Effects of Biostimulants on Alleviating Drought Stress in Isparta Rose

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Drought stress is one of the major abiotic factors limiting the growth and productivity of *Rosa damascena*. This study aimed to evaluate the effects of different biostimulant applications on the antioxidant defense system and biochemical responses of *R. damascena* under varying irrigation levels. Plants were grown under three irrigation regimes (100%, 50%, and 25% of field capacity (FC)) for 28 days, and treatments included vermicompost extract (V), *Bacillus* OSU-142 (B), and an algal extract (A) collected from Eğirdir Lake. Antioxidant enzyme activities, oxidative stress markers (MDA, H_2O_2), and proline content were measured weekly. Results showed that decreasing irrigation levels induced significant increases in antioxidant enzyme activities, MDA, H_2O_2, and proline content, indicating oxidative and osmotic stress responses. Under well-watered conditions (100% FC), biostimulant applications had limited effects on these parameters. However, under moderate (50% FC) and severe drought stress (25% FC), biostimulant treatments effectively reduced MDA, H_2O_2, and proline accumulation, and led to lower antioxidant enzyme activities compared to controls. *Bacillus* OSU-142 and vermicompost were particularly effective treatments in mitigating oxidative damage and maintaining cellular homeostasis under severe water deficit. These findings show that biostimulants reduce ROS accumulation and lipid peroxidation while modulating antioxidant defense in *R. damascena.*

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INTRODUCTION

Rosa x damascena Mill. belongs to the genus *Rosa* within the family Rosaceae. Known worldwide as the Damask rose, *R. x damascena* is a highly valuable aromatic plant due to its essential oil content. In Turkey, it is also referred to as the "pink oil rose," "oil rose," "Kazanlık rose," "Damascus rose," and "Isparta rose" (Tiryaki *et al*. 2023; Çelik and Pepe 2024). The oil-bearing rose is cultivated primarily for industrial rose oil production in countries such as Turkey and Bulgaria, as well as in Morocco, Egypt, Iran, Syria, India, Pakistan, and the Caucasus region. In recent years, its cultivation has also expanded for medicinal purposes.

Stress in plants refers to external environmental conditions that negatively affect their growth, development, or productivity (Verma *et al*. 2013). Such stress factors can alter fundamental metabolic processes, including gene expression, cellular metabolism, growth rate, and yield. Plant stress is often associated with sudden changes in environmental conditions. However, certain plant species with inherent stress tolerance can gradually adapt to these adverse conditions over time, thereby enhancing their resilience (Çelik 2023). Plant stress is generally categorized into two main types: abiotic and biotic stress. Abiotic stress arises from physical or chemical environmental factors such as temperature extremes, drought, or salinity, whereas biotic stress is caused by living organisms, including pathogens and insect pests (Verma *et al*. 2013; Çelik 2023). Depending on the severity and duration, these stressors can disrupt plant metabolism and cause significant damage. While mild or short-term stress may be mitigated through the plant's internal repair mechanisms, severe stress can accelerate senescence, inhibit flowering and seed formation, and ultimately lead to plant death (Verma *et al*. 2013). Such plants are considered stress-sensitive. Conversely, certain species such as desert ephemerals have evolved strategies to completely avoid stress conditions (Zhu 2002).

Abiotic stress factors such as drought (water deficit), excessive irrigation (flooding), extreme temperatures (frost, cold, and heat), salinity, and mineral toxicity adversely affect plant growth, development, yield, and seed quality (Naeem *et al*. 2013). With the anticipated decline in freshwater resources in the future, the intensity and impact of such abiotic stresses are expected to increase (Kijne 2006). Consequently, the development of plant varieties with enhanced tolerance to abiotic stress has become essential for ensuring sustainable agricultural production in the coming years. The root system serves as the first line of defense in plants against abiotic stress. When plants grow in soils that are biologically diverse and well-structured, their chances of surviving under stress conditions significantly improve (He *et al*. 2018).

Plants' abilities to adapt to environmental changes requires the establishment of a new cellular homeostasis, in which multiple signaling and metabolic pathways across different cellular compartments are tightly coordinated. However, this coordination can be disrupted, particularly under water and salt stress conditions (Mittler *et al*. 2004). During stress, high-energy electrons are transferred to molecular oxygen (O_2), leading to the formation of reactive oxygen species (ROS) (Mittler 2004). ROS such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical ($HO\cdot$) are highly reactive molecules that are capable of causing oxidative damage to proteins, DNA, and lipids (Mittler *et al*. 2004). Under optimal growth conditions, ROS are generated at low levels mainly in organelles such as chloroplasts, mitochondria, and peroxisomes. However, under stress conditions, their production increases significantly. The accumulation of reactive oxygen species (ROS) under stress conditions largely depends on the balance between their production and elimination (Mittler *et al*. 2004). Maintaining this balance is closely associated with the plant's ability to rapidly adjust to changes in environmental growth conditions (such as light intensity and temperature), the duration and severity of the stress, as well as the energy status of the tissue. In chloroplasts, the primary cause of ROS generation is the over-reduction of the photosynthetic electron transport chain, often due to insufficient CO_2 fixation. Similarly, in mitochondria, one of the main mechanisms leading to ROS production is the over-reduction of the mitochondrial electron transport chain. In peroxisomes, hydrogen peroxide (H_2O_2) is generated during the oxidation of glycolate to glyoxylate in the process of photorespiration. During normal metabolic activity and especially under stress conditions, the plant employs a defense system comprising non-enzymatic antioxidants such as ascorbic acid (AsA) and glutathione (GSH), as well as enzymatic antioxidants including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PrxR). These protective mechanisms are present in nearly all cellular compartments and play a critical role in limiting oxidative damage and preventing cell death (Mittler *et al*. 2004; Çelik and Karakurt 2022).

In response to the adverse environmental impacts of conventional agriculture, there has been a growing shift toward sustainable farming systems that emphasize environmentally conscious management practices. Such approaches focus on improving nutrient availability and uptake, supporting plant growth and fruit quality, and enhancing resilience to abiotic stresses including low temperatures, salinity, and drought (Mahmoud *et al*. 2024). Moreover, these practices contribute to the suppression of bacterial, fungal, and nematode infestations and may help mitigate viral infections (Siddiqui 2006; Mosa *et al*. 2014; Vishwakarma *et al*. 2024). A range of inputs—such as mycorrhizal inoculants, compost, vermicompost, rhizobacteria, animal manure, algae-derived products, humic substances, leonardite, molasses, and certain minerals such as titanium compounds—are recognized for their roles in promoting environmental sustainability in agriculture (Cankurt and İpek 2023; Zahra *et al*. 2024).

Notably, rhizobacteria, algae, and vermicompost can be applied both to soil and as foliar treatments, acting as biostimulants that exert beneficial effects throughout all stages of plant development, from germination to fruiting. They support nutrient uptake, assimilation, and translocation within plants, and they can improve water-use efficiency while enhancing soil structure and reducing susceptibility to erosion (Dudás *et al*. 2017). Plant growth-promoting rhizobacteria (PGPR) are soil- and root-associated microbes that are increasingly used as biofertilizers because of their capacity to enhance nitrogen fixation, improve water-use efficiency, stimulate phytohormone production, increase phosphorus availability, and lower ethylene levels in plants, thereby promoting growth (Ekici *et al*. 2015; İpek and Esitken 2024). Vermicompost, produced by the aerobic decomposition of organic matter through the activity of earthworms, is rich in beneficial microorganisms and humic substances, offering high water-holding capacity and microbial activity that improve soil quality and plant performance (Atiyeh *et al*. 2000; Kara Özbek and Dalkılıç 2017).

Algae-based products are another important natural input in sustainable agriculture, supplying bioactive compounds that can enhance plant growth, increase sugar content, and improve fruit color and overall quality (Battacharyya *et al*. 2015; Dmytryk and Chojnacka 2018). Overall, the use of biostimulants such as PGPR, vermicompost, and algae contributes to improved plant stress tolerance without disrupting key metabolic processes, while supporting nutrient assimilation, water-use efficiency, and soil health (Dudás *et al*. 2017).

Drought stress has significant negative effects on plant yield and oil quality. Particularly in oilseed plants, water limitation can reduce the synthesis of fatty acid components and volatile oil production. This disrupts the plant’s metabolic processes, limits photosynthesis, and increases oxidative stress, leading to an imbalance in biochemical processes. To mitigate the severity of drought stress, various external applications have been used to enhance plant resistance to drought. The aim of this study was to investigate the effects of different biostimulants (PGPR, vermicompost, and lake algae extract) on alleviating drought stress in Isparta rose (*Rosa x damascena* Mill.) in terms of antioxidant enzyme activities and biochemical compounds. In this context, the study aimed to analyze the changes occurring in the plant’s antioxidant enzyme systems and biochemical contents under drought conditions. Furthermore, this study is one of the first to investigate the effects of such biostimulants on alleviating drought stress in Isparta rose.

EXPERIMENTAL

Material

The plant material used in this study consisted of one-year-old rose *(Rosa x damascena* Mill.) seedlings grown in Isparta (Türkiye). The seedlings were planted in 8-liter polyethylene tubes (25 × 50 cm in size) with holes at the bottom, filled with a mixture of sand, soil, and peat in a 1:2:1 ratio, and transferred to a greenhouse. The growing medium in the experiment was slightly alkaline, with very high lime content, no salt, good organic matter, and a loamy texture. The quality class of the irrigation water, according to the US salinity laboratory classification system, was C2S1, which is suitable for irrigation (Ucar *et al*. 2017).

This study was conducted to investigate the effects of different irrigation levels and biostimulant applications on mitigating drought stress in *R. damascena* Mill. (Isparta rose). The experiment was set up in pots under controlled conditions using a completely randomized design with 12 treatment combinations and three replications per treatment, each containing five plants. The treatments consisted of three irrigation regimes representing pot field capacity (FC): 100% FC (well-watered control), 50% FC (moderate drought stress), and 25% FC (severe drought stress). Within each irrigation level, three biostimulant treatments were applied: vermicompost, *Bacillus* OSU-142 inoculation, and algae extract. The experimental treatments are detailed in Table 1.

Table 1. The Experimental Treatments

Control (100% FC)
Control + Vermicompost (100% FC)
Control + *Bacillus* OSU-142 (100% FC)
Control + Algae Extract (100% FC)
50% FC
50% FC + Vermicompost
50% FC + *Bacillus* OSU-142
50% FC + Algae Extract
25% FC
25% FC + Vermicompost
25% FC + *Bacillus* OSU-142
25% FC + Algae Extract

Methods

Irrigation treatments

Irrigation levels were adjusted to 100%, 50%, and 25% of pot field capacity, based on gravimetric measurements. The FC of the pots was calculated gravimetrically prior to the experiment. During each irrigation event, pots were weighed to determine the precise amount of water required to achieve the target field capacity level, ensuring accurate control of water-deficit treatments. Irrigation treatments began at the beginning of May (05.05.2025) and ended at the beginning of June (02.06.2025). Irrigation was carried out weekly to replenish the water lost from the FC of the pots, and biostimulants were applied during each irrigation.

Biostimulant applications

All biostimulant treatments were prepared in liquid form and applied to the soil together with the irrigation water. For each irrigation level, the total volume of liquid to be applied was calculated in advance based on the corresponding field capacity, and biostimulant solutions were prepared accordingly.

Liquid vermicompost was procured in pure form from a specialized supplier and applied to the soil at a volume corresponding to the designated irrigation amount for the treatment.

The *Bacillus* OSU-142 strain used in the study was acquired from a specialized company at a concentration of 10^8 CFU. Before the bacterial stock solution was added, it was diluted to 3% based on the volume of water lost relative to pot FC and then applied.

Algae (*Chlorella* spp.) collected from Lake Egirdir (Isparta) were processed in the laboratory. The algae extract was prepared following the method described by Sivasankari *et al.* (2006). The algae extract stock solution was diluted to a concentration of 15% based on the volume of water lost relative to the pot field capacity and then applied.

Biochemical and antioxidant enzyme analyses

Catalase (CAT) activity

Ten grams of leaf tissue were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 g polyvinyl polypyrrolidone (PVPP). The homogenates were centrifuged at 27,000 × g for 50 min at 4 °C, and the supernatant was used for analysis. Enzyme activity was determined according to the method of Beers and Sizer (1952) and expressed as units per milligram of protein (U/mg protein).

Superoxide dismutase (SOD) activity:

Ten grams of leaf tissue were homogenized in 100 mM sodium phosphate buffer supplemented with PVPP and processed under the same centrifugation conditions. SOD activity was measured following the protocol of Constantine and Stanley (1977) and expressed as U/mg protein.

Ascorbate peroxidase (APX) activity

Four grams of leaf sample were homogenized in 12 mL of 50 mM potassium phosphate buffer (pH 7.3) containing 1 mM EDTA, 2 mM DTT, and 1 mM ascorbic acid. After centrifugation at 10,000 × g for 15 min at 4 °C, the supernatant was used for enzymatic analysis. APX activity was assayed according to the method of Nakano *et al.* (1981) and expressed as mol/min/g protein.

Peroxidase (POD) and lipid peroxidation (MDA) activity

Ten grams of leaf material were homogenized in cold 100 mM sodium phosphate buffer containing PVPP, followed by centrifugation at 27,000 × g for 50 min at 4 °C. POD and MDA activities were evaluated according to the method described by Jiang *et al.* (2010) and expressed as ΔA_{460} min/mg and nmol/g protein.

Total proline content

One g leaf sample was homogenized with 3 mL of 3% sulfosalicylic acid. The resulting mixture was centrifuged at 12,000 g for 10 min at room temperature to obtain the supernatant. The proline content was determined according to the method described by Bates *et al*. (1973) and expressed as mg/g fresh weight.

Hydrogen peroxide (H_2O_2) content

The amount of hydrogen peroxide (H_2O_2) was determined according to the established method (Sergiev *et al*. 1997). A 500 mg leaf samples were homogenized in 5 mL of 1% TCA and centrifuged at 12,000 rpm for 15 minutes. To 0.5 mL of the supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0), and 1 mL of potassium iodide (KI) were added. Absorbance was measured at 390 nm. Hydrogen peroxide content was calculated using a standard curve and expressed as μmol/g.

Total soluble protein content

Total soluble protein content was determined according to the modified Hartree–Lowry method (1972). Samples (1 g) were treated with cold ethanol, homogenized, and centrifuged (10,000 g, 20 min, 4 °C). Following repeated ethanol washes, the pellet was extracted with protein extraction buffer (50 mM Tris, 1.2 M NaCl, pH 7.0) and incubated on ice. After centrifugation and filtration, a defined volume of the protein extract was reacted sequentially with reagents A, B, and C, and the mixtures were incubated at the specified temperatures and subsequently cooled to room temperature. Following incubation, the color intensity was measured spectrophotometrically at 650 nm, and protein concentrations were calculated using a BSA standard curve.

Statistical Analysis

The study was conducted in a factorial arrangement using a randomized complete block design with three replications. The data were analyzed using one-way analysis of variance (ANOVA) in the Minitab 17 statistical software. Significant differences among treatments were determined using Tukey's multiple comparison test, and mean differences were indicated by different letters.

RESULTS AND DISCUSSION

Catalase (CAT) enzyme activity was measured at weekly intervals over a 28-day period in *R. damascena* plants subjected to different irrigation levels and biostimulant treatments under drought stress conditions. As shown in Fig. 1, CAT activity remained relatively stable under well-watered conditions (100% FC), whereas significant increases were observed under water-deficit treatments. However, the extent of this increase varied substantially depending on both the irrigation level and the type of biostimulant applied. Under optimal irrigation (100% FC), CAT activity showed some fluctuations over time, reaching 11.6 U/mg protein by day 28. Biostimulant applications (vermicompost, *Bacillus OSU*-142, and algal extract) did not produce significant changes compared to the control group at this irrigation level, indicating that under non-stress conditions, their impact on enzymatic antioxidant response was limited. Under moderate drought stress (50% FC), CAT activity increased notably compared to the 100% FC control, suggesting that water deficit induced an upregulation of the plant's antioxidant defense system. In this group, CAT activity showed a steady increase over time and peaked at 35.0 U/mg protein on day 28. Biostimulant-treated plants exhibited slightly lower CAT activity levels, ranging between 33.7 and 34.8 U/mg protein, vermicompost and *Bacillus* OSU-142 treatments showing a modest suppressive effect on CAT activity under moderate stress. The most pronounced effects were observed under severe drought conditions (25% FC). In all treatments, CAT activity increased significantly over time, reaching the highest level recorded in the study (41.1 U/mg protein in the untreated 25% FC group) by day 28. In contrast, biostimulant applications under this severe stress condition resulted in time-dependent reductions in CAT activity. Among them, the algal extract (25% FC + A) was effective in significantly lowering CAT levels compared to the untreated 25% FC control. *Bacillus* OSU-142 and vermicompost treatments also reduced CAT activity, although their effects were less pronounced than that of the algal extract.

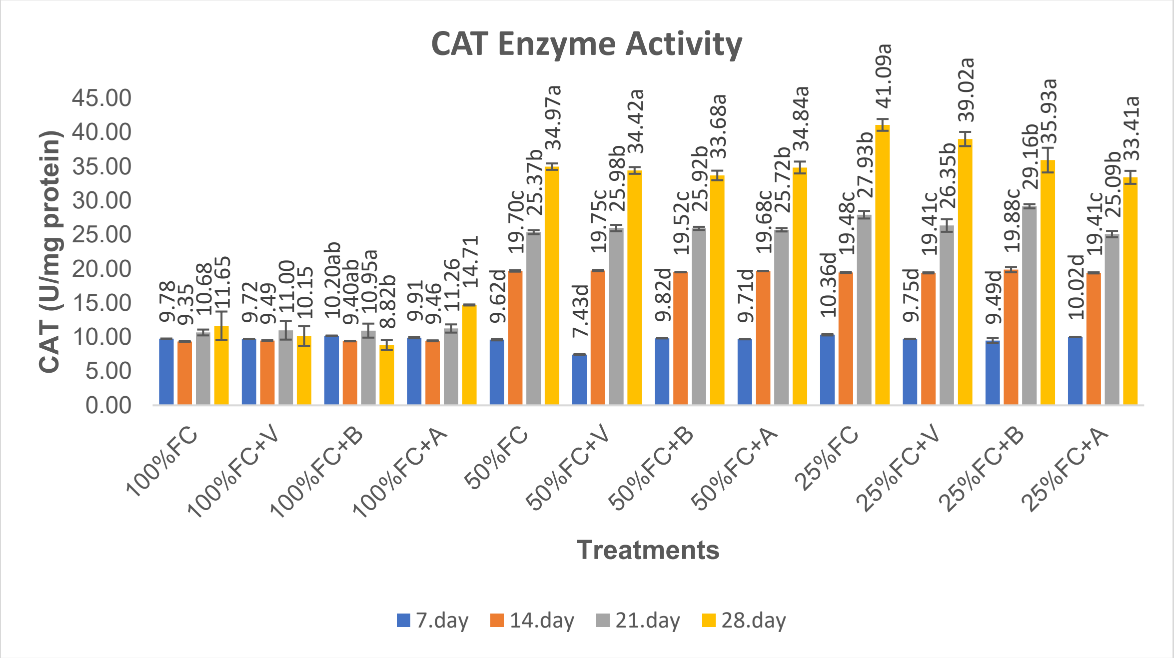

Fig. 1. Effects of biostimulants on CAT enzyme activity (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: $P < 0.01$; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

Superoxide dismutase (SOD) enzyme activity was monitored over a 28-day period in *R. damascena* plants subjected to different irrigation levels and biostimulant treatments, with the results presented in Fig. 2. As shown in Fig. 2, all treatment groups exhibited a gradual increase in SOD activity over time. However, the magnitude of this increase varied depending on both irrigation level and biostimulant application. Under well-watered conditions (100% FC), SOD activity started at relatively low levels (6.84 U/mg protein) and increased to 14.3 U/mg protein by day 28. Biostimulant applications (vermicompost, *Bacillus* OSU-142, and algal extract) produced only limited additional increases at this irrigation level, indicating minimal impact under non-stress conditions. This modest rise in activity was thought to be partly due to the gradual increase in temperature within the polyethylene greenhouse during the experimental period. Under moderate drought stress (50% FC), SOD activity increased more markedly over the 28 days of the experiment. Measurements on days 21 and 28 showed values ranging between 17.6 and 22.2 U/mg protein. Vermicompost (50% FC + V) and *Bacillus* OSU-142 (50% FC + B) treatments significantly reduced SOD activity compared to the untreated 50% FC stress, suggesting a mitigating effect on oxidative stress. The algal extract (A) also led to reduced SOD activity under 50% FC conditions, although its effect was somewhat less pronounced than that of the other biostimulants. The most striking increases in SOD activity were observed under severe drought stress (25% FC). All treatments exhibited their highest SOD activity levels by day 28. Biostimulant applications were particularly effective under these conditions, resulting in lower SOD activity than under the untreated 25% FC stress. Notably, *Bacillus* OSU-142 treatment significantly suppressed SOD activity under severe drought, reducing it to 18.53 U/mg protein on day 28.

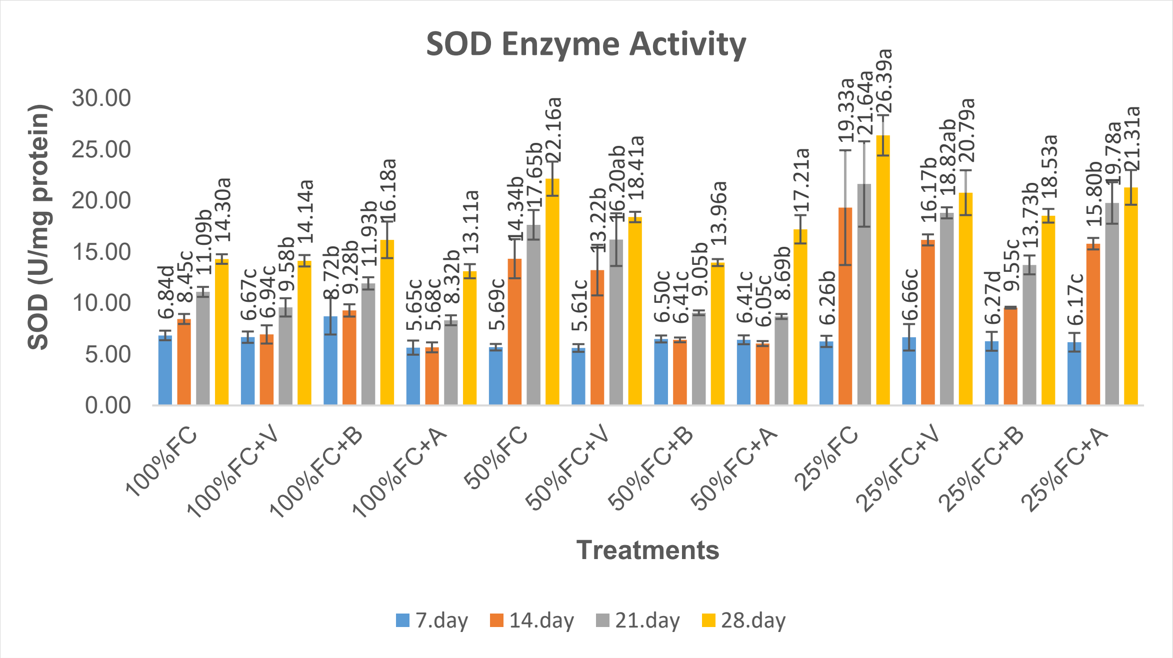

Fig. 2. Effects of biostimulants on SOD enzyme activity (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: $P < 0.01$; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

Ascorbate peroxidase (APX) enzyme activity was evaluated over a 28-day period in *R. damascena* plants subjected to different irrigation regimes and biostimulant treatments. As shown in Fig. 3, the APX activity exhibited a clear, time-dependent increase across all treatment groups; however, the magnitude of this increase varied significantly depending on irrigation level and biostimulant application. Under well-watered conditions (100% FC), the APX activity remained relatively low throughout the 28 days, reaching 11.94 mol/min/g protein by day 28. Biostimulant treatments (vermicompost, *Bacillus* OSU-142, and algal extract) did not produce significant differences compared to the untreated control at this irrigation level, indicating that under non-stress conditions, antioxidant defenses were only minimally activated. Under moderate drought stress (50% FC), APX activity increased substantially over time. By day 28, the untreated 50% FC stress group exhibited an APX activity of 37.0 mol/min/g protein, whereas biostimulant-treated groups showed notably lower values, ranging from 22.2 to 28.6 mol/min/g protein. In particular, *Bacillus* OSU-142 (50% FC + B) treatment resulted in the lowest APX activity among the 50% FC treatments, demonstrating its potential to suppress antioxidant defense responses under moderate drought stress. Vermicompost and algal extract applications also reduced APX activity compared to the untreated 50% FC stress, although their effects were slightly less pronounced than those of *Bacillus* OSU-142. The most pronounced increases in APX activity were observed under severe drought stress (25%

FC). All treatment groups exhibited marked and progressive increases in APX activity over the four-week period, with the untreated 25% FC stress reaching the highest recorded value of 44.5 mol/min/g protein on day 28. Again, biostimulant applications helped suppress APX activity under these severe stress conditions. *Bacillus* OSU-142 treatment (25% FC + B) achieved the lowest APX activity overall (32.4 mol/min/g protein), indicating a substantial reduction in the antioxidant response under severe water stress. Vermicompost and algal extract treatments also resulted in significant reductions in APX activity compared to the untreated 25% FC control.

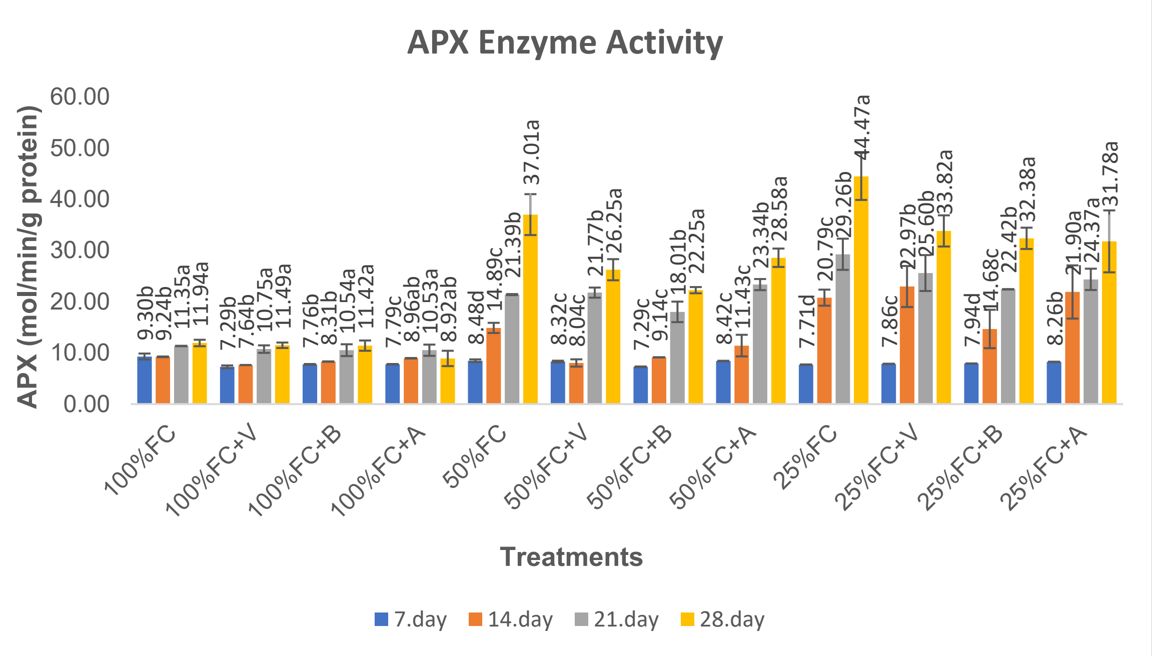

Fig. 3. Effects of biostimulants on APX enzyme activity (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: $P < 0.01$; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

Peroxidase (POD) enzyme activity was evaluated over a four-week period in *R. damascena* leaves subjected to different irrigation levels and biostimulant treatments, with the results presented in Fig. 4. As shown, POD activity increased over time in all treatment groups; however, the extent of this increase varied significantly depending on the irrigation regime and the biostimulant applied. Under well-watered conditions (100% FC), POD activity reached a maximum of 9.94 Δ_{A460}min/mg protein by day 28. Biostimulant applications at this irrigation level did not lead to significant changes in POD activity compared to the untreated control, indicating that under low-stress conditions, the plant's defense system was only minimally activated. Under moderate drought stress (50% FC), POD activity increased substantially in all groups. By day 28, the plants under untreated 50% FC stress exhibited POD activity of 23.14 Δ_{A460} min/mg protein. In contrast, *Bacillus* OSU-142, vermicompost, and algal extract treatments resulted in notably lower POD activity levels of 14.52, 16.87, and 20.77 Δ_{A460} min/mg protein, respectively. Under severe drought stress conditions (25% FC), POD activity reached the highest values recorded across all treatment groups. The untreated 25% FC stress group exhibited activity of 30.20 U/mg protein on day 28. However, biostimulant applications significantly reduced this increase: *Bacillus* OSU-142, vermicompost, and algal extract treatments lowered POD activity to 23.80, 30.43, and 33.39 Δ_{A460} min/mg protein, respectively, by day 28.

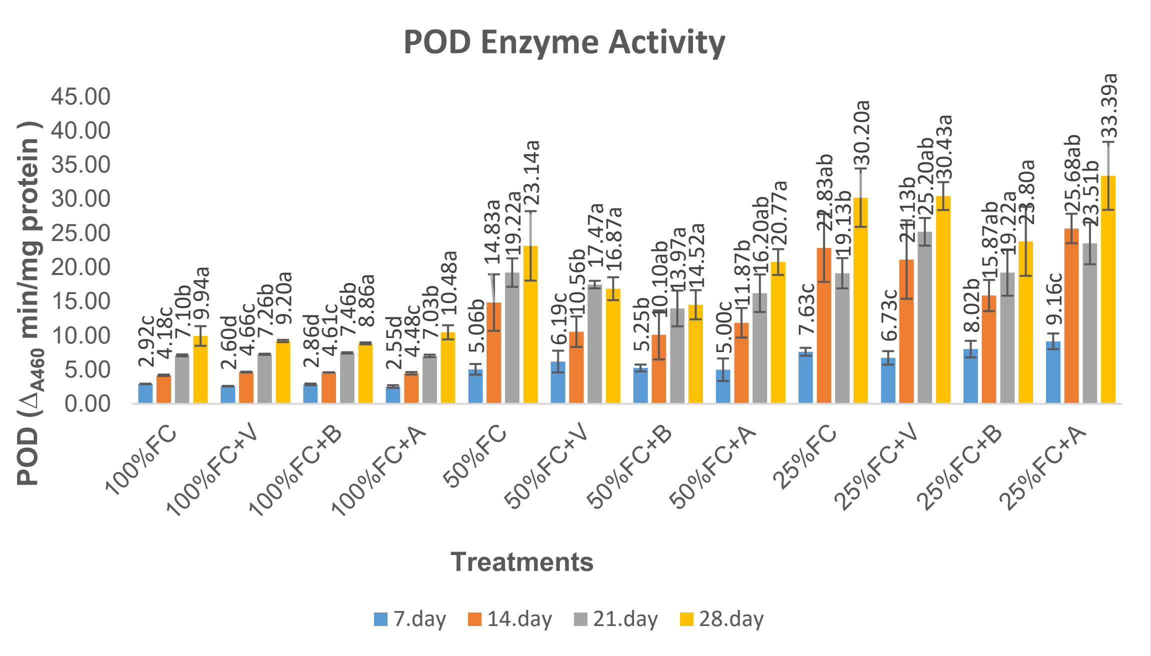

Fig. 4. Effects of biostimulants on POD enzyme activity (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: *P* < 0.01; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

Malondialdehyde (MDA) content was monitored over a 28-day period in *R. damascena* plants subjected to different levels of water stress and various biostimulant treatments. Figure 5 shows that the MDA content exhibited a gradual increase over time across all treatment groups, although the magnitude of this increase differed significantly depending on the level of stress applied and the type of biostimulant used. Under well-watered conditions (100% FC), MDA levels remained relatively low throughout the four-week period, with final values on day 28 ranging from 3.16 to 3.91 nmol/g. Biostimulant applications (vermicompost, *Bacillus* OSU-142, and algal extract) did not cause notable changes in MDA levels compared to the untreated control at this irrigation level. This indicates that under low-stress conditions, lipid peroxidation was limited and membrane integrity was largely maintained. Under moderate drought stress (50% FC), there was a clear increase in MDA content. By day 28, the untreated 50% FC stress group exhibited an MDA accumulation of 7.99 nmol/g. In contrast, *Bacillus* OSU-142, vermicompost, and algal extract treatments reduced this value to 7.35, 7.34, and 7.30 nmol/g, respectively. These results suggest that biostimulant applications effectively mitigated oxidative damage under moderate stress conditions by suppressing lipid peroxidation. Under severe drought stress (25% FC), MDA content increased significantly, reaching a maximum value of 22.9 nmol/g in the untreated 25% FC stress group by day 28. *Bacillus* OSU-142, vermicompost, and algal extract treatments significantly reduced MDA accumulation to 16.5, 20.0, and 22.0 nmol/g, respectively. These reductions highlight the protective effect of biostimulants in maintaining membrane integrity and their potential to limit lipid peroxidation under conditions of oxidative stress.

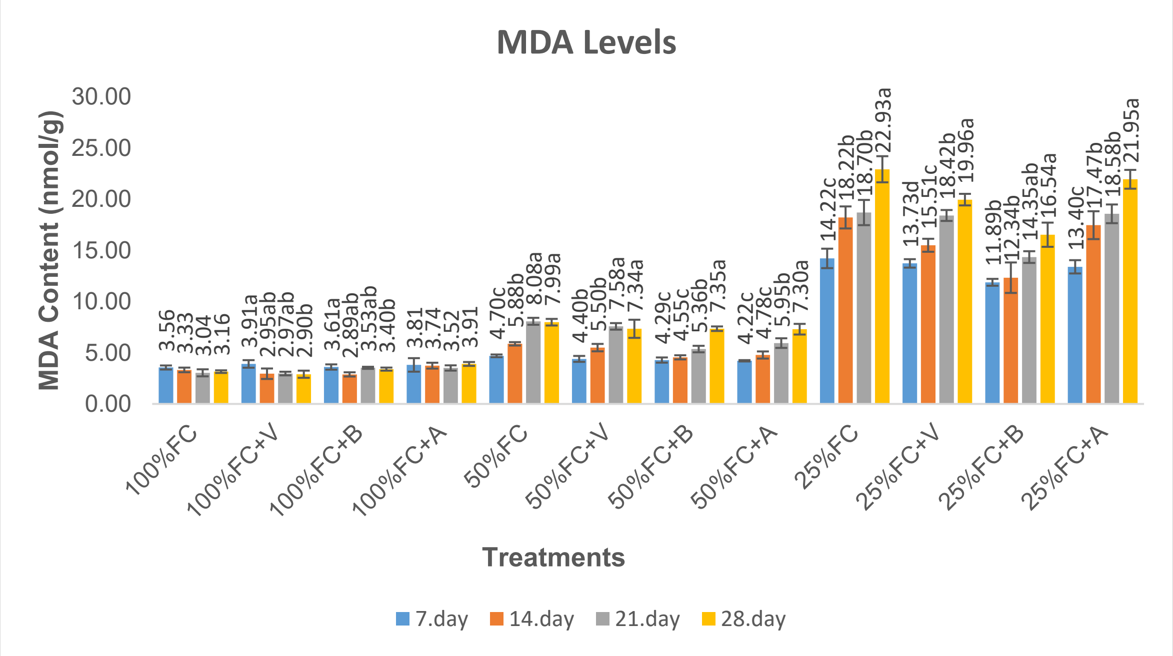

Fig. 5. Effects of biostimulants on MDA levels (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: $p<0.01$; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

Proline content was evaluated over a four-week period in *R. damascena* leaves subjected to different irrigation levels and biostimulant treatments. The results presented in Fig. 6 reveal that proline accumulation increased gradually over time in all treatment groups; however, the rate and extent of this increase varied depending on the irrigation regime and the type of biostimulant applied. Under well-watered conditions (100% FC), proline content stabilized at approximately 21.6 to 25.2 µg/mL by day 28. Biostimulant applications in this irrigation regime showed limited impact on proline accumulation, indicating that osmoregulatory mechanisms were only minimally activated either in the absence of or under low levels of stress. Under moderate drought stress (50% FC), proline accumulation increased markedly. By day 28, the untreated 50% FC stress group exhibited a proline level of 34.6 µg/mL, whereas *Bacillus* OSU-142, vermicompost, and algal extract treatments reduced these values to 29.2, 32.1, and 27.2 µg/mL, respectively. Under severe drought stress (25% FC), proline content reached its highest levels across all treatments. In the untreated 25% FC stress group, proline accumulation was measured at 42.3 µg/mL on day 28. Vermicompost, *Bacillus* OSU-142, and algal extract treatments significantly reduced this value to 36.7, 31.3, and 35.8 µg/mL, respectively. Notably, *Bacillus* OSU-142 treatment was effective in significantly suppressing proline accumulation under severe drought conditions.

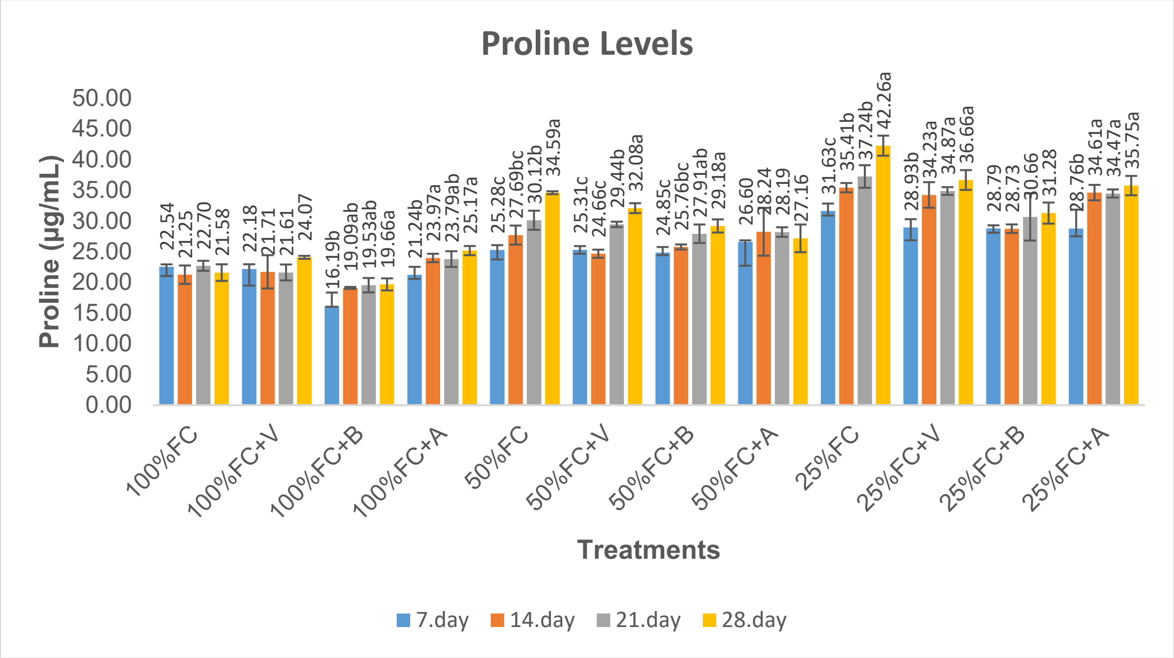

Fig. 6. Effects of biostimulants on Proline levels (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: *P* < 0.01; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

Hydrogen peroxide (H_2O_2) content in *R. damascena* was monitored over a 28-day period under different water stress levels and various biostimulant applications, and the results are presented in Fig. 7. The H_2O_2 levels exhibited a gradual increase over time across all treatment groups; however, the magnitude of this increase differed significantly depending on the severity of water stress and the type of biostimulant applied. Under well-watered conditions (100% FC), H_2O_2 levels remained relatively low throughout the four-week period, reaching values ranging between approximately 2.30 and 2.86 µmol/g by day 28. Biostimulant applications (vermicompost, *Bacillus* OSU-142, and algal extract) did not cause significant changes in H_2O_2 levels compared to the control under these non-stress conditions, indicating that reactive oxygen species (ROS) accumulation was limited, and the cellular redox balance was largely maintained. In contrast, under moderate drought stress (50% FC), a noticeable increase in H_2O_2 content was observed. By day 28, the H_2O_2 concentration in the 50% FC stress group reached 6.02 µmol/g, whereas treatments with vermicompost (5.32 µmol/g), *Bacillus* OSU-142 (4.80 µmol/g), and algal extract (4.90 µmol/g) significantly reduced H_2O_2 accumulation. These findings suggest that biostimulant applications under moderate stress effectively mitigated ROS buildup by enhancing the plant's antioxidative defense mechanisms. Under severe drought stress (25% FC), H_2O_2 levels increased dramatically. The highest value recorded in the study was 13.09 µmol/g in the 25% FC stress group on day 28. Biostimulant treatments with *Bacillus* OSU-142 (11.1 µmol/g), vermicompost (12.26 µmol/g), and algal extract (13.07 µmol/g) significantly reduced H_2O_2 accumulation compared to the non-treated control. Notably, *Bacillus* OSU-142 was the most effective in limiting H_2O_2 buildup under severe water deficit, highlighting its potential role in alleviating oxidative stress through efficient ROS regulation.

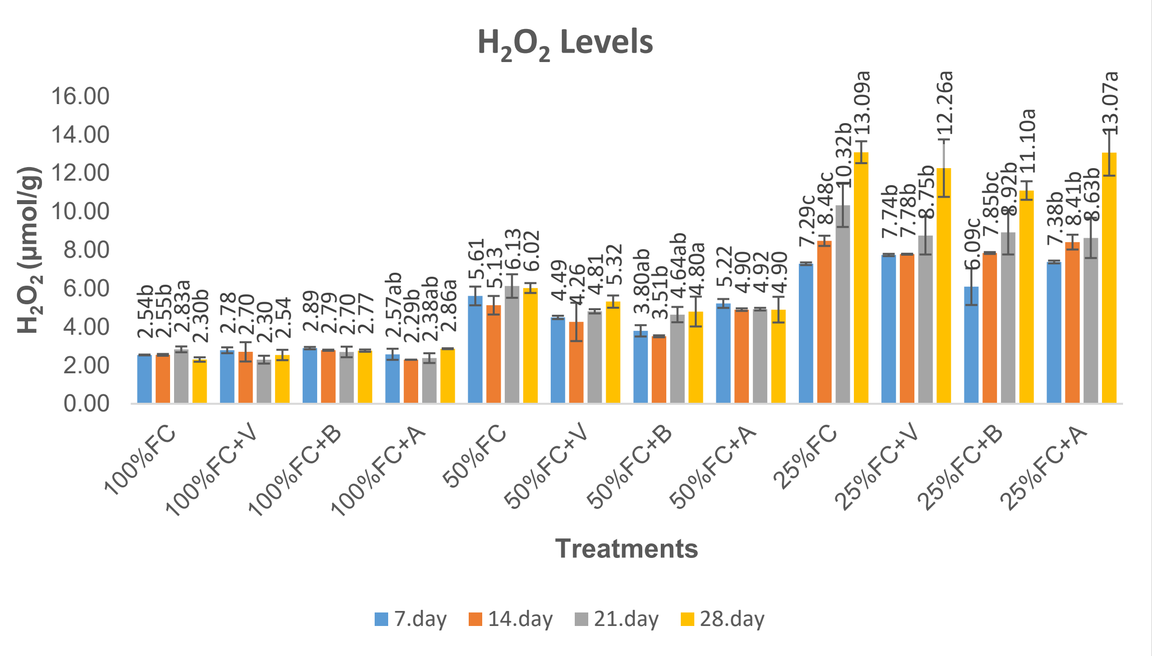

Fig. 7. Effects of biostimulants on H_2O_2 levels (Note: In the statistical analysis, activities were evaluated independently across different periods. Significance level: $P < 0.01$; the colors in the figure indicate the sampling dates of leaf samples collected at different periods. FC: Field capacity, V: Vermicompost, B: *Bacillus* OSU-142, A: Algae extract)

The effects of different irrigation levels and biostimulant applications on the physiological and biochemical responses of *R. damascena* were evaluated over a 28-day period under controlled conditions. Results revealed that drought stress significantly affected antioxidant enzyme activities, oxidative stress markers, and osmo-protectant accumulation, with clear differences observed across irrigation regimes and biostimulant treatments. In general, progressive water deficit (from 100% FC to 25% FC) induced a marked increase in the activities of antioxidant enzymes including CAT, SOD, APX, and POD as well as in the contents of MDA, H_2O_2, and proline. These responses indicate an enhanced activation of the antioxidant defense system in plants exposed to drought-induced oxidative stress.

Under well-watered conditions (100% FC), enzyme activities and oxidative stress markers remained relatively low throughout the experiment, and biostimulant applications (vermicompost, *Bacillus* OSU-142, and algal extract) produced no significant differences compared to the untreated control. This suggests limited induction of oxidative stress and minimal need for an enhanced antioxidant response under optimal irrigation.

In contrast, under moderate drought stress (50% FC), enzyme activities and oxidative damage markers increased significantly. Biostimulant-treated plants exhibited notably lower levels of CAT, SOD, APX, and POD activities compared to plants under 50% FC stress conditions without treatment, indicating reduced oxidative stress. Similarly, MDA and H_2O_2 contents were significantly decreased by biostimulant applications, suggesting mitigation of lipid peroxidation and ROS accumulation. Proline accumulation, which increased under 50% FC stress, was also reduced in biostimulant-treated groups. These findings demonstrate that biostimulants helped limit oxidative damage and osmotic stress under moderate drought conditions.

Under severe drought stress (25% FC), the strongest increases in antioxidant enzyme activities, MDA, H_2O_2, and proline levels were recorded in the 25% FC, reflecting intense oxidative stress and cellular damage. Biostimulant applications significantly reduced enzyme activities (especially CAT, SOD, APX, and POD) and oxidative stress markers compared to the enzyme activities and oxidative stress markers under untreated 25% FC stress. Vermicompost and *Bacillus* OSU-142 achieved the lowest levels of H_2O_2 and MDA accumulation under severe stress, indicating effective suppression of ROS production and protection of membrane integrity. Proline accumulation was also significantly lower in biostimulant-treated plants, suggesting reduced osmotic stress signaling.

Biostimulants are innovative agricultural tools that promote plant growth and development in ways that depend on their concentration and mode of application. When applied to plants or soil, these organic or inorganic products contain biological compounds or microorganisms that, independently of their own nutrient content, enhance plant growth and productivity by increasing nutrient uptake and assimilation efficiency, thereby improving tolerance to abiotic stresses, and enhancing crop quality (Bulgari *et al*. 2019; Rouphael and Colla, 2020). However, the specific mechanisms activated by biostimulants in plants under stress conditions remain only partially understood. Considering the physiological and biochemical changes they induce, it is hypothesized that biostimulants influence specific metabolic reactions. Therefore, the metabolic pathways activated by biostimulants under stress conditions can be strengthened to support plant adaptation to such adverse environments (Staykov *et al*. 2020).

It is well established that biostimulants play a central role in reducing the degenerative effects of free radicals accumulated in plant tissues under abiotic and biotic stress conditions (Omidbakhshfard *et al*. 2020; Alharby *et al*. 2021). Del Buono (2021) reported that certain biostimulants help accumulate osmoprotectants such as proline, sugars, alcohols, nitrates, and abscisic acid under drought conditions, thus enhancing endogenous responses that improve water balance, increase root water uptake capacity, and ultimately reduce water loss. Similarly, Lola-Luz *et al*. (2014) reported significant effects of *Ascophyllum nodosum* seaweed extract on broccoli and spinach under water stress conditions. Their study demonstrated that biostimulant applications with *A. nodosum* improved green color quality by enhancing chlorophyll biosynthesis under stress and enabled the cultivation of greener leafy vegetables while also increasing antioxidant compound content. Alharby *et al*. (2021) found that silymarin-enriched biostimulant applications on maize under cadmium stress significantly reduced oxidative stress markers such as MDA, H_2O_2, and O_2^-, while increasing activities of antioxidant enzymes such as SOD, CAT, and POD. Kovács *et al*. (2024) investigated the effects of biostimulant and growth-promoting applications on antioxidant enzyme activities in *Viburnum opulus* "Roseum" and reported that the Bistep and Yeald Plus treatments produced significantly higher POD activity than the control group, suggesting that these biostimulants trigger an active oxidative defense mechanism in the plant. The researchers concluded that biostimulants activate antioxidant systems in plants and contribute to defense against osmotic or oxidative stress. El-Deeb *et al*. (2012) aimed to identify endophytic bacteria isolated from *R. damascena* trigintipetala during flowering and evaluate their plant growth-promoting traits. They isolated 38 culturable endophytic bacterial strains from healthy root, stem, leaf, and flower tissues, that were closely related to genera such as *Acetobacter*, *Acinetobacter*, *Methylococcus*, *Bacillus*, *Micrococcus*, and *Planococcus*. These bacteria were found to possess potential for direct contributions to root development, nutrient uptake, and plant health through plant hormone production, nutrient solubilization, and microbial enzyme activity. Hessini *et al*. (2022) investigated the effects of three different irrigation regimes (100%, 50%, and 25% FC) on antioxidant enzyme activities and other biochemical parameters in leaf extracts of *R. damascena* Mill. var. trigintipetala, finding that reduced irrigation levels led to increases in both antioxidant enzyme activities and phenolic content. The effects of foliar application of seaweed extract on potato plants under water stress, observing significant increases in activities of key antioxidant enzymes such as SOD, CAT, APX, and POD, alongside reductions in oxidative stress markers such as MDA were also examined (Abd El Baky *et al*. 2016). Yildirim *et al*. (2025) reported that vermicompost, bacteria, and algal extract applications in apple significantly enhanced antioxidant enzyme activities (SOD, CAT, APX) to help combat oxidative stress, while increasing phenolic compound contents to strengthen the plant's overall defense potential. Recent studies also highlight the growing use of plant growth-promoting bacteria (PGPB), vermicompost, and algal applications in drought-prone regions, demonstrating their ability to increase antioxidant enzyme activities and enhance tolerance to the adverse effects of stress (Çakmakçı *et al*. 2007; Sarma and Saikia 2014; Ahmad *et al*. 2024; Alshammari *et al*. 2024; Direk *et al*. 2024; Gharib *et al*. 2024; Lenart *et al*. 2024; Talaat and Abdel-Salam 2024; Heidarpour *et al*. 2025).

In this study, the effects of biostimulant applications on antioxidant enzyme activities were evaluated under different irrigation regimes. The results showed that antioxidant enzyme activities in biostimulant-treated groups were higher than in the fully irrigated 100% FC control, indicating that biostimulant-treated plants exhibit a typical physiological response by activating their defense systems to maintain oxidative balance under stress conditions. These findings align with previous research on other plant species (Abd El Baky *et al*. 2016; Alharby *et al*. 2021; Yildirim *et al*. 2025), confirming the supportive role of biostimulants in enhancing antioxidant defense mechanisms under stress.

Conversely, antioxidant enzyme activities measured in biostimulant-treated groups were found to be lower than those in groups subjected to more intense water stress treatments (50% FC and 25% FC) without biostimulant application. This suggests that biostimulants may reduce oxidative stress levels in plant cells by limiting intracellular ROS accumulation, thus lowering the need for an excessively activated antioxidant defense system. In other words, biostimulants may suppress or limit ROS production in plant cells, leading to a more moderate enzymatic antioxidant response. This finding suggests that biostimulants not only trigger defense responses but also play a regulatory role in maintaining cellular homeostasis and minimizing energy loss.

CONCLUSIONS

The findings from this study clearly demonstrate that biostimulant applications mitigate drought-induced oxidative stress by limiting the accumulation of reactive oxygen species (ROS) within plant tissues.

1. The observation that antioxidant enzyme activities (CAT, SOD, APX, POD) were consistently lower in biostimulant-treated plants under stress conditions compared to the untreated stress controls suggests that these treatments reduce the cellular demand for antioxidative defense, thereby allowing plants to allocate metabolic resources more efficiently. This indicates that biostimulants either suppress ROS production or

enhance its detoxification, thus preserving membrane integrity and maintaining overall cellular homeostasis.

2. Furthermore, the significant reduction in oxidative damage markers such as MDA and H_2O_2 in biostimulant-treated groups confirms the limitation of lipid peroxidation and oxidative injury.
3. The observed decrease in proline accumulation with biostimulant applications also suggests an alleviation of osmotic stress responses.
4. Collectively, these results highlight the potential of vermicompost, *Bacillus* OSU-142, and algal extract treatments as sustainable and environmentally friendly agricultural strategies to enhance drought tolerance, limit oxidative damage, and maintain growth performance in *R. damascena* under water-limited conditions. In this context, this study provides important insights into how biostimulants influence the dynamics of antioxidant systems in *R. damascena* under stress conditions.

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