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Exposure to TiO_2 nanoparticles improves the physiological characteristics of drought-challenged chickpeas (*Cicer arietinum* L.)

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Abstract

Drought stress markedly affects plant growth and crop production. In turn, treatment with some metal-based nanoparticles (NPs) such as TiO_2-NPs could improve the plant tolerance against drought stress. In the present study, the effects of different levels of moisture regime (40%, 60%, and 90% field capacity [FC]) in conjunction with various concentrations of TiO_2-NPs (0, 5, 10, 20, and 40 mg. L^{-1}) on chickpea were studied. Exposure of drought-challenged chickpea plants to TiO_2-NPs raised antioxidant enzyme activity compared with plants grown under drought without TiO_2-NP treatment. The highest activity of ascorbate peroxidase (APX) was observed at 40% FC and application of 40 mg. L^{-1} TiO_2-NPs. Moreover, peroxidase (POX) activity has increased with the enhancing concentration of TiO_2-NPs to 20 mg. L^{-1} at 90% FC. In comparison, the application of 40 mg. L^{-1} TiO_2-NPs and decreasing levels of FC caused a rise in the activity of superoxide dismutase (SOD). Exposure to TiO_2-NPs raised the amount of total phenols and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) at different levels of moisture regime. The content of malondialdehyde (MDA) at 60% FC has decreased by 22% after treatment with 20 mg. L^{-1} TiO_2-NPs compared with control plants. Also, treatment with TiO_2-NPs heightened the proline content, and the highest amount of proline was obtained at 40% FC by applying 20 mg. L^{-1} NPs. The treatment with TiO_2-NPs in the moisture regimes led to higher chlorophyll and carotenoid production in chickpea plants. Taken together, the application of TiO_2-NPs could raise the defense potential of chickpea plants against oxidative stress caused by the generation of reactive oxygen species.

KEYWORDS

antioxidant system, biochemical component, drought stress, reactive oxygen, TiO_2-NPs

1 | INTRODUCTION

Drought stress and water scarcity are the most critical limiting factors for crop production. Different plants respond to drought stress through morphological, biochemical, and metabolic processes (Ganjeali & Kafi, 2007). Water shortage causes a deficiency of moisture that is needed for growth and completion of the life cycle in plants. Plants go through drought stress when the absorption of water through their roots is hardened and the transpiration rate increases. These conditions often occur in dry and semi-dry climates (Jaleel et al., 2008).

Legumes are an important source of proteins and nutrients, essential to the health and livelihood of people, especially in developing countries (Yahara et al., 2013). Chickpea (*Cicer arietinum* L.) from the legume family is one of the oldest popular crops worldwide. Actually, production and farming among legumes come after improvement of cultivation of beans and lentils (Shobeyri et al., 2006). A significant problem for chickpea production in arid and semi-arid areas is water scarcity. Investigations have shown that among the biotic and abiotic stresses, drought stress alone leads to a 50% reduction in chickpea yield (Anbessa & Bejig, 2002). Recently, nanotechnology has been used in modern agriculture to overcome some drought-induced concerns (Navarro et al., 2008). Nanomaterials can be used not only to enhance the nutrient levels of the soil but also to increase the yield of crops (Gao et al., 2008; Hong et al., 2005). It can also prevent plant diseases by modulating the biological and nonenzymatic processes (Nair et al., 2010). Nanofertilizers are reported to be helpful in improving plant nutrition in soil and absorbing water and moisture from the soil (Iqbal et al., 2019).

Nanoparticles (NPs) are solid structures with crystal sizes in the range of nanometer-scale (Sing & Nolwa, 2011). Metal oxide NPs are considered essential tools in addressing current and future obstacles related to crop productivity (Alabdallah et al., 2021). NPs such as AgNPs and TiO2-NPs offer a sustainable and affordable solution for promoting plant growth in drought conditions. These eco-friendly NPs have the potential to alleviate the negative effects of drought on plants (Alabdallah et al., 2021, 2021). TiO_2-NPs as nontoxic nanomaterials exist in three crystalline forms including anatase, rutile, and brookite (Morse & Glorer, 2000). In recent years, there has been a growing interest in utilizing TiO_2-NPs in agriculture as they have the potential to enhance plant growth and productivity. It was reported that treatment by TiO_2-NPs (rutile) increases the photochemical activity in the chloroplast of *Spinacia olevacea*, which stimulates the Hill reaction with an accelerating reduction in Fe_2O_3 and oxygen (Zheng et al., 2005). Therefore, cyclic phosphorylation may occur more effectively after treatment with TiO_2-NPs. In another study, TiO_2-NPs raised the growth rate in spinach by proliferating the concentration of chlorophyll molecules and the consequent increase in photosynthesis rate (Lei et al., 2007). It is noteworthy that anatase TiO_2-NPs optimize the growth and yield of spinach. They enter the plant more quickly than the bulk TiO_2 improving photosynthesis (Owolade & Ogunleti, 2008). They increased the absorption of solar energy by plant cells and enhanced electron transfer chemical reactions in the spinach plant (Lei et al., 2007).

In an experiment conducted on lentils at the research farm in Zanjan, Iran, the application of titanium dioxide NPs affected the growth of lentil plants. The use of NP fertilizers resulted in increased leaf area and improved photosynthesis indices, ultimately leading to an increase in grain yield (Soltani et al., 2014). Similarly, a study reported significant differences in height, weight spike, grain performance, and harvest index in wheat germs when titanium NPs were sprayed with foliar at a concentration of 0.01. These effects were observed under both normal and drought conditions (Mansouri et al., 2017). Furthermore, the results indicated that under drought stress conditions, foliar application of titanium dioxide NPs increased grain yield by 2% to 23% compared with no foliar application (Soltani et al., 2014). Additionally, when titanium dioxide NPs were applied to barley plants in field cultivation, the treatment with 3% titanium dioxide resulted in a 32.21% higher grain yield compared with the control group (Moaveni et al., 2011).

The present study investigates the effect of anatase TiO_2-NPs on the mitigation of the impact of drought stress on growth features and some physiological and biochemical characteristics of chickpea plants. The obtained results may support the influences TiO_2-NPs on physiological and biochemical attributes in chickpea plants in suppression of water stress.

2 | MATERIALS AND METHODS

2.1 | Plant materials and treatments

The experiments were conducted in the Research Center for Plant Sciences, Ferdowsi University of Mashhad. Seeds of chickpea cv. Arman were sterilized and transferred to 3 L pots containing sand and soil in a 1:1 ratio (Table 1). The soil used was sterilized in an autoclave (1.2 bar and 121°C) for 40 min. Pots were transferred to the greenhouse with a temperature of 20 ± 5°C. The factorial experiment was conducted based on the completely randomized design with three replications. Two-week-old plants were exposed to three levels of drought stress, that is, 40%, 60%, and 90% field capacity (FC).

The TiO_2-NPs were obtained in 99% purity from the US Research Nanomaterial Inc. The stock suspension was papered by dissolving 10 mg of TiO_2-NPs in 1 L deionized water and then dispersing in an ultrasonic bath (200 W, 50 KHz) for 40 min. After 10 days of drought stress treatments, seedlings were treated with different concentrations of TiO_2-NPs (0, 5, 10, 20, and 40 mg. L^{-1}). The TiO_2-NPs treatments were continued every 2 weeks until pod formation (Rasouli et al., 2016).

2.2 | Characterization of NPs

The crystal structure and purity of NPs were examined by X-ray diffraction (XRD) (Explore, V Company, GNR, Italy; voltage = 40kv emission current = 30 mA, Anode: Cu and $k = 1.54$). The average size of NPs was determined using the Debye–Sherrer formula (Patterson, 1939). High-resolution images of TiO_2-NPs were obtained by a transmission electron microscope (TEM) (LEO 912 AB, Germany). The average hydrodynamic diameter in an aqueous solution was measured by DLS (dynamic light scatting) at 25°C and pH 6.5 (LEO, Germany) (Figure 1).

2.3 | The enzymatic antioxidant assays

When pods were formed in more than half of the plants (56 days after drought stress), the youngest fully expanded leaves were collected

TABLE 1 Features of the applied soil.

Organic carbon (OC) (%)	Electrical conductivity (EC) (dSm^{-1})	$CaCO_3$ (%)	Phosphorus (P) ($mg.kg^{-1}$)	Potassium (K) ($mg.kg^{-1}$)	Nitrogen (N) (%)	Soil texture
3.5	2.12	13	7	151	0.05	Loam sand

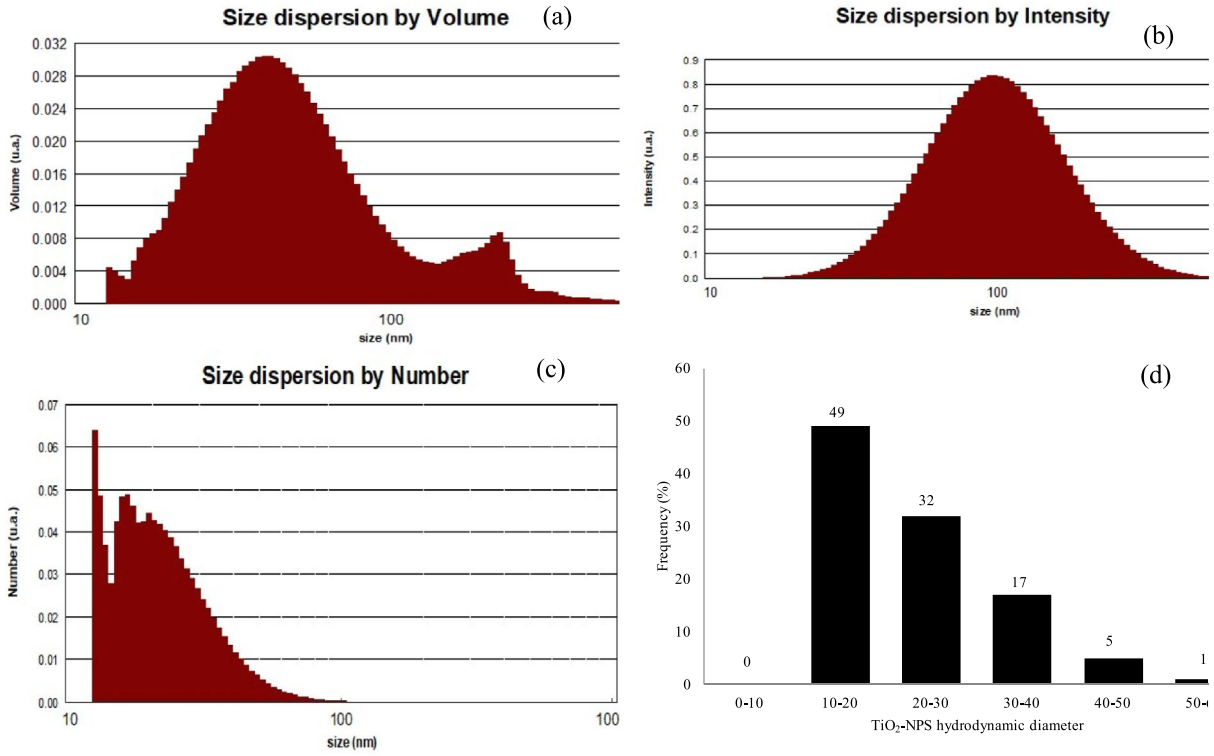

FIGURE 1 Hydrodynamic particle diameter distribution of TiO_2-nanoparticles (NPs) by volume (a). Hydrodynamic particle diameter distribution of TiO_2-NPs by intensity (b). Hydrodynamic particle size distribution by number of TiO_2-NPs (c). Frequency of hydrodynamic particle diameter distribution of TiO_2-NPs (d).

and weighed to determine fresh weight. They were frozen in liquid nitrogen and subsequently used for biochemical assays.

The activity of ascorbate peroxidase (APX) (EC 1.11.1.11) was measured following the previously reported method (Nakano & Asada, 1981). The reaction solution included 2 mM ascorbate, 0.1 mM EDTA, and 0.1 mM H_2O_2 in potassium phosphate buffer (50 mM; pH 7.0). The suspension was centrifuged (13,000 rpm, 20 min, 4°C), and the supernatant was used for enzyme activity analysis. Total APX activity was measured by recording the decline in absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

The activity of peroxidases (POX) (EC 1.11.1.7) was determined by the formerly reported method (Malik & Singh, 1980). The 1 mL reaction mixture contained phosphate buffer (20 mM, pH 6.0), 0.5 mM 2-methoxy phenol (guaiacol), and 1 mM hydrogen peroxide with an appropriate aliquot of enzyme extract. The absorbance was recorded during 2 min at 470 nm ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$).

For assessment of superoxide dismutase (SOD) (EC 1.15.1.11) activity, 1 mL reaction mixture was prepared containing 50 mM Hepes (pH 7.6), 0.1 mM EDTA, 50 mM Na_2CO_3, 13 mM methionine, 0.025% (w/v) Triton X-100, 75 μM nitroblue tetrazolium, and 2 μM riboflavin and an appropriate aliquot of enzyme extract. Microtubes were placed at a distance of 30 cm from a light source (120 V) for 15 min. The absorbance of the sample was then recorded at 569 nm. The amount of enzyme that caused 50% interdiction was considered as a unit. The enzyme activity was calculated as the enzyme unit per g of fresh weight for all the samples (Giannopolitis & Ries, 1977).

2.4 | Determination of the proline content

Proline concentration was measured by a previously reported method (Bates et al., 1973). One hundred milligrams of fresh leaves was homogenized in 3% sulfosalicylic acid. The extract was centrifuged at 4000 rpm for 10 min. An aqueous supernatant was mixed with ninhydrin acid reagent (ninhydrin, phosphoric acid 6 M, and glacial acetic acid) and glacial acetic acid. Then, the test tubes were kept in boiling water bath (100°C) for 30 min. After cooling, absorption was recorded at 520 nm by a spectrophotometer. Proline content in fresh tissues was calculated using a standard proline curve in a concentration range of 0 to 25 mg. L^{-1}.

2.5 | Measurement of phenol content

Total phenol content was measured by using Folin–Ciocalteu reagent (Singleton & Rossi, 1965). About 100 mg of fresh leaves was homogenized in liquid nitrogen and then 1 mL of ethanol as solvent was added to the sample. About 50 µL of extract was mixed with 1 mL of deionized water, 0.05 mL of Folin–Ciocalteu reagent, and 0.30 mL of sodium carbonate (20%). After 30 min, the absorbance was read at 765 nm. Gallic acid was applied as a standard.

2.6 | Measurement of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) activity

One hundred milligrams of fresh leaves was homogenized in liquid nitrogen and then 1 mL of ethanol was added to the homogenate. The supernatant (50 µL) of extract was mixed with 0.95 mL of DPPH and was kept at room temperature in darkness for 30 min. The decrease in absorbance of DPPH solution was evaluated at 517 nm. Ascorbic acid was applied as a standard (Lichtenthaler, 1987).

2.7 | Assessing the lipid peroxidation

Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) content in plant material according to the method reported by Heath and Packer (1968). The samples were homogenized with 0.1% (W/V) trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 5 min. Subsequently, 0.5 mL of supernatants was mixed with 2 mL of 20% TCA containing 0.5% of 2-thiobarbituric acid, and the resulted mixture was heated for 30 min in hot water at 95°C. Aliquots were immediately transferred to an ice bath and then centrifuged at 10,000 rpm for 15 min. Finally, the absorbance of supernatants was recorded at 532 and 600 nm. MDA concentration was calculated based on the difference of two absorbance values.

2.8 | Determination of the pigment content

An amount of 100 mg fresh leaf was crushed in 0.05 mL of 96% ethanol in a mortar. After five min vortexing, the sample was retained in the dark and cold for 24 h. The solution was centrifuged for 5 min at 4000 rpm. The absorption of supernatant was measured at the wavelengths of 648, 664, and 470 nm, respectively. The concentration of chlorophyll a, chlorophyll b, and carotenoids was calculated using the following equations (Wellburn, 1994):

$$C_a = 13.36 \times A_{664} - 5.19\, A_{648}$$
$$C_b = 27.43 \times A_{648} - 8.12 \times A_{664}$$
$$C_{x+c} = (1000 \times A_{470} - 2.13 \times C_a - 97.64 \times C_b)/209$$

where C_a, C_b, and C_{x+C} indicate the content of chlorophyll *a*, *b*, and carotenoids in mg/g FW.

2.9 | Assessing the soluble protein content

The concentration of proteins was measured calorimetrically using Bradford method (Bradford, 1976). One hundred milligrams of fresh leaves was homogenized in 1 mL phosphate buffer (pH 6.8) and centrifuged at 13,000 rpm for 15 min. Then, 0.45 mL of Bradford reagent was added to 50 µL of samples. The absorption of solutions was read at 595 nm, and the concentration of protein was determined using a standard curve prepared using bovine serum albumin (BSA).

2.10 | Measurement of the soluble carbohydrates

Soluble carbohydrates were determined based on the modified phenol sulfuric assay. Accordingly, 100 mg of fresh leaves was homogenized in liquid nitrogen. Afterward, 500 µL of 70% ethanol was added to the sample. The reaction mixture was centrifuged at 3500 rpm for 10 min. Finally, supernatant, chloroform, and deionized water (in a ratio of 2:2:1) were mixed. After a 10 s shaking, phenol (4:1 W/V) and sulfuric acid were added to the appropriate aliquot of the light phase. After a 30 min heating in boiling water, the absorbance of samples was recorded at 480 nm. Soluble sugar concentration was calculated using a standard curve prepared by glucose.

2.11 | Statistical analysis

The experiment was conducted as a factorial arrangement in a completely randomized design with three replications. Analysis of variance and means comparison was performed by Minitab version 17 statistical software. Means were compared with Tukey's test at a 5% probability level.

3 | RESULT AND DISCUSSION

3.1 | Characteristics of the TiO_2-NPs

XRD analysis was performed to determine the crystalline structure of NPs (Figure 2). The peaks of samples established an anatase structure at $2\theta = 25.3°$, $37.8°$, and $48.1°$ with four-faced tetragonal structure (Movafeghi et al., 2018). The anatase TiO_2–NPs increase the level of contact with materials due to their specific area and small particle size and have a more significant effect due to their lower oxygen absorption capacity. According to Debye–Scherrer (Equation (1)) (Debye & Scherrer, 1916), the mean crystallite size of NPs was estimated to be 13 nm. According to DLS analysis, the hydrodynamic diameter distribution profile of TiO_2-NPs (> 60%) was found to be between 12 and 25 nm at 25°C and pH = 6.5 (Figure 1). Hydrodynamic particle diameter distribution of TiO_2-NPs by volume (Figure 1a), hydrodynamic particle diameter distribution of TiO_2-NPs by intensity (Figure 1b), hydrodynamic particle size distribution by number of TiO_2-NPs (Figure 1c), and frequency of hydrodynamic

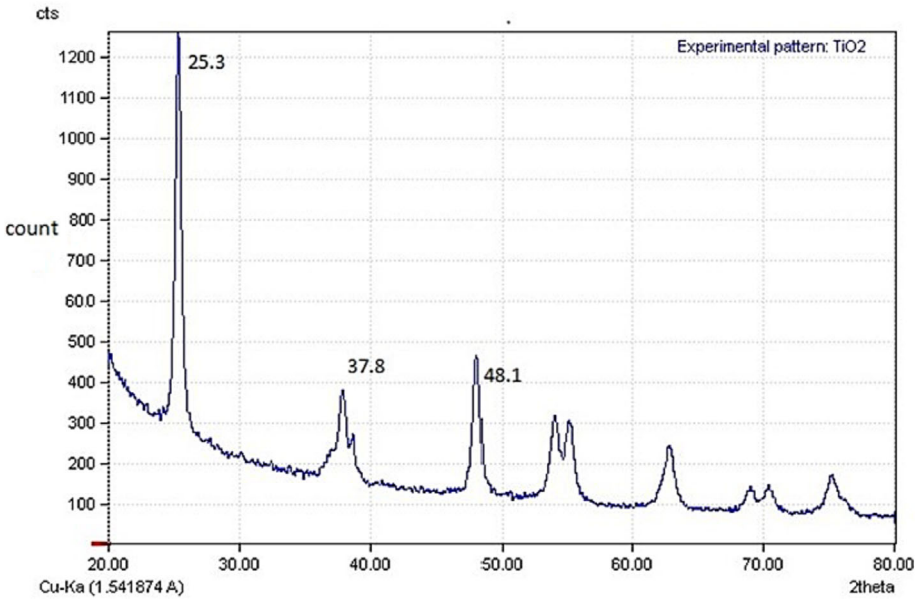

FIGURE 2 XRD pattern of TiO_2-nanoparticles (NPs) confirming the crystalline structure of TiO_2-NPs.

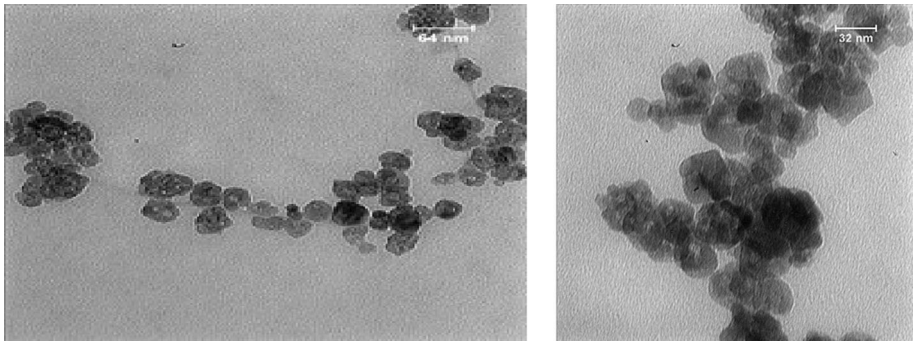

FIGURE 3 Transmission electron microscope (TEM) images of TiO_2-nanoparticles (NPs) with different magnifications confirming their size.

particle diameter distribution of TiO_2-NPs (Figure 1d) provided. The diameter and potential energy on the surface of suspended particles determine the degree of stability of suspension (Verwey et al., 1948). With an increase in diameter, the change in the electrostatic forces between particles rises, which also increases the sedimentation rate (Lebovka et al., 2014). DLS and XRD results were endorsed by TEM images (Figure 3), which confirmed the size of TiO_2-NPs. The result of the zeta-colloid formed in the sample was 13.96 ± 6.67 mV, and this can take into consideration as evidence of the presence of titanium dioxide NP ions. When the zeta potential is between 10 and 30 mV, the stability of NPs is low (Navarro et al., 2008). For this reason, the nanoparticles were sprayed on plant leaves.

$$D = 0.9\ \lambda/\beta\ \mathrm{Cos}\theta \quad (1)$$

3.2 | Antioxidant enzymes' activity

The activity of APX in chickpeas was significantly affected by drought stress and TiO_2-NPs ($P < 0.01$) as well as their interaction. The combined effect of two factors induced a rise in the activity of APX with TiO_2-NPs up to 40 mg. L^{-1}, regardless of soil moisture regimes (Figure 4). The maximum concentration of APX was observed as exhibited with moisture regime at 40% FC and 40 mg. L^{-1} TiO_2-NPs. However, the minimum concentration was observed by exposure to 90% FC and without the application of NPs. Further, applying TiO_2-NPs with the low moisture regime increased the activity of APX in chickpeas. Ascorbate is an important antioxidant that works as an enzyme co-factor in the transmission of electrons in the plasma membrane or in the chloroplasts (Shigeoka et al., 2002). Under environmental stress, plants accumulate reactive oxygen species (ROS) produced in chloroplast and mitochondria, which increase usually through enzymatic sequences in tandem (Tuna et al., 2008). Antioxidant enzymes collect oxygen radicals and convert them into H_2O_2, and then hydrogen is produced and accumulated by enzymes such as APX and catalase (Sairam & Tyagi, 2004). APX, POX, and catalase play overlapping roles in the plants' defense system. However, their unified role is most prudent regarding detoxification and decomposition of hydrogen peroxide produced in the cells (Sofo et al., 2015). Previously, TEM analysis has confirmed the ability of TiO_2-NPs to enter the plant cells. As reported, TiO_2-NPs showed the ability to reinforce the antioxidant system and suppress the adverse effects of water stress. In other words, the use of TiO_2-NPs increases the enzymatic antioxidant activity and diminishes the effects of ROS under drought conditions.

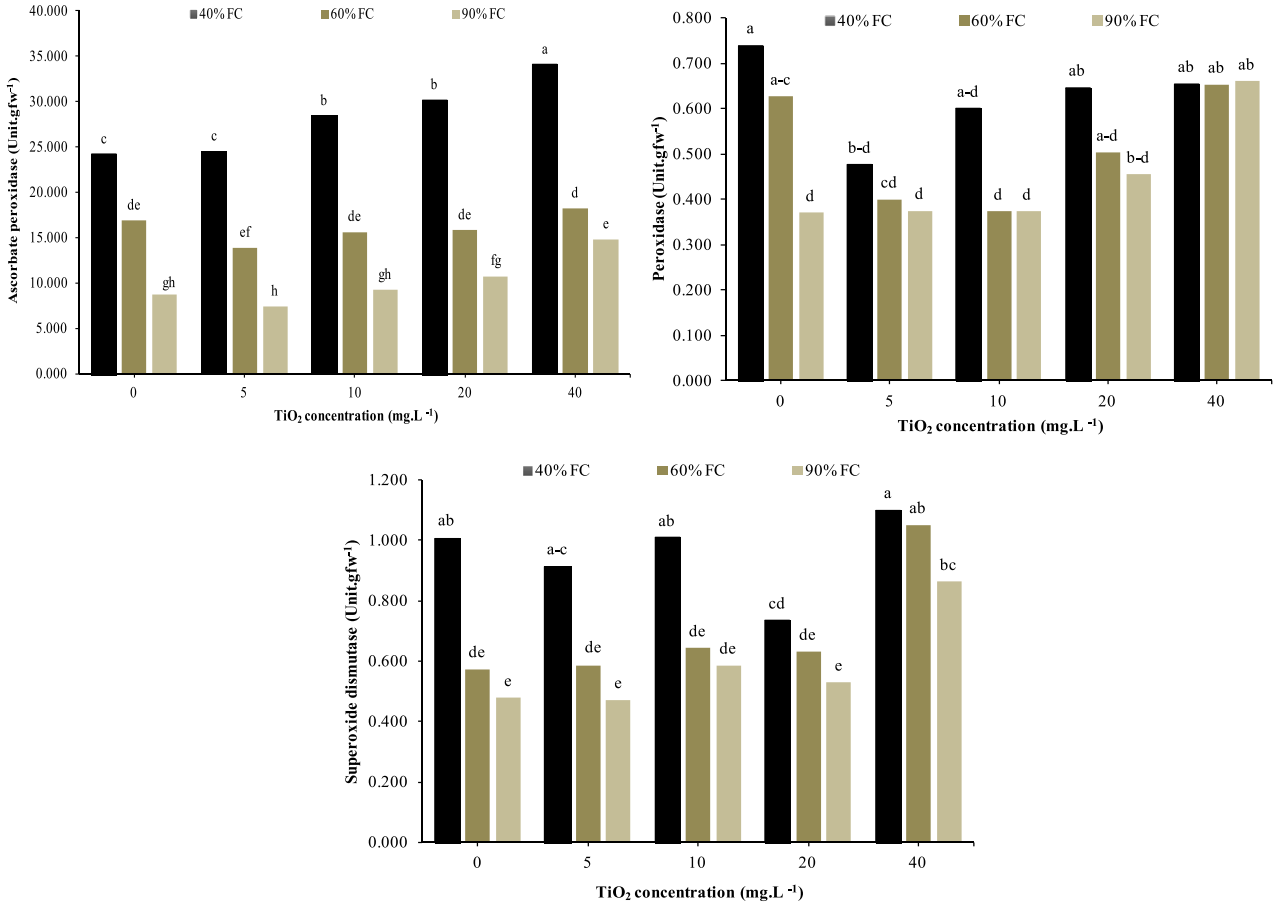

FIGURE 4 The effects of TiO_2-nanoparticles (NPs) application on ascorbate peroxidase (APX), peroxidase (POX), and superoxide dismutase (SOD) activity of plants subjected to different moisture regimes. Values are means ($n = 3$) ± SE. Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to the Tukey's multiples test.

In our study, the activity of POX was influenced by moisture regimes, TiO_2-NPs, and their interaction ($P < 0.05$). With an increase in the concentration of TiO_2-NPs up to 20 mg. L^{-1}, POX activity in chickpeas has enhanced at 90% FC. At 40% and 60% FC moisture levels, with no application of NPs, POX activity was increased by 14% at 40% and 24% at 60% FC on plants treated with 20 mg. L^{-1}. POX level was the highest at 40% FC condition with no application of NPs. The lowest value of POX was observed at 90% FC in the absence of TiO_2-NPs. These results showed that the application of TiO_2-NPs enhanced POX activity in severe drought stress (40% FC) (Figure 4). However, the application of TiO_2-NPs produced a slight difference between moisture regimes. Previous research has shown that TiO_2-NPs can reinforce the antioxidant system and suppress the adverse effects of drought stress leading to plant cell death (Morteza et al., 2013). Application of TiO_2-NPs could also markedly drop the amount of electrolyte leakage. When the size of NPs is more minor, their response is more conspicuous, and response distinguishing ability is more predictive (Zheng et al., 2005).

In this study, the maximum SOD activity was achieved with the increasing concentration of TiO_2-NPs. The application of 40 mg. L^{-1} TiO_2-NPs with the decreasing level of moisture regime caused a rise in the activity of SOD (Figure 4). It was reported that the 10% to 30% concentration of TiO_2-NPs caused a continuous increase in the activities of SOD and CAT in calendula (*Calendula officinalis* L.) (Moaveni et al., 2011). Studies on nanomaterial treatment in chickpea plants have shown that scarce concentrations do not sufficiently enhance the activity of SOD and APX enzymes in response to drought (Sairam & Tyagi, 2004). A 0.05% concentration of TiO_2-NPs significantly reduced the impact of drought stress on the SOD level in *C. officinalis* (Lu et al., 2002).

3.3 | Proline content

The proline content in the leaves of chickpea plants was influenced by moisture regimes, TiO_2-NPs, and their interactions ($P < 0.05$). The proline content of chickpea leaf was enhanced at all three levels of moisture regimes. Application of 5 mg. L^{-1} of TiO_2-NPs at 40 FC level led to a 23% decline in the amount of proline concerning the control. At all levels of the moisture regime, the use of 40 mg. L^{-1} NPs caused a more significant decline compared with 20 mg. L^{-1} TiO_2-NPs. The highest amount of proline was obtained at 40% FC by applying 20 mg. L^{-1} TiO_2-NPs. In contrast, the lowest amount was achieved at 90% FC level without the application of TiO_2-NPs (Figure 5). In

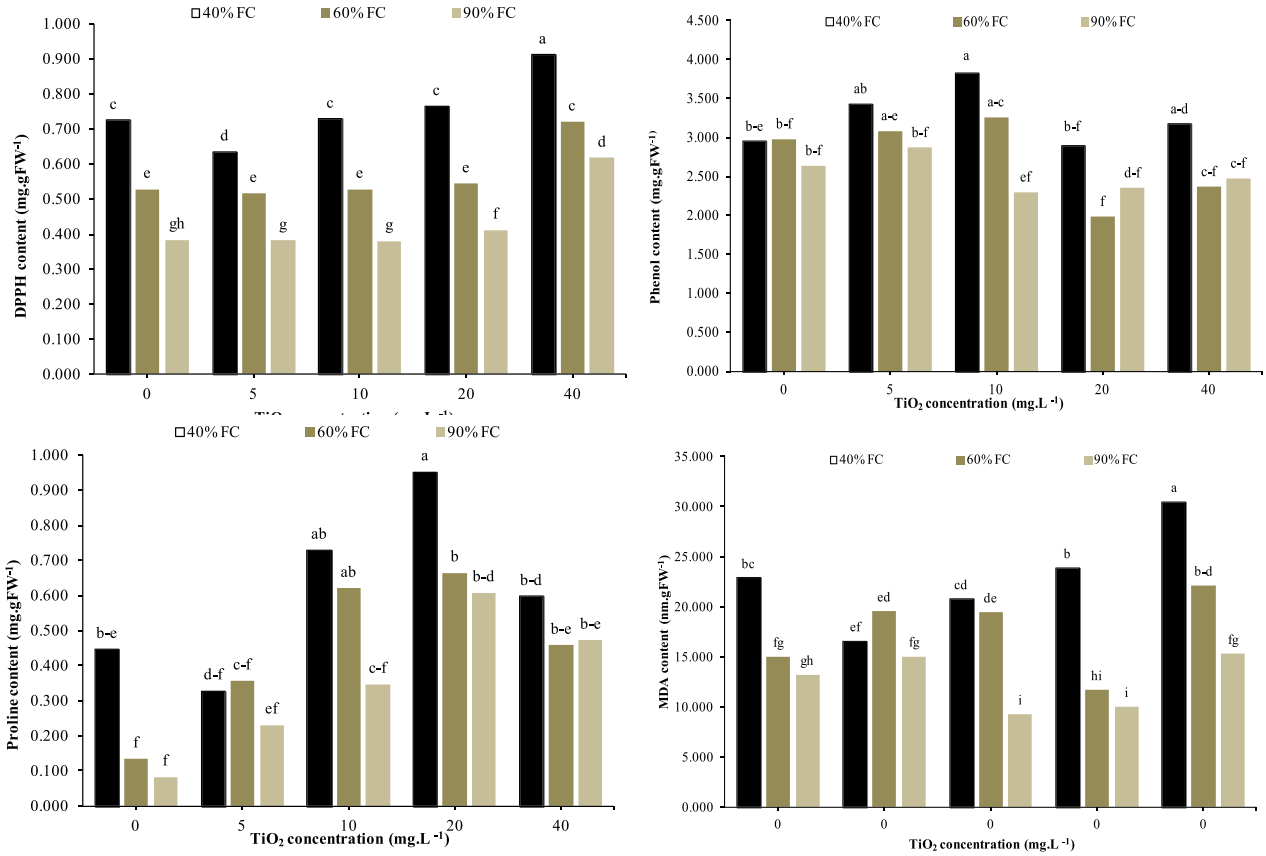

FIGURE 5 The effects of TiO_2-nanoparticles (NPs) application on 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), phenol, proline, and malondialdehyde (MDA) content of plants subjected to different moisture regimes. Values are means ($n = 3$) ± SE. Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to the Tukey's multiples test.

general, application of TiO_2-NPs at reduced moisture regimes would increase proline content in chickpea leaves. In response to drought and salinity stress, plant cells begin to synthesize and accumulate some protective molecules such as proline, proteins, sugars, alcoholic compounds, and organic acids (Aref et al., 2016; Arshi et al., 2010; Hasegawa et al., 2000; Umar et al., 2018). Biosynthesis of proline is an initial response of plants to environmental stresses (Anjum et al., 2014) such as salinity (Hayat et al., 2012; Munns, 2005), water deficit (Aref et al., 2016), low temperature (Naidu et al., 1991), heavy metals (Sharma & Dietz, 2006), and UV radiation (Saradhi et al., 1995). Proline can protect plants against environmental stresses by influencing electron transmit in membrane protein complexes (Hossain et al., 2014) and enzymes such as RUBISCO (Holmström et al., 2000). In the present study, the amount of proline has increased under drought stress that was heightened by application of TiO_2-NPs. However, it should be mentioned that TiO_2-NPs can cause toxicity at high concentrations (Singh & Singh, 2004).

3.4 | Content of phenols, DPPH, and MDA

The content of phenols in chickpea plants was noticeably influenced by the applied factors ($P < 0.01$). TiO_2-NPs increased the concentration of total phenols at all levels of the moisture regime. Interaction of moisture regimes and TiO_2-NPs indicated the increase of phenol content at 40% and 60% FC using 10 mg. L^{-1} of TiO_2-NPs. In comparison, at these moisture regimes, the use of 40 mg. L^{-1} concentration of NPs reduced the phenol content (Figure 6). The highest phenol content (3.9 mg.g^{-1} fresh weight of leaves) was observed after treatment with 10 mg. L^{-1} TiO_2-NPs at 40% FC level. The lowest value was found after exposure to 10 mg. L^{-1} of TiO_2-NPs at 90% FC moisture regime (Figure 5). The ROS-scavenging potential of plants is directly connected to their antioxidant defense system (Cuin & Shabala, 2008). The production of secondary metabolites such as phenolic compounds as nonenzymatic antioxidants is an identified response to abiotic stress (Close & McArthur, 2002). Our results have shown that application of TiO_2-NPs at the severity of drought stress (40% FC) could raise the content of phenols, which was in fair agreement with the findings of some earlier studies (Khater & Osman, 2015; Zheng et al., 2005). Chlorogenic acid and germanic acid are the major phenolic compounds produced by the spray of 36 mg. L^{-1} TiO_2-NPs on the leaves of *Dracocepha lummodavica* (Kamali Zadeh, 2016). Phenolic compounds enable plants to neutralize free radicals, form complexes with metal ions, and stop triple oxygen molecules (Peksel et al., 2010). Moreover, phenolics inhibit lipid oxidation reactions through electron-free radicals.

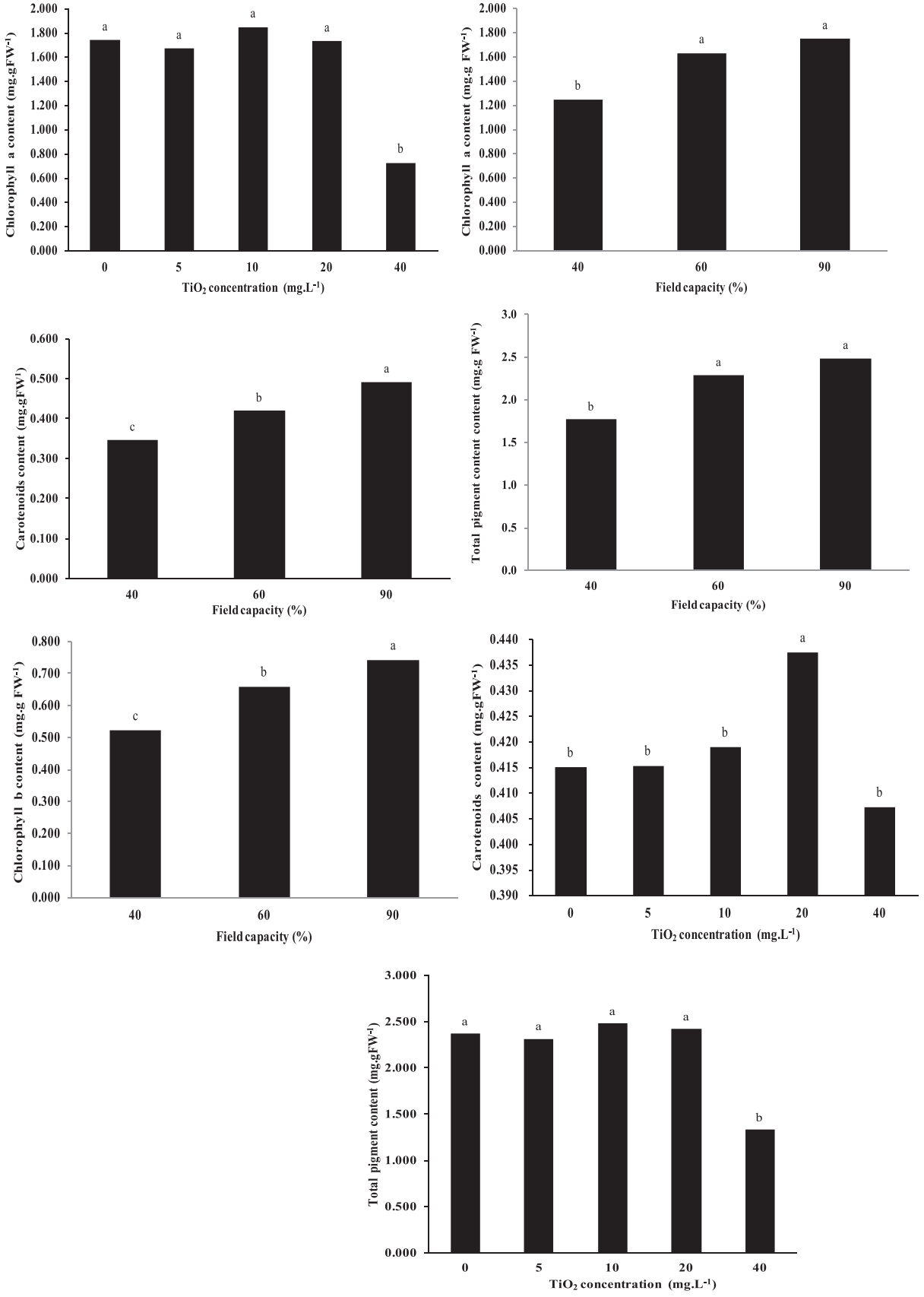

FIGURE 6 The effects of TiO_2-nanoparticles (NPs) application on chlorophyll a, b, total chlorophyll, and carotenoid content of plants subjected to different moisture regimes. Values are means ($n = 3$) ± SE. Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to the Tukey's multiples test.

The obtained data indicated that the concentration of DPPH in chickpeas was primarily affected by various moisture regimes, TiO_2-NPs, and their interaction ($P < 0.01$). By elevating the concentration of TiO_2-Nps up to 20 mg. L^{-1}, the amount of DPPH has increased at all levels of the moisture regime. However, 5 mg. L^{-1} TiO_2-NPs used at 40% FC reduced DPPH concentration by 31% compared with the control plants. Interestingly, adding 40 mg. L^{-1} TiO_2-NPs at all levels of the moisture regimes enhanced the DPPH level (Figure 5). The maximum concentration of DPPH was obtained with 40 mg. L^{-1} TiO_2-NPs at 40% FC. In contrast, the minimum amount was achieved by 10 mg. L^{-1} of TiO_2-NPs at 90% FC condition. In general, the increasing concentration of TiO_2-NPs in drought stress conditions raised the DPPH concentration of chickpeas. Oxygen radicals are produced mainly in chloroplasts and mitochondria, causing oxidative damage to lipids, proteins, and nucleic acids, impairing vital processes of respiration and photosynthesis, and thereby inhibiting plant growth (Qureshi et al., 2013). Thus, production of oxygen radicals is one of the significant reasons for tissue damage in plants exposed to environmental stress (Küçük et al., 2007).

The MDA content was influenced by both applied factors ($P < 0.01$). At 60% FC, a notable difference between the treatments of 0 and 20 mg. L^{-1} TiO_2-NPs was observed. MDA content was decreased by 22% in control plants which can be confirmed by testing the activity of peroxidase when 20 mg. L^{-1} TiO_2-NPs are applied at 60% FC. However, at 40% FC and applying 20 mg. L^{-1} TiO_2-NPs, no significant effect was detected. The highest MDA was seen at 40 FC and 40 mg. L^{-1} TiO_2-NPs (Figure 5). The high cost of peroxidase antioxidants has led to the removal of ROS and active oxygen species and has suppressed peroxidation within the lipid of the cell membrane (Cavalcanti et al., 2007). As reported, drought stress causes an increase in the antioxidant system and specially POX to decrease the destruction of the cell membrane in *Lycopresicum esculantum* (Ünyayar et al., 2006).

3.5 | Pigment content

The chlorophyll b content was significantly affected by moisture regimes ($P < 0.01$). Compared with 40% of FC, chlorophyll b was significantly increased by 35% at 90% of FC in comparison with the control group (Figure 6). Changes in the concentration of TiO_2-NPs did not cause considerable variation in chlorophyll a content at different levels of FC (Figure 6). The chlorophyll a content has increased at 7% at 90% FC after application of 20 mg. L^{-1} of TiO_2-NPs. Also, the application of 20 mg. L^{-1} of TiO_2-NPs at all moisture regimes boosted the total chlorophyll by 10% at 40% FC, 7% at 60% FC, and 5% at 90% FC compared with the control plants (Figure 6). The concentration of chlorophyll a is an indicator of photosynthesis as a process of carbon supply (Herzog, 1986). Under drought conditions, chlorophyll formation was abruptly discontinued due to reduced cellular metabolism (Ashraf et al., 1994). A decline in chlorophyll concentration under drought stress was observed owing to reduced activity of chlorophyllase, POX, and phenolic compounds, eventually leading to chlorophyll degradation. However, it was reported that there were no influences of drought stress on chlorophyll concentration in wheat cultivars (Kulshreshtha et al., 1987). It seems that the condition of the experiment, for instance, duration and manner of application of treatments, would lead to different effects (Jagtap et al., 1998). Titanium plays a significant role in photosynthesis via increased accumulation of fructose 1,6-bisphosphate in the Calvin cycle, stimulation of glucogenic enzymes, alternation in the pentose phosphate pathway, and participation in carbohydrate metabolism. It can also stimulate chlorophyll synthesis, collectively contributing to increased chlorophyll synthesis (Kiss et al., 1985). Alteration in the concentration of TiO_2-NPs in moisture regimes led to a 1.4 times higher carotenoid contents in chickpeas. The comparison of the obtained means of parameters of the moisture regimes and TiO_2-NPs showed that with the decreasing moisture levels and the increasing concentration of TiO_2-NPs, the carotenoid content was elevated. In agreement with our finding, a previous study showed

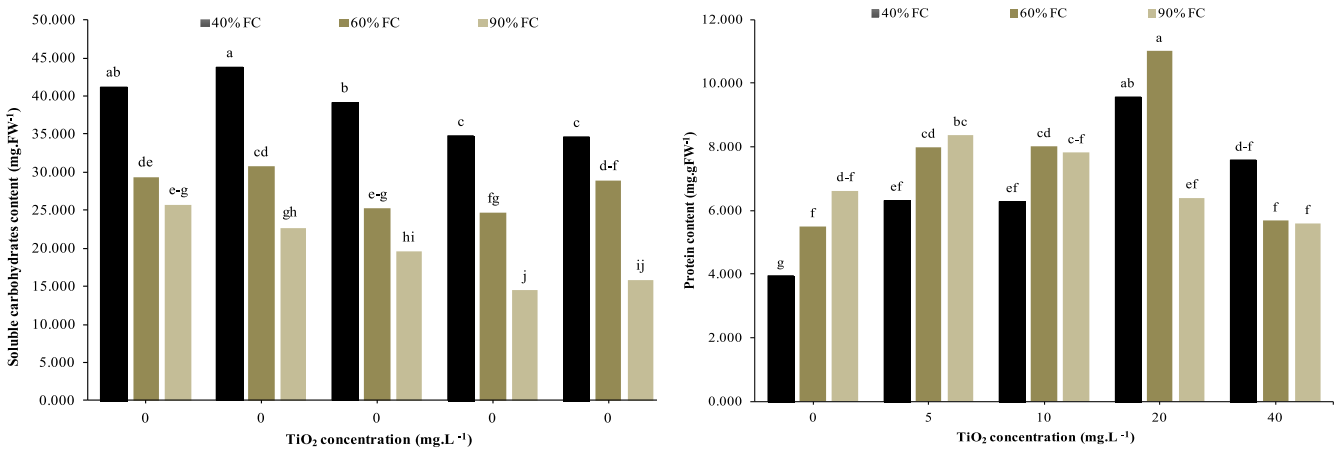

FIGURE 7 The effects of TiO_2-nanoparticles (NPs) application on the protein and soluble carbohydrate content of plants subjected to different moisture regimes. Values are means ($n = 3$) ± SE. Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to the Tukey's multiples test.

that the application of 0.01% TiO_2-NPs in corn significantly raised the content of carotenoids (Morteza et al., 2013).

3.6 | Protein and carbohydrate content

In this study, moisture level, TiO_2-NPs, and their interaction showed a significant influence on protein content ($P < 0.01$). The maximum protein content was observed with 20 mg. L^{-1} TiO_2-NPs and 60% FC (Figure 7). Reduction in the concentration of proteins under oxidative stress caused by drought conditions is a common reaction in plants (Mafakheri et al., 2010). The amount of protein in Pirouz, IIC482, and Bivaniej cultivars of chickpeas exposed to drought stress was declined during the vegetative as well as reproductive phases (Mafakheri et al., 2010). Moreover, the protein content of grain significantly dropped in five cultivars of wheat under drought stress (Parchin & Shaban, 2014).

The concentration of soluble carbohydrates was significantly ($P < 0.01$) affected by both the TiO_2-NPs concentration and the moisture regimes. The effect of moisture regimes on the amount of soluble carbohydrates was higher than that of TiO_2-NPs (Figure 7). Carbohydrates, which act as the source of energy as well as signaling molecules, help plants to resist environmental stresses (Asch et al., 2001). The amount of soluble sugar in sugar beet was increased by the addition of ammonium titanyl sulfate to soil or foliar application of TiO_2-NPs in *Foeniculum vulgare* (Khater, 2015).

4 | CONCLUSION

In this study, it was clearly shown that the activity of some antioxidant enzymes such as SOD and POX in drought-challenged chickpea plants has increased by TiO_2-foliar treatments. Spraying TiO_2-NPs, especially at low concentrations, has stimulated the antioxidant enzymes and consequently lessened the disturbing effect of drought in chickpea plants. Concurrently, treatment with TiO_2-NPs raised the amounts of proline and phenols at different moisture regimes, which are defensive components against oxidative stress. Application of TiO_2-NPs has resulted in the improvement of some growth parameters, such as the content of the photosynthetic pigment in drought-stressed plants. Overall, the application of TiO_2-NPs could considerably improve the plant tolerance against the harmful impact of drought in chickpea plants.

AUTHOR CONTRIBUTIONS

Roya Ghorbani: Writing—original draft. **Ali Ganjeali:** Project administration. **Ali Movafeghi:** Project administration.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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