NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 43, 2026
www.incda-fundulea.ro; doi.org/10.59665/rar4396 First Online: August, 2026. DII 2067-5720 RAR 2026-187

Alleviation of Water Deficit Stress in Sunflower Using Glycine Betaine and Titanium Dioxide Nanoparticles: Impacts on Oxidative Stress and Gas Exchange Processes

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ABSTRACT

Water stress due to drought leads to oxidative damage, loss of cell membrane stability, reduced gas exchange, and inhibition of growth and development. The current research evaluated the potential benefits of GB and TiO_2-NPs applied alone or in combination at 100 mM L^{-1} and 2 mM L^{-1}, respectively, for mitigating oxidative stress in sunflower plants under conditions of prolonged irrigation periods (20 days) as compared to the optimal period (14 days) over the years 2024 and 2025 seasons. It was found that continuous irrigation greatly elevated the contents of H_2O_2, $O_2{\bullet}^-$, and MDA, whereas the membrane stability was considerably reduced. On the other hand, spraying of GB and TiO_2-NPs alone or in combination ameliorated these unfavorable impacts through reduction in ROS and MDA concentrations, increasing the membrane stability, and accumulation of phenolics in plants exposed to water shortage conditions during two consecutive growing periods. Also, the foliar application resulted in an increase in total phenolics and activation of antioxidant enzymes (SOD, CAT, POD, APX, and GR), and the synergistic effects were more pronounced than the effects caused by the isolated application of the mentioned compounds. In contrast to the vast majority of past studies on individual impacts of GB or TiO_2-NPs, the current investigation proved that their combination is a better choice for improving drought tolerance, providing a promising approach to improve water use efficiency and stress tolerance in sunflower crops.

Keywords: antioxidant enzymes, hydrogen peroxide, membrane stability index, net photosynthesis, stomatal conductance, superoxide, transpiration rate.

INTRODUCTION

Drought is a major constraint limiting plant growth, yield, and agricultural productivity (Munns, 2011), and its frequency and severity are expected to increase under climate change (IPCC, 2023). Meanwhile, the global population is projected to rise from 7.6 to 9.8 billion by 2050 (United Nations, 2017), increasing pressure on food systems already threatened by water scarcity. Addressing this challenge requires developing drought-tolerant crops through better understanding of plant responses to drought stress (Earley et al., 2024). *Helianthus annuus* L. (sunflower), one of the world's major vegetable oil crops cultivated on about 27 million hectares globally (FAO, 2019), is highly affected by water deficit, which significantly reduces yield and oil quality (Ghaffari et al., 2023). Although relatively drought tolerant, sunflower productivity, grain yield, and oil content are markedly reduced under water stress conditions (Chimenti et al., 2002; Todorovic et al., 2009; Ghaffari et al., 2012; Akcay and Dagdelen, 2016).

Drought-induced oxidative stress results from excessive ROS production that exceeds the antioxidant defense system, leading to

Received 14 May 2026; accepted 18 August 2026.

damage of lipids, proteins, and cellular membranes (Yang et al., 2021). This impairs photosynthesis, accelerates leaf senescence, and reduces crop productivity. Drought also restricts CO_2 uptake, disrupting photosynthesis and respiration and increasing ROS accumulation such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) due to over-reduction of the chloroplast electron transport chain. In addition, metabolic disturbances and energy deficiency further enhance oxidative stress (Haghpanah et al., 2024).

Glycine betaine (GB) and titanium dioxide nanoparticles (TiO_2 NPs) alleviate oxidative damage and improve stomatal conductance (SC) under water deficit stress (de Oliveira Maia Júnior et al., 2020; Abdelhameed et al., 2025). Glycine betaine enhances osmotic adjustment by stabilizing proteins and membranes, boosts antioxidant enzymes including superoxide dismutase (SOD), and catalase (CAT), as well as reduces ROS accumulation. It also improves photosynthesis by supporting chlorophyll synthesis, protecting photosystem II, and regulating stomatal behavior to balance CO_2 uptake and water loss. Additionally, GB influences drought-responsive gene expression, and its external application is often necessary due to limited endogenous synthesis (Singh et al., 2025). Overall, it strengthens antioxidant capacity and increases phenolic compounds, improving tolerance to drought stress (Jarin et al., 2024; Basit et al., 2025).

Titanium dioxide nanoparticles (TiO_2 NPs) enhance drought tolerance by strengthening antioxidant defense systems and maintaining cellular integrity. They stimulate antioxidant enzymes such as SOD, peroxidase (POD), and CAT, thereby reducing ROS accumulation (El-Beltagi et al., 2023). TiO_2 NPs also protect photosynthetic pigments, preserve membrane stability, and improve SC and gas exchange. Green synthesis using plant extracts such as Aloe vera enables sustainable production of TiO_2 NPs, which improve crop productivity under drought and salinity stress, particularly in soybean plants (Abdelhameed et al., 2025). Furthermore, TiO_2 NPs mitigate oxidative stress (OS) by lowering ROS levels and increasing phenolic compound accumulation, thereby enhancing tolerance to abiotic stresses, including salinity and temperature fluctuations (Gohari et al., 2020; Chandoliya et al., 2024).

New research indicates that the separate treatment of GB and TiO_2 NPs could increase the tolerance of plants to drought stress (Basit et al., 2025; Singh et al., 2025; Turan and Yağci, 2025). However, limited studies have investigated their combined effects on reducing oxidative stress across different crops. In particular, the synergistic role of GB and TiO_2 NPs in mitigating oxidative stress and enhancing plant physiological performance under water deficit conditions remains poorly understood, especially in sunflower. Therefore, this study aims to evaluate the individual and combined effects of GB and TiO_2 NPs on reducing oxidative stress and improving the physiological functions of sunflower plants under drought stress.

MATERIAL AND METHODS

Study site, experimental treatments and design

The field experiment was conducted during the 2024 and 2025 summer seasons at the Sakha Agricultural Research Station (31°07′N, 30°05′E), Egypt. Glycine betaine (GB; 100 mM L^{-1}) and TiO_2 nanoparticles (TiO_2 NPs; 2 mM L^{-1}) were applied as foliar sprays individually or in combination under prolonged irrigation intervals of 20 days compared with the optimal interval of 14 days in sunflower plants. Sprays were applied until complete leaf wetness without runoff, twice during growth: at stem elongation (25 days after planting) and at flowering (50 days after sowing). TiO_2 NPs were obtained from Pishgaman Nano Materials Co. and dispersed in triple-distilled water using ultrasonic dispersion according to the supplier's instructions. The experiment was arranged in a randomized complete block design with four replicates.

Field preparation and agricultural practices

The field was prepared after harvesting wheat from the previous season. The soil was ploughed twice, then harrowed and ridged at

0.7 m spacing, and divided into 42 m² plots (10 rows per plot; each row 6 m long and 0.7 m wide). To prevent cross-contamination of water and treatments, plots were separated by 1.5 m wide ditches. Sunflower seeds were sown in hills spaced 25 cm apart along one side of each ridge and later thinned to one plant per hill before the first irrigation (21 days after sowing).

Each plot received 40 kg P_2O_5/ha in the form of calcium superphosphate (15.5% P_2O_5). This was done when preparing the soil before planting. The recommended nitrogen application rate was 60 kg N per feddan, applied as urea. Planting occurred on the 15th and 18th of May, and harvests took place on the 2nd and 6th of September for the 2024 and 2025 season, respectively. Agronomic standards, such as the control of weeds and pests, were strictly followed according to the Agricultural Research Center guidelines of Giza, Egypt.

Physiological and biochemical traits

Physio-biochemical traits were measured in the third leaf from the plant tip, 70 days after sowing.

Hydrogen peroxide and superoxide anion

The hydrogen peroxide concentration was measured using the procedure described by Sergiev et al. (1997). This involved grinding 0.5 g of fresh plant material in 1% trichloroacetic acid (TCA) followed by centrifugation at 12,000 × g for 20 minutes. The supernatant was subsequently added to 0.7 mL of 10 mM phosphate buffer (pH 7.0) and 1.4 mL of 1 M potassium iodide. Absorbance was measured at 390 nm. Production of the superoxide radical ($O2^{\bullet -}$) was evaluated according to the method described by Acharya et al. (2011). Leaves were treated with reaction mixture consisting of 50 mM Tris-HCl buffer (pH 6.4), 0.2 mM nitroblue tetrazolium (NBT), 0.2 mM NADH, and 250 mM sucrose. The mixture was placed under vacuum infiltration for 10-15 minutes, after which the sample was exposed to illumination (200 µmol m^{-2} s^{-1}) for 24 hours. Spectrophotometric analysis of absorbance was carried out at 530 nm, whereas superoxide radicals were quantified based on extinction coefficient of 12.8 mM^{-1} cm^{-1}.

Malondialdehyde content and membrane stability index

The malondialdehyde (MDA) level was estimated via the thiobarbituric acid (TBA) assay as per Du and Bramlage (1992). Leaf tissue samples (0.4 g) were ground in liquid nitrogen and extracted with 80% ethanol, followed by centrifugation at 6000 rpm for 5 min. For MDA determination, 0.7 mL of the extract was mixed with 0.7 mL of 0.65% TBA in 20% TCA containing 0.01% BHT, while control samples contained extract with 20% TCA and BHT only. Samples were incubated at 95°C for 25 min, cooled, and re-centrifuged if necessary. Absorbance was recorded at 440, 532, and 600 nm, and MDA content was calculated using an extinction coefficient of 157 mM^{-1} cm^{-1}, expressed as nM g^{-1} fresh weight. Membrane stability was assessed in leaves at 70 days after sowing according to Lutts et al. (1996).

Total phenols content

Total phenolic compounds were assessed following the method of Bessada et al. (2016).

Preparation of enzyme extract

Frozen soybean leaves were ground in liquid nitrogen for antioxidant enzyme extraction. The powdered tissue was homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5% sucrose and 0.1% 2-mercaptoethanol at a 3:1 buffer-to-tissue ratio. The homogenate was centrifuged at 10,000 × g for 20 min, and the resulting supernatant was collected for further analysis. All procedures were performed under refrigerated conditions to maintain enzyme stability and activity.

Estimation of enzymes activity

Catalase activity (CAT, EC 1.11.1.6) was measured using Aebi's (1984) procedure based on the decrease in H_2O_2 absorbance at 240 nm. The determination of Peroxidase activity (POD, EC 1.11.1.7) followed the procedure used by Polle et al. (1994). A single unit of enzyme activity is defined as the amount of enzyme required to oxidize 1 μmol of ascorbate per minute per milligram of protein. Superoxide dismutase activity (SOD, EC 1.15.1.1) was measured using the nitro blue tetrazolium (NBT) photoreduction technique of Giannopolitis and Ries (1977). The quantity of SOD activity was calculated as the enzyme quantity needed for 50% inhibition of NBT photoreduction at 560 nm.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined following Nakano and Asada (1981) by monitoring the decrease in absorbance at 290 nm due to ascorbate oxidation, using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Glutathione reductase (GR; EC 1.6.4.2) activity was measured spectrophotometrically based on NADPH oxidation at 340 nm according to Foyer and Halliwell (1976). The reaction mixture contained phosphate buffer, EDTA, NADPH, oxidized glutathione (GSSG), and enzyme extract, with an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

The activities of all enzymes were quantified based on protein content (U/mg of protein). The total amount of soluble protein was measured through the Bradford assay (Bradford, 1976) using bovine serum albumin (BSA) as the reference protein.

Stomatal conductance, net photosynthesis and transpiration rate

The SC, transpiration, and photosynthesis rates were determined by using a steady-state porometer (LI-COR LI-1600, Lincoln, NE, USA) having a lithium chloride sensor. The porometer was calibrated according to the technique provided by Krishnan et al. (2011). The readings were recorded from the fully developed leaves from each repetition. The SC and photosynthesis were presented in μmol m^{-2} s^{-1} and transpiration in mmol m^{-2} s^{-1}.

Statistical Analysis

The collected data were analyzed using analysis of variance (ANOVA), and treatment effects were considered significant at $p \leq 0.05$. All analyses were performed using the COSTAT statistical software (CoHort Software, 1986). Following the detection of significant differences, treatment means were compared via Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Oxidative stress indicators

Figures 1 (a-d) illustrate that prolonging the irrigation interval from 14 days to 20 days increased the concentration of ROS *i.e.* H_2O_2 and $O_2{\bullet}^-$ in sunflower plants when compared to the optimal irrigation interval of 14 days in the seasons of 2024 and 2025. There were increases in hydrogen peroxide concentration of 355.9% and 292.3%, and increases in $O_2{\bullet}^-$ concentration of 328.5% and 328.2% in 2024 and 2025, respectively. Nevertheless, spraying with GB and TiO_2 NPs, regardless of whether they were used individually or in conjunction, successfully inhibited the generation of H_2O_2 and $O_2{\bullet}^-$ under prolonged irrigation intervals during the two seasons. Out of all the individual applications, GB was found to be more effective than TiO_2 NPs in alleviating ROS accumulation, but the combination therapy showed the greatest efficacy in lowering H_2O_2 and $O_2{\bullet}^-$ concentrations. Glycine betaine was able to inhibit H_2O_2 concentration by 48.8% in the two seasons, while TiO_2 NPs were able to inhibit H_2O_2 concentration by 37.6% and 29.7% in 2024 and 2025, respectively. Notably, the combined treatment resulted in the highest reduction, lowering H_2O_2 by 62.9% and 61.7% and $O_2{\bullet}^-$ by 72.2% and 66.8% in the two respective seasons.

Malondialdehyde content and membrane stability index

As illustrated in Figures 1 (e-h) that the increase in the irrigation interval caused the increment in malondialdehyde (MDA) content and the reduce in membrane stability index in sunflower plants when compared to control plants (14-day irrigation interval) for two seasons. The increase in MDA content was by 189.3% and 178.4%, whereas membrane stability index decreased by 48.3% and 43.3% for the 2024 and 2025 seasons, respectively. On the contrary, foliar spraying of GB and TiO_2 nanoparticles separately or together was capable of overcoming such negative effects under the extended irrigation interval in both seasons. In comparison to TiO_2 nanoparticles, GB proved to be better for the reduction in MDA and improvement in membrane stability, although their combination was the most efficient in this regard. Specifically, the combined treatment reduced MDA levels by 68.1% and 67.5% and increased membrane stability by 85.9% and 72.8% in the 2024 and 2025 seasons, respectively.

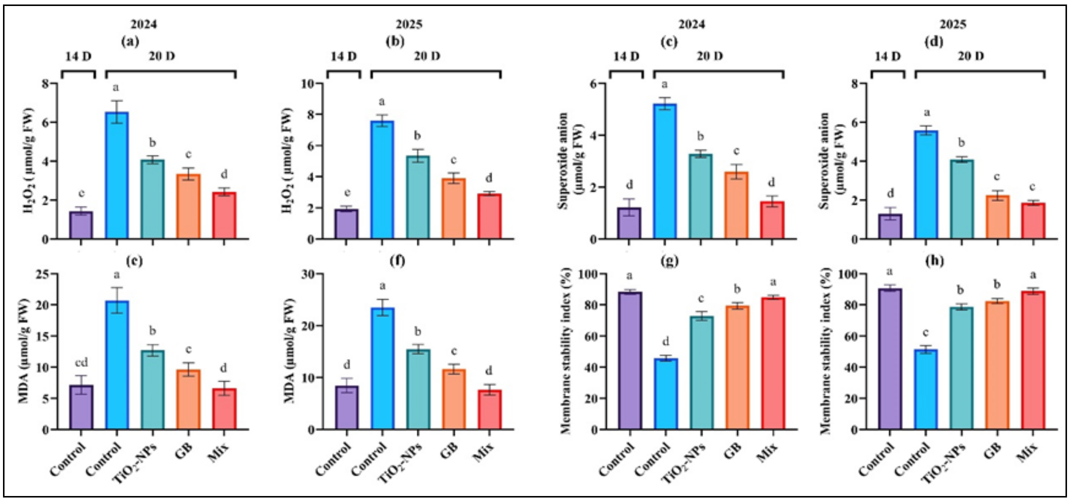

Figure 1. Effect of foliar spraying of GB and TiO_2 NPs, applied separately or together (Mix), on OS indicators and membrane stability in sunflower plants under a 20-day irrigation interval compared with the optimal 14-day interval during the 2024 and 2025 seasons. The assessed parameters include hydrogen peroxide content (a, b), superoxide anion (c, d), malondialdehyde (e, f), and membrane stability (g, h). Different letters above the bars indicate significant differences among treatments at $p \leq 0.05$ using Duncan's multiple range test.

Antioxidant enzymes activity

From Figure 2, it is evident that increasing the irrigation interval greatly boosted AOE activities like SOD, CAT, POD, APX, and GR, in comparison with the optimal irrigation interval during both seasons of growth. However, an enhancement in enzyme activities was also achieved after spraying GB and TiO_2 NPs, either alone or together. From the individual treatments, GB was more successful in increasing the activities of the enzymes than TiO_2 NPs. When both substances were applied together, the activities of the enzymes were higher than when other treatments were applied. In particular, the combined application elevated the activities of SOD by 89.4% and 86.8%, CAT by 104.6% and 96.3%, POD by 87.2% and 126.9%, APX by 92.6% and 74.0%, and GR by 213.9% and 132.1% during the 2024 and 2025 seasons, respectively.

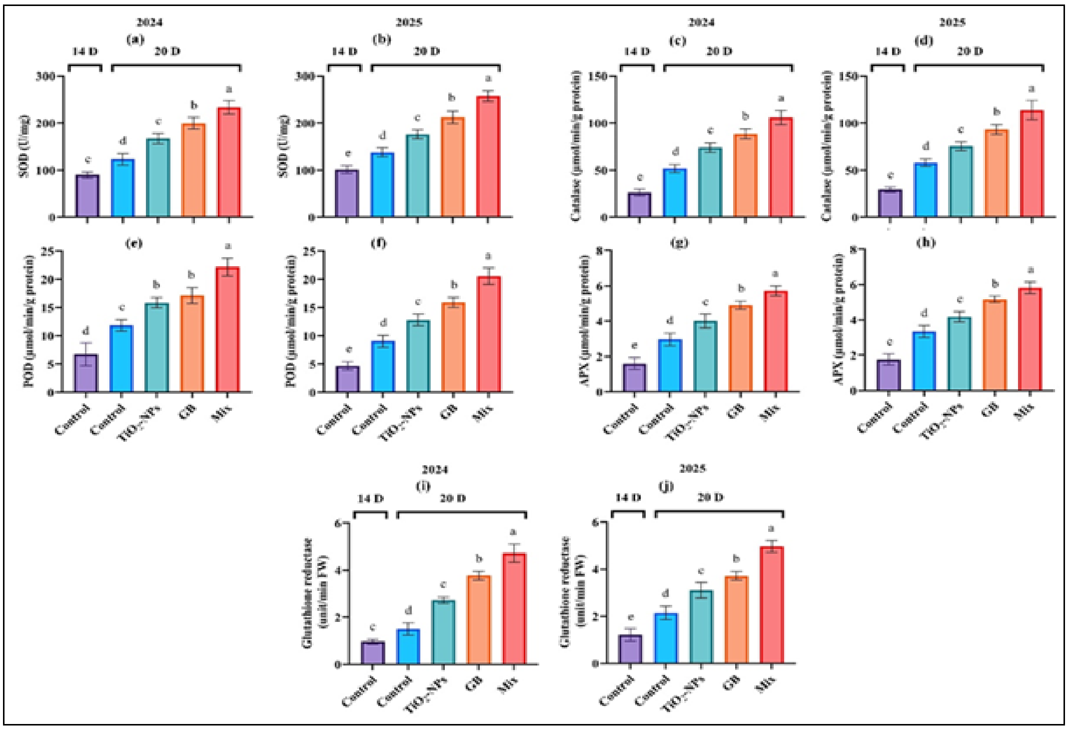

Figure 2. Effect of foliar use of GB and TiO_2 NPs, applied either alone or in combination (Mix), on AOE activities in sunflower plants under a 20-day irrigation interval compared with the optimal 14-day interval during the 2024 and 2025 growing seasons. The evaluated enzymes include SOD (a, b), CAT (c, d), POD (e, f), APX (g, h), and GR (i, j). Bars annotated with different letters reflect significant differences among treatments at $p \leq 0.05$ based on Duncan's multiple range test.

Stomatal conductance, net photosynthesis and transpiration rate

As indicated in Figure 3, prolonged intervals between watering greatly reduced the values of SC, transpiration rate (TR) and net photosynthesis (NP) when compared to well-irrigated plants (14 days interval). For the 2024 and 2025 growing seasons, reductions were observed at 81.6% and 87.4% for SC, 65.2% and 72.8% for NP, and 72.8% and 75.6% for TR. As opposed to this, use of GB and TiO_2 NPs (alone or together) enhanced the levels of gas exchange parameters under prolonged irrigation intervals. For the treatments applied separately, it is worth mentioning that GB gave better results than TiO_2 NPs in terms of enhancement of SC, photosynthesis rate, and transpiration. When combined, the results indicated maximum improvement compared to other treatments. For the 2024 and 2025 growing seasons, improvements were seen at 357.7% and 660.9% for SC, 156.1% and 227.7% for NP, and 204.0% and 255.8% in the 2024 and 2025 seasons, respectively.

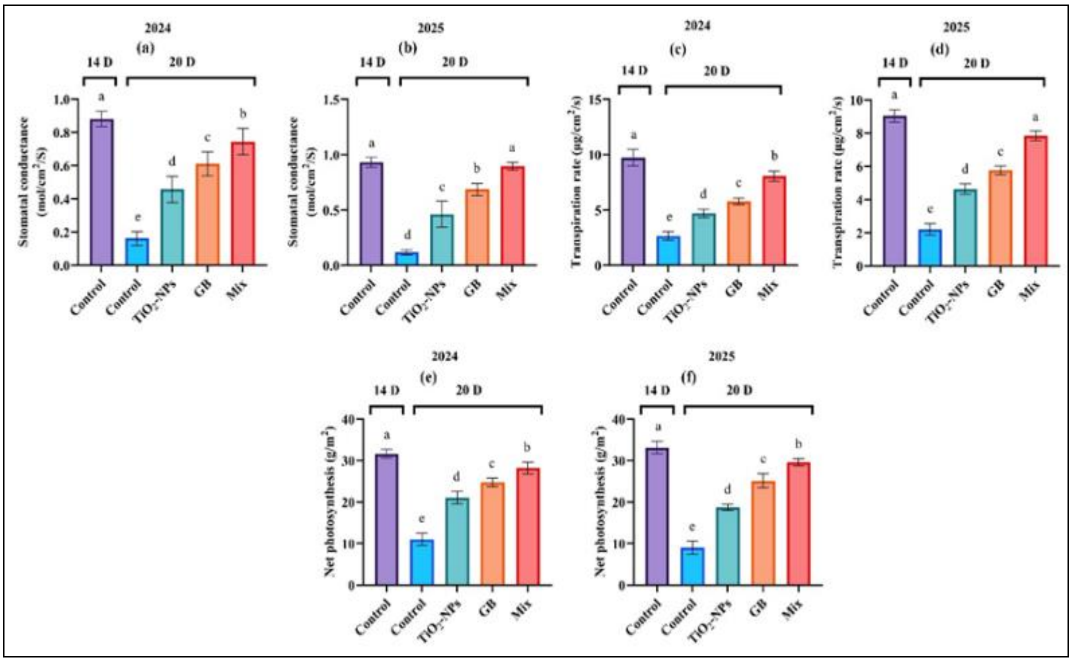

Figure 3. Impact of foliar spraying of glycine betaine (GB) and titanium dioxide nanoparticles (TiO_2 NPs), applied alone or together (Mix), on gas exchange parameters in sunflower plants under a 20-day irrigation interval compared with the optimal 14-day interval during the 2024 and 2025 growing seasons. The measured traits include SC (a, b), transpiration rate (c, d), and net photosynthetic rate (e, f). Bars annotated with different letters reflect significant differences among treatments at $p \leq 0.05$ based on Duncan's multiple range test.

Total phenols content

Figure 4 reveals that an increase in the irrigation interval greatly increased the total phenolic content, when compared to the well-irrigated treatment during both growing seasons. The total phenol content further increased after the foliar spraying of GB and TiO_2 NPs, which could be performed either alone or together. Out of the two individual treatments, GB was better at promoting total phenols content when compared to TiO_2 NPs; however, the mixture of the two treatments gave the maximum total phenolic content during both growing seasons. Specifically, the combined treatment increased total phenolic content by 104.4% and 108.3% in the 2024 and 2025 seasons, respectively.

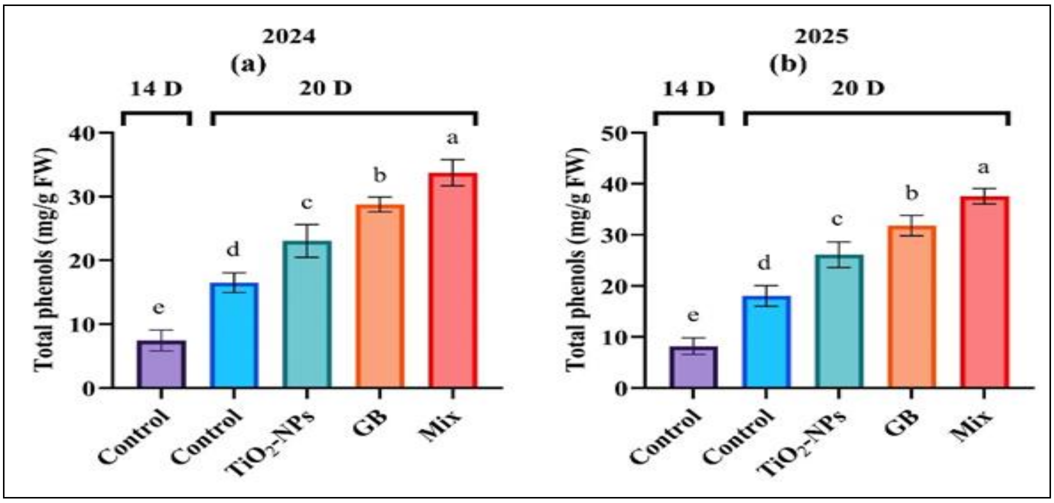

Figure 4. Impact of foliar use of glycine betaine (GB) and titanium dioxide nanoparticles (TiO_2 NPs), applied individually or in combination (Mix), on phenolic content in sunflower plants under a 20-day irrigation interval compared with the optimal 14-day interval during the 2024 and 2025 growing seasons. Bars annotated with different letters reflect significant differences among treatments at $p \leq 0.05$ based on Duncan's multiple range test.

Water deficit induces oxidative stress by enhancing ROS production, which causes lipid peroxidation and increases MDA levels, ultimately reducing membrane stability, growth, and productivity. In this study, extending irrigation intervals from 14 to 20 days elevated H_2O_2 and $O2^{\bullet-}$ accumulation in sunflower due to disrupted electron transport in chloroplasts and mitochondria, leading to electron leakage. These ROS subsequently damage lipids, proteins, and nucleic acids, impairing cellular functions and organelle integrity (Hussain et al., 2019; Yang et al., 2021; Haghpanah et al., 2024). Although antioxidant enzymes (SOD, CAT, POD, APX, and GR) and non-enzymatic antioxidants such as phenolics increased, severe drought exceeded the capacity of these defense systems. Drought stress reduces stomatal conductance, net photosynthesis, and transpiration through ABA-induced stomatal closure, limiting CO_2 uptake. Stomatal regulation is further modulated by hormones such as salicylic acid, jasmonates, ethylene, and cytokinins (Rhaman et al., 2025). Reduced CO_2 availability, along with photooxidative damage and Rubisco inhibition, further suppresses photosynthetic activity. Although decreased transpiration helps conserve water, it compromises leaf cooling. Ultimately, loss of turgor pressure and impaired xylem transport shift plant metabolism from growth toward survival under drought conditions (Wang et al., 2026). Compared with water deficit stress (WDS) alone, application of GB and/or TiO_2 NPs to sunflower plants under prolonged irrigation intervals alleviated the detrimental effects of drought by reducing ROS accumulation and MDA content, while enhancing membrane stability, antioxidant activity, and total phenolic concentrations. Glycine betaine (GB) protects photosystem II and thylakoid membranes by reducing electron leakage and ROS formation, while stabilizing membrane phospholipids through osmotic regulation, thereby decreasing electrolyte leakage and maintaining membrane integrity under water deficit conditions (Annunziata et al., 2019; Nawaz and Wang, 2020). The reduction in MDA content under GB reflects lower lipid peroxidation and oxidative damage (Nawaz and Wang, 2020). Glycine betaine also enhances antioxidant enzyme activities (SOD, CAT, POD, APX) and promotes accumulation of non-enzymatic antioxidants such as phenolics and flavonoids, improving ROS scavenging efficiency (Jarin et al., 2024). In addition, it increases glutathione reductase (GR) activity, strengthening the glutathione ascorbate cycle, stabilizing redox balance, and improving drought tolerance (Jarin et al., 2024; Basit et al., 2025). However, GB can reduce stomatal conductance (SC), net photosynthetic rate (NP), and transpiration due to osmotic adjustment and stomatal regulation, which limits CO2 diffusion despite protecting the photosynthetic machinery. Accordingly, Shemi et al. (2021) and Khalid et al. (2022) noted that although GB enhances antioxidant and osmotic defenses under drought stress, restricted CO_2 availability may still limit photosynthetic activity.

Titanium dioxide nanoparticles (TiO_2 NPs) mitigate drought-induced oxidative damage by regulating photosynthetic electron transport, thereby reducing ROS generation and membrane oxidation. This is reflected in decreased MDA levels, indicating reduced membrane damage, alongside improved membrane stability due to lower electrolyte leakage and preserved lipid bilayer integrity (Rasheed et al., 2022; Daler et al., 2024). TiO_2 NPs also enhance antioxidant enzyme activities (SOD, CAT, APX, and GR), promoting efficient ROS detoxification and maintaining glutathione in its reduced form, while increasing non-enzymatic antioxidants such as phenolics and flavonoids, thereby strengthening overall stress tolerance (Ramadan et al., 2022; Daler et al., 2024). In addition, they improve drought tolerance by enhancing stomatal conductance (SC), photosynthesis, and transpiration through improved guard cell function and water balance. TiO_2 NPs further protect photosystem II (PSII) by stabilizing chloroplast membranes and enhancing electron transport, as evidenced by increased Fv/Fm ratios in sweet corn under deficit irrigation (Karvar et al., 2022). Overall, TiO_2 NPs help maintain gas exchange and osmotic balance under drought conditions, thereby improving photosynthetic efficiency (Daler et al., 2024).

Glycine betaine (GB) exhibited greater effectiveness than TiO_2 NPs in alleviating drought-induced oxidative and physiological stress due to its dual role as an osmoprotectant and molecular chaperone, whereas TiO_2 NPs mainly act through indirect enhancement of antioxidant defense systems. GB reduces ROS generation and lipid peroxidation, thereby lowering MDA accumulation and improving membrane integrity and ionic balance (Shemi et al., 2021; Singh et al., 2025). In contrast, the effects of TiO_2 NPs depend largely on stimulation of antioxidant defenses and may vary with the applied dose (Chandrashekar et al., 2023). Glycine betaine also provides superior membrane protection through its incorporation into lipid bilayers, enhancing membrane fluidity and integrity, while stimulating antioxidant enzymes such as SOD, CAT, APX, and GR, in addition to non-enzymatic antioxidants including phenolics, for efficient ROS scavenging. Regarding gas exchange, GB ensures more stable osmoregulation and stomatal regulation, whereas TiO_2 NPs show relatively variable effects on SC and photosynthetic activity. Overall, GB was more effective in improving plant growth and survival under water-deficit conditions. However, the combined application of GB and TiO_2 NPs produced the best results due to their complementary modes of action, where GB directly regulates osmotic balance and ROS detoxification, while TiO_2 NPs enhance antioxidant enzyme activity and photosynthetic performance. This synergistic interaction improved cellular water status and biochemical protection simultaneously, resulting in greater membrane stability, lower oxidative damage, and enhanced carbon assimilation under prolonged irrigation intervals.

As illustrated in Figure 5, foliar spraying of GB (100 mM L^{-1}) and TiO_2-NPs (2 mM L^{-1}), whether applied separately or together to sunflower plants at 25 and 50 days after sowing, under an extended irrigation interval of 20 days compared with the optimal 14-day interval, effectively alleviated the harmful effects of WDS. These treatments reduced the production of H_2O_2 and $O_2^{\bullet-}$ as well as MDA accumulation. In addition, they enhanced membrane stability, improved gas exchange traits (SC, NP, and TR), and increased total phenolic content along with the activity of antioxidant enzymes, including SOD, CAT, POD, APX, and GR. Notably, the combined treatment proved more effective than the individual applications.

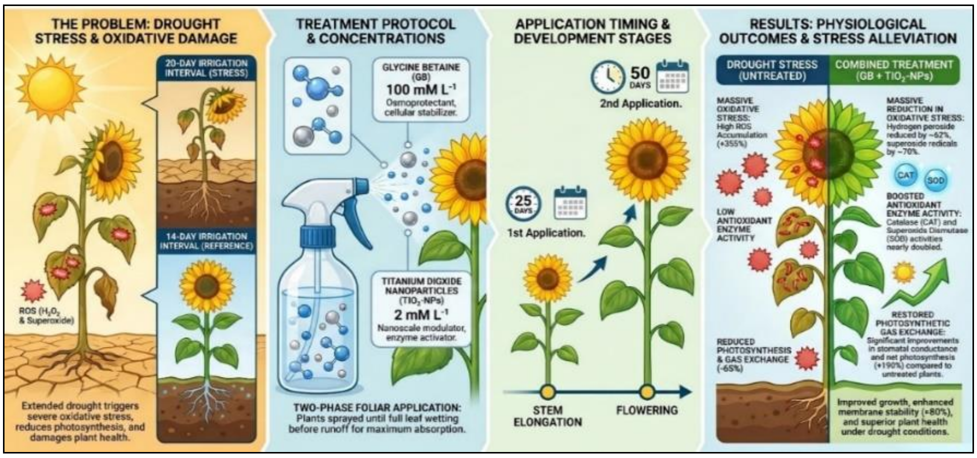

Figure 5. Schematic representation illustrating the positive impact of foliar use of glycine betaine (100 mM L^{-1}) and titanium dioxide nanoparticles (2 mM L^{-1}), applied individually or in combination to sunflower plants at 25 and 50 days after sowing, under a prolonged irrigation interval of 20 days compared with the optimal interval of 14 days. These treatments decreased the generation of H_2O_2 and $O_2^{\bullet-}$ as well as MDA accumulation, while enhancing membrane stability index, gas exchange traits (SC, net photosynthetic rate, and transpiration), total phenolic content, and the activity of AOE including SOD, CAT, POD, APX, and GR. Notably, the combined treatment exhibited greater effectiveness than the individual applications.

CONCLUSIONS

It was revealed that when applied simultaneously in sunflower plants, glycine betaine and titanium dioxide nanoparticles had a synergic impact on the antioxidant activity of the plants and were thus better at alleviating oxidative stress, stabilizing the cell membranes, increasing the phenol content, and facilitating gas exchange in long intervals of irrigation (20 days). While earlier studies have mostly concentrated on the effects of glycine betaine and titanium dioxide nanoparticles separately on the antioxidant mechanisms of plants, their simultaneous application seems to be an effective strategy to increase water use efficiency and plant tolerance to stress under water deficit conditions.

FUNDING

Financial assistance for this work was granted by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research at King Faisal University, Saudi Arabia, under Grant No. KFU262285.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial assistance provided by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research at King Faisal University, Saudi Arabia (Grant No. KFU262285).

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