SHST
Horticulture and Forestry Society of Transylvania

Ozsan Kilic T and Onus AN (2023)
**Notulae Scientia Biologicae**
Volume 15, Issue 3, Article number 11652
DOI:10.15835/nsb15311652
Research Article

NSB
Notulae Scientia Biologicae

# Effects of pre-harvest chitosan application on growth parameters and total phenolic - antioxidant contents of bitter gourd (*Momordica charantia* L.)

Tugce OZSAN KILIC*, Ahmet Naci ONUS

*Akdeniz University, Faculty of Agriculture, Department of Horticulture, Dumlupınar Bulvarı, 07070 Antalya, Turkey; tugceozsan@akdeniz.edu.tr (*corresponding author); onus@akdeniz.edu.tr*

## Abstract

Bitter gourd (*Momordica charantia* L.) plant is cultivated for both culinary and medicinal purposes in the world due to its valuable benefits for health-promoting properties. The current study aimed to reveal the effects of chitosan applied at three different concentrations (50 ppm, 100 ppm, and 150 ppm) in three different treatments with 21 days intervals on growth parameters and total phenolic and antioxidant contents of bitter gourd. The findings of the study demonstrated that the foliar treatment with 100 ppm chitosan at the second and third treatments had positive effects on the majority of growth parameters examined and on increasing total phenol and antioxidants in bitter gourd. As a result, chitosan could be a possible substance to be used to enhance bitter gourd plants' growth and development as well as in several industrial fields such as pharmaceuticals and agriculture.

***Keywords:*** agrochemical; bitter gourd; elicitors; foliar treatment; health properties; natural products

## Introduction

Global population expansion has made it crucial to find innovative ways to enhance human health by preventing oxidative stress and its damaging effects through metal chelating and/or free radical - scavenging processes (Pereira *et al.*, 2007). It has been proposed that bioactive compounds with antioxidant activity, such as certain vitamins and polyphenols, are primarily responsible for the preventive role since oxidative damage is one of the key processes in the genesis of many illnesses (Wang *et al.*, 2011; Viacava *et al.*, 2018). Therefore, to promote public health via diet, studies have recently concentrated on ways to increase the bioactive compounds of plant-based products. Because of their health-promoting properties, it is critical to increase our consumption of these bioactive compounds in daily nutrition.

Antioxidants can be significantly improved in terms of their pharmacological and nutritional qualities by extracting them from plant-based sources. Tropical plants, among other natural resources, have gained great interest as potentially rich sources of antioxidant chemicals with medicinal and nutritional benefits (Chong *et al.*, 2015). As a possible source of beneficial bioactive chemicals, bitter gourd (*Momordica charantia* L.) has a great potential. It is a popular tropical vegetable, belongs to *Cucurbitaceae* family, produced in different part of

*Received: 08 Aug 2023. Received in revised form: 10 Sep 2023. Accepted: 22 Sep 2023. Published online: 26 Sep 2023.*
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

the world for both culinary and medical uses because of its benefits for human health (Grover and Yadav, 2004; Braca *et al.*, 2008; Tanwar *et al.*, 2022). In traditional medicine, bitter gourd's leaves are used as a tea for diabetes, to treat intestinal gas, to encourage menstruation, and as an antiviral for measles, hepatitis, and other ailments. Due to health-promoting qualities of fruits and some other vegetative parts particularly anti - oxidative, antidiabetic, anti - inflammatory, anticancer, antiobesity, as well as antibacterial activities it has gained increasing interest on a global scale (Kulkarni *et al.*, 2021; Bora *et al.*, 2023).

To increase the synthesis and accumulation of bioactive compounds in plants, several approaches have been thoroughly adopted to increase their levels in plants. Elicitation, the improved or induced production of certain bioactive compounds, ensures plant competitiveness as well as survivability. Elicitors are exogenous molecules that induce secondary metabolic processes, which in turn trigger a variety of defensive responses in plants, comprising physiological and morphological adaptations as well as the accumulation of bioactive components beneficial for human health (Viacava *et al.*, 2018). Elicitors have recently been found to be an effective technique to increase plant growth and bioactive compounds synthesis in plants. They can perform this role thanks to a variety of their unique characteristics, which include antifungal, antibiotic, as well as antiviral capabilities. These chemicals also have a variety of economic uses, including as bioactive substances in agriculture, cosmetics, pharmaceuticals, and other fields. Pre-harvest elicitor applications on plants could be a one of the realistic strategies to promote bioactive components' biosynthesis (Pérez-Balibrea *et al.*, 2011).

In this regard, chitosan, a natural biopolymer, might provide a sustainable alternative to traditional agrochemicals for such a safer agricultural system (Maluin and Hussein, 2020). A natural elicitor, chitosan, is known as a biopolymer having several uses in industry, pharmaceuticals, and agriculture. Chitosan and its nanoparticles may function in agriculture as a powerful antibacterial agent, plant growth promoter, and agrochemical carrier (Gȯrnik *et al.*, 2008; Maluin and Hussein, 2020; Mirheidari *et al.*, 2022). The use of natural elicitors like chitosan might be a crucial part of sustainable agriculture, according to the ample evidence that suggests plants might well strategy achieves tolerance to a wide range of pathogenic microorganisms and enhance growth and development after the implementation of chitosan (Chakraborty *et al.*, 2020). A stimulating effect of chitosan on the accumulation of phenolics was reported in various plant species by several researchers such as in *Prunus armeniaca* L. (Ghasemnezhad *et al.,* 2010), *Fragaria* × *ananassa* cv. Qingxiang (He *et al*., 2018), *Stevia rebaudiana* Bertoni (Mehregan *et al*., 2017), *Salvia officinalis* L. (Vosoughi *et al*., 2018), *Mentha piperita* L. (Salimgandomi and Shabrangy, 2016), *Ocimum basilicum* L. and *Melissa officinalis* L. (Hawrylak-Nowak *et al*., 2021).

The aim of the current study is revealing the effect of foliar chitosan treatment growth parameters and total phenolic and antioxidant contents of bitter gourd's vegetative plant parts.

## Materials and Methods

### *Plant cultivation*

This study was carried out in greenhouses of Akdeniz University, Faculty of Agriculture, and Department of Horticulture. Bitter gourd fruits were harvested from Antalya, Turkey (36°53'10.1" N 30°45'23.4"E) roughly at the same size, appearance, and maturity. To remove debris, chaff, and other foreign objects, fruits were washed under tap water. In order to conduct the trials, damaged, broken, and hollow seeds were manually removed from the cleaned fruits. Three seeds used in the study were sown in plastic pots at the size of 13 × 35 cm filled with peat: perlite mortar mixture at the ratio of 2:1.

### *Morphological characteristics*

The uniform bitter gourd plants were taken from each plastic pot at the 4-5 leafy stage (approximately 30 days from sowing) and transferred to new plastic pots filled with the same peat: perlite mixture ratio to measure morphological characteristics of number of leaves, number of leeches, length of leeches, length of petioles, the thickness of petioles, length of internodes, the thickness of internodes, number of internodes, lengths of the plants. In addition; fresh and dry weights of leaves, and SPAD (Konica Minolta SPAD-502Plus) values were also measured. Plant height measurements were taken from the emerging point of soil surface to the top of the main stem of plants. Length measurements were conducted with a ruler and a measuring tape, while thickness measurements were performed with a digital caliper (Figure 1).


**Figure 1.** Bitter gourd plants at different growth and development stages after chitosan elicitor treatments

All above stated morphological features were recorded before applications and after 21 days of the last chitosan treatment. After twenty-one days from the last chitosan treatment, the fresh leaf samples from each chitosan application were harvested. After weighing up the fresh leaves, leaves were kept at 70 °C thorough 72 hours in an incubator. At the end of the 72 hours leaves' dry weights were also measured.

*Preparation and application of chitosan*

While chitosan was dissolved in 0.1 M HCl solution at various concentrations (50, 100, 150 ppm) and supplemented with 0.5% Tween-20, the control plants treated with distilled water with 0.5% Tween-20.

Considering the developmental stages and starting from the first true leaf formation of the plants, the different chitosan dose applications were conducted thrice with 21 days intervals as follows; first chitosan dose application was done to the first 5 leaves, second application was to the first 10 leaves and the third application was the first 15 leaves of plants. All chitosan treatments were conducted before noon by spraying 5 mL onto each leaf with a hand sprayer.

*Total phenolic content determination*

By utilizing the Folin-Ciocalteu technique as described by Škerget *et al.* (2005), the total phenolic content was calculated. The sample (1.0 g) is extracted in 100 mL of the extraction solution. The 2.5 mL Folin-Ciocalteu reagent, which had been diluted 10 times with water, was combined with a volume of 0.5 mL of sample. With the addition of 2.0 mL sodium carbonate (75.0 g $L^{-1}$), the mixture was kept at 50 °C for five minutes before being cooled. At 760 nm, the absorbance values of the samples were determined. The total phenolic content was expressed as mg gallic acid equivalent per 100 g dry weight of leaves.

*Total antioxidant activity determination*

According to Fernández-León *et al.* (2013), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity was examined. With the aid of ultraturrax, a 1.0 g dry sample was dissolved for 1 minute in a 20 mL extraction solution (80% methanol). For analysis, 950 mL of diluted DPPH was added to 50 mL of the diluted extract in an Eppendorf tube, which was then vortexed for 30 minutes while being maintained in the dark. In order to measure the absorbance of the DPPH solution, spectrophotometric measurements at a wavelength of 515–517 nm were taken. The total antioxidant activity was expressed as mg trolox equivalent per 100 g dry weight of leaves.

*Statistical analysis*

The current study's experimental treatments were carried out in triplicate and four plants in each replicate using a completely randomized factorial design. The acquired data were subjected to variance analyses using the JMP package software, and the differences between means were identified using the least significant difference (LSD) test; the differences were determined statistically significant at $p < 0.05$.

## Results and Discussion

The results of the experimental study influence on the bitter gourd plants' morphological characteristics and total phenolic and antioxidant contents are represented in Figures 2 to 5, and Table 1. Data shown in Figure 2 clearly demonstrate that the numbers of leaves, leeches, and internodes were affected by chitosan concentrations and treatment number.

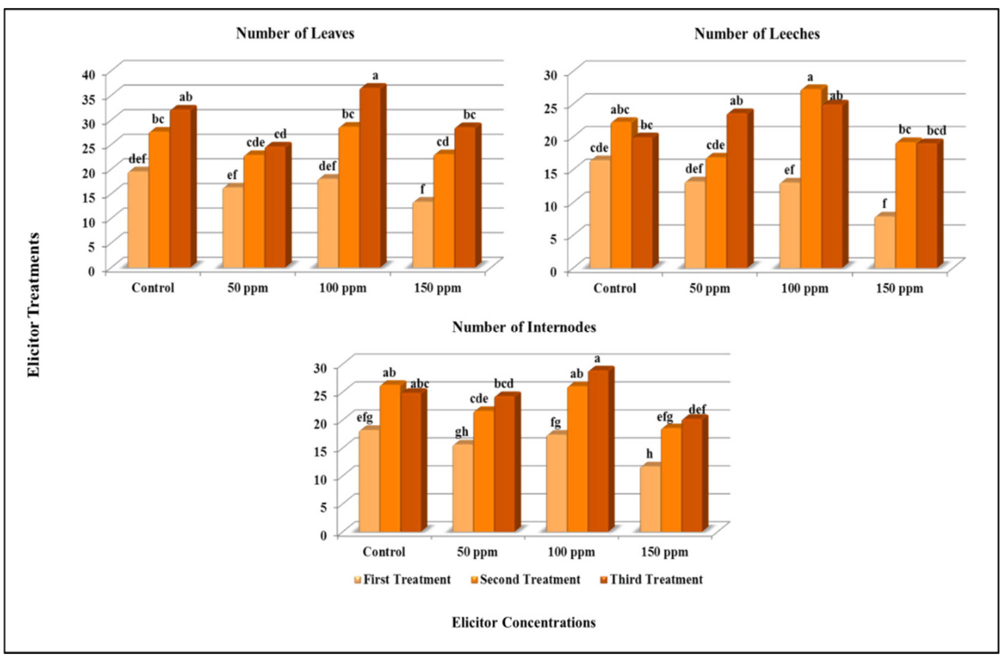


**Figure 2.** Effects of number of chitosan treatments at different dosages on number of leaves, leeches and internodes

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05;

Number of leaves: LSDc*= 3.805; LSDt*= 3.295; LSDc×t= N.S.;

Number of leeches: LSDc*= 3.429; LSDt*= 2.970; LSD c×t= N.S.;

Number of internodes; LSDc*= 2.424; LSDt*= 2.099; LSDc×t= N.S.; (c: cultivar; t: treatment; c×t: cultivar×treatment)

Experimental results also showed that 150 ppm chitosan concentration and the first treatment did not have any positive effect results on the number of leaves, leeches, and internodes. It was determined that the most suitable chitosan concentration was 100 ppm, while the appropriate treatment number was 3. Similar results were also reported by several researchers. For example, Nahar *et al.* (2012), Tantasawat *et al.* (2010) and Lee *et al.* (2005) showed the stimulative effects of chitosan on *Cymbidium*, *Dendrobium* and soy seedling growth, respectively. In another study, it was reported that foliar spray treatment of chitosan at 0.4 g $L^{-1}$ concentration led to a substantial improvement under normal or stressful conditions when compared with untreated control plants for plant growth properties (Ghasemi Pirbalouti *et al.*, 2017).

When the chitosan elicitor concentration applied and number of treatments were evaluated on petiole length, it was determined that the 100-ppm application dose provide the most ideal results at the 1$^{st}$ or 2$^{nd}$ treatment. In terms of leech length, the 100-ppm chitosan treatment concentration stood out, while the 2$^{nd}$ or 3$^{rd}$ application times showed statistically significant differences in terms of number of treatments. Similarly, the effects of the 100-ppm chitosan treatment concentration at the 3$^{rd}$ treatment were statistically prominent in terms of plant lengths. Unlike its effects on other morphological characteristic parameters, it was determined that the effect of 150 ppm chitosan concentration on internode lengths of bitter gourd plant was statistically the most ideal at the third application time (Figure 3).

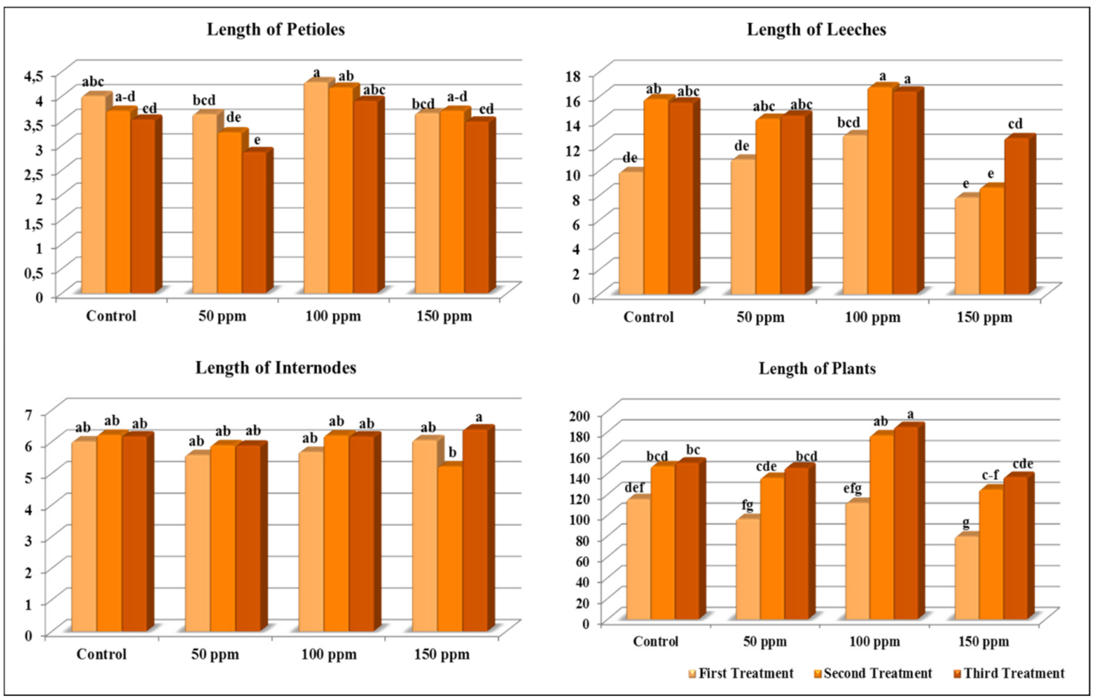


**Figure 3.** Effects of number of chitosan treatments at different dosages on length of petioles, leeches, internodes and length of plants (cm)

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***:$p<0.001$; **:$p<0.01$; *:$p<0.05$;

Length of petioles: LSDc*= 0.351; LSDt*= 0.304; LSDc×t= N.S.;

Length of leeches: LSDc*= 1.776; LSDt*= 1.538; LSD c×t= N.S.;

Length of internodes; LSDc= N.S.; LSDt= N.S.; LSDc×t= N.S.;

Length of plants; LSDc*= 18.785; LSDt*= 16.268; LSDc×t= N.S.; (c: cultivar; t: treatment; c×t: cultivar×treatment)

Regarding petiole and internode thickness, chitosan treatments except 3$^{rd}$ application at 150 ppm concentration increased petiole and internode thickness (Figure 4). It has been reported that chitosan accelerated and prompted the plant's growth and morphological characteristics of various plants such as gerbera

(Wanichpongpan *et al.*, 2000), soybean sprouts (Lee *et al.*, 2005), and sweet basil (Kim, 2005). Luan et al. (2005) demonstrated the stimulating effect of chitosan on shoot lengths and fresh biomass in several plants such as *Chrysanthemum morifolium*, *Limonium latifolium*, *Eustoma grandiflora*, and *Fragaria ananasa*. The research conducted by Salachna and Zawadzińska (2014) has shown that chitosan can be used as a bio-stimulator on freesia plants. According to these researchers, the chitosan-treatments had positive effects on leaves and shoots of freesia plants. Several other studies also reported positive effects of chitosan treatment on plant growth and development (Chen and Xu, 2005; Gornik *et al.*, 2008; Ghasemi Pirbalouti *et al.*, 2017). The results of a study conducted on okra showed that chitosan treatment boosted plant height and leaf number per plant in both pot and field conditions (Mondal *et al.*, 2012). The improved growth and development of plants after chitosan treatment was attributed to the stimulation effects of chitosan on important enzyme activities of plant nitrogen metabolism and transportation of the nitrogen in the leaves (Uthairatanakij *et al.*, 2007). It has been also demonstrated that chitosan might activate a signaling pathway associated with auxin biosynthesis, which might promote the formation of plant hormones like gibberellins and enhance growth and development (Uthairatanakij *et al.*, 2007).

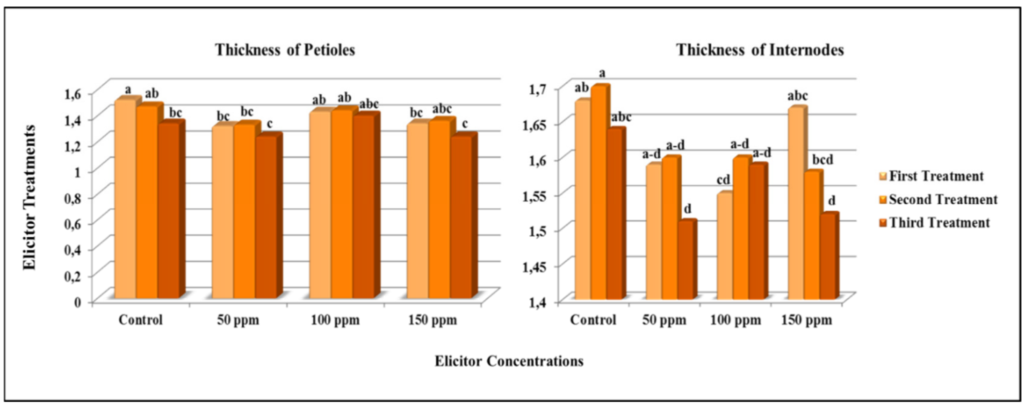


**Figure 4.** Effects of number of chitosan treatments at different dosages on petiole and internode thickness (mm)

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05;

Thickness of petioles: LSDc*= 0.097; LSDt*= 0.084; LSDc×t= N.S.;

Thickness of internodes; LSDc*= 0.068; LSDt= N.S.; LSDc×t= N.S.; (c: cultivar; t: treatment; c×t: cultivar× treatment)

In the current study, the unsatisfied results in terms of the relative chlorophyll content (SPAD) values were obtained from the 150-ppm chitosan concentration and the 3rd treatment, while the most ideal values were obtained in the first treatment of 100 ppm chitosan treatment or in the second treatment of 150 ppm chitosan concentration (Figure 5). Several researchers have demonstrated that the usage of chitosan increased the amount of relative chlorophyll (SPAD) in plants. In the research reported by Dzung *et al.* (2011), spraying coffee plants with chitosan increased the relative chlorophyll content as well as carotenoid content. Similarly, Salachna and Zawadzińska (2014) showed that the chitosan-treated freesia plants had more relative chlorophyll content than the control. In this respect, Nguyen Van *et al.* (2013) showed that a rise in the quantity of nitrate, phosphorus, and potassium in leaves was reported after spraying the seedlings with chitosan three times, while they also demonstrated that plants spraying 10-50 ppm chitosan had the higher photosynthetic rates. According to these researchers, it is possible to say that plants' improved nutrient absorption is led to the rise in chlorophyll content resulted from chitosan application. Khan *et al.* (2002) reported that foliar chitosan treatment enhanced the rate of net photosynthetic of plants associated with increases in stomatal conductance and transpiration rate, without affecting the intercellular $CO_2$ concentration.

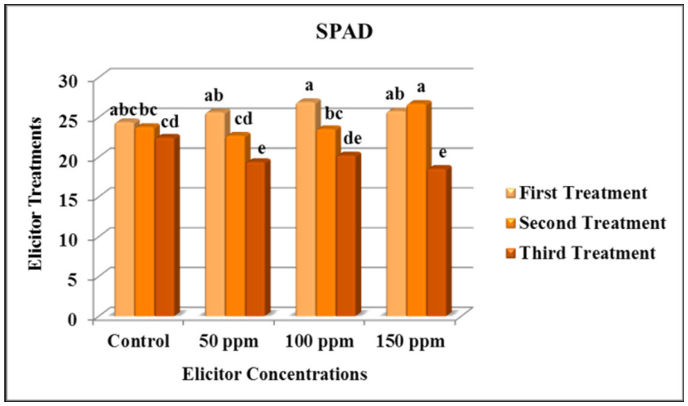


**Figure 5.** Effects of number of chitosan treatments at different dosages on SPAD values
(1): Differences between the means are shown with separate letters
(2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05;
SPAD value: LSDc= N.S.; LSDt*= 1.389; LSDc×t= 2.779; (c: cultivar; t: treatment; c×t: cultivar×treatment)

At the end of the chitosan treatments, the leaves of the bitter gourd plants were harvested and weighed. The best leaf fresh weights were obtained from 150 ppm and 100 ppm chitosan treatments. However, there was no statistically significant difference between chitosan treatments at different concentrations in terms of leaf dry weights (Table 1). In a study conducted on strawberries by Abdel-Mawgoud *et al.* (2010), chitosan treatment increased plant height, leaf number, fresh and dried leaf weights, and crop yield. Ghasemi Pirbalouti *et al.* 2017 reported that the highest fresh and dry weights of basil plants were obtained from chitosan treated plants.

**Table 1.** Effects of number of chitosan treatments at different dosages on fresh-dry weights and total polyphenol and antioxidant contents

| Mean differences values - applications of | Elicitor concentrations | | | |
|---|---|---|---|---|
| | Control | 50 ppm | 100 ppm | 150 ppm |
| Weight of fresh leaves | 26.28 AB | 19.81 B | 31.04 A | 32.43 A |
| Weight of dry leaves | 5.89 | 4.52 | 5.85 | 5.80 |
| Total polyphenol | 210.86 B | 236.67 B | 281.37 A | 294.10 A |
| Total antioxidant | 115.94 *B* | 142.16 *A* | 146.84 *A* | 144.12 *A* |
| LSD values of elicitor concentrations | $LSD_{\text{Weight of Fresh Leaves}} = 4.252$ $LSD_{\text{Weight of Dry Leaves}} = 0.948$ $LSD_{\text{Total Polyphenol}} = 24.499$ $LSD_{\text{Total Antioxidant}} = 12.944$ | | | |

Different letters between fresh – dry weights and total polyphenol and antioxidant contents denote statistically significant differences (LSD test, $p < 0.05$).

The effects of chitosan treatment at different concentrations on total phenol and total antioxidant contents of bitter gourd leaves were also evaluated. Accordingly, it was determined that concentrations at 150 ppm and 100 ppm of chitosan treatment affected the total polyphenol content positively, while the control treatment and 50 ppm treatment concentration did not positively affect the total polyphenol content. On the other hand, it was determined that three different chitosan concentrations tried in the study had a positive effect on the total antioxidant content of bitter gourd leaves, but there was no difference between the treatment concentrations. Therefore, it can be said that chitosan treatment at different concentrations is more effective than the control group without any treatment on the antioxidant contents of bitter gourd plants (Table 1). In previous studies it has been also demonstrated that the total phenolic content of the extracts of two species of basil and *Vitis vinifera* was considerably influenced by the varied chitosan concentrations (Cai *et al.*, 2012; Ghasemi Pirbalouti *et al.*, 2017). Plant extracts and soluble phenolic contents are both responsible for the antioxidant properties of plants. It is known that chitosan most likely has the ability to control antioxidant enzyme activity (Ghasemi Pirbalouti *et al.*, 2014).

## Conclusions

The purpose of the present study was to optimize the concentration of foliar chitosan elicitor treatment and determine the appropriate number of treatments in bitter gourd plants. It is recommended that treatment with chitosan, which is an affordable and readily accessible shellfish by-product from the production of seafood, might be a potential material utilized to improve the growth and development of bitter gourd plants. The findings of the current study revealed that the foliar treatment of 100 ppm chitosan and $2^{nd}$ or $3^{rd}$ treatment is highly effective in most growth parameters. Finally, it is demonstrated that chitosan has a good impact on increasing total polyphenol and antioxidant content of bitter gourd which may promote the health of humankind.

## Authors' Contributions

Conceptualization: TOK and ANO; Investigation: TOK; Methodology: TOK and ANO; Supervision: ANO; Writing - original draft: TOK and ANO; Writing - review and editing: TOK and ANO. Both authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

## Acknowledgements

Thanks to Assoc. Prof. Mehmet Ali SARIDAS from Çukurova University for his contribution to statistical analysis.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

Abdel-Mawgoud AMR, Tantawy AS, El-Nemr MA, Sassine YN (2010). Growth and yield responses of strawberry plants to chitosan application. European Journal of Scientific Research 39(1):161-168.

Bora AFM, Eric-Parfait Kouame KJ, Li X, Liu L, Pan Y (2023). New insights into the bioactive polysaccharides, proteins, and triterpenoids isolated from bitter melon (*Momordica charantia*) and their relevance for nutraceutical and food application: A review. International Journal of Biological Macromolecules 231:123-173. *https://doi.org/10.1016/j.ijbiomac.2023.123173*

Braca A, Siciliano T, D'Arrigo M, Germanò MP (2008). Chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil. Fitoterapia 79(2):123-5. *https://doi.org/10.1016/j.fitote.2007.11.002*

Cai Z, Kastell A, Mewis I, Knorr D, Smetanska I (2012). Polysaccharide elicitors enhance anthocyanin and phenolic acid accumulation in cell suspension cultures of *Vitis vinifera*. Plant Cell Tissue Organ Culture 108:401-409. *https://doi.org/10.1007/s11240-011-0051-3*

Chakraborty M, Hasanuzzaman M, Rahman M, Khan MAR, Bhowmik P, Mahmud NU, Tanveer M, Islam T (2020). Mechanism of Plant Growth Promotion and Disease Suppression by Chitosan Biopolymer. Agriculture 10:624. *https://doi:10.3390/agriculture10120624*

Chen HP, Xu LL (2005). Isolation and characterization of a novel chitosan-binding protein from non-heading Chinese cabbage leaves. Journal of Integrative Plant Biology 47:452-456.

Chong YM, Chang SK, Sia WCM, Yim HS (2015). Antioxidant efficacy of mangosteen (*Garcinia mangostana* Linn.) peel extracts in sunflower oil during accelerated storage. Food Bioscience 12:18-25. *https://doi.org/10.1016/j.fbio.2015.07.002*

Dzung NA, Khanh VTP, Dzung TT (2011). Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. Carbohydrate Polymers 84:751-755. *https://doi.org/10.1016/j.carbpol.2010.07.066*

Fernández-León MF, Fernández-León AM, Lozano M, Ayuso MC, Amodio ML, Colelli G, González-Gómez D (2013). Retention of quality and functional values of broccoli 'Parthenon' stored in modified atmosphere packaging. Food Control 31:302-313. *https://doi.org/10.1016/j.foodcont.2012.10.012*

Ghasemi Pirbalouti A, Malekpoor F, Salimi A, Golparvar A (2017). Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. Scientia Horticulturae 217:114-122. *https://doi.org/10.1016/j.scienta.2017.01.031*

Ghasemi Pirbalouti AG, Samani MR, Hashemi M, Zeinali H (2014). Salicylic acid affects growth, essential oil and chemical compositions of thyme (*Thymus daenensis* Celak.) under reduced irrigation. Plant Growth Regulation 72:289-301. *https://doi.org/10.1007/s10725-013-9860-1*

Ghasemnezhad M, Shiri MA, Sanavi M (2010). Effect of chitosan coatings on some quality indices of apricot (*Prunus armeniaca* L.) during cold storage. Caspian Journal of Environmental Sciences 8:25-33.

Grover J, Yadav S (2004). Pharmacological actions and potential uses of *Momordica charantia*: A review. Journal of Ethnopharmacology 93(1):123-132. *https://doi.org/10.1016/j.jep.2004.03.035*

Górnik K, Grzesik M, Romanowska-Duda B (2008). The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. Journal of Fruit and Ornamental Plant Research 16:333-343.

Hawrylak-Nowak B, Dresler S, Rubinowska K, Matraszek-Gawron R (2021). Eliciting effect of foliar application of chitosan lactate on the phytochemical properties of *Ocimum basilicum* L. and *Melissa officinalis* L. Food Chemistry 342:128358. *https://doi.org/10.1016/j.foodchem.2020.128358*

He Y, Bose SK, Wang W, Jia X, Lu H, Yin H (2018). Pre-harvest treatment of chitosan oligosaccharides improved strawberry fruit quality. International Journal of Molecular Sciences 19:2194. *https://doi.org/10.3390/ijms19082194*

Khan WM, Prithiviraj B, Smiyh DL (2002). Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. Photosynthetica 40:621-624. *https://doi.org/10.1023/A:1024320606812*

Kim HJ, Chen F, Wang X, Rajapakse NC (2005). Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). Journal of Agricultural and Food Chemistry 53:3696-3701. *https://doi.org/10.1021/jf0480804*

Kulkarni P, Lohidasan S, Mahadik K (2021). Isolation, characterisation and investigation of in vitro antidiabetic and antioxidant activity of phytoconstituents from fruit of *Momordica charantia* Linn. Natural Product Researches 35:1035–1037. *https://doi.org/10.1080/14786419.2019.1613400*

Lee SJ, Umano K, Hibamoto ST, Lee KG (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chemistry 91:131-137. https://doi.org/10.1016/j.foodchem.2004.05.056

Lee YS, Kim YH, Kim SB (2005). Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. HortScience 40:1333-1335. https://doi.org/10.21273/HORTSCI.40.5.1333

Luan LQ, Ha VTT, Nagasawa N, Kume T, Yoshii F, Nakanishi TM (2005) Biological effect of irradiated chitosan on plants *in vitro*. Biotechnology and Applied Biochemistry 41(1):49-57.

Maluin FN, Hussein MZ (2020). Chitosan-based agronanochemicals as a sustainable alternative in crop protection. Molecules 25:1611. https://doi.org/10.3390/molecules25071611

Mehregan M, Mehrafarin A, Labbafi MR, Naghdi Badi HA (2017). Effect of different concentrations of chitosan biostimulant on biochemical and morphophysiological traits of stevia plant (*Stevia rebaudiana* Bertoni). Journal of Medicinal Plants 16:169-181.

Mirheidari F, Hatami M, Ghorbanpour M (2022). Effect of different concentrations of IAA, $GA_3$ and chitosan nano-fiber on physio-morphological characteristics and metabolite contents in roselle (*Hibiscus sabdariffa* L.). South African Journal of Botany 145:323-333. https://doi.org/10.1016/j.sajb.2021.07.021

Mondal MMA, Malek MA, Puteh AB, Ismail MR, Ashrafuzzaman M, Naher L (2012). Effect of foliar application of chitosan on growth and yield in okra. Australian Journal of Crop Science 6(5):918-921.

Nahar SJ, Shimasaki K, Haque SM (2012). Effect of different light and two polysaccharides on the proliferation of protocorm-like bodies of *Cymbidium* cultured *in vitro*. Acta Horticulturae 956:307-313. https://doi.org/10.17660/ActaHortic.2012.956.35

Nguyen Van S, Dinh Minh H, Nguyen Anh D (2013). Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatalysis and Agricultural Biotechnology 2(4):289-294. https://doi.org/10.1016/j.bcab.2013.06.001

Pereira JA, Oliveira I, Sousa A, Valentão P, Andrade PB, Ferreira ICFR, Estevinho L (2007). Walnut (*Juglans regia* L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food and Chemical Toxicology 45(11):2287-2295. https://doi.org/10.1016/j.fct.2007.06.004

Pérez-Balibrea S, Moreno DA, García-Viguera C (2011). Improving the phytochemical composition of broccoli sprouts by elicitation. Food Chemistry 129:35-44. https://doi.org/10.1016/j.foodchem.2011.03.049

Salachna P, Zawadzińska A (2014). Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of Ecological Engineering 15(3):97-102. https://doi.org/10.12911/22998993.1110223

Salimgandomi S, Shabrangy A. (2016). The effect of chitosan on antioxidant activity and some secondary metabolites of *Mentha piperita* L. Journal of Pharmaceutical and Health Sciences 4(2):135-142.

Škerget M, Kotnik P, Hadolin M, Hraš AR, Simonič M, Knez Z (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry 89:191-198. https://doi.org/10.1016/j.foodchem.2004.02.025

Tantasawat P, Wannajindaporn A, Chantawaree C, Wangpunga C, Poomsom K, Sorntip A (2010). Chitosan stimulates growth of micropropagated *Dendrobium* plantlets. Acta Horticulturae 878:205-212. https://doi.org/10.17660/ActaHortic.2010.878.24

Tanwar S, Dhakad P, Dhingra G, Tanwar K (2022). A review on salient pharmacological features and chemical constituents of Bitter Melon. Biological Sciences 02(02):229-239. https://doi.org/10.55006/biolsciences.2022.2207

Uthairatanakij A, Teixeira JA, Obsuwan K (2007). Chitosan for improving orchid production and quality. Orchid Science and Biotechnology 1:1-5.

Viacava GE, Goyeneche R, Goñi MG, Roura SI, Agüero MV (2018). Natural elicitors as preharvest treatments to improve postharvest quality of Butterhead lettuce. Scientia Horticulturae 228:145-152. https://doi.org/10.1016/j.scienta.2017.10.018

Vosoughi N, Gomarian M, Pirbalouti AG, Khaghani S, Malekpoor F (2018). Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies. Industrial Crops and Products 117:366-374. https://doi.org/10.1016/j.indcrop.2018.03.021

Wang C, Lu J, Zhang S, Wang P, Hou J, Qian J (2011). Effects of Pb stress on nutrient uptake and secondary metabolism in submerged macrophyte *Vallisneria natans*. Ecotoxicology and Environmental Safety 74(5):1297-1303. *https://doi.org/10.1016/j.ecoenv.2011.03.005*

Wanichpongpan P, Suriyachan K, Chandrkrachang S. (2000). Effects of chitosan on the growth of Gerbera flower plant (*Gerbera jamesonii*). In: Uragami T, Kurita K, Fukamizo T (Eds). Chitin and Chitosan in Life Science. Proceedings of the Eighth International Chitin and Chitosan Conference and Fourth Asia Pacific Chitin and Chitosan Symposium, Yamaguchi, Japan, 21-23 September 2000; Kodansha Scientific: Tokyo, Japan, pp 198-201.