Optimization of Foliar Chitosan Doses as a Biofertilizer for Enhanced Pepper Cultivation

Boran İkız*[a], H. Yıldız Daşgan[b], Cumali Yeniay and Muhammet Taş
Çukurova University, Department of Horticulture, 01330 Balcalı, Adana, Turkey

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Abstract: Chitosan is a water-soluble aminopolysaccharide obtained by the deacetylation of chitin, which is the second most abundant biopolymer after cellulose. Chitin is primarily found in the exoskeletons of shellfish, and chitosan is produced by converting the acetyl groups in the chemical structure of this natural polymer into amine groups. The study aimed to evaluate the effects of different chitosan doses on plant growth, yield and fruit properties of pepper. The experiment included a control group with no chitosan application, as well as treatments with 150 ppm, 300 ppm, and 450 ppm of chitosan. The applications were made foliar once a week The 150 ppm chitosan treatment increased yield by 67.4%, and the 300 ppm treatment by 44.9%, compared to the control. Chitosan treatments also significantly and positively influenced plant growth parameters, such as plant height, plant width, and leaf number. Additionally, the chitosan applications increased, vitamin C, total phenols and total flavonoids, EC (electrical conductivity) in the fruit juice. This study's objectives are to ascertain the proper chitosan dosage for application and to track the beneficial benefits of employing chitosan as a biostimulant.

1 INTRODUCTION

Chitosan is a naturally occurring biopolymer with a wide range of possible uses in agriculture. In particular, chitosan is employed as a rhizosphere modulator, plant growth regulator, and biopesticide. When used as a biopesticide, chitosan effectively combats bacteria, fungus, and viruses (Badawy et al., 2011). It strengthens the structure of the soil, makes it more capable of retaining water, and encourages microbial activity in the rhizosphere. It prevents harm from heavy metals, which enhances soil health (Naeem ve et al., 2018). Because it encourages cell division and plant growth, chitosan plays a significant role in boosting fruit and vegetable output yields (Khan et al., 2009). It is well known that the food and agricultural industries also employ chitosan as a film coating. Biodegradable and antibacterial, chitosan-based films are also kind to the environment. According to Rihm et al. (2020), this is an additional alternative for food preservation and plant protection. It can also have an effect on agriculture's sustainability by being economical and environmentally benign (Malerba et al., 2021). Pepper (Capsicum annuum L.) is an economically important crop grown for its nutritional value, bioactive compounds, antioxidant properties, and natural colors (Santosh, 2013). Pepper is widely consumed worldwide both as a fresh vegetable and as a processed product. Varieties of peppers include sweet peppers (e.g., bell peppers) and hot peppers (e.g., chili peppers). With its high content of vitamin C, vitamin A, and antioxidants, pepper is considered a highly nutritious vegetable and is therefore regarded as an important source for a healthy diet (Sidhu et al., 2019). According to 2022 FAO data, pepper is one of the most important vegetables, cultivated on 2,020,816 hectares with a production of 36,972,494 tons (FAO, 2018).

Pepper is also used as a model plant for biotechnological and biostimulant applications that enhance resistance to various biotic (pathogens, insects) and abiotic (drought, salinity) stress factors

[a] https://orcid.org/0000-0003-3012-4533
[b] https://orcid.org/0000-0002-0403-1627
* Correspondence Author

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(Triverdi et al., 2020). Pepper is a plant that performs best under warm climate conditions. Factors such as water stress and high temperatures have significant impacts on the productivity of pepper cultivation. To enhance the plant's resilience to these environmental stresses, various biostimulants and water management strategies are employed.

There are over 200 common names used 2nda re Capsicum annuum species. Among the most common are chili pepper, red pepper (sweet varieties), bell pepper, red pepper, jalapeños, and chiltepin (hot varieties), as well as Christmas peppers (ornamental) (Latham et al., 2009; Zhang et al., 2002). n the past, some kinda forms of this species were referred to as C. Frutescens, but the characteristics used to distinguish these 2nda re2e observed in many populations of C. Annuum, and there is no consistently recognizable C. Frutescens species (Zhang et al., 2002). t can be difficult to differentiate Capsicum annuum from cultivated C. Chinense (the hottest peppers) and C. Frutescens (tabasco peppers), as their morphological traits may overlap. These three species share the same ancestral gene pool 2nda re sometimes referred to as the "annuum-chinense-frutescens complex (Araceli et al., 2009).

*Capsicum annuum*L. Contains capsaicinoids (capsaicin, dihydrocapsaicin), carotenoids (lutein, zeaxanthin, capsorubin, β-carotene), flavonoids (quercetin, kaempferol, catechin, epicatechin, rutin, luteolin), and steroidal saponins (capsicoside E, F, G, and capsicidin). The main components of C. Annuum essential oil, identified using GC/MS, are capsamide and acetic acid. C. Annuum has been reported to possess various biological activities, including antioxidant, antibacterial, antiviral, antiproliferative, anti-adipogenic, antimutagenic, enzyme inhibitory, anti-inflammatory, hepatoprotective, antidiabetic, renoprotective, hypocholesterolemic, antitumor, anti-obesity, analgesic, appetite suppressant, and anti-reflux properties (Yuca, 2002).

2 MATERIALS AND METHODS

The research took place during the summer growing season of 2024 at the University of Cukurova, Adana, Turkiye (36°59′N, 35°18′E, 20 m above sea level). The temperature values in the region fluctuated throughout the days, with daytime temperatures ranging between 35-40°C. The row spacing was set to 50 cm, and the spacing within the rows was 90 cm. Chitosan (Adaga-NanoWet) was applied to the plants at doses of 150 ppm, 300 ppm, and 450 ppm per week to determine the optimal dose after 15 days first planting and per one week. The pepper seeds used in this study were of the Semerkant F1 variety, developed by the Nunhemscompany. After being grown into seedlings, they were transplanted into the soil.). Harvesting of the plants began 45 days after planting and was carried out five times.

Plant height, plant width, and plant canopy measurements were taken using a meter, while fruit length, fruit diameter, and fruit width were measured using calipers. Color measurements were performed using the FRU Precise color reader WR-18 color space system. pH and EC measured by portable WTW phmeter. Chlorophyll content was measured using a handheld SPAD meter from the Minolta brand. Vitamin C content was carried out using the adapted approach outlined in Elgailani et al. (2017)'s study.

A randomized block experiment was conducted using a four-replicate design, with 15 plants each replicate. n the context of fruit analysis, measurements were performed on 10 fruits per repeat. Furthermore, at the time of harvest, 10 plants were measured in each replication. Statistical analyses were performed with the JMP software. The aim of this study is to observe the ameliorative effects of using chitosan as a biostimulant and to determine the appropriate chitosan dosage for application.

3 RESULTS AND DISCUSSION

3.1 Plant Height, Plant Diameter and Stem Diameter

When examining the plant height in pepper plants, it was observed that the use of chitosan as a biostimulant increased plant height. Plant height increased in all three doses compared to the control plants; however, there was no statistically significant difference between the treatments. n the control plants, the plant height was 48.08 cm, while the plants treated with 300 ppm chitosan as a biostimulant increased to 55.25 cm. Similarly, plants treated with chitosan exhibited a greater stem diameter compared to the control plants. Although the difference was not statistically significant, the plant diameter was found to be highest in the 300 ppm chitosan treatment, consistent with other parameters. Salachna and Zawadzinska (2014) demonstrated that chitosan, with varying molecular weights, can be used as a biostimulant in the cultivation of Freesia plants in pots. They observed that, regardless of the compound's molecular weight, plants treated with chitosan had more leaves and shoots, flowered earlier, and produced more flowers and bulbs. Additionally, Kumaraswamy et al. (2021)

reported that the application of chitosan-silicon nano-fertilizer on corn plants enhanced growth and yield. They observed that these biostimulants increased leaf area as well as stem and root length. Perez et al. (2024) conducted foliar applications of chitosan, brassinosteroids, and thidiazuron on strawberries, demonstrating that chitosan treatment increased plant height, leaf count, leaf area, and fruit firmness.

Table 1: Effects of chitosan doses on plant growth measurements in pepper.

Apps.	Plant-Diameter (cm)	Plant Height (cm)	Stem diameter (mm)
Control	45.54	48.08 b	12.83 b
150 ppm Chitosan	53.46	55.00 a	17.42 a
300 ppm Chitosan	55.08	55.25 a	19.70 a
450 ppm Chitosan	52.00	54.50 a	17.76 a
P0.05	N.S	0.02	0.002
LSD	N.S	6.14	2.80

There is no significant difference between means with the same letter in the same column, LSD the least significant difference, NS: Non significant, Apps: Applications.

3.2 Yield

The 150 ppm chitosan treatment increased yield by 67.4%, the 300 ppm treatment by 44.8%, and the 450 ppm treatment by 17.7%, compared to the control (Figure 1). Abdel-Mawgoud et al. (2010) applied chitosan to strawberry plants and observed an increase in the number of fruits per plant as well as improvements in yield parameters per plant. Plant growth parameters, chlorophyll content, and consequently photosynthetic capacity, as well as Vitamin C content an important factor in pepper plants showed better results in all chitosan treatments compared to the control.

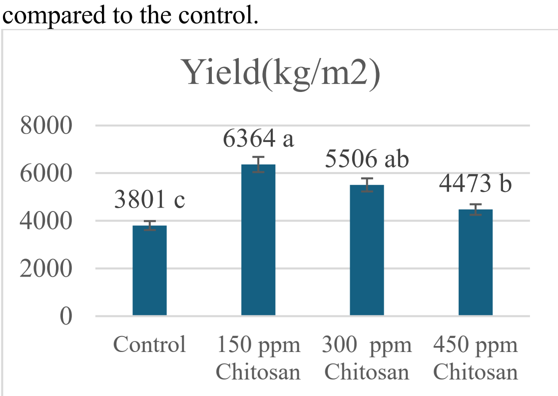

Figure 1: Effects of chitosan doses on yield in pepper.

However, it was observed that higher chitosan doses led to a reduction in yield. Using the lowest dose of chitosan is also economically advantageous. When examining other parameters, no significant differences were observed among the different chitosan treatments.

3.3 Chlorophyll Content

In terms of chlorophyll content, the use of chitosan, bio-fertilizer yielded higher results compared to the control plants. Since an increase in chlorophyll content is associated with photosynthesis, it was observed that chitosan enhances photosynthesis, which in turn influences the increase in growth parameters such as plant height and stem diameter. İnam et al. (2024) investigated the effects of chitosan and zinc oxide fertilizers on alleviating the severe impacts of drought on corn plants. They observed that the application of 1000 µg/L chitosan produced better results than both the control and zinc oxide treatments. Similarly, in their study on strawberries, Perez et al. (2024) suggested that the combined application of chitosan and brassinosteroids significantly promoted crown diameter, photosynthetic pigments, carotenoids, and the fresh and dry weights of both roots and above-ground parts, as well as maturation. In their study, Tamer Khalifa et al. (2024) investigated the interactive effects of soil mulching materials (non-mulched, white plastic, rice straw, and sawdust) and chitosan foliar spray applications (control, 250 mg L^{-1} regular chitosan, 125 mg L^{-1} nano-chitosan, and 62.5 mg L^{-1} nano-chitosan) on the biochemical soil properties and productivity of common beans grown in clay-saline soil. Their research emphasized the adoption of eco-friendly strategies to enhance the sustainability of agricultural ecosystems. The higher chlorophyll content indicates enhanced photosynthesis, which in turn promotes increased plant growth and development. This also explains the higher yield observed in chitosan applications compared to the control.

3.4 Fruit Properties

When examining fruit width, it is observed that the use of chitosan shows an increase compared to the control. The use of 150 ppm chitosan increased by 8.12% compared to the control, 300 ppm by 26.78%, and 450 ppm by 11.22%. The use of chitosan has generally increased the fruit width. Fruit size has also shown a significant increase in chitosan usage, just like fruit width. The fruit size increased by 9.39% in the 150 ppm chitosan application compared to the control, 21.74% in the 300 ppm application, and 7.65% in the 450 ppm application. No correlation has been established between chitosan application and dry matter production percentage (Table 3). With the use of chitosan as a biostimulant, not only quality parameters but also physical measurements of the fruit show improvement (Rahman et al., 2018).

Table 2: Effects of chitosan doses on chlorophyll content in pepper.

Apps.	Chlorophyll Content(SPAD)
Control	49.14 b
150 ppm Chitosan	54.49 a
300 ppm Chitosan	50.97 a
450 ppm Chitosan	55.55 a
$P_{0.05}$	0.01
LSD	5.001

There is no significant difference between means with the same letter in the same column, LSD the least significant difference

Table 3: Effects of chitosan doses on fruit properties in pepper.

Apps.	Fruit Width (mm)	Fruit Height (cm)	Dry Matter (%)
Control	25.5 b	11.50 b	5.31
150 ppm Chitosan	27.57 b	12.58 ab	5.24
300 ppm Chitosan	32.33 a	14.00 a	5.36
450 ppm Chitosan	28.36 ab	12.38 ab	5.44
$P_{0.05}$	0.03	0.02	N.S
LSD	4.43	1.47	N.S

There is no significant difference between means with the same letter in the same column, LSD the least significant difference

3.5 Fruit Colour

The LAB color model includes L (lightness), A (green to red scale), and B (blue to yellow scale). Across all treatments, the lightness (L) values are similar, with a slight increase observed with chitosan applications. The highest lightness occurs at 300 ppm (46.81), while the control group has the lowest value (45.19). Although the differences in lightness between the control and chitosan-treated groups are 4ort, they suggest a modest improvement in brightness with chitosan (Table 4).

The A parameter, representing the green-red scale, shows a gradual increase with higher chitosan concentrations. The control group has an A value of -5.18, while the maximum shift toward red occurs at 300 ppm (-6.92). This indicates that the 300 ppm chitosan application slightly enhances red pigmentation compared to other treatments and the control (Table 4).

4ort he B parameter (blue-yellow scale), the highest value is recorded at 300 ppm (18.20 a), with both 300 ppm and 450 ppm treatments yielding statistically significant increases in yellow pigmentation compared to the control (16.34 b). This suggests that chitosan, particularly at 300 ppm, enhances the yellow coloration of the fruit, potentially contributing to a more visually appealing appearance (Table 4).

The application of 300 ppm chitosan is the most efficacious in affecting fruit coloration, resulting in substantial increases in the yellow (B) and red (A) scales, thereby improving overall color characteristics. This concentration has the greatest lightness, rendering the fruit more luminous. Chitosan treatments, particularly around 300 ppm, significantly improve the aesthetic quality of the fruits. Dulta et al. (2022) examined the impact of ZnO nanoparticle-infused chitosan coating on the post-harvest quality of eggplants. Improvements in both the texture and color of the fruits were noted in comparison to the control.

Table 4: Effects of chitosan doses on fruit colour in pepper.

Apps.	L(Fruit)	A(Fruit)	B(Fruit)
Control	45.19	-5.18	16.34 b
150 ppm Chitosan	46.61	-5.35	18.57 a
300 ppm Chitosan	46.81	-6.92	18.20 a
450 ppm Chitosan	46.16	-6.69	17.15 a
$P_{0.05}$	N.S	N.S	0.01
LSD	N.S	N.S	1.98

There is no significant difference between means with the same letter in the same column, LSD the least significant difference, NS: Non significant.

3.6 Quality Paramaters

3.6.1 pH and EC in Fruit Juice

Although there is a noticeable increase in pH value, no statistically significant increase has been observed. However, the EC value decreased as the chitosan dose increased, specifically showing a reduction at the 450 ppm application (Table 5). In their study published, Daşgan et al. (2022) found the effects of biostimulant application on EC and pH within the same group.

Table 5: Effects of chitosan doses on pH and EC in pepper.

Apps.	pH	EC(dSm^{-1})
Control	5.54	4.99 a
150 ppm Chitosan	5.86	5.06 a
300 ppm Chitosan	5.87	5.11 a
450 ppm Chitosan	5.76	4.47 b
$P_{0.05}$	N.S.	0.01
LSD	N.S.	0.51

(There is no significant difference between means with the same letter in the same column, LSD the least significant difference, NS: Non significant).

3.6.2 Vitamin C

The control group exhibits the lowest Vitamin C content at 69.6 (b), signifying that in the absence of chitosan treatment, Vitamin C levels are inferior to those in treated groups. The 150 ppm chitosan treatment demonstrates a considerable elevation in Vitamin C content to 76.1 (a), which is statistically superior to the control, underscoring the beneficial impact of chitosan at this dose. At 300 ppm, the Vitamin C concentration attains 73.0 (ab), surpassing the control but not exhibiting a statistically significant difference from the 150 ppm treatment. The 450 ppm chitosan concentration yields the greatest Vitamin C content at 78.2 (a), exhibiting a statistically significant difference from the control, indicating that elevated chitosan concentrations may further enhance Vitamin C levels. The Vitamin C value showed an increase of 9.34% at 150 ppm, 4.47% at 300 ppm, and 12.36% at 450 ppm compared to the control (Table 6).

The study demonstrates that chitosan treatments markedly increase Vitamin C content. While 450 ppm produces the maximum concentrations, 150 ppm provides a significant enhancement with only one-third the quantity of chitosan relative to 450 ppm. In light of the absence of a statistically significant difference, 150 ppm may be regarded as the more effective option. Plants subjected to chitosan exhibit enhanced synthesis of secondary metabolites, including polyphenolics, lignin, flavonoids, and phytoalexins, alongside Vitamin C, resulting in improved product quality. Chitosan nanoparticles of varying sizes have been employed to encapsulate Vitamin C, prolong its shelf life, and investigate its distribution.

3.6.3 Total Phenols and Total Flavanoids Compounds

Total phenol and flavonoid contents in the fruit are directly associated with quality, and it has been observed that the use of chitosan in pepper fruit increases these substances (Gholamipour et al., 2009). In our study, when examining the effect of chitosan use on the total phenolic content in pepper plants, an increase of 15.69% was observed at a 150 ppm dose, 14.81% at a 300 ppm dose, and 12.39% at a 450 ppm dose. Even though there is no statistical difference, there is a decrease in the total phenol content as the dose increases. When the total flavonoid content was examined, compared to the control, 150 ppm chitosan showed an increase of 22.21%, 300 ppm an increase of 11.31%, while the application at the 450 ppm dose showed a decrease of 0.44%. A high dose of chitosan has caused a decrease in the total flavonoid content (Table 7).

Table 6. Effects of chitosan doses on Vitamin C in pepper.

Apps.	Vit C (mg 100 g FW^{-1})
Control	69.6 b
150 ppm Chitosan	76.1 a
300 ppm Chitosan	72.9 ab
450 ppm Chitosan	78.2 a
$P_{0.05}$	0.01
LSD	4.41

There is no significant difference between means with the same letter in the same column, LSD the least significant difference.

Table 7. Effects of chitosan doses on total phenols and flavanoids in pepper.

Apps.	Total phenol (mg GA/100 g FW)	Total flavonoids (mg RU/100 g FW)
Control	74.39 b	126.22 b
150 ppm Chitosan	86.06 a	154.27 a
300 ppm Chitosan	85.41 a	140.50 ab
450 ppm Chitosan	83.61 a	125.66 b
$P_{0.05}$	0.008	0.001
LSD	4.31	14.64

There is no significant difference between means with the same letter in the same column, LSD the least significant difference.

4 CONCLUSIONS

This study conclusively reveals that the application of chitosan greatly influences the growth, physical characteristics, and yield of pepper plants. The use of 150 ppm chitosan produced the maximum yield, suggesting that lower concentrations are more efficacious in improving yield results in pepper growing. Although 300 ppm chitosan was ideal for enhancing fruit characteristics including breadth, height, and color pigmentation, it did not exceed 150 ppm in overall productivity. Moreover, elevated doses such as 450 ppm shown no substantial advantages and could even detrimentally affect yield. The results indicate that chitosan is an excellent biostimulant for pepper growth, with 150 ppm identified as the optimal concentration for enhancing yield and crop quality. This research underscores the promise of chitosan as an economical and environmentally sustainable biostimulant for improving pepper yield. Future investigations should examine the impact of chitosan across diverse environmental conditions and various pepper cultivars to enhance application methodologies.

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