measurements were made from 3 different points on the outer 2nd and 3rd leaves. Chlorophyll and color values were determined by using the Minolta SPAD-502 chlorophyll meter and the Minolta CR-400 model color device, respectively. Color results were given in terms of CIE L^*, a^*, and b^*. Chroma (C^*) and hue angle (h°) values were calculated according to a^* and b^* values [$C^* = \sqrt{(a^{*2} + b^{*2})}$; h^o= arctan (b^*/a^*)] (McGuire 1992).

Total soluble solid (TSS %)

After the outer 2nd and 3rd leaves of lettuce plants were homogeneously shredded, the juices were obtained and the TSS was determined using a digital refractometer (Hanna HI96801, Hanna Instruments, Cluj-Napoca, Romania).

Dry matter (%)

Samples taken from the harvested lettuce plants were dried in an oven at 65 °C after determining their wet weights. After reaching constant weight, the samples were weighed on a precision balance, and dry weight values were recorded. The results were calculated according to Kul (2014) and expressed as a percentage.

Ascorbic acid (mg/100 g)

The ascorbic acid content in lettuce samples was determined according to the oxalic acid method described by Cemeroğlu (2013). A homogeneous mixture was obtained with the help of a blender with 2% oxalic acid solution as much as the amount of sample taken from lettuce leaves. The known amount of sample taken from this mixture was made up to 100 mL with oxalic acid and filtered. About 10 mL of the filtrate was titrated with the 2,6-dichlorophenol-indophenol solution. Results are given in mg/100 g.

Total phenolic (mg/100 g)

For the extraction process, 5 g of lettuce sample was homogenized in 20 mL of 95% ethyl alcohol for 2 min. After boiling for 10 min, the homogeneous mixture was centrifuged at 8000 rpm and filtered through a filter paper. Then, 20 mL of 80% ethyl alcohol was added to the samples and boiled for 10 min. The mixture was made up to 100 mL with 80% ethyl alcohol. After extraction, total phenolic matter analysis was completed using the Folin-Ciocalteu reagent according to the method described by Coseteng and Lee (1987), and the results were given in mg/100 g.

Antiradical activity (%)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Dorman *et al*. 2003) was used to determine antiradical activity in lettuce leaves. Pureed samples were passed through a filter and centrifuged. About 50 µL of this filtrate was taken, and 450 µL of Tris-HCl buffer (50 mM, pH 7.4) and 1.0 mL of DPPH solution (0.10 mM, in methanol) were added. After the samples were kept in the dark for 30 min, absorbance readings were taken at a wavelength of 517 nm in a spectrophotometer (Boeco-S200 VIS; Bremen Optical Engineering Company, Hamburg, Germany model).

Nitrate accumulation (mg/kg)

Nitrate accumulation in leaves was determined by the salicylic acid method (Cataldo *et al*. 1975). After adding 10 mL of distilled water to 1.0 g of the sample, the mixture was kept in an incubator at 45 °C for 1 h and centrifuged at 5000 rpm for 15 min. About 0.8 mL of 5% salicylic acid dissolved in sulfuric acid was added to 0.2 mL of the sample. After incubation at room temperature for 20 min, 19 mL of 2 N NaOH solution was added to the samples. Absorbance readings were taken at 410 nm wavelength.

Statistical Analysis

The data were processed by analysis of variance using Minitab (17) Inc. (MINITAB LTD., Coventry, UK) package program. Significant differences between means were revealed using Tukey Test ($P < 0.05$).

RESULTS AND DISCUSSION

Total Yield

The results showed that chitosan applications significantly affected the total yield of lettuce ($P < 0.05$). Compared to the control, 300 ppm chitosan application increased the total yield value 8.52% (Table 2). Various studies have reported that chitosan applications significantly increased yield values in pepper (Chookhongkha *et al*. 2012), okra (Mondal *et al*. 2012), tomato (Rahman 2015), and in rocket (Özkan 2021). Costales-Menéndez and Falcón-Rodríguez (2020) reported that the yield increase was due to the stimulatory effect of chitosan on plant growth by increasing nutrient availability and absorption as well as photosynthesis through the accumulation of metabolites and increased leaf pigmentation.

Table 2. Effects of Chitosan Applications on Total Yield and Growth Characteristics in Lettuce

Applications	Total Yield (kg/da)	Plant Height (cm)	Plant Diameter (cm)	Root Collar Diameter (mm)	Number of Leaves per Plant	Leaf Length (cm)	Leaf Width (cm)
Control	3032.5b*	31.30 n.s.	23.84b*	21.40ab*	36.53ab*	24.17b*	11.30b*
75 ppm	2970.1b	31.48	23.43b	21.94a	35.80b	25.77a	11.48b
150 ppm	3069.3b	31.29	24.75a	20.60b	36.33ab	25.50a	11.27b
300 ppm	3290.9a	32.00	23.32b	20.81b	37.73a	25.67a	12.33a

*: Means with different letters differ significantly ($P < 0.05$), n.s. = not significant

Plant Height

When Table 2 is examined, although the effects of the chitosan applications on the plant height of the lettuce were found to be insignificant ($P < 0.05$), it was determined that at 300 ppm chitosan dose increased the plant height 2.24% compared to the control. This increase can be explained by the fact that chitosan promotes plant growth by influencing plant physiological processes such as protein synthesis, enzyme activation, nutrient uptake, cell division, and cell elongation (Chakraborty *et al.* 2020). In different studies, chitosan applications increased plant height values in tomato (Rahman 2015), and spinach (Dewi *et al*. 2020), whereas Jan Mohammadi *et al*. (2014) found that the effect of chitosan applications on plant length values in lentil was not significant.

Plant Diameter

The highest plant diameter value among the applications was obtained in the 150 ppm chitosan dose, and it was determined that this dose increased the plant diameter value 3.8% compared to the control. Pincay-Manzaba *et al*. (2021) and Abd El-Aziz *et al*. (2019) both reported that chitosan treatments increased diameter values in tomato and onion, respectively. Mondal *et al*. (2012) reported that chitosan positively affected plant growth and development by increasing the activities of key enzymes in nitrogen metabolism and improving nitrogen transport in leaves (Table 2).

Root Collar Diameter

Table 2 shows that the effects of the chitosan applications on root collar diameter were significant ($P < 0.05$). According to Riseh *et al.* (2023), chitosan can enter the vascular system of plants and activate plant metabolic pathways by forming a complex with other compounds. As well as providing the plant with the nutrients it needs, it can form bonds with other natural polymers and combine with fertiliser and other nutrients to improve the texture of the soil. This report may explain the reason for the 2.5% (75 ppm chitosan application) increase in root collar diameter compared to the control. Supporting this study, Abd El-Aziz *et al.* (2019) reported that chitosan increased the neck diameter of onion.

Number of Leaves per Plant

Table 2 demonstrates that the effect of chitosan application on leaf number was positive. Abd El-Aziz *et al*. (2019) in onion, Abdelaal *et al*. (2021) in garlic, and Nurliana *et al*. (2022) in lettuce reported that chitosan applications increased the number of leaves in plants. Supported by these reports, in this study, it was determined that the leaf numbers increased 3.2% in the 300 ppm chitosan application compared to the control. According to Vasconsuelo *et al*. (2004) chitosan applications increased the lignification of cell walls and lignin biosynthesis in plants and thus triggered shoot formation in plants.

Leaf Length

The effects of chitosan applications on the leaf length of lettuce were significant ($P < 0.05$). It was observed that chitosan applications increased leaf length 5.5% (150 ppm), 6.2% (300 ppm), and 6.6% (75 ppm) compared to the control (Table 2). In agreement with the current findings, Sultana *et al*. (2015) and Dewi *et al*. (2020) reported that chitosan increased leaf length in strawberry, and spinach, respectively. Furthermore, Saharan and Pal (2016) reported that chitosan application can enhance plant growth by increasing auxin hormone synthesis. However, the mechanism by which this hormone synthesis is increased is not yet understood.

Leaf Width

In this study, it was observed that chitosan applications (especially 300 ppm chitosan dose) had a positive effect on leaf width compared to the control (Table 2). Similarly, Dewi *et al*. (2020) in spinach, and Nurliana *et al*. (2022) in lettuce reported that chitosan applications increased leaf width.

Chlorophyll

Table 3 shows that the effects of chitosan applications on chlorophyll values were significant ($P < 0.05$). The highest values were found in 300 ppm chitosan application and control. Different from the current findings, Mondal *et al*. (2012) reported that chitosan applications increased chlorophyll content in okra, Xu and Mou (2018) in lettuce, and Özkan (2021) in rocket.

Table 3. Effects of Chitosan Applications on Chlorophyll, TSS, and Dry Matter in Lettuce

Applications	Chlorophyll (SPAD)	TSS (%)	Dry Matter (%)
Control	41.6a*	3.89 ns	5.35 n.s.
75 ppm	38.4b	4.10	5.66
150 ppm	38.1b	3.99	5.05
300 ppm	41.8a	4.06	5.50

*: Means with different letters differ significantly (P < 0.05), n.s. = not significant

TSS

The effect of chitosan on the TSS of lettuce was insignificant (P < 0.05) (Table 3). The lowest value was found in the control (3.89%), while the highest value was obtained from the 75 ppm chitosan application. Similarly, Ibraheim and Mohsen (2015) in zucchini, and Özkan (2021) in rocket reported that chitosan applications increased the amount of TSS, but this increase was statistically insignificant.

Dry Matter

When the results on dry matter values were analyzed, despite being insignificant, the 75 and 300 ppm chitosan doses gave better values (Table 3). According to various studies on chitosan, it increases the dry matter content of okra (Mondal *et al*. 2012) and rocket (Özkan 2021).

Leaf Color

In this study, L^* values ranging from 45.3 to 46.5, a^* values from -17.2 to -18.0, b^* values between 26.2 and 27.5, C^* values from 31.3 to 32.8, and *h*° values between 123.7 and 123.6 were obtained (Table 4). Although the effects of chitosan treatments on leaf color values were insignificant, it was determined that chitosan caused relatively better results compared to the control. Chookhongkha *et al*. (2012) reported positive effects of chitosan applications on leaf color values in pepper and Özkan (2021) in rocket.

Table 4. Effects of Chitosan Applications on Leaf Color Values in Lettuce

Applications	*L**	*a**	*b**	*C**	*h*°
Control	45.4 a*	-17.2a	26.2a	31.3a	123.3a
75 ppm	45.3a	-18.0a	27.2a	32.6a	123.6a
150 ppm	46.5a	-17.9a	27.5a	32.8a	123.1a
300 ppm	46.3a	-17.8a	27.0a	32.3a	123.3a

*Means in the same column followed by the same letter are not significantly different (P < 0.05)

Ascorbic Acid

The effects of the chitosan applications on ascorbic acid in lettuce were insignificant (P < 0.05) (Fig. 1). Ascorbic acid values ranged between 7.24 and 7.54 mg/100 g. However, when the data were examined, it was determined that chitosan applications decreased ascorbic acid values compared to the control. A similar situation, *i.e.*, decrease in ascorbic acid values with increasing chitosan doses, was reported by Sultana *et al*. (2017) and Özkan (2021). Zoldners *et al*. (2005) described that as the presence of chitosan significantly accelerates ascorbic acid oxidation and this effect varies depending on the chitosan/ascorbic acid ratio.

Total Phenolic Content

The results showed that chitosan applications significantly affected the total phenolic content of lettuce ($P < 0.05$). A 300 ppm chitosan application showed the highest total phenolic value. This application was followed by the 150 ppm application. The lowest total phenolic values were found in 75 ppm chitosan and control applications (Fig. 1).

Plants need constitutive defense and induced defense to regulate their basal metabolism and thus accelerate secondary metabolic processes. It has been demonstrated that chitosan is an elicitor (stimulant) that causes a variety of biological reactions in some plants (Zheng *et al*. 2021). Kurtuluş and Vardar (2020) stated that chitosan is used as an abiotic elicitor in the production of secondary metabolites in plant cell and tissue cultures, increases defense responses against biotic and abiotic stresses in plants, and increases the amount of phenolic compounds and proteins by activating some genes in signaling pathways. Furthermore, in studies conducted by different researchers, it was found that chitosan applications increased phenolic matter content in *Hypericum perforatum* (Brasili *et al*. 2014), tea (Srisornkompon *et al*. 2014), and rocket (Özkan 2021). These reports further support the results from this study.

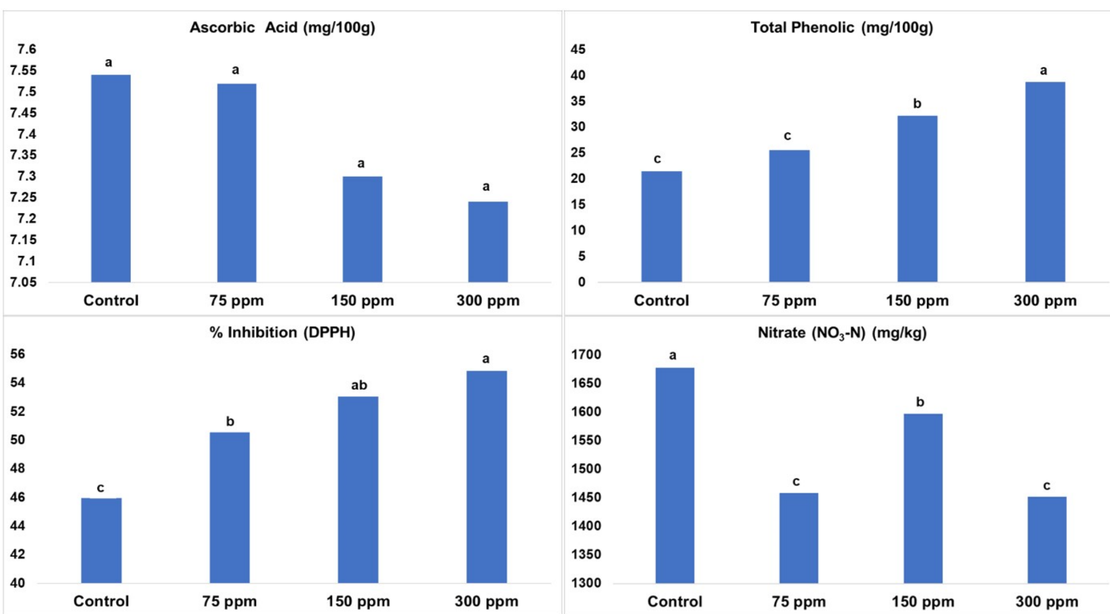

Fig. 1. Effects of chitosan applications on ascorbic acid, total phenolic, antiradical activity, and nitrate accumulation in lettuce

Antiradical Activity

When Fig 1 is examined, it is seen that the effects of the chitosan applications on antiradical activity are significant ($P < 0.05$). It was determined that chitosan applications increased the antiradical activity 10% (at 75 ppm chitosan dose), 15% (at 150 ppm chitosan dose), and 19% (at 300 ppm chitosan dose) compared to the control. In support of these results, different researchers reported that chitosan applications significantly increased antioxidant activity in lettuce sprouts (Viacava and Roura 2015), spinach (Singh 2016), tomato (Hernández-Hernández *et al*. 2018), and rocket (Özkan 2021).

Nitrate Accumulation

The effects of different doses of chitosan applications on nitrate content in lettuce were significant. The highest nitrate value was found in the control (1680 mg/kg), while the lowest nitrate content was obtained from the 300 ppm dose of chitosan application (1450 mg/kg) (Fig. 1).

According to the Turkish Food Codex 2008 data, the highest acceptable nitrate value for lettuce grown in open conditions harvested between October 1 and March 31 is 4000 mg/kg, while this value is 4500 mg/kg for lettuce grown under cover (Karademir 2019). The current study was below these limits, and it was determined that chitosan applications reduced nitrate content 4.4% (at 150 ppm chitosan dose), 13.05% (at 75 ppm chitosan dose), and 13.4% (at 300 ppm chitosan dose), respectively, compared to the control.

In support of the current findings, Chung *et al*. (2001) and Özkan (2021) found that chitosan applications reduced nitrate content in lettuce and rocket 14% to 18% and 4% to 9%, respectively, compared to the control group. Similarly, Mondal *et al*. (2012, 2016) reported that chitosan treatments increased nitrate reductase activity leading to reduced nitrate accumulation.

CONCLUSIONS

1. In this study, lettuce yield varied between 2970 and 3290 kg/da, and it was determined that 300 ppm chitosan application increased the yield 8.5% compared to the control. In addition, 300 ppm chitosan application had a positive effect on leaf number, leaf width, chlorophyll and total phenolic contents, and antiradical activity values. While 75 ppm chitosan application had the highest root collar diameter value, 150 ppm chitosan application had the highest plant diameter value.
2. It was determined that all chitosan doses increased leaf length and decreased nitrate accumulation compared to the control.
3. As a result, when all parameters were evaluated, it was observed that chitosan application, especially 300 ppm dose, made positive contributions not only to yield and yield parameters but also to bioactive compounds (total phenolic and antiradical activity) and nitrate, which are of great importance for human health.
4. In addition, the fact that the best results were generally determined at the highest dose among the applications in the study reveals the necessity of new studies at higher levels of this dose.

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