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TAGETES ERECTA MEDICINAL VALUE UNDER WATER-STRESS AND OLIGOSACCHARIDE (COS) TREATMENT

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Abstract

Water limitation significantly impacts ornamental plant production, particularly in arid regions. *Tagetes erecta* L. is well known for its decorative value and therapeutic importance as well as its sensitivity to low water availability. This study explores the application of chitosan oligosaccharides (COS) sprays at different levels (50, 200, and 500 ppm) in enhancing plant resilience to drought stress while maintaining their optimal physiological performance. The plants were watered on two different schedules (every two days and every six days). Drought conditions (every six days) resulted in decreased plant growth, chlorophyll composition, carbohydrate levels, and increased proline accumulation, and oxidative stress. The application of COS, particularly at moderate concentrations (50–200 ppm), improved plant performance. The treated plants showed enhanced growth, increased nutrient levels (carbohydrates, k and Ca), and retained photosynthetic pigments. Concurrently, COS reinforced the antioxidant defense system of the plant, enhancing the activity of crucial enzymes (SOD, CAT, and APX) and diminishing oxidative damage (H_2O_2). Drought alone stimulated phenolic compound accumulation and antioxidant activity. The COS application resulted in stronger antimicrobial properties of plant extracts against several pathogenic microorganisms. Overall, the results indicate that COS provides benefits beyond protecting stressed plants. This process enables them to adapt more efficiently and enhances their biochemical and medicinal properties. The dual benefit of COS shows that it could be a long-term strategic application for growing *T. erecta* in arid areas.

Key words: Tagetes; Drought; Antioxidants; Oligosaccharide; Foodborne pathogens

Introduction

Environmental factors, especially water availability, have a significant effect on the growth and quality of *Tagetes erecta* L. (Asteraceae), even though it can grow in different locations. These impacts are important for improving farming methods and making sure the flowers look good, especially when the watering circumstances change. Recent research indicates that proper irrigation strategies can enhance the ornamental traits and physiological responses of *T. erecta*, making it vital for effective cultivation under water stress conditions (Marković *et al*., 2024). Horticultural crops such as *T. erecta* still have a hard time with drought, especially in dry or semi-dry ecosystems found in Saudi Arabia. Photosynthesis, and cellular metabolism are essential metabolic processes that are adversely affected by water deficiency. Consequently, resulting in reduced biomass and decelerated plant growth. Drought conditions induce oxidative stress through the excessive production of reactive oxygen species (ROS), which can be detrimental to cellular integrity if not adequately regulated (García-García *et al*., 2023). Plants react to water stress via many mechanisms, including the buildup of osmoprotectants like proline and the activation of antioxidant pathways (Chawla *et al*., 2022). Plant defensive mechanisms are essential for managing drought stress and encompass both enzymatic and non-enzymatic processes (Zulfiqar *et al*., 2020). Stress-induced responses frequently increase secondary metabolite levels, enhancing the medicinal value of the plant (Pagare *et al*., 2015). *T. erecta* biochemical adaptations have been linked to increased antioxidant and antimicrobial properties (Kim *et al*., 2024; Siddiqa *et al*., 2025).

The scientific and agricultural communities are becoming more interested in eco-friendly ways to make plants more resistant to abiotic stressors without lowering yields. From this point of view, biostimulants have become a potential way to manage stress. Chitosan and its derivatives, especially chitosan oligosaccharides (COS), have received considerable attention owing to their biodegradability, low toxicity, and capacity to act as plant signaling molecules (Salachna & Łopusiewicz, 2022). Oligosaccharides represent a class of biostimulants synthesized commercially through the application of elevated temperatures to chitin, followed by an alkaline deacetylation process aimed at eliminating proteins and calcium (Magnabosco *et al*., 2023). These oligosaccharides can be prepared in either liquid form or as a soluble powder in water. They are extensively utilized as plant elicitors to enhance the synthesis of secondary metabolites, several studies suggest that it may improve crop yield and enhance stress tolerance (García-García *et al*., 2023; Rojas-Pirela *et al*., 2024). Additionally, COS can trigger antioxidant mechanisms and promote secondary metabolite accumulation, suggesting a dual role in stress reduction and quality enhancement (Kappel *et al*., 2022).

Foodborne infections, including diarrheal and emetic symptoms, are critically significant in the agricultural

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sector, particularly in global milk processing (Wróbel *et al.*, 2025). These disorders are caused by several microorganisms, including the bacterium *Bacillus cereus* and *Listeria monocytogenes*, and the severity of the associated diseases can result in human mortality. Fungi result in significant agricultural losses, jeopardize human food storage systems, and produce mycotoxins that induce carcinogenic diseases and neurological impairments (Dabuo *et al.*, 2022). The fungus include *Aspergillus niger*, which induces black mold on many horticulture crops, and *Aspergillus ochraceus*, which contaminates human food; both species have evolved resistance to antifungal drugs (Ransom, 2000). Likewise, pathogenic bacteria have developed significant resistance to antibiotics, necessitating the search for natural alternatives that are more efficacious in managing foodborne diseases. To mitigate losses in the food sector and ensure food security, synthetic food preservatives were introduced, despite their long-term adverse consequences on human health. These conditions directed the pursuit of natural bioactive substances capable of controlling foodborne bacteria.

This study offers a novel perspective by linking drought stress management with the enhancement of medicinal quality in *T. erecta*. Unlike conventional approaches that focus mainly on mitigating growth reduction under water deficit, this work demonstrates that chitosan oligosaccharides (COS) can simultaneously sustain plant growth and stimulate the accumulation of bioactive compounds. By integrating physiological, biochemical, and antimicrobial responses within a single framework, the study reveals a dual-function strategy in which stress is not only alleviated but also strategically leveraged to improve plant value. This combined approach provides new insight into the use of biostimulants as tools for optimizing both productivity and phytochemical potential under water-limited conditions.

This study explores the utilization of COS to enhance drought resistance in *T. erecta* by analysing morphological, physiological, and biochemical responses. The effect of COS treatment on antioxidant capacity and antimicrobial activity under water-deficient conditions was also investigated. This work offers a more comprehensive insight into managing stress using biostimulants to optimize both yield and quality by correlating plant performance with bioactive potential.

Materials and Methods

Plant material and experimental design of plants: Uniform young plants (approximately 10 cm in height) of *T. erecta* L. were obtained from commercial nurseries in Riyadh, Saudi Arabia, during spring 2025. The experiment was conducted in a controlled glasshouse at the University of King Saud. Plants were transplanted into 2.1 L pots containing a mixture of peat and perlite (3:1, w/w). Following growth establishment in 1 week, the plant were supplemented with a balanced fertilizer (Crystalon® 20:20:20 N:P:K) at 2 g L^{-1}. Plants were acclimatized for 3 weeks under controlled environmental conditions (15.1°C–27.5°C, 58%–67% relative humidity, and ~1000 µmol m^{-2} s^{-1} PAR at midday), consistent with the optimal greenhouse cultivation parameters for ornamental species. During this period, irrigation (38–50 mL per plant) was applied daily.

After acclimatization, the plants underwent two irrigation regimes: watering every 2 days (2DWI) and every 6 days (6DWI) for six weeks to replicate well-watered and drought stress, respectively. Chitosan oligosaccharide solutions with a deacetylation degree greater than 95% were applied to leaves at concentrations of 50, 200, and 500 ppm until runoff, two weeks before stress was imposed, in accordance with previously described methods for applying biostimulants (Rojas-Pirela *et al.*, 2024). Foliar application volumes were standardized across treatments The untreated plants were used as controls.

The plants were arranged in a split-plot design employing a randomized complete block design (RCBD). The irrigation treatments allocated to main plots and oligosaccharide concentrations were the subplots. Each treatment contained five replicates, with three blocks in total.

Morphological and Physiological Measurements: Plants were harvested after six weeks of treatment. Growth parameters, including plant height and leaf number, were recorded. The leaf area was determined using digital image analysis with AutoCAD software. The total dry biomass was obtained after oven-drying the plant samples at 30°C to a constant weight. The total carbohydrate content was quantified in fresh leaves following Dubois *et al*., (1956). Mineral elements (K^+ and Ca^{2+}) were determined from leaf sap extracts using inductively coupled plasma spectrophotometry. The proline composition in leaves was determined using a spectrophotometer at 520 nm according to Bates *et al*., (1973).

Antioxidants, chlorophyll, phenolics, and enzyme activities: Leaf samples were air-dried, ground into a fine powder, and extracted in methanol (99%) for 24 h under dark conditions, following established extraction protocols for phenolic compounds (Elansary *et al*., 2020). The DPPH radical scavenging experiment (Brand-Williams *et al*., 1995) and the β-carotene–linoleic acid bleaching method (Marco, 1968) were used to test the antioxidant activity. The absorbance was measured at 517 and 470 nm. IC_{50} values were calculated from the inhibition curves. Butylated hydroxytoluene (BHT) was used as a positive control.

The Folin–Ciocalteu method was utilized to quatify the total phenolic content (Singleton & Rossi, 1965), expressed as gallic acid equivalents (mg GAE g^{-1} extract).

Chlorophyll content was measured spectrophotometrically following Moran & Porath (1980).

The activities of antioxidant enzymes, including catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), and hydrogen peroxide (H_2O_2) content, were determined according to established spectrophotometric methods (Nakano & Asada, 1981; Aebi, 1984; Elansary *et al*., 2020).

Antimicrobial assays and medicinal properties: The antimicrobial activity of methanolic leaf extracts against selected gram-positive and gram-negative bacteria and fungal strains was evaluated. The selected bacteria for the analyses included *Listeria monocytogenes (clinical isolate), Bacillus cereus (ATCC 14579), Staphylococcus aureus (ATCC 6538), Micrococcus flavus (ATCC 10240), Pseudomonas aeruginosa (ATCC 27853), and Escherichia*

coli (ATCC 35210). An economic group of fungi were tested in this experiment and included *Candida albicans* (ATCC 12066), *Penicillium ochrochloron* (ATCC 48663), *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), and *A. flavus* (ATCC 9643). The microdilution method (Elansary *et al*., 2018) was used to test the antibacterial and antifungal effects. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited visible microbial growth, while the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined as the lowest concentrations that resulted in a 99.5% reduction in the initial inoculum. Positive controls included streptomycin and ampicillin for antibacterial assays and fluconazole and ketoconazole for antifungal assays, whereas 5% dimethyl sulfoxide was used as a negative control. All experiments were conducted in triplicate and repeated twice.

Statistical analysis

Data are expressed as mean values ± standard deviation. One-way analysis of variance (ANOVA) was used to do the statistical analysis, and the least significant difference (LSD) test at $p<0.05$ was used to separate the means. We used SPSS (PASW Statistics, version 21) to do the analyses.

Results

Vegetative growth and biochemical responses to irrigation intervals and oligosaccharides: Table 1 presents the effects of irrigation interval (2DWI vs. 6DWI) and oligosaccharide (COS) treatments on the vegetative growth traits of *T. erecta*, including leaf number, leaf area, plant dry weight, and plant height. The application of COS at 50 and 200 ppm under well-watered conditions (2DWI) significantly enhanced all growth parameters compared with the untreated control. The highest values were consistently observed at 200 ppm, indicating optimal plant growth stimulation. In contrast, the highest concentration (500 ppm) showed no improvement and often reduced growth.

All growth parameters decreased significantly under 6DWI conditions, indicating the harmful effects of drought stress. COS treatments, particularly those at 50 and 200 ppm, partially alleviated this reduction, significantly improving leaf number, biomass, and plant height compared to untreated stressed plants. Moderate COS concentrations alleviate drought-induced growth inhibition, whereas excessive COS concentrations, specifically 500 ppm, are ineffective or harmful. Table 2 presents the biochemical effects of COS treatments on carbohydrates, K, Ca, and proline compositions. Under normal irrigation conditions, COS concentrations of 50 and 200 ppm significantly increased carbohydrate accumulation and mineral content (K and Ca), indicating enhanced metabolic activity and nutrient uptake. The proline content increased slightly with COS. Proline accumulation was significantly higher across all treatments under drought stress, confirming its role as an indicator of stress. COS treatments, particularly at 50 and 200 ppm, significantly increased proline levels, indicating a greater capacity for osmotic adjustment. The COS also enhanced the levels of carbohydrates and minerals under stress conditions, suggesting a better physiological state and greater stress resilience. Moderate doses were more effective than 500 ppm.

Table 1. Irrigation intervals and oligosaccharide effects on leaf number and area, dry weight, and plant height in *T. erecta*. Values are expressed as means (± sd).

Water interval	Treatment with oligosaccharides (ppm)	Leaf number (leaf plant^{-1})	Leaf area (cm^2 plant^{-1})	Plant dry weight (g plant^{-1})	Plant height (cm)
2DWI	0	12.5 ± 0.1b*	511.3 ± 15.1b	10.6 ± 0.3b	30.3 ± 0.1b
	50	13.2 ± 0.3a	555.3 ± 11.2a	11.5 ± 0.2a	32.8 ± 0.1a
	200	13.3 ± 0.2a	567.2 ± 14.1a	11.8 ± 0.1a	32.9 ± 0.3a
	500	12.1 ± 0.1b	501.3 ± 17.7b	10.7 ± 0.2b	30.5 ± 0.1b
6DWI	0	5.2 ± 0.1e	218.4 ± 13.2d	5.6 ± 0.1d	17.6 ± 0.1d
	50	6.2 ± 0.1d	269.2 ± 10.3c	6.3 ± 0.3c	21.7 ± 0.3c
	200	7.1 ± 0.2c	276.1 ± 10.1c	6.3 ± 0.2c	21.9 ± 0.1c
	500	5.2 ± 0.1e	224.2 ± 13.1d	5.3 ± 0.2d	17.9 ± 0.5d

*Means followed by different letters within columns are significantly different, based on the least significant difference test ($p\leq0.05$).

Table 2. Irrigation intervals and oligosaccharide effects on total carbohydrate, K, Ca, and proline composition in the leaves *of T. erecta leaves.* Values are means (± sd).

Water interval	Oligosaccharides treatment (ppm)	Total carbohydrates (% DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Proline (mg g^{-1} DW)
2DWI	0	13.04 ± 0.1b	23.7 ± 0.1c	3.41 ± 0.05b	1.59 ± 0.03c
	50	13.81 ± 0.0a	28.1 ± 0.1b	3.75 ± 0.03a	1.67 ± 0.05b
	200	14.12 ± 0.3a	28.6 ± 0.2b	3.81 ± 0.05a	1.70 ± 0.05b
	500	13.35 ± 0.3b	24.6 ± 0.1c	3.53 ± 0.01b	1.62 ± 0.01c
6DWI	0	12.06 ± 0.3c	26.7 ± 0.2b	3.47 ± 0.05b	1.73 ± 0.03b
	50	13.25 ± 0.1b	29.6 ± 0.6a	3.74 ± 0.01a	1.90 ± 0.02a
	200	13.34 ± 0.5b	29.9 ± 0.5a	3.75 ± 0.03a	1.92 ± 0.01a
	500	12.04 ± 0.3c	27.8 ± 0.3b	3.46 ± 0.01b	1.75 ± 0.01b

*Means followed by different letters within columns are significantly different, based on the least significant difference test ($p\leq0.05$)

Table 3. DPPH and β-Carotene-linoleic acid in leaf extracts, phenolic composition, and total chlorophyll of *T. erecta*. Values are means of triplicate determinations ±sd.

Water interval	Oligosaccharides treatment (ppm)	Free radical scavenging activity of DPPH (IC50, µg ml^{-1})	β-Carotene-linoleic acid assay (IC_{50}, µg ml^{-1})	Total phenolic content (mg GAE g^{-1})	Total chlorophyll content (mg g^{-1} FW)
2DWI	0	8.1 ± 0.3a	9.2 ± 0.1a	12.3 ± 0.1 c	0.71 ± 0.01b
	50	6.4 ± 0.03b	8.0 ± 0.03b	13.3 ± 0.3 b	0.75 ± 0.02a
	200	6.3 ± 0.01b	8.1 ± 0.01b	13.4 ± 0.2 b	0.76 ± 0.01a
	500	7.3 ± 0.03a	9.4 ± 0.02a	13.0 ± 0.1 b	0.71 ± 0.01b
6DWI	0	6.2 ± 0.02b	9.2 ± 0.03a	13.3 ± 0.2 b	0.64 ± 0.01c
	50	4.2 ± 0.02c	7.1 ± 0.03c	14.1 ± 0.5 a	0.69 ± 0.02c
	200	4.0 ± 0.07c	7.1 ± 0.02c	14.3 ± 0.1 a	0.69 ± 0.02a
	500	5.9 ± 0.03b	8.2 ± 0.01b	13.4 ± 0.3b	0.65 ± 0.03b

*Means followed by different letters within columns are significantly different based on the least significant difference test ($p \leq 0.05$)

Table 4. MIC and MBC of *T. erecta* leaf extracts (mg^{-1}mL) against bacteria.

Water interval	Treatment with oligosaccharides (ppm)	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Listeria monocytogenes*
2DWI	0	0.21 ± 0.2	0.11 ± 0.3	0.8 ± 0.3	0.11 ± 0.01	0.10 ± 0.02	0.18 ± 0.01
		0.41 ± 0.01	0.27 ± 0.01	0.16 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.34 ± 0.01
	200	0.19 ± 0.01	0.10 ± 0.04	0.7 ± 0.00	0.8 ± 0.03	0.11 ± 0.02	0.15 ± 0.02
		0.40 ± 0.01	0.27 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.31 ± 0.01
	500	0.18 ± 0.01	7.10 ± 0.03	0.6 ± 0.03	0.8 ± 0.02	0.08 ± 0.01	0.14 ± 0.01
		0.37 ± 0.01	0.24 ± 0.01	0.13 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.31 ± 0.01
6DWI	0	0.19 ± 0.01	0.11 ± 0.02	0.7 ± 0.01	0.7 ± 0.04	0.11 ± 0.01	0.15 ± 0.02
		0.39 ± 0.01	0.24 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.21 ± 0.01	0.30 ± 0.01
	200	0.17 ± 0.03	0.8 ± 0.07	0.6 ± 0.02	0.7 ± 0.02	0.9 ± 0.03	0.14 ± 0.01
		0.36 ± 0.01	0.21 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.17 ± 0.01	0.31 ± 0.01
	500	0.16 ± 0.01	0.72 ± 0.03	0.5 ± 0.03	0.6 ± 0.01	0.8 ± 0.02	0.14 ± 0.01
		0.34 ± 0.01	0.19 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.17 ± 0.01	0.26 ± 0.01
	Streptomycin	0.9 ± 0.01	0.21 ± 0.01	0.04 ± 0.01	0.11 ± 0.001	0.08 ± 0.00	0.15 ± 0.01
		0.41 ± 0.01	0.42 ± 0.01	0.13 ± 0.01	0.18 ± 0.002	0.13 ± 0.01	0.32 ± 0.01
	Ampicillin	0.23 ± 0.01	0.11 ± 0.01	0.10 ± 0.003	0.11 ± 0.002	0.13 ± 0.01	0.15 ± 0.01
		0.43 ± 0.01	0.14 ± 0.01	0.17 ± 0.003	0.15 ± 0.003	0.21 ± 0.01	0.27 ± 0.01

Table 5. MIC and MBC of *T. erecta* leaf extracts (mg^{-1}mL) against fungi.

Water interval	Treatment with oligosaccharides (ppm)	*Aspergillus niger* MIC MFC	*Aspergillus ochraceus* MIC MFC	*Aspergillus flavus* MIC MFC	*Penicillium ochrochloron* MIC MFC	*Candida albicans* MIC MFC
2DWI	0	0.16 ± 0.01	0.15 ± 0.1	0.13 ± 0.02	0.20 ± 0.01	0.10 ± 0.01
		0.32 ± 0.01	0.35 ± 0.01	0.25 ± 0.01	0.43 ± 0.01	0.23 ± 0.01
	200	0.15 ± 0.01	0.16 ± 0.02	0.10 ± 0.00	0.21 ± 0.01	0.11 ± 0.01
		0.31 ± 0.01	0.33 ± 0.01	0.24 ± 0.01	0.42 ± 0.01	0.23 ± 0.01
	500	0.14 ± 0.01	8.15 ± 0.02	0.11 ± 0.01	0.20 ± 0.02	0.08 ± 0.01
		0.30 ± 0.01	0.28 ± 0.01	0.20 ± 0.01	0.41 ± 0.01	0.17 ± 0.01
6DWI	0	0.15 ± 0.01	0.16 ± 0.02	0.10 ± 0.01	0.21 ± 0.01	0.11 ± 0.01
		0.31 ± 0.01	0.33 ± 0.01	0.23 ± 0.01	0.40 ± 0.01	0.21 ± 0.01
	200	0.15 ± 0.01	0.14 ± 0.01	0.11 ± 0.02	0.18 ± 0.03	0.10 ± 0.03
		0.31 ± 0.01	0.27 ± 0.01	0.21 ± 0.01	0.37 ± 0.02	0.19 ± 0.01
	500	0.11 ± 0.01	0.13 ± 0.03	0.10 ± 0.01	0.18 ± 0.01	0.10 ± 0.02
		0.24 ± 0.01	0.26 ± 0.01	0.19 ± 0.01	0.36 ± 0.01	0.18 ± 0.01
	FLZ	0.14 ± 0.01	0.21 ± 0.01	0.12 ± 0.01	0.20 ± 0.01	0.11 ± 0.01
		0.29 ± 0.02	0.35 ± 0.01	0.23 ± 0.01	0.34 ± 0.01	0.22 ± 0.01
	KTZ	0.11 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.21 ± 0.01
		0.22 ± 0.01	0.41 ± 0.01	0.40 ± 0.01	0.40 ± 0.01	0.41 ± 0.01

Antioxidant activity and phytochemical traits: Table 3 shows the antioxidant activity (DPPH and β-carotene assays), total phenolic content, and total chlorophyll content. Under 2DWI, COS treatments at 50 and 200 ppm significantly improved antioxidant activity (lower IC50 values), increased total phenolics, and enhanced chlorophyll content compared to control plants. Under 6DWI, antioxidant activity further increased (lower IC50 values), particularly at 50 and 200 ppm, indicating stress-induced enhancement of antioxidant defense. The TPC was the highest under drought combined with COS, especially at 200 ppm. Conversely, the chlorophyll content decreased under drought conditions, although COS partially mitigated this decline.

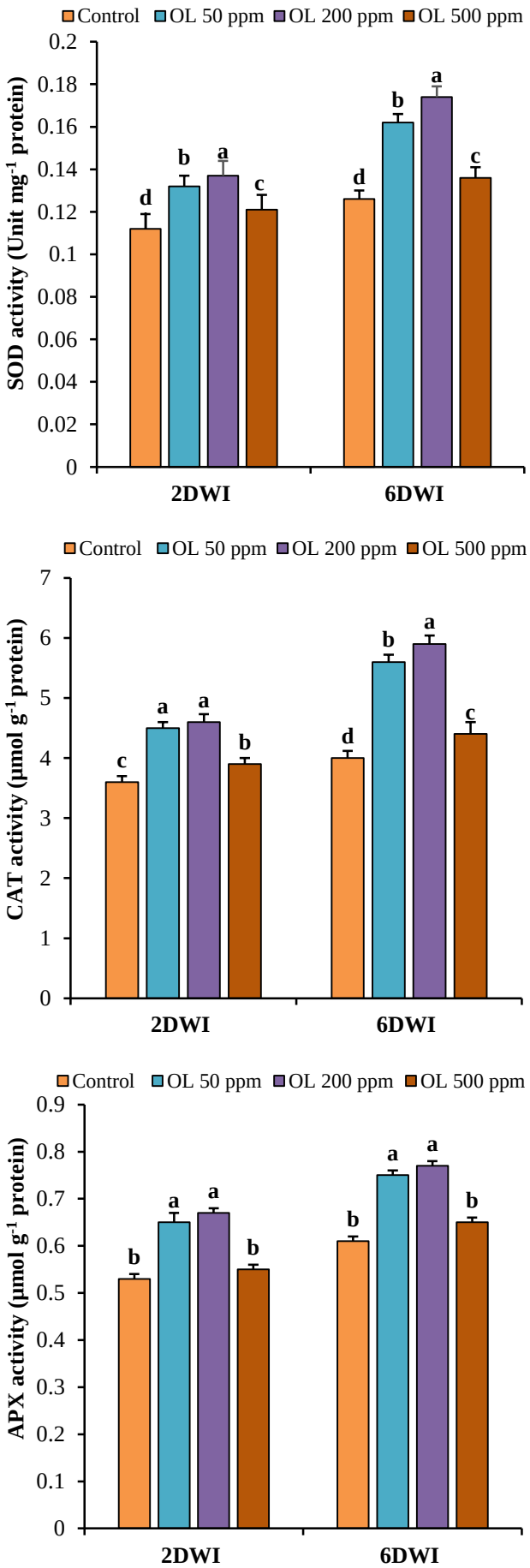

Fig. 1. SOD, CAT, and APX activities in *T. erecta* subjected to prolonged irrigation intervals and different OL concentrations.

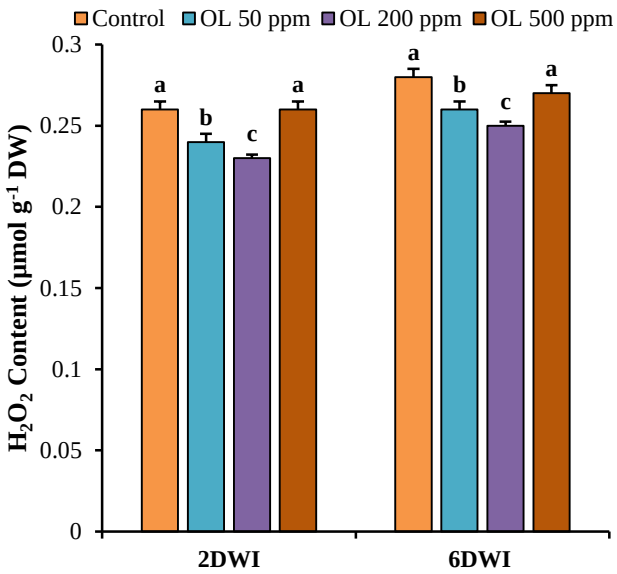

Fig. 2. H_2O_2 content in *T. erecta* plants subjected to prolonged irrigation intervals and different OL concentrations.

Antioxidant enzyme activities (SOD, CAT, and APX): Figure 1 shows the activities of the following key antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). Under water stress (6DWI), enzyme activities were significantly increased compared with those of well-watered plants, reflecting the activation of the antioxidant defense system. COS treatments, particularly at 50 and 200 ppm, further enhanced enzyme activities, indicating improved ROS-scavenging capacity. The highest enzyme activities were observed under combined drought stress and 200 ppm COS treatment, indicating a strong synergistic effect. Conversely, 500 ppm showed reduced effectiveness, aligning with other parameters. Figure 2 shows H_2O_2 accumulation as an indicator of oxidative stress.

Significant increases in H_2O_2 levels, as measured by the 6DWI, confirmed enhanced oxidative stress. COS treatments decreased H_2O_2 accumulation, especially at 50 and 200 ppm. The lowest H_2O_2 levels under stress were recorded at 200 ppm COS, corresponding with the highest antioxidant enzyme activities (Fig. 1. In contrast, 500 ppm was less effective in reducing oxidative stress.

Antibacterial and antifungal activities: The antibacterial activity of *T. erecta* extracts against several pathogenic bacteria is shown in Table 4. Extracts from COS-treated plants generally exhibited stronger antibacterial activity (lower MIC and MBC values) than untreated controls. The most pronounced effects were observed at 200 and 500 ppm, particularly under drought stress (6DWI), indicating that stress-induced phytochemical accumulation enhances antimicrobial properties.

Pseudomonas aeruginosa and *Escherichia coli* showed relatively lower MIC values, whereas *Bacillus cereus* was more resistant. In several cases, the activity of COS-treated extracts was comparable to that of standard antibiotics (streptomycin and ampicillin), highlighting their potential medicinal value.

The antifungal activity of T. erect leaves extract against multiple fungal strains is shown in Table 5. Similar to antibacterial results, COS treatment enhanced antifungal

activity, particularly at 200 and 500 ppm. Under drought stress, the extracts exhibited lower MIC and MFC values, indicating stronger antifungal effects. *Aspergillus flavus* and *Candida albicans* were particularly sensitive to the extracts, whereas *Penicillium ochrochloron* exhibited relatively higher resistance. Under water stress, COS-treated plants consistently produced extracts with improved antifungal efficacy, in some cases comparable to those of standard antifungal agents (fluconazole and ketoconazole). Antimicrobial assays were performed using independent biological replicates.

Discussion

This study indicated that water deficiency severely hampers the vegetative growth of *T. erecta*, whereas COS alleviated these negative effects in a concentration-dependent way. The decreases in leaf number, leaf area, plant height, and biomass observed with prolonged irrigation intervals (Table 1) were consistent with previous research indicating that drought stress limits cell expansion, reduces stomatal permeability, and hampers photosynthetic processes (García-García *et al*., 2023; Rojas-Pirela *et al*., 2024). Environmental stress significantly impaired the physiological processes of *T. erecta*, thereby reducing growth performance and biomass accumulation (Keshavarz *et al*., 2025; Molnár *et al*., 2025).

Improvements observed with COS application at concentrations of 50–200 ppm is in agreement with previous work showing that chitosan derivatives can augment crop growth through optimized nutrient intake, augmented photosynthesis, and phytohormone regulation (García-García *et al*., 2023; Rojas-Pirela *et al*., 2024). The growth and pigment profiles of *T. erecta* have been enhanced under optimized environmental conditions and biostimulant treatments (Keshavarz *et al*., 2025). The decline at 500 ppm suggests a threshold beyond which COS exerts inhibitory effects, a phenomenon also observed in recent studies on elicitors (Rojas-Pirela *et al*., 2024).

Biochemically, drought stress significantly increased proline accumulation while reducing carbohydrate content (Table 2), confirming its role in osmotic adjustment and stress tolerance. Proline is a well-known marker for drought stress in plants; its accumulation is considered an adaptive response to water deficit, which enhances the osmoregulation and stabilization of cellular structures (García-García *et al*., 2023). Stress-induced metabolic adjustments in *T. erecta* have been linked to enhanced osmolyte and stress-responsive compound synthesis (Rivas-García *et al*., 2023). COS treatment further enhanced the carbohydrate content and mineral accumulation (K and Ca), indicating improved metabolic efficiency and ion homeostasis under stress conditions. These findings were consistent with recent reports that COS improves nutrient assimilation and osmotic regulation under abiotic stress (Rojas-Pirela *et al*., 2024). The buildup of potassium and calcium correlates with carbohydrate accumulation in stressed plants, hence enhancing plant performance under stress and improving cell turgor pressure (Shaaban *et al.*, 2023). The buildup of K and Ca in plants under stress conditions enhances the photosynthetic rate, resulting in higher chlorophyll content (a mechanism for drought resistance) and carbohydrate accumulation, as reported herein, which contributes to improved plant performance during stress.

The enhancement of antioxidant activity and phenolic content (Table 3) under drought stress and COS treatment highlights a key adaptive mechanism in *T. erecta*. The oxidative stress resulting from drought conditions may stimulate the secondary metabolites' production in plants, such as phenolic compounds (García-García *et al*., 2023). In marigold, phenolic compounds were major contributors to its medicinal value, and their accumulation is highly responsive to environmental conditions (Rivas-García *et al*., 2023; Domínguez-Niño *et al*., 2025). We noted a substantial enhancement in the phenolic composition of leaves under water stress circumstances, which was further elevated in plants treated with oligosaccharides. The augmentation in total phenolic content in leaves corresponded with an enhancement in antioxidant activity, as assessed by the DPPH and linoleic acid assays. Furthermore, oligosaccharide concentrations of 50 and 200 ppm significantly elevated phenolic content in leaves compared to control plants; these findings align with previous work (Yin *et al.*, 2011), which indicated increased polyphenol levels in *Origanum vulgare* subjected to oligosaccharide treatment.

Excess reactive oxygen species, such as H_2O_2, O_2, and OH^-, are generated in plants during water stress circumstances, resulting from an imbalance between electron generation and use (Sood, 2025). This situation may result in damage and potentially cell death if reactive oxygen species (ROS) are not efficiently eliminated. The antioxidant defense system in plants comprises enzymatic and non-enzymatic components that function to preserve intracellular redox equilibrium during stress situations. Non-enzymatic agents encompass secondary metabolites, including total and free ascorbate, along with phenolic compounds and their derivatives (e.g., flavanones and anthocyanins). Phenols are crucial in eliminating reactive oxygen species (ROS) in stressed plants and significantly influence the antioxidant assessments of DPPH and linoleic acid assays, which primarily quantify hydroxyl (OH^-) free radicals. COS further enhanced antioxidant activity, as evidenced by lower IC_{50} values, indicating a synergistic interaction between stress and elicitation. Similar synergistic effects have been reported where COS acts as a signalling molecule that activates phenylpropanoid pathways and secondary metabolism (García-García *et al*., 2023).

Under drought conditions it's common to see a reduction in chlorophyll composition, which might be attributed to the damage in the photosynthetic apparatus. However, COS partially alleviated this decline, indicating a protective role in maintaining photosynthetic integrity. These results are in agreement with a previous report showing that biostimulants may enhance the photosynthetic efficiency as well as chlorophyll stability under drought conditions (Keshavarz *et al*., 2025).

The antimicrobial results (Tables 4 and 5) provided further evidence of the enhanced medicinal value of stress and COS treatment. The antibacterial and antifungal properties of the treated plants were enhanced, which can be linked to the higher concentration of bioactive compounds, including phenolics and flavonoids. *T. erecta* possesses strong antibacterial, antifungal, and antiviral properties

associated with the phytochemical constituents (Kim *et al*., 2024; Vaz *et al*., 2024). Marigold polyphenol-rich extracts have demonstrated significant pharmacological activities, including osteogenic and therapeutic potential (Sanjaya *et al*., 2024). The observed enhancement under drought conditions supports the concept that abiotic stress can increase the medicinal quality of plants by stimulating secondary metabolism (Rivas-García *et al*., 2023). The enhanced antibacterial and antifungal activity seen in leaf extracts from plants exposed to extended watering intervals and oligosaccharide treatments is linked to the accumulation of phenols in the treated specimens. The accumulation of phenols significantly inhibits the growth of bacteria and fungus (Rao *et al.*, 2010).

There were increases in the antioxidant enzyme activities of SOD, CAT, and APX under drought conditions (Figure 1), which point to the activation of ROS-scavenging mechanisms. This response is well documented in plants exposed to oxidative stress (García-García *et al*., 2023). COS application further enhanced these enzyme activities, suggesting improved redox homeostasis. Similar findings have been reported in which COS enhances antioxidant enzyme systems and improves stress tolerance (Rojas-Pirela *et al*., 2024). Studies have shown that alginate-derived oligosaccharides (ADO) can significantly alleviate cadmium stress in wheat seedlings, leading to increased shoot and root lengths, as well as improved chlorophyll content and photosynthetic rates (Ma *et al.*, 2010). Also, ADO treatment has been shown to make tomato seedlings more resistant to drought stress by changing the activities of antioxidant enzymes and lowering oxidative damage (Liu *et al*., 2009). This dual role of oligosaccharides not only shows how important they are for optimizing enzymes, but it also shows how they could be used as biostimulants in sustainable agriculture, where making plants more resistant to stress can lead to higher crop yields and less reliance on chemical fertilizers.

H_2O_2 levels increased under drought stress, but COS treatments significantly reduced them (Fig. 2), consistent with these results. This reduction reflects enhanced ROS detoxification and confirms the protective role of COS in mitigating oxidative damage. The reduction in antioxidant enzyme activity is generally as H_2O_2 levels go up, showing how the COS-activated defence system interacts within plant biology. It is critical to understand how oligosaccharides and H_2O_2 levels in leaves are related during drought stress because these substances are critical for plant health. Recent studies have shown that oligosaccharide application can lead to an increase in reactive oxygen species, notably hydrogen peroxide, which acts as a signalling molecule to enhance the plant's stress response mechanisms (Ji *et al.*, 2023). This suggests that while oligosaccharides contribute to stress tolerance by promoting water retention, they may simultaneously trigger oxidative responses that necessitate a delicate balance within the plant's physiological processes.

The findings indicated a distinct interaction between drought stress and COS application in affecting the growth, physiology, and medicinal attributes of *T. erecta*. Drought stress independently enhanced secondary metabolism; however, COS further refines this response by improving physiological performance and augmenting phytochemical accumulation, leading to increased resilience against drought and enhanced therapeutic properties of *T. erecta*. The combined effect presents a promising strategy for increasing *T. erecta* yield and medicinal value, especially under water-limited conditions. The novelty of this study lies in how it brings together two aspects that are often investigated separately: stress tolerance and the enhancement of medicinal value. Most studies focused on minimizing growth losses; in contrast, this work goes a step further by showing that stress, when carefully managed, can actually be used to improve the functional quality of *T. erecta*. More importantly, the study demonstrates that chitosan oligosaccharides do not simply alleviate the negative effects of water deficit—they actively reshape the plant's response. COS strikes a balance between survival and production by keeping growth going and boosting antioxidant defenses while at the same time increasing the buildup of bioactive molecules. This dual role is particularly significant because it highlights a practical strategy where plant stress is not only mitigated but also leveraged to increase value. The integration of physiological performance, biochemical responses, and antimicrobial activity within a single framework further distinguishes this work, offering a more complete understanding of how biostimulants can be used to optimize both yield and quality under water-limited conditions.

Limitations: This study was conducted under controlled greenhouse conditions, which may not fully reflect the variability of field environments, including fluctuations in climate, soil, and biotic factors. Additionally, the experiment was limited to a single season, location, and a specific range of chitosan oligosaccharide (COS) concentrations, which may affect the generalization of the results. The study also did not investigate underlying molecular mechanisms. Therefore, further field-based and multi-season studies are needed to confirm the broader applicability of these findings.

Conclusion

The current investigation revealed that the application of chitosan oligosaccharides mitigated the negative impacts of drought by promoted the plant vegetative growth (plant height, leaf number and area and plant dry weight), enhanced photosynthesis (total chlorophyll), improved nutrient uptake (Ca, K, and carbohydrates) and fortified antioxidant defences (proline accumulation, antioxidants). The treatment amplified phenolic compound accumulation and enhanced antimicrobial activity, thereby improving the overall functional value of the plant. The results also indicate that chitosan oligosaccharides affect in a dose-dependent matter in T. erecta ranging from 50 to 200 ppm. Optimizing application rates is crucial for achieving the most effective results. This study provides a valuable approach for managing ornamental crops in water-scarce environments. Integrating chitosan oligosaccharides into cultivation practices enables not only sustained plant growth but also enhanced *T. erecta* quality and bioactivity. This offers the potential for more resilient and value-added production systems, especially in areas where water scarcity is a persistent issue.

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