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FOLIAR APPLICATION OF NANO TITANIUM DIOXIDE MITIGATES DROUGHT STRESS IN *MORINGA PEREGRINA*

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Abstract

Drought stress is one of the most significant environmental factors limiting plant growth and productivity. In this regard, applying nano-fertilizers is a favorable emerging method for reducing abiotic stresses. The present study aimed to investigate the effect of different concentrations of TiO_2 NPs on the morphological, physiological, and antioxidative response of *Moringa peregrina* grown under drought stress. The suspensions of nano-TiO_2 in the varying concentrations (D=0, T1=50, T2=100 and T3=200 mg/L) were applied as foliar spray to *M. peregrina* seedlings after 4 weeks of emergence for three continuous days. The seedlings were irrigated with distilled water every 3 days until the relative soil water content reached approximately 30%. Untreated pots (control) were irrigated with tap water continuously. Later, data for different morphological and biochemical parameters were determined in triplicate. The results so obtained showed that the treatment of *M. peregrina* under drought stress with TiO_2 NPs enhanced the total chlorophyll content, total soluble proteins, proline content, and antioxidant enzyme activities in dose-dependent manner compared to those of non-treated plants. It is concluded from the present study that drought stress-induced damages, such as oxidative stress and membrane impairment, can be ameliorated by foliar application of TiO_2 NPs. Therefore, an appropriate concentration of TiO_2 NPs can be used as an exogenous stimulus for enhancing morphological and biochemical performances in *M. peregrina* under drought stress.

Key words: Antioxidant activities; Lipid peroxidation; *Moringa peregrina*; Proline; Reactive oxygen species; Titanium dioxide

Introduction

It is anticipated that climate change will bring increases in average global temperatures (1.4ºC-5.8ºC by 2100) and precipitation levels to varying degrees around the globe. The current water crisis in the Middle East is substantial, impacting Saudi Arabia and the other nations within the Gulf Cooperation Council (GCC), due to the hot climate marked by scarce rainfall and an elevated rate of evaporation. (Hassen & Bilali, 2022). The limited availability of water, in contrast to energy resources, renders the GCC among the most arid areas globally (Anon., 2018).

Plants encounter a variety of environmental stressors that can significantly affect and reduce plant growth. (Zhou *et al*., 2025). Among all the abiotic limiting factors, drought stress is probably the major barrier to crop productivity and quality around the world (Zhang *et al*., 2022). Drought stress occurs when the water requirements of the plant cannot be fulfilled, as a result of inadequate soil retention or a low groundwater level (Parkash & Singh, 2016). The austerity and incidence of drought stress will rise in the upcoming days, which will cause severe threats to crop productivity (Chapman *et al*., 2021).

Drought stress negatively affects crop development and production worldwide (Seleiman *et al*., 2021). It inhibits seed germination, photosynthesis, and hormonal activities in plants (Shah *et al*., 2022), reduces chlorophyll and plant metabolic activities (Morales *et al*., 2020), decreases membrane permeability, and increases the generation of reactive oxygen species (ROS) (Rao & Chaitanya, 2019). To mitigate the detrimental effects, plants effectively control ROS production through the synthesis of both enzymatic and non-enzymatic antioxidants. (Sachdev *et al*., 2021).

In addition to improving crop production and to the deleterious effects of drought stress, several strategies such as screening of tolerant cultivars, application of osmolytes, hormones, and microbes can be exploited to increase crop productivity. (Rasheed *et al*., 2022).

Nanotechnology has arisen as a promising tool, usually applied in the food, agricultural, and medical fields (Alabdallah & Hasan, 2021). Several nanoparticles (NPs), including zinc oxide (ZnO), iron oxide (Fe_3O_4), copper (Cu-NPs), titanium dioxide (TiO_2), and silicon oxide (SiO_2), have gained considerable attention and application in agriculture. (Hashem *et al*., 2021). Reportedly, NPs have improved plant performance against several stresses. In addition, nanotechnology can reduce nutrient losses from fertilizers and increase crop production (Saranya *et al*., 2019).

Moringa peregrina, also known as the frankincense tree, is a plant of high economic value. (Ghodsi *et al*., 2014), widely cultivated in semi-arid countries, distributed along the Red Sea to northern Somalia, the Arabian Peninsula, the Persian Gulf, and the Red Sea coast (Padayachee & Baijnath, 2012). The Kingdom of Saudi Arabia is one of the main local distribution areas for *Moringa peregrina* (Robiansyah *et al*., 2014). *M. peregrina* is recognized as one of the plant species

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possessing exceptionally high nutrient levels, and thus is considered an important future crop in semi-arid and arid regions (Mahmoud & Gairola, 2013).

For several economic uses, and the little available information about this species under water scarcity conditions, an attempt was made to evaluate the morphological and biochemical responses as a marker of *M. peregrina* subjected to drought stress, and also to study its response towards TiO_2 nanoparticles as an anti-stress material.

Material and Methods

Characterization of (TiO_2) Titanium dioxide nanoparticles: Titanium dioxide-Nano powder < 50 nm, product no. 30446 from the Sigma-Aldrich brand, (primary γ) crystals with a molecular weight (79.866 g/mol, were characterized by using the scanning electron microscope (SEM). Fig. 1 shows that the maximum of the TiO_2 NPs was in a crystalline shape. The particle size ranges between 15-30 nm, scanned by JSM-7610F.

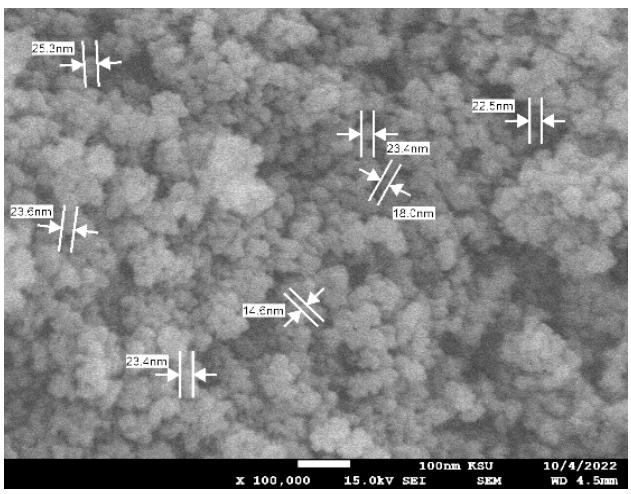

Fig. 1. Scanning electron microscopy (SEM) showing the diameter of TiO_2 NPs.

Preparation of TiO_2 NPs treatment: TiO_2 NPs were prepared at three different concentrations, 50, 100, and 200mg/L (w/v) in DDW. The suspensions were stored in dark vials at a temperature below 25°C and were later used for foliar application.

Experimental design and treatment: *M. Peregrina* seeds were collected from Hail and Al Madinah regions (Kingdom of Saudi Arabia). The seeds were gathered in sealed plastic bags and subsequently taken to the laboratory at King Saud University. Later, seeds were surface sterilized with 1% (w/v) sodium hypochlorite (NaOCl) for 5 min after washing, the seeds were soaked overnight in distilled water and planted in plastic pots containing 1.1 kg of soil. The suspensions of nano-TiO_2 in the following concentrations (D=0, T1=50, T2=100, and T3=200 mg/L) were given as foliar spray to *M. peregrina* seedlings after 4 weeks of emergence for three continuous days. Seedlings were irrigated with distilled water every 3 days until the relative soil water content reached approximately 30%. Untreated pots (control) were irrigated with tap water continuously. Later, morphological and biochemical parameters were studied in triplicate.

Data recorded

Morphological growth: As described by (Gbadegesin *et al*., 2021), fresh weights (FW) of leaves, shoots, and roots were weighed on an electronic top pan balance (Model PW 184, Adam Equipment, UK). For dry weight (DW) determination, samples were oven dried at 65°C ± 20°C for 72 h for 72 h and then weighed independently. FW and DW were expressed in grams (g) per plant. Plant length was measured with a metric scale and expressed in centimeters (cm).

Extraction and estimation of chlorophyll content: Chlorophyll (chl) content was estimated in the fresh leaf samples by the method of Arnon (1949) as mentioned in (Hiscox & Israelstam, 1979). The methodology involves the estimation of plant pigments without maceration. Leaves kept in moist filter paper in an icebox were washed with cold DDW and chopped. 0.1 g of the chopped leaf samples were taken in triplicate vials containing 5 mL of dimethyl sulfoxide (DMSO). The vials were then incubated in the oven at 65ºC for 1 h, for a complete leaching of the pigments. The absorbance of DMSO, containing the pigments, was recorded at 663 and 645 nm, using a UV-Vis spectrophotometer (Libra S22, Biochro Ltd, England). Values of optical densities (ODs) were used to compute the chlorophyll a, chlorophyll b, and total chlorophyll contents with the following formula given by Arnon (1949).

Lipid peroxidation (MDA content): The lipid peroxide in leaves was estimated as malondialdehyde (MDA) content by the method given by Heath & Packer (1968). Fresh tissues were macerated in 0.1% TCA using a mortar and pestle and centrifuged at 10,000 × *g* for 5 min. Then 1.0 mL of the supernatant was taken into a separate test tube, 4.0 mL of 0.5% TBA was added, and the concoction was heated at 95°C for 30 minutes, then cooled rapidly in an ice bath and re-centrifuged at 5000 × *g* for 5 min to suspend the turbidity. The absorbance was recorded at 532 nm and 600 nm and corrected for nonspecific turbidity by subtracting the value at 600 nm. 0.5% TBA reagent was used as a blank.

Estimation of proline content: Estimation of proline was carried out by the method of Bates *et al*., (1973). 0.5 g of fresh leaf tissues were homogenized in 10 ml of 3% sulphosalicylic acid and then centrifuged for 10 min at 10,000 × *g*. In a test tube, 2 mL of supernatant, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid were taken, the concoction was kept in an oven for 1 h at 100°C, and the ongoing reaction was terminated by transferring the tubes to an ice bath. Once the incubation was complete, the mixture was separated using 4 mL of toluene and was vigorously vortexed. The fraction having chromatophore was then extracted from the aqueous phase, and the absorbance was determined at 620 nm using a spectrophotometer (l BIO 20, Perkin Elmer, Germany). The amount of proline was expressed as nmol g^{-1} FW.

Soluble protein content: Soluble protein content was calculated by the method of Bradford (1976). 0.5 g of the fresh leaf sample was chopped and homogenized in extraction buffer (5 mL of 0.1M phosphate buffer) using a pre-cooled mortar and pestle. The homogenate was centrifuged for 10 min at 5000 × *g*, to 1.0 mL of supernatant, 1.0 mL of 10% TCA was added, and again

centrifuged at 3300 × *g* for 10 min. The obtained pellet was then dissolved in 1 mL of 0.1 N NaOH. 1.0 mL of an aliquot in test tubes was vortexed, mixed, and left for 10 min for optimum color development. The absorbance was recorded at 595 nm using a UV-Vis spectrophotometer (Libra S22, Biochro Ltd, England). The protein content was expressed in mg /g FW.

Enzyme assays: Measurement of SOD activity was carried out by the method of (Beyer & Fridovich, 1987). 0.1 g of the plant tissue was homogenized in 1 mL of the extraction buffer with the help of a mortar and pestle. The process was carried out under cool conditions (4°C). The mortar and pestle were kept in ice during homogenization; the homogenate was centrifuged for 20 min at 10,000 × *g* at 4°C. The SOD activity was assayed to inhibit the photo reduction of NBT to form blue formazan by super oxide radicals. The assay mixture, consisting of 1 mL of reaction buffer, 1 M sodium bicarbonate, 200 mM methionine, 3 mM EDTA, 60 µM riboflavin, and 100 µl of enzyme extract, was taken in a test tube and incubated in the light of 15 W fluorescent lamps at 25/28°C for 10 min. A blank containing all the above substances of the reaction mixture, except the enzyme extract, was placed in the light along with the samples. The reaction was stopped by switching off the light, and the tubes were covered with a black cloth. Absorbance of the samples along with the blank was recorded at 560 nm. Measurements for this experiment are referred to as (B) and (A). The same experiment was performed in isolation from light, and the absorbance of the samples, along with the blank, was read at 560 nm. The difference in percentage reduction in color between "B" and "A" was then calculated. One unit of SOD is defined as the volume of the enzyme required to cause photo inhibition of NBT by 50%. The activity was expressed in Enzyme units.

In vitro assay of ascorbate peroxidase activity was estimated by the method of Nakano & Asada (1981). 0.1 g of the Extraction: plant material was ground in 1 mL of the extraction buffer and centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was collected and used for the assay immediately. Reaction mixture containing 1.5 mL of the buffer, 0.1 mL of 0.3% H_2O_2, 0.1 mL of 0.5 mM Ascorbate, 3 mM 0.1 mL of EDTA, and 100µl of the enzyme extract was allowed to run for 3 min at 25°C. The oxidation rate of ascorbic acid was estimated following a decrease in the absorbance at 290 nm on a UV-Vis spectrophotometer (Libra S22, Biochro Ltd, England). The enzyme activity was calculated from the initial rate of reaction using the extinction coefficient of 2.8 mM of ascorbate. One enzyme^{-1} cm^{-1} unit determines the amount of enzyme necessary to decompose 1µmol ascorbate per mg of protein per min at 25°C.

In vitro catalase activity was determined by the method of (Aebi, 1984) to the equation given by (Chen *et al*., 2016). 0.1 g of the fresh leaf tissue was homogenized in 1 mL of the extraction mixture under cold conditions. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4°C. The supernatant was used for a quick assay. Catalase activity was determined by monitoring the disappearance of H_2O_2, measuring a reduction in the absorbance at 240 nm on a UV-Vis spectrophotometer (Libra S22, Biochro Ltd, England). The reaction was carried out in a mixture containing 1.0 mL of the reaction buffer, 0.1 mL of EDTA, 0.1 mL of the enzyme extract, and 0.1 mL of H_2O_2, and allowed to run for 3 min. One enzyme unit (EU) determines the amount of enzyme necessary to decompose 1 µmol of H_2O_2 per mg protein per min at 25°C and expressed as units (U).

Statistical analysis

All data were statistically subjected to a one-way analysis of variance using the Statistical Package for the Social Sciences (SPSS) software (Chicago, Illinois, USA, version 22; SPSS Inc.) and the mean values for each treatment were compared using Duncan's test at the $p<0.05$ confidence level.

Results

Growth attributes: Data on the shoot and root length of *M. peregrina* as affected by different levels of TiO_2 NPs are presented (Table 1). A significant reduction in the plant root length of 16% was observed in treatment D over the control. Whereas a dose-dependent increment in root length of *M. peregrina* was observed in response to TiO_2 NPs. The highest increase in plant root length (22%) was recorded at T3, followed by T2 (10.4%) and T1 (6%) when compared to the control. A significant decrease in plant shoot length was observed at all treated samples compared to the control. However, maximum reduction (12%) in plant shoot length was observed in treatment D, whereas minimum decline of 3% was observed at the T3 level of TiO_2 NPs. The number of leaves of *M. peregrina* affected by various concentrations of TiO_2 NPs is shown in Table 1. A significant decrease was observed in the number of leaves at all treated samples compared to the control. However, maximum decline (13%) was recorded at treatment D, followed by T1 (8%). Whereas T2 and T3 equally showed a reduction of 5% over the control. Maximum reduction in plant FW was recorded in treatment D (21%). Whereas a significant difference in plant fresh weight was observed in nano TiO_2 exposure under drought stress. The minimum reduction of 4% was observed at T3. Whereas reduction in plant fresh weight was (8%) in T2 and (10%) in T1, respectively, when compared to the control (Table 1). There was a significant decrease in dry weight at all treatment levels compared to the control. Maximum reduction in plant dry weight by 88% was observed at D.

Table 1. Effect of TiO_2 NPs treatment on growth parameters of *M. peregrina* under drought stress.

Treatments	Root length (cm)	Shoot length (cm)	No. of leaves	Plant fresh weight (FW g)	Plant dry weight (DW g)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Control	$8.500^{a} \pm 0.289$	$16.267^{a} \pm 0.024$	$40^{a} \pm 0.577$	$4.275^{a} \pm 0.067$	$1.998^{a} \pm 0.074$
Drought (D)	$7.167^{b} \pm 0.294$	$14.272^{b} \pm 0.086$	$35^{b} \pm 2.082$	$3.370^{b} \pm 0.039$	$0.235^{b} \pm 0.027$
T1 (TiO_2 NPs) 50mg/L	$9.000^{ac} \pm 0.115$	$15.177^{c} \pm 0.033$	$37^{ab} \pm 2.082$	$3.838^{c} \pm 0.039$	$1.529^{ac} \pm 0.026$
T2 (TiO_2 NPs) 100mg/L	$9.383^{c} \pm 0.088$	$15.335^{c} \pm 0.477$	$38^{ab} \pm 3.055$	$3.932^{c} \pm 0.098$	$1.688^{ac} \pm 0.022$
T3 (TiO_2 NPs) 200mg/L	$10.356^{d} \pm 0.195$	$15.833^{ac} \pm 0.007$	$38^{ab} \pm 3.055$	$4.124^{ad} \pm 0.016$	$1.714^{ac} \pm 0.174$

Whereas the nano TiO_2 treated plant under drought conditions was reduced by T1 (23%), T2 (16%) T3 (14%), respectively, over the control (Table 1).

Chlorophyll content: Total chlorophyll content in *M. peregrina* as influenced by different doses of TiO_2 NPs treatments are presented in (Fig. 2). Various concentrations of TiO_2 NPs resulted in enhancement of total chlorophyll content in M. peregrina leaves. Whereas decline in total chlorophyll content by (6.3%) was observed in treatment D over control. Significant enhancement in total chlorophyll content was observed in M. peregrina under TiO_2 NPs in dose dependent manner. The highest increment (27%) was observed at T3 followed by T2 (6%) and T1 (4%) respectively when compared to control.

The malondialdehyde (MDA) content: The level of lipid peroxidation in leaf samples of *M. peregrina,* as determined in terms of Malondialdehyde (MDA) content, is presented in Fig. 3. The highest level of MDA content was recorded in treatment D with a percent increase of 67.3%. However, the increase in MDA content at T1 level was (46.78 %) followed by T2 with the enhancement of (31.78 %), and T3 with (13.23%), respectively, when compared to the control (Fig. 3).

Soluble protein content: Soluble protein content of *M. peregrina* as influenced by various doses of TiO_2 NPs treatments is presented (Fig. 4). In the observed results, treatment D caused a significant reduction in soluble protein content when compared to the control. Whereas a significant increment in soluble protein content was observed in response to TiO_2 NPs in a dose-dependent manner in *M. peregrina.* The highest increase in soluble protein content was recorded at T3 by (91.7%), followed by T2 (72.9%) and T1 with the percentage increase of (42.7%), respectively, over the control.

Proline content: Proline content in *M. peregrina* was affected in response to all the TiO_2 NPs treatments, as well as under treatment D. However, the highest level of proline content was observed in treatment T3 (82%), and the minimum was observed in treatment D (47.2%). Whereas the increment in proline content in T2 was 66%, and T1 was 59%, respectively, over the control (Fig. 5).

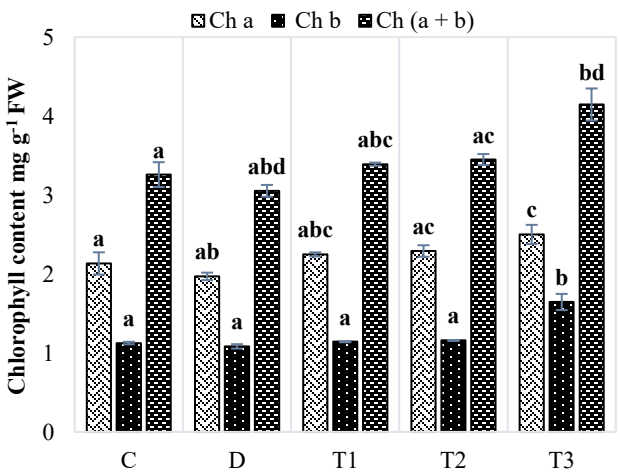

Fig. 2. Effect of TiO_2 NPs treatment on chlorophyll A and B pigments and total chlorophyll of *M. peregrina* under drought stress. Bars with the same letters are not significantly different at $p<0.05$ using Duncan's test.

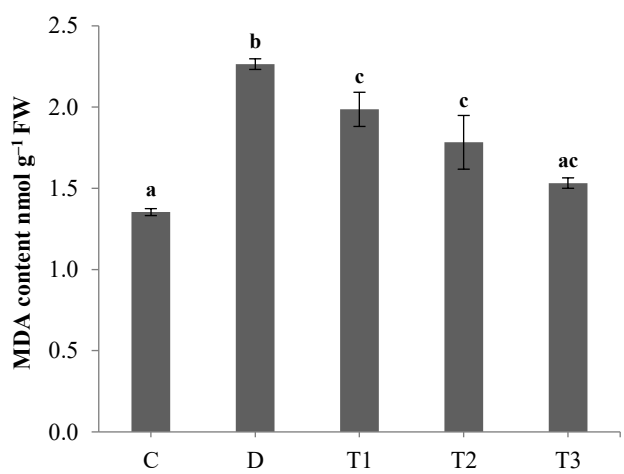

Fig. 3. Effect of TiO_2 NPs treatment on MDA content of *M. peregrina* under drought stress. Bars with the same letters are not significantly different at $p<0.05$ using Duncan's test.

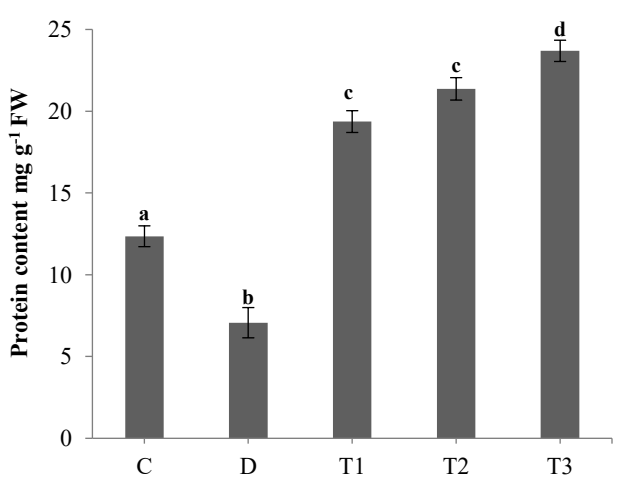

Fig. 4. Effect of TiO_2 NPs treatment on soluble protein content of *M. peregrina* under drought stress. Bars with the same letters are not significantly different at $p<0.05$ using Duncan's test.

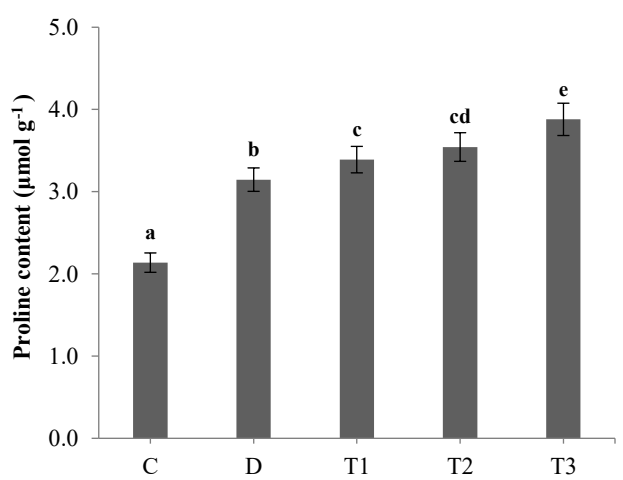

Fig. 5. Effect of TiO_2 NPs treatment on proline content of *M. peregrina* under drought stress. Bars with the same letters are not significantly different at $p<0.05$ using Duncan's test.

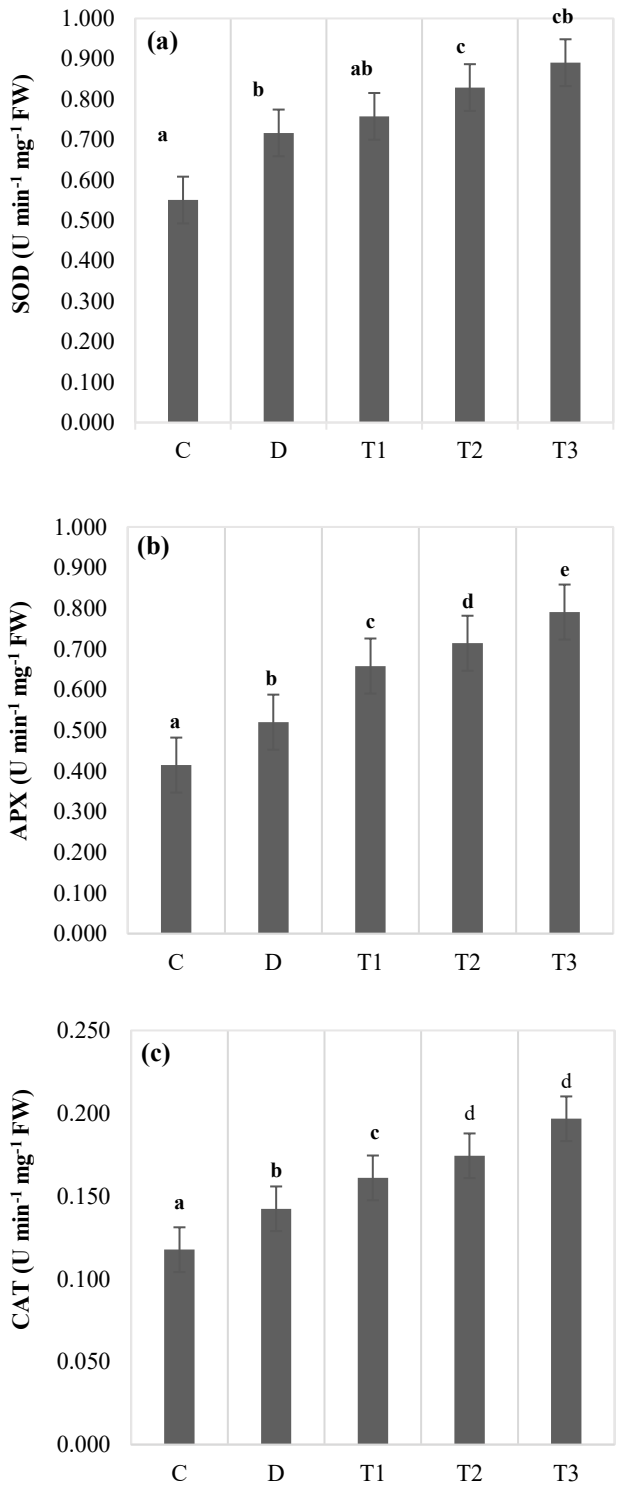

Fig. 6. Effect of TiO_2 NPs treatment on the activities of antioxidant enzymes of *M. peregrina* under drought stress (a) Superoxide dismutase (b) Ascorbate peroxidase (c) Catalase. Bars with the same letters are not significantly different at $p<0.05$ using Duncan's test.

Enzymatic Antioxidants

(SOD) Superoxide Dismutase Enzyme: Activity of SOD exhibited significant variation in *M. peregrina* at diverse levels of TiO_2 NPs treatments. The highest SOD activity by (62%) was recorded at T3, followed by (50%) at T2 and T1 with the increment of (38%) respectively over control. However, the minimum SOD activity (30%) was observed at D treatment when compared to control (Fig. 6a).

(APX) Ascorbate Peroxidase Enzyme: Results on the differences in antioxidant enzyme (APX) of *M. peregrina* under different levels of TiO_2 NPs treatments are shown (Fig. 6b). The maximum APX activity (91.1%) was documented at the T3 dose of TiO_2 NPs treatment compared to the control, followed by (72%) at T2 and (72.5%) at T3 concentration, respectively, compared to the control. Whereas the minimum increase (26%) in APX activity was noticed in the D treatment over the control.

(**CAT) Catalase Enzyme:** Data on the (CAT) activity of *M. peregrina* as affected by various concentrations of TiO_2 NPs treatments are presented (Fig. 6c). Different doses of TiO_2 NPs treatment resulted in the enhancement of Catalase activity (CAT). However, the highest level of CAT (67.3 %) was reported at the T3 level of treatment, followed by T2 (47.4%) and T3 (36.4%), respectively, when compared to the control. However, the minimum CAT activity (20.3%) was shown by treatment D compared to the control.

Discussion

Data presented in Table 1 showed the plant FW and DW of *M. peregrina* of stressed *M. peregrina* as influenced by different doses of TiO_2 NPs. Application of TiO_2 NPs showed positive effects on the plant FW and DW under drought stress conditions and significantly improved the negative effects of drought. Approximately all TiO_2 concentrations reversed the adverse effects of drought stress by enhancing the FW and DW of *M. peregrina*.

Growth is a major process significantly influenced by drought (Saikia *et al*., 2018). Inadequate water availability may result in retarded plant growth as cell division and elongation are the most sensitive plant functions which are vulnerable to water availability. Sensitivity of the cell might be attributed to the drop in turgor pressure and disruption of water movement through the xylem to the adjacent elongating cells (Azam *et al*., 2019). Besides these, reduced leaf area, root expansion, and elongation in search of water, metabolic changes, and oxidative damage are also caused by drought, resulting in poor plant growth (Farooq *et al*., 2017).

NPs, according to their unique properties, size, surface charge, shape, and potential interaction with plants, could help to decrease the drought effect. (Tarafdar *et al*., 2014). The influence of NPs on plants is highly reliant on their concentration as well. In this study, the most effective concentration against drought-induced effects in terms of plant FW and DW in *M. Peregrina* was spraying with 200 mg/ml. Similar to our findings increase in spinach dry mass and mung bean in response to TiO_2 NPs has been reported by Raliya *et al*., (2015). Our results are also consistent with the study of Gohari *et al*., (2020) in *D. moldavica.* Reportedly, foliar application of TiO_2 NPs can penetrate

the leaves through stomatal openings and then be transported to various tissues via symplast or apoplast pathways (Larue *et al*., 2012). In addition, NPs can accumulate nutrient elements on their surface and act as a nutrient reserve to the plants (Zulfiqar *et al*., 2019), the possible reasons behind the increase in FW and DW of treated *M. peregrina* plants. Similarly, TiO_2 NPs may act as a nano supplement to increase biomass production by promoting plant metabolic activities (Adeel *et al*., 2020).

In the conducted experiments TiO_2 treated *M. peregrina* seedlings, compared to untreated ones, were less negatively affected by drought stress in terms of plant root length, shoot length, and number of leaves. (Table 1). The significant increase was observed at all the levels of treatments. However, the maximum enhancement in all the morphological parameters was observed at T3 (200mg/L) level, respectively, compared to non-treated plants. Reportedly, NPs can enter the leaves via stomatal openings through the cell wall intercellular spaces (Adeel *et al*., 2020). To the contrary, TiO_2 NPs generate hydroxyl radicals; these radicals have been defined as a potential cell wall-loosening agent by unspecific cleavage of polysaccharides (Mohammadi *et al*., 2016). The TiO_2 NPs, which are passed through the apoplast in an appropriate concentration, would thus possibly loosen cell wall structure indirectly, which may stimulate cell enlargement and growth of the treated plant. Our result coincides with the findings of Gohari *et al*., (2020), who reported the positive effects of TiO_2 NPs on the number of leaves and plant height in response to salt stress. Rahneshan *et al*., (2018) reported that TiO_2 application improved the absorption rate of macro and micronutrients, which in turn improved plant growth attributes (e.g., plant height, leaf number) and reduced negative effects of stress. Similar to our results, different concentrations of TiO_2 NPs improved seed germination, plant biomass, and plant root length in *Lathyrus sativus* (Hojjat, 2020). TiO_2 NPs enhanced wheat plant growth and yield components in water-deficient conditions. (Jaberzadeh *et al*., 2013).

Foliar application of *M. peregrina* with different concentrations of TiO_2 NPs (50, 100, and 200) under drought conditions increased chlorophyll a, b, and total chlorophyll contents more than the control plants. This increase in pigment contents of *M. peregrina* was diverse between treatments. The highest increment in total chlorophyll content by 27% was observed in treatment T3 of TiO_2 NPs, followed by T2 and T1. Degradation of the light-receiving pigments would reduce photosynthetic rate and subsequently biomass production. (Aghdam *et al*., 2016). This may explain why DW in the present study was significantly reduced due to drought stress. Additionally, morphological characteristics such as the number of leaves on the plant also decreased.

The observed data suggest that chlorophyll may be related to growth attributes of *M. peregrina*, but the other factors could also be significant. TiO_2 is documented as a beneficial component for essential processes in plants, including photosynthesis, particularly at low concentrations (Ahmad *et al.*, 2018). According to Amaral *et al*., (2024), Rubisco plays a vital role in carbon fixation during photosynthesis and significantly impacts crop yield under diverse growth conditions. TiO_2 nanoparticles have been shown to notably enhance both the activity of Rubisco activase and its mRNA expression (Dias *et al.*, 2019).

Similarly, TiO_2 NPs can significantly improve the photochemical activity of photosystem II as well as stimulate energy transfer inside the photosystem (Su *et al.*, 2017). Reportedly, under stressed conditions, foliar application of TiO_2 NPs increased chlorophyll content in *Solanum lycopersicum* (Tiwari *et al*., 2017*) Vetiveria zizanioides* (Shabbir *et al.*, 2019) *Zea mays*, (Karvar *et al*., 2022), *Brassica napus* (Sehrish *et al*., 2023), *Mentha piperita* (Mohammadi *et al*., 2023).

MDA serves as an indicator to estimate the degree of lipid peroxidation and injury to the plasmalemma, along with organelle membranes, resulting from the damage caused by ROS due to environmental stresses (Nadarajah, 2020). According to Taibi *et al*., (2016), the assessment of lipid peroxidation also serves as an indicator of abiotic stress. Reduced MDA production signifies improved integrity of cell membranes. (Aghdam *et al*., 2016). The highest (67.3%) level of MDA content was verified in the untreated stressed (D) plants, whereas the TiO_2 NPs were found to lower the negative effect of drought stress in the treated plants in a dose-dependent manner. Our results are consistent with the study of (Alabdallah *et al*., 2021), who documented that the exposure of TiO_2 NPs decreases the MDA content in *Triticum aestivum* plants. In the conducted study, applying TiO_2 NPs in *M. peregrina* ameliorates the negative impact of drought stress by showing lower MDA content than the non-treated stressed plants. It has been highlighted that the application of exogenous TiO_2 NPs lowers the H_2O_2 levels by enhancing the activities of antioxidant enzymes and leads to a reduction in MDA content (Gohari *et al.,* 2020).

Nano-TiO_2 treatments caused an increase in total proline contents of *M. peregrina* plants under drought compared to control plants under normal irrigation. Proline is recognized as a multi-functional molecule (Sertan Cevik, 2022). Acts as an osmoprotectant, accumulating during drought stress to support cellular functions and maintain osmotic equilibrium (Bushra *et al*., 2023). Also, proline might protect cells by improving their ability to absorb water and aiding in the activation of enzymes (Hosseinifard *et al.*, 2022). While functioning as an osmolyte, proline is also observed as a strong antioxidative defense agent, a metal chelator, a ROS neutralizer, and an inhibitor of programmed cell death (Adejumo *et al.*, 2021). In the conducted experiment, the induction of proline accumulation might be due to the stimulation of proline synthesis through the glutamate pathway. Considerable evidence proves the positive association between the proline accumulation and enhanced stress tolerance in plants (Sadeghipour *et al*., 2020). Our results correlate with the findings of Shallan *et al*., (2016) & Ramadan *et al*., (2022). Higher levels of proline in TiO_2 NPs-treated plants have been shown to stabilize the protein structures and protein complexes, and act as ROS scavengers to counter the detrimental effects of different

abiotic stresses (Annunziata *et al*., 2019). The synthesis of excess proline to mitigate oxidative stress in plants exposed to metallic nanoparticles has been reported by numerous researchers. (Wang *et al*., 2020, Ahmed *et al*., 2021, Kumar *et al*., 2023).

Soluble proteins are important biomolecules of plant active substances. Accumulation of soluble proteins in different plant parts encourages the stimulation of metabolic enzymes, antioxidant enzymes, detoxification enzymes, and also some functional components for eradicating free radicals in plants under various stress conditions (Zhang *et al*., 2013). The data revealed that the total soluble proteins were enhanced significantly in *M. peregrina* leaves and observed the maximum percentage increase (91.7%) was observed after treating with nano-TiO_2 at 200 mg/L, while the lowest soluble protein content was recorded in drought-stressed plants without TiO_2 treatment. Similarly, in previous studies, an increase in protein content with the application of TiO_2-NPs has been observed for wheat (Ullah *et al*., 2020), tomato (Sertan Cevik, 2020) rice (Iqbal *et al*., 2023). These results indicate that TiO_2-NPs boosted the yield of soluble protein content in different crops by improving the uptake and accumulation of essential nutrients (Iqbal *et al*., 2023). Moreover, the increased soluble protein levels associated with the greater NPs concentrations may be linked to the synthesis of antioxidant enzymes to mitigate the induced stress (Hu *et al*., 2018).

Catalase (CAT), Ascorbate peroxidase (APX) and superoxide dismutase (SOD) are antioxidant enzymes that defend cells against oxidative stress of highly reactive free radicals. (Moustafa *et al*., 2020). The incomplete reduction of oxygen under drought stress conditions triggers the reactive oxygen species (Azam *et al*., 2019). ROS generation is considered as an oxidative impairment that is hazardous to the cell membrane, affecting protein, deoxyribonucleic acid, lipid molecules, and the generation of protease-resistant aggregates (Nita & Grzybowski, 2016). In this regard, resistance of plants to drought can be correlated to the efficacy of the antioxidant system to detoxify ROS (Lum *et al*., 2014).

Our study showed that the foliar application of nano-TiO_2 on *M. peregrina* under water deficit conditions augmented the activities of SOD, APX and CAT enzymes compared to control plants irrigated under normal conditions. The obtained results revealed that under drought stress, spraying of TiO_2 NPs at (T3) concentration 200 mg/L was more effective in enhancing the activities of antioxidant enzymes than other concentrations (50 and 100 mg/L) in *M. peregrina*. Reportedly, CAT is largely responsible for removing H_2O_2 from the peroxisomes. APX is the key enzyme for the elimination of H_2O_2 from the chloroplasts and SOD for catalyzing the dismutation of O^{-2} to O_2 and H_2O_2. (Shallan *et al*., 2016). Application nano-TiO_2 improve the activities of antioxidant enzymes such as CAT, APX, SOD, in plants under stress (Iqbal *et al*., 2023). Our results corroborate with the findings of Da Costa & Sharma (2016), Asl *et al*., (2021), Gonzalez-García *et al*., (2021), Mustafa *et al*., (2021), Karvar *et al*., (2022), who examined that NPs acquire the capability to augment the antioxidant enzymatic activities.

Conclusions

To our knowledge, this is an initial report on the assessment of TiO_2 NPs effects in *M. peregrina* under drought stress. In the present study, growth attributes of *M. peregrina* were decreased due to scarce water conditions, which consequently decreased the photosynthetic pigments, proline content, and antioxidant activities in non-treated plants. Our result showed that under drought stress, foliar application of TiO_2 NPs with different concentrations (50, 100, 200 mg/L) in *M. Peregrina* resulted in enhancement of growth parameters and increased the chlorophyll content, MDA content, proline content, total soluble proteins and antioxidant enzymatic activities as compared to untreated plants in dose dependent manner. It can be concluded from the obtained results, that the foliar application of TiO_2 NPs at higher concentration could significantly enhance the morphological attributes and biochemical activities in *M. peregrina* as it shows that plants tried to counter imposed stress by activated antioxidant defense system which was sufficient to defend the plants from detrimental effects of drought stress. Therefore, substantial use of TiO_2 NPs to decrease the adverse effects of drought stress in economically important plant such as *M. peregrina* is worth to be more investigated in detail.

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