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CHITOSAN ENHANCES DROUGHT TOLERANCE IN *DIANTHUS CARYOPHYLLUS* VIA ANTIOXIDANT REGULATION AND OSMOTIC ADJUSTMENT

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Abstract

Severe water shortages limit horticultural crop growth and development, compromising production. A thorough understanding of novel tools to enhance stress tolerance in crops is crucial for coping with the growing environmental challenges to world crop production in this century. Chitosan water solutions at concentrations of 0, 0.5, 2, and 5 µM were applied to *Dianthus caryophyllus* (carnation) plants that were watered every two days or every six days for six weeks. Morphological, physiological, and metabolic indicators related to plant responses to water deficit were investigated. Chitosan-treated plants (0.5 and 2 µM) showed significant improvements in all growth features compared with the untreated plants during both regular and longer watering periods. Growth improvements in chitosan-treated plants were linked to higher chlorophyll, carbohydrate, proline, potassium, and calcium levels. Increases in phenol and free and total ascorbate levels were observed in the treated plants. Plants also had higher levels of antioxidants, such as superoxide dismutase, catalase, and ascorbate peroxidase, in their leaves. The H_2O_2 accumulated to a lesser degree. Longer gaps between watering led to smaller plant sizes, which was considered a drought-avoidance response, linked to higher levels of carbohydrates, potassium, calcium, proline, and chlorophyll, helping the plants manage water pressure and stay hydrated. Reactive oxygen species (ROS) accumulation was controlled by enzymatic and non-enzymatic methods. Plant response mechanisms for enhanced drought resistance interact under chitosan treatment to improve plant performance under stress conditions.

Key words: Antioxidants; Carnation; Chitosan; *Dianthus caryophyllus*; Drought mechanisms

Introduction

Water scarcity is a substantial challenge to agricultural productivity, particularly amid expanding industrial water demands, a growing global population, and climate change (Calvin *et al*., 2023; Mohanty *et al*., 2024). Ultimately, diminished crop yields are the result of physiological alterations in plants, including changes in leaf number and size, carbohydrate and ion composition, enzyme activity, and the expression of genes associated with free radical defense. Water stress activates these changes. These physiological adaptations are associated with resistance mechanisms to water stress, such as escape strategies, avoidance, and tolerance (Nilsen & Orcutt, 1996; Yanqing *et al*., 2024).

Osmotic control is an important way that plants adapt in response to dry conditions, since it helps them keep their cells turgid and their metabolic activities going. This process requires the accumulation of solutes, including proline and soluble carbohydrates, as well as potassium and calcium (Pessarakli, 2021; Kumar *et al*., 2024). Employing mechanisms such as the reduction of leaf size and the expansion of root systems, drought avoidance strategies boost water acquisition and minimize water loss (Soufan *et al*., 2023). In response to arid stress, plants frequently increase the expression of specific proteins, assimilate stress-related nutrients, such as potassium, and accumulate specific antioxidants that counteract the adverse effects of reactive oxygen species. Potassium is essential for the regulation of stomata and the facilitation of enzymatic processes, while calcium is involved in signal transduction and the maintenance of membrane stability during stress. Concurrently, antioxidant defense systems are activated to mitigate oxidative damage and counteract reactive oxygen species, including enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Rao *et al*., 2025; Soostani *et al*., 2025). The success of these integrated responses ultimately determines a plant's capacity to endure drought stress.

Global concerns regarding the development of innovative tools to assist horticultural crops in mitigating the impacts of water stress are increasing, including the application of chitosan (Rojas-Pirela *et al*., 2024). Chitosan is a biostimulant commercially synthesized by exposing chitin to elevated temperatures, thereafter undergoing deacetylation in alkaline conditions to eliminate proteins and calcium (Das *et al*., 2024). Chitosan oligosaccharides can be characterized as a solution or a soluble powder in water. They are widely used as plant elicitors of secondary metabolite production (Soostani *et al*., 2025), particularly polyphenols (Yin *et al*., 2012). Several studies have shown that it may improve agricultural yield (El-Araby *et al*., 2024; Balusamy *et al*., 2022). Faluku *et al*., (2024) reported that synthesized fulvic acid-releasing chitosan nanoparticles stimulated rice growth in drought conditions. Nevertheless, the mechanism via which chitosan improves water stress tolerance in horticultural crops remains largely unexplored.

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Carnation (*Dianthus caryophyllus*) is an important plant in ornamental horticulture all over the world. People value it for its beauty, cultural significance, and medical benefits (Whealy, 1992). This plant, part of the Caryophyllaceae family, originates from the Mediterranean area of southern Europe (Fassou *et al*., 2022). Carnation is mainly a herbaceous perennial, while it is often grown as an annual in commercial horticulture settings. The flower's unique scent comes mostly from essential oils such as eugenol, benzyl benzoate, and methyl benzoate (Wang *et al*., 2025). Previous studies have demonstrated that Carnation and its associated species possess several medicinal attributes, including antioxidant, antibacterial, insecticidal, anticancer, and anti-inflammatory effects (Chandra *et al*., 2016; Survase *et al*., 2024). These effects are associated with diverse phytochemical molecules, highlighting the plant's potential. Carnations generally have difficulties growing because they are sensitive to water stress conditions, which may significantly affect vegetative growth, flower quality, and market value. While biostimulants are becoming more common in horticulture, there isn't sufficient research concerning the way chitosan may assist Carnation plants cope with drought, especially when it comes to how it affects both their physiological and biochemical responses.

This study addresses a key gap in ornamental horticulture by explaining how chitosan improves drought-resistant properties in Carnation. In contrast to other studies that focused on specific physiological or biochemical characteristics, the current research concurrently connects growth performance with osmotic management and antioxidant defense under varying watering regimens. This study presents an integrated investigation of mineral composition, osmolyte accumulation, and the detoxification of reactive oxygen species, thereby establishing a structural framework for understanding chitosan-mediated stress resilience. These findings provide innovative perspectives on the application of biostimulants to enhance ornamental crop performance in water-scarce environments and endorse the development of sustainable production techniques in arid and semi-arid areas.

Material and Methods

Plant material and treatments used: Young carnation plants, measuring 10 cm in height, were procured from local commercial nurseries in January 2023 and 2024. Plants were cultivated in a glass greenhouse at the College of Food and Agricultural Sciences, King Saud University, Saudi Arabia. The plants were relocated to 2.1 L pots filled with a blend of brown peat and perlite (3:1 w/w), enriched with Crystalon® (20% N: 20% P: 20% K at two g/L medium). The plants were cultivated for three weeks at temperatures between 15.2°C and 27.6°C, with relative humidity ranging from 58% to 68%, photosynthetically active radiation around 1000 µmol m^{-2} s^{-1} at noon, and daily irrigation of 38–50 mL per plant. Plants were categorized into two groups: one group received irrigation every two days (2DWI), while the other group was irrigated every six days (6DWI) for a duration of six weeks.

A water solution of chitosan oligosaccharide (with more than 95% deacetylation, in powder form from Aldebeiky Group Co., Cairo, Egypt; 100 kDa (1×10^5 g mol^{-1})) was sprayed on the plants at concentrations of 0, 0.5, 2, and 5 µM until two weeks before the watering schedule changed; The experiment used a split-plot design. Irrigation intervals constituted the primary plot, whereas chitosan treatments represented the secondary subplot. Plants were organized into three blocks (n = 3), each comprising at least 10 replicates per treatment, resulting in a total of 270 plants in the randomized complete block design (RCBD).

Morphological and physiological parameters: Plants were collected following six weeks of stress exposure. At that juncture, plant height and leaf count were recorded. The leaf area was promptly computed utilizing a scanner and the AutoCAD software. The total dry weight was ascertained by dehydrating cleaned plants to a consistent weight in an oven at 70°C. Total carbohydrates, potassium (K^+), calcium (Ca^{2+}), and proline were quantified in plant leaves at the conclusion of the experiment. After freeze-drying the samples, they were ground, sieved, and stored at -20°C until subsequent analysis. Total carbs were measured according to Dubios *et al*., (1956) and reported as a percentage. One gram of frozen leaves was used to get the cell sap, which was then diluted (1:100, v/v) to measure the amounts of K^+ and Ca^{2+} using an inductively coupled plasma spectrophotometer. The proline leaf concentration was measured using a spectrophotometer at 520 nm (Torello & Rice, 1986; Bates *et al*., 1973).

Antioxidants, chlorophyll, phenolic compounds, and enzymatic activity: Air-dried leaves were pulverized into a fine powder; 0.25 g from each sample was dissolved in 3 mL of 99% methanol while being stirred at low speed on a magnetic agitator in the dark for 24 hours at ambient temperature. Methanolic extracts were centrifuged for five minutes at 10,000 RPM (7,000 × g) in a cold environment. The supernatant that came out (~2.7 mL) was then evaporated using a rotary evaporator to make a semisolid extract, which was then saved for later antioxidant research. The antioxidant abilities of all samples were checked using the DPPH and β-carotene-linoleic acid methods, which measure how well they can fight against harmful hydroxyl radicals, based on Elansary (2017). In the DPPH method, samples were incubated for 30 minutes, followed by absorbance measurement at 517 nm. The β-Carotene-linoleic acid experiment measured absorbance at 470 nm. Researchers ascertained the concentration of the sample necessary to inhibit 50% of DPPH/β-carotene-linoleic acid (IC_{50} in µg/mL) by graphing the percentage of inhibition against extract concentration. BHT served as a positive control, and the trials were conducted twice in duplicate. The total phenolic content in methanolic leaf extracts was measured using the Folin-Ciocalteau color test, comparing it to gallic acid, and the results were given as the amount of gallic acid in milligrams per gram of extract. The total chlorophyll content in fresh leaves was measured following the methodology of Moran & Porath (1980).

Frozen ground leaves were used to measure total and free ascorbate following the methodology of Elansary *et al*., (2017). To summarize, 0.5 grams of ground-frozen leaf tissues were mixed with 8 mL of cold trichloroacetic acid (TCA, 5%, w/v); then, the mixture was spun in a centrifuge. The supernatant was treated with a PBS (200

mM, pH 7.4) and dithiothreitol (DTT, 1.5 mM) mixture for 50 minutes. N-ethylmaleimide (NEM, 200 µL, 0.5%, w/v) was used to eliminate excess DTT. The solution was then combined with TCA (1 mL, 10%), o-phosphoric acid (800 µL, 42%), and 2,2-dipyridyl. The absorbance of the combination was quantified at 525 nm. To find the amount of free ascorbate, the same method was used, but instead of DTT and NEM, 400 µL of deionized water was added, and the levels of free and total ascorbate were determined following Elansary *et al*., (2017). The levels of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), along with the buildup of H_2O_2, were measured following Elansary *et al*., (2017).

Statistical analyses

The data gathered in the 2023 and 2024 growing seasons were shown as averages, and the smallest important difference (LSD) was determined using the one-way ANOVA test in SPSS (PASW Ver. 21) at $p<0.05$.

Results

Morphological responses to irrigation frequencies and chitosan application: Increasing the time between watering from two to six days significantly reduced the morphological parameters of carnation plants, such as the number of leaves, the area of the leaves, the dry weight of the plants, and the height of the plants (Table 1). For example, during the 2016 season with longer irrigation intervals, the control plants lost leaves (from 30.8 to 14.1 leaves per plant), leaf area (from 326.3 to 152.2 cm² per plant), dry weight (from 5.3 to 3.6 g per plant), and height (from 14.3 to 8.6 cm).

When plants were watered at the regular interval (2DWI), applying chitosan at 0.5 and 2 µM significantly increased the number and size of leaves, the weight of the plants, and the height of the plants. For instance, applying chitosan at 2 µM (with regular watering) rendered the plant's leaves grow from 30.8 to 34.1 leaves per plant, the leaf area grew from 326.3 to 358.1 cm² per plant, and the dry weight grow from 5.3 to 6.2 g per plant. In addition, when the watering intervals were longer (6DWI), plants treated with chitosan at 0.5 and 2 µM demonstrated major improvements in all growth aspects that were measured compared to plants treated with chitosan at 5 µM and plants that weren't treated.

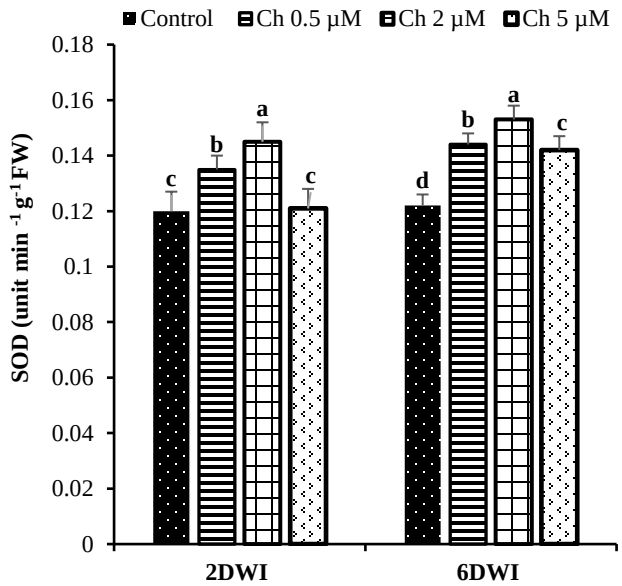
Impact of irrigation frequency and treatments on plant physiological metrics: Longer gaps between watering (6DWI) led to lower amounts of total carbohydrates, potassium (K^+), calcium (Ca^{+2}), and proline in plants compared to regular watering (Table 2). In both 2DWI and 6DWI, the leaves of plants treated with chitosan at 0.5 and 2 µM had much higher amounts of total carbohydrates, K, Ca, and proline than the control plants and those treated with 5 µM chitosan. For example, using chitosan at 2 µM (with watering every 6 days) increased total carbohydrates (from 14.51% to 15.71%), potassium (from 18.5 to 24.9 mg per gram of dry weight), calcium (from 3.53 to 4.05 mg per gram of dry weight), and proline (from 1.25 to 1.37 mg g^{-1} dry wt.) in the 2023 season.

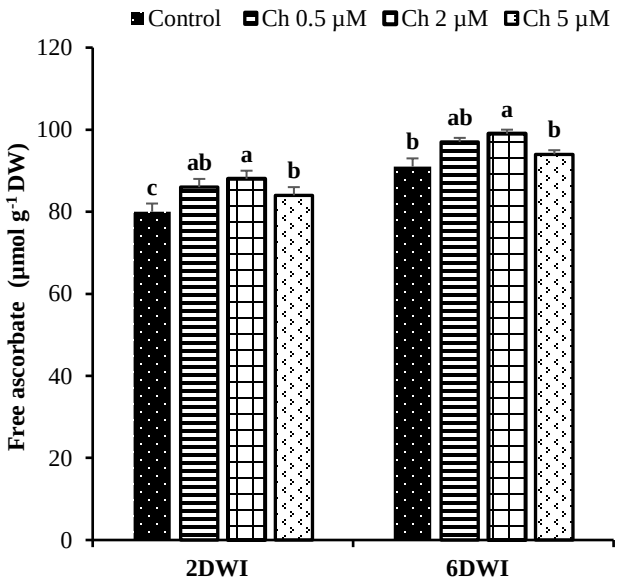
Antioxidants, phenolic compounds, and chlorophylls: Extension of the irrigation interval from two to six days resulted in a considerable enhancement of DPPH free radical scavenging activity in carnation plants (Table 3). The DPPH (IC50) of plants decreased from 9.3 to 7.1 µg ml^{-1} after the start of 6DWI treatment in the first season (2023), indicating an enhancement in scavenging activity. A comparable trend was observed in the second season. The scavenging activity of leaf extracts was observed under water stress conditions, as demonstrated by the β-Carotene-linoleic acid assay. Carnation plants subjected to standard irrigation (2DWI) and extended irrigation (6DWI) exhibited a marked enhancement in leaf extract scavenging activity after the application of chitosan at concentrations of 0.5 and 2 µM, in comparison to control groups and the 5 µM chitosan treatment during both the 2023 and 2024 seasons. Carnation plants treated with 2 µM chitosan exhibited enhanced DPPH (IC50) free radical scavenging activity, increasing from 11.1 to 10.0 µg ml^{-1} and from 9.3 to 7.1 µg ml^{-1} during 2- and 6-day irrigation intervals, respectively, during the 2023 season.

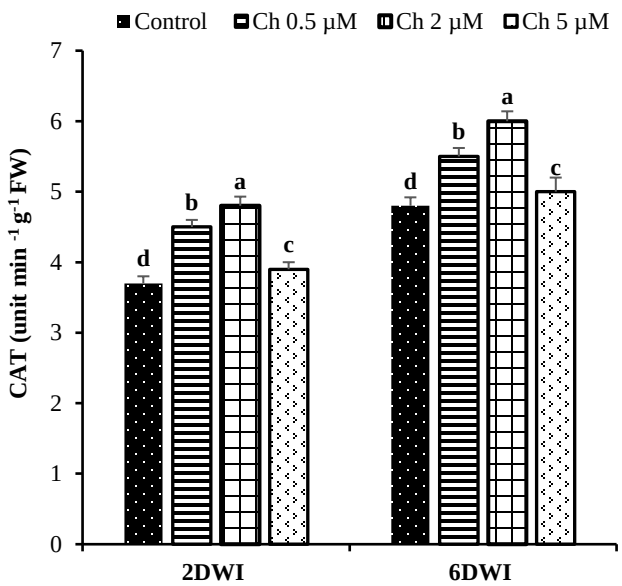
Similarly, a notable increase in the total phenolic content in carnation plants was observed when the irrigation interval was extended over the two growing seasons examined (Table 3). Chitosan treatment significantly enhanced the phenolic content, especially in plants treated with 0.5 and 2 µM. In 2023, carnation leaf extracts exhibited an increase in phenolic content from 11.1 to 11.9 mg GAE g^{-1} and from 11.9 to 12.7 mg GAE g^{-1} in plants treated with 2DWI and 6DWI, respectively. The total phenolic content increased a lot in plants treated with 0.5 and 2 µM chitosan compared to the control and 5 µM chitosan treatments. The total chlorophyll content in carnation considerably decreased in control plants exposed to 6DWI, from 0.70 to 0.66 mg g^{-1} DW during the first season (Table 3). Chitosan application resulted in a notable enhancement of chlorophyll content in treated plants at 0.5 and 2 µM, relative to the control and 5 µM chitosan, throughout both watering intervals during the two assessed growth seasons. For instance, total chlorophyll increased from 0.70 to 0.75 and from 0.66 to 0.70 mg g^{-1} DW in plants cultivated under 2DWI and 6DWI, respectively.

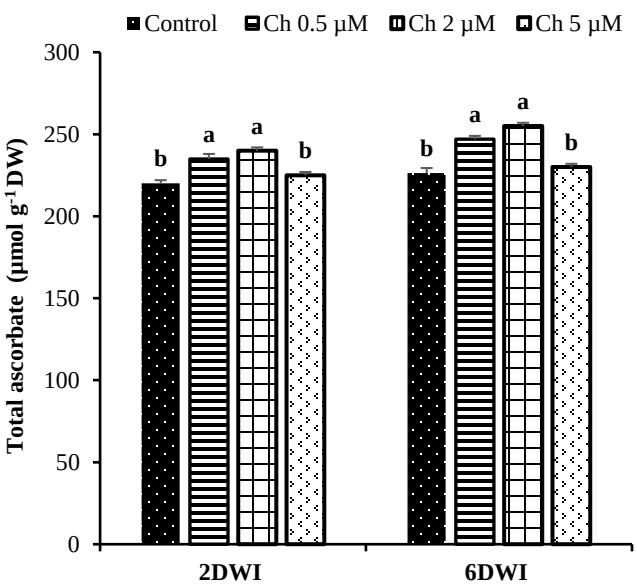
Antioxidant enzyme activity: The activities of the major antioxidants SOD, CAT, and APX exhibited significant increases in carnation plants treated with chitosan at concentrations of 0.5 and 2 µM compared with those of plants treated with chitosan at 5 µM and the control under both regular and extended irrigation intervals (Fig. 1). The application of chitosan at 200 ppm yielded the highest activity of SOD, CAT, and APX enzymes observed under both 2DWI and 6DWI across both seasons examined.

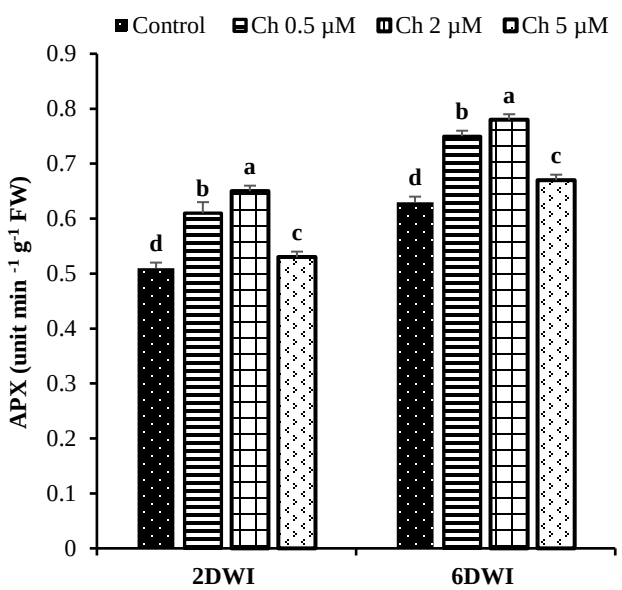
Free and total ascorbate (non-enzymatic antioxidants) exhibited a substantial increase in chitosan-treated plants at 0.5 and 2 µM compared to those treated with chitosan at 5 µM and control treatments under both regular and extended irrigation intervals (Fig. 2). Simultaneously, there were notable decreases in H_2O_2 levels in chitosan-treated plants at 0.5 and 2 µM, in comparison to the 5 µM dosage and the control treatment, across both seasons (Fig. 2).











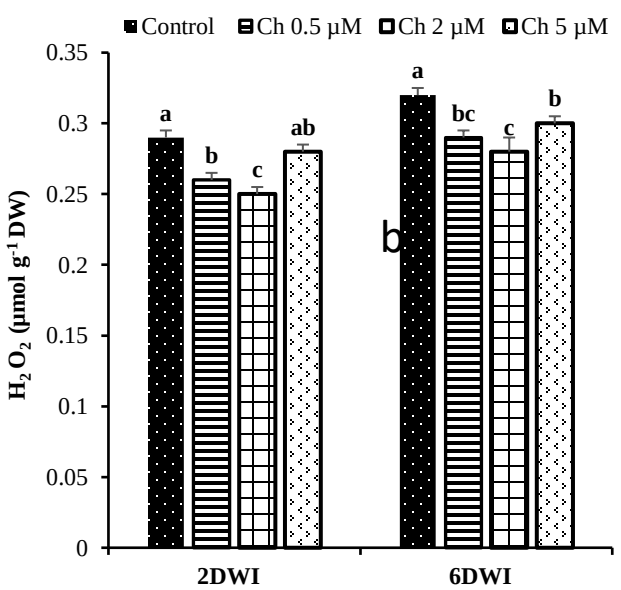


Fig. 1. Activities of SOD, CAT, and APX in carnations exposed to extended watering intervals and varying chitosan concentrations.

Fig. 2. Free and total ascorbate and hydrogen peroxide levels in carnation plants exposed to extended irrigation intervals and varying chitosan concentrations.

Table 1. This study examined the effects of water deficiency and chitosan application on leaf count, leaf surface area, plant biomass, and plant height in carnations after a six-week treatment period. Values are presented as means (± standard deviation).

Water interval	Chitosan treatment (µM)	Number of leaves (plant^{-1})		Leaf area (cm^2 plant^{-1})		Plant dry weight (g plant^{-1})		Plant height (cm)	
		2023	2024	2023	2024	2023	2024	2023	2024
2DWI	0	30.8 ± 0.1b*	30.3 ± 0.2b	326.3 ± 21.3b	325.1 ± 14.5b	5.3 ± 0.2b	5.3 ± 0.2b	14.3 ± 0.1b	14.9 ± 0.1b
	0.5	32.2 ± 0.3ab	33.2 ± 0.2a	347.6 ± 13.1a	345.4 ± 11.5a	6.1 ± 0.1a	6.0 ± 0.1a	16.1 ± 0.3a	15.8 ± 0.2a
	2	34.1 ± 0.2a	34.1 ± 0.1a	358.1 ± 14.5a	357.4 ± 16.3a	6.2 ± 0.2a	6.1 ± 0.2a	16.3 ± 0.2a	15.9 ± 0.2a
	5	30.6 ± 0.1b	30.5 ± 0.3b	321.3 ± 12.6b	323.5 ± 21.5b	5.2 ± 0.1b	5.3 ± 0.1b	15.1 ± 0.3b	14.5 ± 0.1b
6DWI	0	14.1 ± 0.2d	14.2 ± 0.1d	152.2 ± 11.1d	151.6 ± 14.6d	3.6 ± 0.1d	3.5 ± 0.1d	8.6 ± 0.3d	8.8 ± 0.2d
	0.5	16.6 ± 0.1 cd	16.5 ± 0.1 cd	176.1 ± 17.6c	176.4 ± 14.1c	4.1 ± 0.1c	4.1 ± 0.1c	10.1 ± 0.1c	9.7 ± 0.1c
	2	18.1 ± 0.2c	18.0 ± 0.1c	184.6 ± 15.4c	179.3 ± 10.3c	4.2 ± 0.1c	4.1 ± 0.1c	10.2 ± 0.3c	9.0 ± 0.2c
	5	14.4 ± 0.1d	14.3 ± 0.2d	149.4 ± 10.2d	150.3 ± 11.1d	3.4 ± 0.1d	3.3 ± 0.1d	8.7 ± 0.2 cd	8.1 ± 0.1e

*Means denoted by distinct letters within columns are substantially different according to the LSD test ($p<0.05$)

Table 2. The study examines the effects of irrigation intervals and chitosan application on the levels of total carbohydrate, potassium, calcium, and proline in carnation leaves over the course of two consecutive seasons. Values represent means (±sd).

Water interval	Chitosan treatment (µM)	Total carbohydrates (% (DW)		K^+ (mg g^{-1} DW)		Ca^{2+} (mg g^{-1} DW)		Proline (mg g^{-1} DW)	
		2023	2024	2023	2024	2023	2024	2023	2024
2DWI	0	14.51 ± 0.1b*	14.35 ± 0.1b	18.5 ± 0.3d	18.1 ± 0.5d	3.53 ± 0.05b	3.52 ± 0.03b	1.25 ± 0.01c	1.22 ± 0.01c
	0.5	15.53 ± 0.2a	15.21 ± 0.3ab	23.3 ± 0.1b	22.7 ± 0.1b	4.03 ± 0.3a	4.01 ± 0.1a	1.34 ± 0.03b	1.31 ± cb
	2	15.71 ± 0.1a	15.42 ± 0.2a	24.9 ± 0.2b	23.8 ± 0.1b	4.05 ± 0.05a	4.08 ± 0.04a	1.37 ± 0.01b	1.34 ± 0.01b
	5	14.64 ± 0.1b	14.45 ± 0.1b	18.8 ± 0.0d	18.5 ± 0.1d	3.63 ± 0.03b	3.59 ± 0.03b	1.27 ± 0.01c	1.25 ± 0.02c
6DWI	0	13.08 ± 0.2c	13.01 ± 0.2c	20.4 ± 0.1b	20.1 ± 0.3c	3.40 ± 0.02b	3.45 ± 0.03b	1.38 ± 0.02b	1.36 ± 0.01ab
	0.5	13.78 ± 0.1 cb	13.53 ± 0.1c	26.1 ± 0.3a	25.7 ± 0.1a	3.91 ± 0.03a	3.93 ± 0.01a	1.46 ± 0.03a	1.43 ± 0.02a
	2	13.87 ± 0.1 cb	13.86 ± 0.2bc	26.8 ± 0.4a	26.5 ± 0.4a	3.94 ± 0.03a	3.91 ± 0.02a	1.48 ± 0.02a	1.45 ± 0.02a
	5	13.30 ± 0.1c	13.23 ± 0.1c	21.1 ± 0.2c	20.7 ± 0.2c	3.51 ± 0.04b	3.50 ± 0.01 lb	1.39 ± 0.03ab	1.37 ± 0.01ab

*Means denoted by distinct letters within columns are substantially different, according to the LSD test ($p<0.05$)

Table 3. Antioxidant activity, total phenolic content, and total chlorophyll concentration in methanolic extracts of carnation leaves values are the means of triple measurements ± standard deviation.

Water interval	Chitosan treatment (µM)	DPPH free radical scavenging activity (IC50, µg ml^{-1})		β-Carotene-linoleic acid assay (IC_{50}, µg ml^{-1})		Total phenolic content (mg GAE g^{-1} FW)		Total chlorophyll content (mg g^{-1} FW)	
		2023	2024	2023	2024	2023	2024	2023	2024
2DWI	0	11.1 ± 0.02a*	10.9 ± 0.03a	12.3 ± 0.01a	12.8 ± 0.01a	11.1 ± 0.2 c	10.7 ± 0.1 c	0.70 ± 0.03b	0.68 ± 0.03bc
	0.5	10.2 ± 0.08b	9.9 ± 0.03b	11.3 ± 0.03b	11.6 ± 0.03b	11.8 ± 0.1 b	11.4 ± 0.1 b	0.74 ± 0.04a	0.72 ± 0.01a
	2	10.0 ± 0.05b	9.8 ± 0.02b	11.4 ± 0.04b	11.8 ± 0.05b	11.9 ± 0.0 b	11.5 ± 0.1 b	0.75 ± 0.01a	0.73 ± 0.01a
	5	10.7 ± 0.04a	10.5 ± 0.01a	12.1 ± 0.02a	12.6 ± 0.03a	11.4 ± 0.2 c	11.0 ± 0.1 c	0.73 ± 0.01ab	0.70 ± 0.02ab
6DWI	0	9.3 ± 0.05c	8.8 ± 0.06c	10.4 ± 0.01c	11.1 ± 0.06c	11.9 ± 0.4 b	11.4 ± 0.2 b	0.66 ± 0.02c	0.65 ± 0.03c
	0.5	7.4 ± 0.01d	6.9 ± 0.00d	8.5 ± 0.02d	9.1 ± 0.01d	12.6 ± 0.3 a	12.2 ± 0.1 a	0.70 ± 0.01b	0.69 ± 0.01b
	2	7.1 ± 0.01d	6.8 ± 0.02d	8.2 ± 0.03d	9.0 ± 0.02d	12.7 ± 0.1 a	12.3 ± 0.2 a	0.70 ± 0.01b	0.70 ± 0.02ab
	5	9.0 ± 0.01c	8.3 ± 0.03c	10.1 ± 0.04c	10.9 ± 0.01c	12.0 ± 0.2 b	11.5 ± 0.3 b	0.67 ± 0.01c	0.66 ± 0.01c

*Means denoted by distinct letters within columns are substantially different, according to the LSD test ($p<0.05$)

Discussion

The increasing demand for irrigation water across the world has rendered it essential to have longer irrigation intervals given that there isn't enough water. This is without a doubt one of the most significant challenges that agriculture, especially horticulture, is facing at present (Mohanty *et al*., 2024; Rojas-Pirela *et al*., 2024). A significant reduction in morphological indices, such as plant height, the number of leaves, leaf area, and dry weight, was attributed to the prolongation of the irrigation interval. The observed changes indicated a drought avoidance strategy in plants (Nour *et al*., 2024), which aligned with significant physiological alterations, including variations in carbohydrate, potassium, calcium, proline, chlorophyll, and antioxidant levels (El-Esawi *et al*., 2017; Sardans &Peñuelas, 2021). Chitosan treatments, applied at selected concentrations, promoted the growth of carnation plants under both standard and prolonged watering regimes, as demonstrated by increased vegetative growth. Previous research (Bistgani *et al*., 2017) has shown similar effects for chitosan treatments on the production of dry matter and essential oils in Thymus daenensis Celak. The study's authors suggested that the increase in dry matter and essential oil production under mild stress could be attributed to higher proline levels and lipid peroxidation. Soostani *et al*. (2025) indicated that chitosan treatments on *Brassica napus* L. augmented the proline composition by 743% relative to the control, under saline stress conditions. The buildup of organic (e.g., proline) and inorganic (e.g., sugars and ions) substances is a recognized mechanism of osmotic adjustment connected to drought tolerance in plants (Khatun *et al*., 2021). Proline buildup in a plant's vegetative tissues can be a very important sign of how effectively it tolerates stress since it helps control vacuolar ion osmotic pressure and helps the plant take up water. In this study, proline concentrations increased with longer watering intervals, suggesting that the plant tissues were

under moderate water stress. Additionally, the buildup of proline was increased in plants treated with chitosan at doses of 0.5 and 2 µM. The novel finding of this study is the observation of enhanced leaf proline content at typical watering intervals when subjected to moderate dosages of 0.5 and 2 µM chitosan, which had not been described before in Carnation. The observed increase of carbohydrates in the Carnation plants studied could indicate a vital stress tolerance mechanism in plants, maybe through osmotic adjustment and the neutralization of reactive oxygen species (Kumar *et al*., 2024).

Further, the buildup of potassium and calcium ions in plant leaves is a popular method for plants to regulate their osmotic balance when they are under stress, like when they are dehydrated or exposed to salt. This accumulation is related to the building of glucose in plants that are under stress. This makes plants function more efficiently under stress and raises cell turgor pressure (Kumar *et al*., 2024).

Plants may endure drought better when they accumulate up potassium and calcium under stress. This is because it boosts up photosynthesis, which in turn raises chlorophyll levels. This is a mechanism for plants to perform more efficiently during periods of stress. When chitosan was added at a low concentration, it rendered the potassium and calcium levels in the leaves much higher. This helped the plants adjust osmotically in situations where there is not enough water.

Whenever plants are subject to water stress, they produce excessive amounts of reactive oxygen species, especially H_2O_2, O_2, and OH^-. This is since they make more electrons than they can use. If these reactive oxygen species are not adequately eliminated, this imbalance may cause damage to cells and potentially lead to cell death (Sood, 2025).

Plants are equipped with an antioxidant defense system constructed of both enzymes and non-enzymatic components. These parts work together to keep the balance of oxidation and reduction in cells when they are under stress. Secondary metabolites, such as total and free ascorbate (as shown in this study), phenolic compounds and their derivatives, such as flavanones and anthocyanins (Andrés *et al*., 2024), are examples of non-enzymatic components. There are several enzymes which contribute to the enzymatic components, but SOD, CAT, and APX are the most essential for keeping hydrogen peroxide (H_2O_2) levels in plants under control.

The rise in the activity of these enzymes observed in this study is consistent with prior research on several plants (Soostani *et al*., 2025; Rojas-Pirela *et al*., 2024).

We identified a substantial increase in the phenolic composition of leaves subjected to water stress, which was further increased in chitosan-treated plants. The increase in total phenolic content in leaves was linked to an increase in antioxidant activity, as shown by the DPPH and linoleic acid analysis. In addition, chitosan doses of 0.5 and 2 µM significantly increased the phenolic content in leaves compared to control plants; these results are consistent with those of Yin *et al*. (2012), who reported elevated polyphenol levels in *Origanum vulgare* plants treated with chitosan. Phenols are essential for the elimination of ROS in stressed plants and have a substantial impact on the antioxidant analysis of DPPH and linoleic acid assays, which predominantly measure hydroxyl-free radicals.

Non-enzymatic antioxidants, such as total and free ascorbate, were increased in both non-stressed and stressed plants treated with chitosan compared to untreated plants. The changes associated with the accumulation of H_2O_2 in stressed plants indicate that chitosan significantly affects the non-enzymatic antioxidant mechanisms in carnation. The buildup of H_2O_2 was linked to an increase in the activity of several antioxidant enzymes, such as SOD, CAT, and APX. Faluku *et al*. (2024) found that synthesized fulvic acid-releasing chitosan nanoparticles promoted rice growth under drought conditions by enhancing antioxidant defense, as evidenced by the lowest levels of H_2O_2 and MDA and the highest levels of APX, which is in agreement with the findings of the present study.

The present study revealed that chitosan sprays improved the morphological and physiological responses of carnations subjected to water stress resulting from extended irrigation intervals. Multiple stress tolerances and avoidance mechanisms were profoundly associated with antioxidant responses at both morphological and physiological levels. The results obtained in this study regarding water stress and chitosan treatments have been confirmed by various parameters including leaf number, leaf area, plant height, and plant dry weight, collectively considered a drought avoidance approach. Morphological alterations occurred along with physiological osmotic modifications, as indicated by the accumulation of carbohydrates, chlorophyll, proline, potassium, and calcium, which form the organic foundation of an intricate drought tolerance mechanism. We examined the overall antioxidant activity, phenolic content, total and free ascorbate levels, hydrogen peroxide concentration, and the activities of the SOD, CAT, and APX enzymes to find out if antioxidants contribute to stress tolerance in Carnation subjected to chitosan. Applying chitosan throughout extended intervals of irrigation might decrease the impact of drought stress on carnations. A thorough comprehension of the mechanisms that facilitate stress resistance may allow for the development of innovative approaches to mitigate the detrimental impacts of climate change and water scarcity.

Conclusion

To our knowledge, this study constitutes a unique and thorough analysis of drought avoidance and tolerance mechanisms at both morphological and physiological levels in carnations subjected to prolonged watering intervals and chitosan treatments. Morphological and physiological data were examined to elucidate the correlation between stress tolerance and avoidance mechanisms in carnation plants exposed to water and chitosan. This study's results showed that several mechanisms work together to improve plant growth when there isn't enough water. Additionally, the foliar application of chitosan significantly mitigated the detrimental effects of water stress on plant growth, indicating its potential as an effective means to enhance water stress tolerance in horticultural crops and to inform the future development of innovative strategies to alleviate these negative impacts.

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