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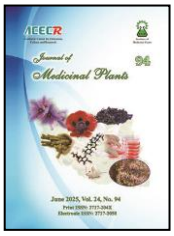

Research Article

Effect of nano chitosan particles on physiological and phytochemical parameters of tea plant (*Camellia sinensis* (L.) Kuntze) Kashef cultivar under drought stress

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ABSTRACT

Background: Tea cultivation faces growing challenges due to drought, a problem worsened by climate change. One potential method to enhance drought tolerance is the foliar application of chitosan and nano chitosan. **Objective:** This study investigates the effects of nano chitosan particles (NCP) on mitigating drought stress in tea plants (Kashef cultivar) while maintaining quality. **Method:** Four concentrations of NCP solution (0, 25, 50, and 100 mg L^{-1}) were applied twice during the dry season in summer 2021 at the Lahijan Tea Research Centre in Iran. Physiological, biochemical, and metabolic parameters were measured under severe drought conditions (25% soil field capacity) and compared to control plants (no drought stress or NCP treatment). **Results:** The data showed that NCP increased the total polyphenol and flavonoid content, except for catechin. Under drought conditions, NCP treatment significantly enhanced relative water content (RWC), total chlorophyll, shoot numbers, green leaf yield, proline, and protein levels. Antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) were activated to counter oxidative stress. Application of the highest NCP concentration (100 mg L^{-1}) significantly enhanced polyphenol accumulation, contributing to improved drought tolerance and tea quality. **Conclusion:** These findings suggest that NCP could be an eco-friendly and effective tool for improving drought resilience in tea plants.

1. Introduction

Climate change and global warming have intensified drought stress, affecting cell development and plant growth, ultimately reducing crop yields. In response, plants activate defense mechanisms, including the synthesis of osmolytes and antioxidants, to counteract the negative impacts of drought [1].

Abbreviations: NCP, Nano chitosan particles; CAT, Catalase; SOD, Superoxide dismutase; EC, Epicatechin; EGCG, Epigallocatechin gallate; EGC, Epigallocatechin; GCG, Gallocatechin gallate; TCA, Epicatechin gallate (ECG), Reactive oxygen species (ROS), Relative water content (RWC), Trichloroacetic acid; BSA, Bovin serum albumin; NBT, Nitro blue tetrazolium; HPLC, High Performance Liquid Chromatography; FESEM, Field emission scanning electron microscopy; XRD, X-ray diffraction; IPGRI, International Plant Genetic Resources Institute

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However, these mechanisms are often insufficient under severe water scarcity, necessitating the external application of certain compounds [2].

Nanotechnology offers promising solutions for agriculture, particularly in enhancing crop production and stress tolerance. Chitosan-based nanoparticles have shown various biological activities that improve plant growth and nutrient uptake [3, 4]. Nano chitosan, a biodegradable material, has demonstrated significant potential in enhancing plant metabolism and promoting stress resilience [5]. Previous research has shown that NCP increases phenolic compounds, flavonoids, and specific antioxidants in crops like *Vicia faba* and mung bean [5, 6].

The use of NCP has been shown to increase the levels of specific compounds, such as epigallocatechin gallate (EGCG), epicatechin (EC), gallic acid (GA), epicatechin gallate (ECG), and gallocatechin gallate (GCG) in *Camellia sinensis* (L.) Kuntze (tea plant) [7].

Tea (*Camellia sinensis*) is a popular beverage worldwide, valued for its health benefits. Its quality is determined by the presence of key metabolites such as catechins and flavonoids, which are influenced by environmental factors like drought. Drought stress reduces the photosynthesis rate, negatively affecting plant growth [8]. Recent studies have shown that catechins act as antioxidants and help mitigate oxidative stress in tea plants under drought conditions [9].

Catechins have recently been recognized as exogenous antioxidants that can reduce plant oxidative stress [10]. However, there are few comprehensive studies on the mechanisms by which catechins scavenge reactive oxygen species (ROS) in tea plants. Drought stress also alters the activity of CAT, SOD in *Camellia sinensis* [11, 12].

Although various strategies have been explored to alleviate drought stress in plants, using nano chitosan particles (NCP) in tea plants under severe drought conditions remains largely underexplored. This study offers a novel assessment of NCP at different concentrations on the drought-sensitive *Camellia sinensis* cultivar Kashef, investigating its physiological, biochemical, and metabolic responses under 25% soil field capacity. It uniquely demonstrates the protective role of NCP in enhancing both growth and quality parameters, providing a comprehensive insight that has not been previously reported.

Given the growing incidence of drought stress in regions like Iran, the application of NCP is a promising approach to improve plant resilience. The foliar use of NCP presents an innovative strategy to mitigate the adverse effects of water deficit, potentially enhancing tea plant performance and quality. This research specifically focuses on the physiological and biochemical implications of NCP, aiming to strengthen drought tolerance and improve overall tea productivity.

2. Materials and methods

2.1. Plant Samples and Treatments

The experiment was set up in a randomized complete block design with three replicates. The Kashef tea cultivar (*Camellia sinensis*, herbarium code: HSBU-2025112) was used at the Lahijan Tea Research Center, Iran. After the first harvest and at the onset of drought stress (25% soil field capacity), the plants were divided into four subplots. Nano chitosan particles were applied at concentrations of 0, 25, 50, and 100 mg L^{-1} through foliar spraying in early July 2021. The nano chitosan particles used were supplied by Nanoshel Company with

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a molecular weight of 161 g mol^{-1}, particle size of 80–100 nm, and purity of 99%.

For characterization, field emission scanning electron microscopy (FE-SEM) was performed using a TESCAN MIRA3 device. X-ray diffraction (XRD) analysis confirmed the crystalline structure of nano chitosan using a STOE model diffractometer. NCP was dissolved in 1% acetic acid. Following two foliar applications at a two-week interval, young branches (two leaves and one bud) were collected for metabolic analysis. In contrast, the third and fourth leaves were harvested for physiological and biochemical studies.

For metabolic analysis of the samples after harvesting, green leaves were dried in an oven at 110°C for one hour and then at 80°C for 10 hours [13]. The dried leaves were ground and stored in impermeable containers in a dark, dry place for polyphenol extraction.

2.2. Metabolic analysis

2.2.1. Total polyphenol

The total polyphenol content in tea was determined using the standard method outlined by ISO14502-1[14]. A 0.2 g sample was mixed with 5 mL of 70% methanol and incubated in a 70°C water bath for 25 minutes. The supernatant was collected, and the total polyphenol concentration was calculated using a standard gallic acid solution and a spectrophotometer at 765 nm.

The polyphenol concentration of the samples was calculated from the standard curve using the following equation:

$$P = \frac{A_{sample} * V_{sample} * d * 100}{S_{std} * m_{sample} * 1.000.000}$$

Where P= total polyphenol percentage, A_{sample}= the absorbance of the tested sample, V_{sample}= the volume of the tested sample, d= the dilution factor (which was considered 100 in this experiment), S_{std}= the slope of the standard calibration curve, m_{sample} = the mass of the tested sample (g).

2.2.2. Catechin Analysis by HPLC

Catechin content was analyzed using high-performance liquid chromatography (HPLC) following ISO14502-2 [15]. Samples were injected into a Phenomenex Luna 5 µm Phenyl-Hexyl column and detected at 278 nm. The concentrations of catechins, including epigallocatechin (EGC), gallocatechin (GC) and catechin, were determined and expressed in milligrams per gram of dry weight (mg g^{-1} DW).

Data collection and integration were performed using Millennium 32 software.

2.3. Physiological measurements

2.3.1. Relative water content (RWC)

Relative water content was measured by weighing leaf discs, first for fresh weight (FW), then after immersion in water for 24 hours for turgid weight (TW), and finally after drying at 70°C for 48 hours for dry weight (DW) [16]. The RWC was calculated using the equation:

RWC (%) = [(FW-DW)/(TW-DW)×100

2.3.2. Chlorophyll content

Chlorophyll content was determined using the Arnon (year) method [17]. Ground leaf samples were mixed with 80% cold acetone, incubated, and centrifuged. Absorbance was measured at 645 nm and 663 nm to quantify chlorophyll *a*, chlorophyll *b*, and total chlorophyll content using standard equations.

Chl a (mg/mL) = 12.7 * A_{663} – 2.69 *A_{645}

Chl b (mg/mL) = 22.9 * A_{645} – 4.68 *A_{663}

Total chloraphyll (mg/mL) = chloraphyll a + chloraphyll b

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2.3.3. Shoot number and green leaf yield

To determine the number of shoots per unit area, three random placements of a 25 x 25 cm box were made within each plot for each treatment. Shoots containing one bud and two to three leaves were counted, and the average was calculated per unit area following the methodology outlined in the International Plant Genetic Resources Institute (IPGRI), tea trait measurement guidelines.

To measure the yield of green leaves per unit area, the fresh weight of the harvested shoots was recorded using a calibrated precision scale. The calculation was based on the IPGRI guidelines [18] and expressed Kg/m².

2.3.5. Measurement of proline content

To measure the proline content, following the method of Bates et al. [19], 10 ml of 3% sulfosalicylic acid was added to the samples. After homogenization, acetic acid and ninhydrin reagent were added to the filtered extracts, and the mixture was placed in a 100°C water bath for 1 hour. After adding toluene, the proline concentration of the samples was measured using a spectrophotometer at a wavelength of 520 nm, and the proline concentration was reported in mg/g fresh weight using a standard curve.

2.4. Biochemical analysis

2.4.1. Total protein concentration

Total protein concentration was determined using a modified Bradford assay [20]. Initially, 0.1 mL of the enzymatic extract was mixed with 0.1 mL of 20% trichloroacetic acid (TCA). The residue was dissolved in 0.5 mL of 1 N sodium hydroxide solution. Bovine serum albumin (BSA) served as the standard protein solution. After preparing the samples, their absorbance was measured using a spectrophotometer at 595 nm.

2.4.2. Enzyme assay

2.4.2.1. Antioxidant enzymes activity

Catalase (CAT) activity was assessed following the method described by Aebi [21], which involves measuring the decomposition of hydrogen peroxide (H2O2) by catalase, and the absorbance was read at 240 nm. Enzyme activity was calculated using the following equation:

$$U/mg = \frac{(A0 - A180) * Vt}{Z240 * d * Vs * Ct * 0.001}$$

Where A0-A180= the difference between the initial and final absorbance, Vt= the final reaction volume (3 mL), Z240= the molar extinction coefficient for H_2O_2 in OD_{240} (34.9 mol cm^{-1}), d= the cuvette optical width (1cm), Vs= the sample volume (mL) and Ct= the sample protein concentration (mg mL^{-1})

SOD activity was determined using a modified method based on previous works [22] involving the reduction of nitro blue tetrazolium (NBT). The reaction mixture was prepared, and the samples were incubated under white light for 15 minutes. Subsequently, the absorbance of the samples was measured at 560 nm. The enzyme efficiency in NBT reduction was calculated using the following equation:

$$Inhibition\ of\ NBT\ reduction\ by\ SOD\% = \frac{(A_{560}\ Control - A_{560}\ Test) \times 100}{A_{560}\ Control}$$

2.6. Statistical analysis

Each experiment was conducted using a randomized complete block design (RCBD) with a minimum of three replicates. Statistical analysis was performed using SAS software version 9.4, and graphs were created using Excel 2016. Analysis of variance regression model (GLM) was utilized to interpret the data and identify significant differences. Tukey's test was applied to compare the means at a 5% probability level.

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3. Results

3.1. Nano Chitosan Characterization

Figure 1a presents the X-ray diffraction (XRD) analysis of the chitosan nanoparticles (NCP), confirming their crystalline structure. The characteristic peak of chitosan was observed at 2θ = 10.12°, with an additional broad peak at 2θ = 20.14°, indicating higher crystallinity (Fig. 1b).

Furthermore, two peaks were observed at 2θ = 44° and 2θ = 64.2°, which are likely related to chitosan nanoparticles. The scanning electron microscope (FE-SEM) image analysis showed that the NCP particles were within the nanometer range, with diameters between 30 and 80 nm (Fig. 1c).

3.2. Effect of nano chitosan on total polyphenol content and flavonoid compounds

The results indicated highly significant differences among the various treatments in terms of total polyphenol content, catechin, and epigallocatechin ($P < 0.05$), whereas gallocatechin did not show a significant difference (Table 1).

Applying nano chitosan (NCP) significantly influenced the polyphenol and catechin content in tea leaves under drought stress. Total polyphenol content increased in response to NCP treatments, with notable enhancements at 50 mg L^{-1} (25.45%) and 100 mg L^{-1} (26.54%) compared to control plants (18.10%).

Catechin content decreased under drought stress, declining from 4.708 mg g^{-1} dry weight (DW) in control plants to 0.480 mg g^{-1} DW with 100 mg L^{-1} NCP treatment. In contrast, gallocatechin levels increased with NCP application, reaching a maximum of 1.648 mg g^{-1} DW at 100 mg L^{-1}. Similarly, epigallocatechin (EGC) content rose in response to NCP, with the highest value of 45.036 mg g^{-1} DW observed at 100 mg L^{-1}, compared to 39.543 mg g^{-1} DW in control plants (Table 2).

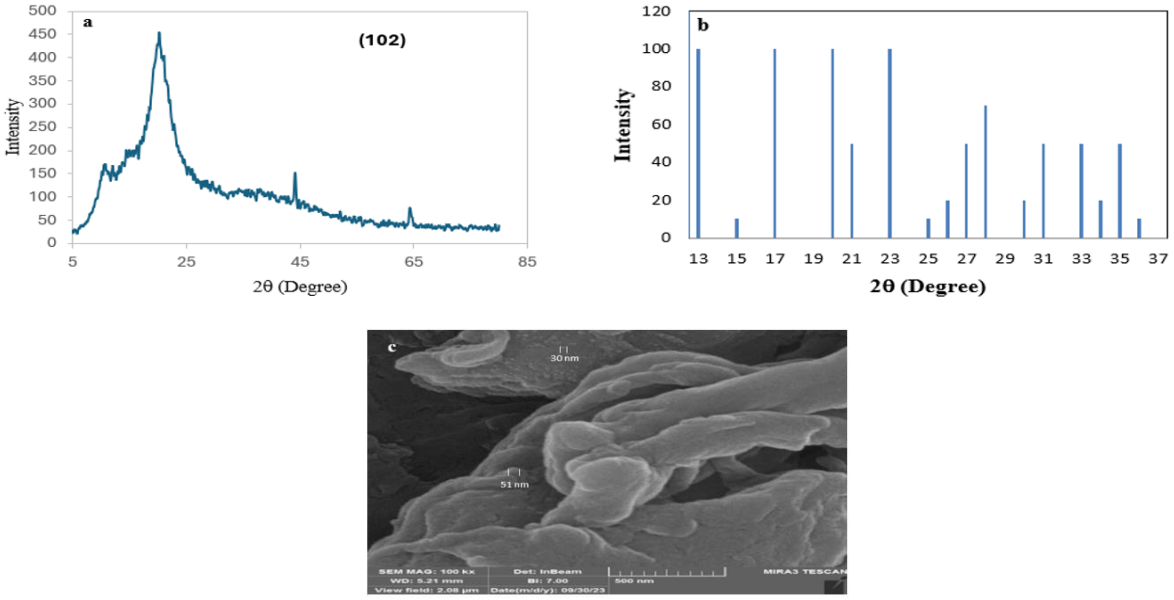

Fig.1. Characterization of NCP. X-ray diffraction spectrum for chitosan structure and NCP (a), X-ray powder diffraction patterns for JCPDS file No. 39-1894 (b) FESEM image of NCP (c) The dimensions of the chitosan nanoparticles are about 30 to 80 nm.

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Table 1. Mean squares and significance levels from ANOVA for the effects of different NCP treatments under drought conditions on various physiological and biochemical parameters. C: control treatment without drought stress and without NCP amendment.

Source	d.f	Mean of squares							
		RWC (%)	Chlorophyl (mg/gFW)	Number of shoots (m2)	Green leaf yield (kg/m^2)	Protein (µg/gFW)	Proline (mg/gFW)	Catalase (U/mgFW)	SOD (U/mgFW)
Replication	2	0.8547^{ns}	0.033^{ns}	66.200^{ns}	0.0148^{ns}	0.000001^{ns}	0.1618^{ns}	66.20^{ns}	0.8364^{ns}
Treatment	4	85.4142*	6.369*	5335.33*	1.200*	0.000163*	151.433*	5335.33*	828.71*
Error	8	0.7662	0.0525	60.0333	0.0135	0.0000	0.1733	193.406	0.2796
C.V		0.982	1.999	8.705	8.705	7.469	3.757	2.583	1.081

Source	d.f	Mean of squares			
		Total Polyphenol (gDW/%)	Catechin (mg/gDW)	Gallocatechin (mg/gDW)	Epigallocatechin (mg/gDW)
Replication	2	2.75*	0.046^{ns}	0.152^{ns}	0.2169^{ns}
Treatment	4	32.40*	51.43*	0.228^{ns}	611.414*
Error	8	0.26	0.959	0.437	2.195
C.V		0.8796	10.569	14.99	1.686

^{ns} and *: non-significant and significant differences at 5% probability level.

Table 2. Effects of different concentration of nano chitosan on total polyphenols and some catechin compounds content in leaves of *C. sinensis* Kashef cultivar under drought stress. C: control treatment without drought stress and without NCP amendment. Different letters in the same column refer to significant differences between treatments according to Tukey's test at $P < 0.05$. Each value is the mean of three replicates (mean ± SD, n = 3).

NCP mg L^{-1}	Total polyphenol (%)	Catechin (mg/gDW)	Gallocatechin (mg/gDW)	Epigalocatechin (mg/gDW)
C	18.1 ± 0.60^{c}	4.708 ± 0.40^{a}	1.266 ± 0.15^{c}	39.543 ±0.70^{b}
0	21.095 ± 0.87^{b}	0.53 ± 0.60^{b}	1.267 ± 0.19^{c}	13.360 ± 0.34^{c}
25	17.024 ± 0.81^{d}	0.42 ± 0.00^{c}	1.440 ± 0.46^{b}	39.240 ± 0.46^{b}
50	25.45 ± 0.85^{a}	0.45 ± 0.08^{c}	1.480 ± 0.26^{b}	18.110 ± 0.68^{c}
100	26.54 ± 0.85^{a}	0.48 ± 0.00^{c}	1.648 ± 0.17^{a}	45.036 ± 0.20^{a}
C.V. (%)	0.8796	10.569	14.990	1.686

3.3. Effect of nano chitosan on RWC and chlorophyll content

Statistical analysis revealed significant differences among treatments in both relative water content (RWC) and chlorophyll concentration ($P < 0.05$) (Table 1).

RWC decreased in untreated drought-stressed plants, dropping from 96.89% in control plants to 83.25% without NCP. However, NCP treatment improved RWC, with the highest RWC recorded at 100 mg L^{-1} (89.87%) (Fig. 2a).

Chlorophyll content also decreased under drought conditions, with untreated plants dropping from 262 mg gFW^{-1} in control plants to 180 mg gFW^{-1}. NCP treatments helped recover chlorophyll content, with the highest recovery seen at 100 mg L^{-1} NCP (246 mg gFW^{-1}) (Fig. 2b).

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3.4. Effect on shoot numbers and green leaf yield

The results showed significant differences among the treatments in terms of shoot numbers and green leaf yield ($P < 0.05$) (Table 1). Drought stress significantly reduced the number of shoots per unit area in untreated plants to 43; however, NCP treatments mitigated this effect, resulting in a marked increase in shoot production. The highest number of shoots (101) was observed in plants treated with 100 mg L^{-1}NCP (Fig. 3a).

Similarly, green leaf yield was reduced in untreated drought-stressed plants (0.185 kg/m²) but increased with NCP application. Plants treated with 100 mg L^{-1}NCP showed a green leaf yield of 0.428 kg/m², close to that of fully irrigated control plants (0.665 kg/m²) (Fig. 3b).

3.5. Protein and proline content

The results showed significant variation among the treatments in terms of protein and proline content ($P < 0.05$) (Table 1).

Drought stress reduced total protein content in untreated plants, but NCP treatments restored protein levels. Application of 100 mg L^{-1} NCP resulted in the highest protein content, which was significantly greater than that observed in the control plants (Fig. 4a).

Proline content, a vital osmolyte involved in the drought stress response, increased significantly following NCP treatment. In plants treated with 100 mg L^{-1} NCP, proline levels were approximately six times higher than untreated control plants (Fig. 4b).

3.6. Antioxidant Enzyme Activity

The results demonstrated significant differences in antioxidant enzyme activity among the treatments ($P < 0.05$) (Table 1).

The highest catalase activity was observed at 100 mg L^{-1} NCP (Fig. 5a). At the same time, SOD activity also increased substantially with higher NCP concentrations (Fig. 5b). This increase in antioxidant activity suggests that NCP helps mitigate oxidative stress caused by drought.

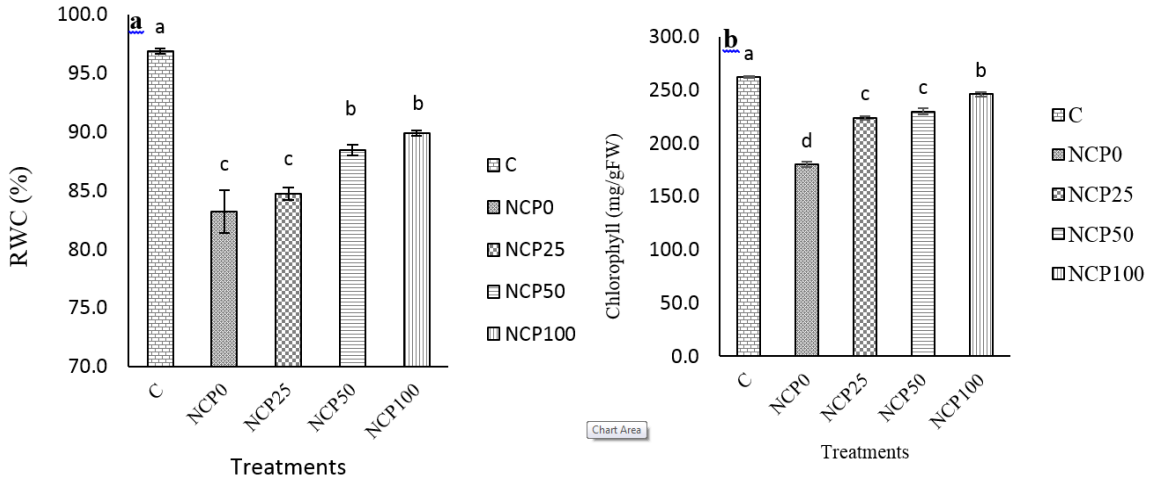

Fig. 2. Effects of different concentration of nano chitosan on RWC content and chlorophyll content (a+b) in leaves of *C. sinensis* Kashef cultivar under drought stress. Control: without nano chitosan and without drought stress, NCP0: 0 mg L^{-1}, NCP25: 25 mg L^{-1} nano chitosan particle, NCP50: 50 mg L^{-1} nano chitosan particle and NCP100: 100 mg L^{-1} nano chitosan particle. Values represent three replicates' means ± SD. Letter indicates significant differences from nano chitosan treatments at $P < 0.05$ according to Tukey test.

[DOI: 10.61882/jmp.24.94.31]

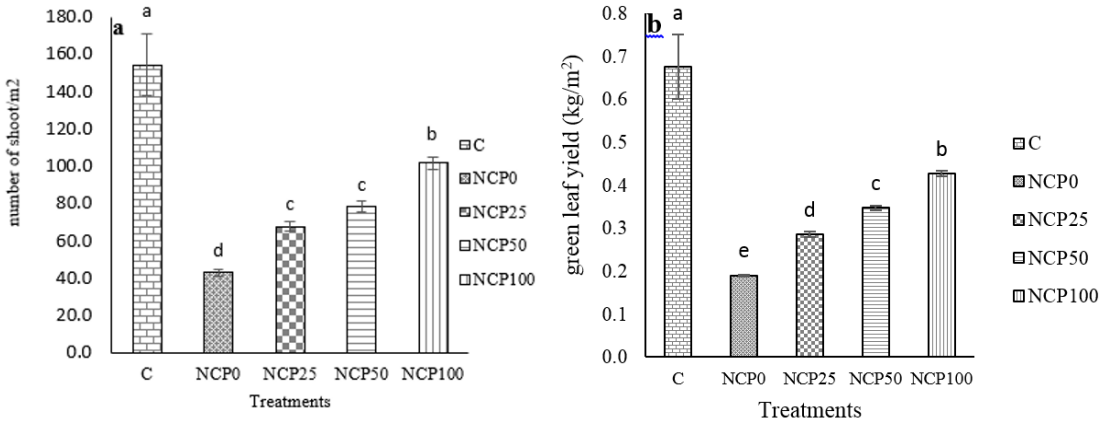

Fig. 3. Effects of different concentration of nano chitosan on shoots number (a) and green leaf yield (b) in *C. sinensis* Kashef cultivar under drought stress. Control: without nano chitosan and without drought stress, NCP0: 0 mg L^{-1}, NCP25: 25 mg L^{-1} nano chitosan particle, NCP50: 50 mg L^{-1} nano chitosan particle and NCP100: 100 mg L^{-1} nano chitosan particle. Values represent three l replicates' means ± SD. Letter indicates significant differences from nano chitosan treatments at $P < 0.05$ according to Tukey test.

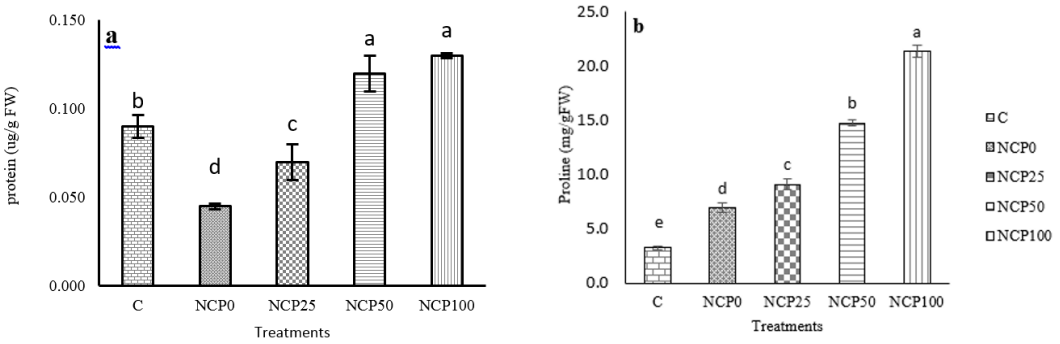

Fig. 4. Effects of different concentration of nano chitosan on total protein and proline content in leaves of *C. sinensis* Kashef cultivar under drought stress. (a) total protein (b) proline. Control: without nano chitosan and without drought stress, NCP0: 0 mg L^{-1}, NCP25: 25 mg L^{-1} nano chitosan particle, NCP50: 50 mg L^{-1} nano chitosan particle and NCP100: 100 mg L^{-1} nano chitosan particle. Values represent three replicates' means ± SD. Letter indicates significant differences from nano chitosan treatments at $P < 0.05$ according to Tukey test.

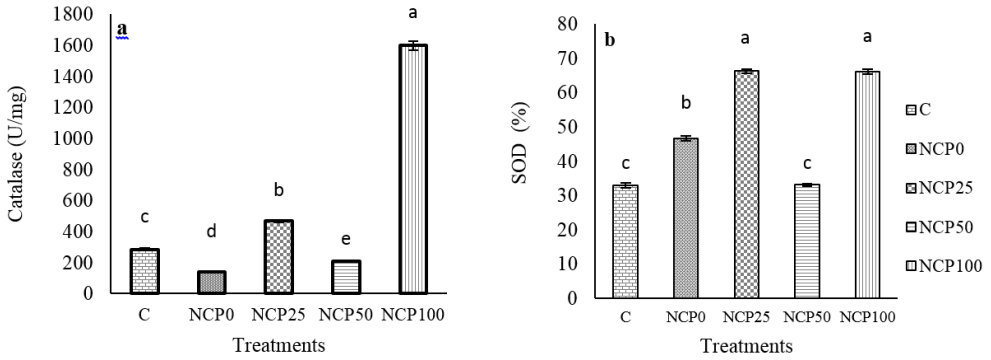

Fig. 5. Effects of different concentration of nano chitosan on antioxidant enzymes in leaves of *C. sinensis* Kashef cultivar under drought stress. (a) Catalase and (b) SOD. Control: without nano chitosan and without drought stess, NCP0: 0 mg L^{-1}, NCP25: 25 mg L^{-1} nano chitosan particle, NCP50: 50 mg L^{-1} nano chitosan particle and NCP100: 100 mg L^{-1} nano chitosan particle. Values represent three replicates' means ± SD. Letter indicates significant differences from nano chitosan treatments at $P < 0.05$ according to Tukey test.

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4. Discussion

Plant growth is significantly constrained by both biotic and abiotic stresses, with drought stress recognized as one of the most severe abiotic challenges. It disrupts cellular processes, notably inhibiting cell proliferation, which leads to substantial reductions in plant growth and yield, effects that are particularly pronounced in tea (*Camellia sinensis*) cultivation. The use of nanomaterials, such as nano chitosan particles (NCP), has emerged as a promising approach to mitigate the detrimental impacts of drought, contributing to more resilient and sustainable tea production. Comprehensive analysis of plant responses at physiological, biochemical, and molecular levels provides critical insights into the extent of stress-induced damage and the potential of applied treatments to enhance plant tolerance [23].

The increase in total polyphenol content with NCP treatments, particularly at 50 mg L^{-1} and 100 mg L^{-1} concentrations, suggests that nano chitosan can enhance the antioxidant capacity of tea plants under drought stress. Polyphenols, known for their role in mitigating oxidative stress, are essential in drought conditions due to the increased production of reactive oxygen species (ROS). The observed rise in polyphenols with NCP treatment aligns with previous studies demonstrating that polyphenols help protect plants from oxidative damage [24]. Moreover, the fluctuations in catechin levels, particularly the decrease in catechin content with drought stress, followed by a significant increase in epigallocatechin (EGC) and gallocatechin levels under NCP treatment, highlight the complex relationship between drought stress and secondary metabolite biosynthesis. EGC and gallocatechin are essential antioxidants in tea plants, and their increase under NCP treatment suggests that nano chitosan may promote the synthesis of specific flavonoids critical for drought tolerance.

The observed improvement in RWC with NCP treatment indicates that nano chitosan enhances the water retention capability of tea plants under drought stress. Maintaining a higher RWC is crucial for plants to sustain cellular functions during water scarcity. This effect can be attributed to NCP's role in stabilizing cell membranes and enhancing water uptake efficiency. The restoration of RWC at 100 mg L^{-1} NCP suggests that this concentration is particularly effective in mitigating drought-induced water loss. Several studies have demonstrated that a reduction in the relative water content (RWC) of tea leaves is closely associated with decreased soil moisture levels [7, 8, 25-28].

Similarly, the recovery of chlorophyll content with NCP treatment underscores the importance of maintaining photosynthetic capacity under drought conditions. Drought typically causes a decline in chlorophyll due to oxidative stress and the degradation of photosynthetic pigments [25]. However, the application of NCP protects chloroplasts, thereby preserving chlorophyll levels and promoting sustained photosynthetic activity. This is crucial for maintaining plant growth and productivity under stress conditions.

The significant increase in shoot numbers and green leaf yield observed with NCP treatment further demonstrates the growth-promoting effects of nano chitosan under drought conditions. Drought stress typically reduces both parameters due to limited water availability, which inhibits cell division and expansion. The fact that NCP-treated plants, particularly those treated with 100 mg L^{-1}, showed higher shoot numbers and leaf yield

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suggests that NCP enhances drought tolerance by improving water retention, photosynthesis, and overall plant vigor. The positive impact of NCP on green leaf yield is particularly important for tea plants, where yield is directly tied to economic value. By mitigating the adverse effects of drought stress on yield, NCP offers a potential solution for maintaining tea production in drought-prone regions. In many studies, the impact of drought stress on reducing the number of harvestable shoots and increasing the number of stagnant shoots in tea plants has been reported [29-31]. The yield reduction of tea under drought stress has been reported by various researchers [32, 33].

Drought stress reduces total protein content in plants, as seen in untreated tea plants in this study. However, the significant recovery of protein levels with NCP treatment, particularly at 100 mg L^{-1}, suggests that nano chitosan helps to maintain or enhance protein synthesis under stress conditions. Proteins are vital for various metabolic processes, and their preservation under drought is essential for plant survival. NCP has been shown to increase total protein content under stress conditions. The effect of nano chitosan on protein content depends on the nitrogen content in chitosan, which plays an essential role in protein synthesis [34]. Moreover, chitosan could reduce the degradation of soluble proteins by increasing the expression of proteinase and protease inhibitor genes [35]. The role of nano chitosan in increasing protein content in *Salvia abrotanoides* [36] and *Triticum aestivum* [37] has been proven under stress conditions, consistent with our findings.

Another important observation is the increase in proline content following NCP treatment, indicating its potential role in the plant's response to drought stress. Proline is an osmoprotectant, stabilizing cellular structures and maintaining osmotic balance during drought. The six-fold increase in proline content with 100 mg L^{-1} NCP demonstrates the efficacy of nano chitosan in promoting osmotic adjustment, which is crucial for enhancing drought tolerance in tea plants. This aligns with previous studies that highlight proline accumulation as a key mechanism for drought resilience [6, 38, 39].

The enhancement of catalase (CAT) and superoxide dismutase (SOD) activity with NCP treatment indicates that nano chitosan boosts the plant's antioxidant defense system. Drought stress induces the production of ROS, which can cause significant cellular damage if not effectively neutralized. By increasing the activity of CAT and SOD, NCP helps tea plants to scavenge ROS more efficiently, reducing oxidative damage and enhancing stress tolerance. The highest antioxidant enzyme activities were observed at 100 mg L^{-1} NCP, suggesting that this concentration is optimal for inducing antioxidant defenses. These findings align with previous research demonstrating nano chitosan's ability to activate reactive oxygen species (ROS)-scavenging enzymes in various plant species, further supporting its effectiveness in mitigating abiotic stress [25, 40].

Given that tea is a C3 plant, increased catalase activity might eliminate the hydrogen peroxide produced by airway cells and lower the rate of photorespiration [41]. Studies by Khesali Langaroudi et al., Liu et al., Panda et al., and Upadhyaya et al. have shown that compared to other tea genotypes, drought-resistant tea genotypes have increased catalase activity [42-44]. In response to biotic and abiotic challenges, plants develop a set of defensive enzymes called peroxidases, which reduce H2O2 in cells and eliminate the production of ROS [45]. Chitosan

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has been shown to accumulate hydrogen peroxide under stress conditions [46] and increase the expression of several defence enzymes in the ROS scavenging system [47]. The activity of catalase and peroxidases has been attributed to the nitric oxide pathway due to nano chitosan [48], indicating that plants treated with NCP have a higher efficiency in removing H2O2 and preventing membrane lipid peroxidation. The results suggest that NCP could induce the pathway of antioxidant enzymes, which could remove ROS, maintain membrane integrity, and ultimately make the plant resistant to drought [25, 40, 49].

5. Conclusion

This study demonstrates the potential of nano chitosan particles (NCP) to mitigate drought stress in tea plants by enhancing physiological and biochemical parameters. NCP treatment significantly improved relative water content, chlorophyll levels, shoot numbers, and green leaf yield under drought conditions. It also increased the total polyphenol content and stimulated the activity of key antioxidant enzymes, such as catalase and superoxide dismutase, which help combat oxidative stress. Furthermore, NCP application led to a substantial rise in protein and proline levels, suggesting that it can improve drought tolerance by stabilizing cell structures and promoting osmotic adjustment.

The findings indicate that NCP can be a viable, eco-friendly solution for inducing drought tolerance in tea plants, improving plant productivity, and maintaining tea quality under stress. Future research should explore long-term field applications of NCP and its effects on other crops, offering a sustainable approach to addressing the challenges of climate change.

Author contributions

All phases of the project were developed by A. Z. and K. F. carried out the experiments in the field, while A. B. served as an advisor and R. A. G. developed the plan and experiments. The experiments were conducted in the lab and the field by M. P. provided assistance with laboratory tests and data analysis. The final manuscript was read and approved by all the authors.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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