

Improvement in Drought Tolerance of Dragonhead, *Dracocephalum moldavica* L. under the Sodium Nitroprusside Effects on Polyethylene Glycol

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ABSTRACT

Dracocephalum moldavica L. (Lamiaceae), an annual herb native to central Asia and naturalized in parts of Europe. The aerial parts and volatile constituents of this plant are widely utilized for their medicinal and aromatic properties. This study investigates the regeneration of *D. moldavica* under *in vitro* drought stress induced by varying concentrations of polyethylene glycol (PEG) (0%, 10%, 15%, and 20%) and evaluates the mitigating effects of sodium nitroprusside (SNP), a nitric oxide donor. SNP treatments (0, 100, and 200 μ M) were incorporated into Murashige and Skoog (MS) medium to assess their impact on morpho-physiological traits under PEG-induced drought conditions. After four weeks of cultivation, several growth parameters were measured, including the number and length of shoots, number of leaves, root characteristics, and survival rates. Results revealed that 10% PEG exhibited the least adverse impact on morphological traits compared to higher PEG concentrations, suggesting this level as optimal for inducing drought stress without excessive damage. SNP at 100 μ M significantly improved morphological and physiological parameters compared to the untreated control and 200 μ M SNP. The application of 100 μ M SNP enhanced shoot and root growth, increased antioxidant enzyme activities, and reduced hydrogen peroxide (H_2O_2) accumulation, thus mitigating oxidative stress. These findings highlight the potential of SNP to alleviate drought-induced damage in *D. moldavica*, particularly at 100 μ M, which proved to be the most effective concentration for improving plant growth and resilience. This research provides valuable insights for optimizing the cultivation of drought-tolerant medicinal plants in arid and semi-arid regions, with implications for enhancing essential oil yields under water-deficit conditions.

Keywords: Antioxidant enzymes, *Dracocephalum moldavica* L., Drought stress, Nitric oxide (NO), Polyethylene glycol (PEG), Sodium nitroprusside (SNP).

INTRODUCTION

The genus *Dracocephalum*, belonging to the Lamiaceae family, encompasses approximately 45 species of flowering plants distributed across various regions, particularly Central Asia and parts of Europe. Among these species, *Dracocephalum*

moldavica L., commonly known as Moldavian balm or dragonhead, is an herbaceous annual plant native to Central Asia and naturalized in Eastern and Central Europe (Hashemian Ahmadi and Hadipanah, 2014). This plant is widely recognized for its medicinal and aromatic properties, with its aerial parts and essential oil being used in traditional medicine and various industries. The major volatile constituents of *D. moldavica* include geranial, geranyl acetate, neral, and geraniol, which are responsible for its characteristic aroma and bioactivity (Abdossi *et al.*, 2015; Golparvar *et al.*, 2016). These constituents have demonstrated significant antibacterial, antifungal, antioxidant, and antirheumatic properties, further emphasizing the therapeutic potential of this plant (Aprotosoiaie *et al.*, 2016; Aćimović *et al.*, 2022).

Plants frequently encounter various environmental stresses, including drought, soil salinity, heavy metal toxicity, and extreme temperatures, which adversely affect their growth, development, and productivity. Among these, drought is a particularly severe abiotic stress that limits water availability, disrupts photosynthetic activity, impairs nutrient uptake, and inhibits physiological processes such as cell division and expansion. These impacts cumulatively reduce plant performance and yield (Seleiman *et al.*, 2021; El Haddad *et al.*, 2023; Hadipanah *et al.*, 2025). In response to drought stress, plants activate complex signaling networks involving molecules such as abscisic acid (ABA), calcium ions (Ca^{2+}), reactive oxygen species (ROS), and nitric oxide (NO). These signals regulate the expression of functional genes (e.g., those related to proline, glycine betaine, soluble sugar, aquaporin, and late embryogenesis abundant proteins) and regulatory genes (e.g., CDPKs and MAPKs), enabling morphological and physiological adaptations to stress (Anjum *et al.*, 2017; Yang *et al.*, 2021).

Previous research has demonstrated that exogenous applications of sodium nitroprusside (SNP) (Pradhan *et al.*, 2020), salicylic acid (Chamani *et al.*, 2025), brassinosteroids (Jangid and Dwivedi, 2017), and polyamines (Sundararajan *et al.*, 2022) can alleviate the negative effects of drought stress by modulating physiological and biochemical pathways.

SNP, a well-known nitric oxide (NO) donor, has been widely used in studies to investigate the protective role of NO under various abiotic stresses, including drought. SNP releases NO upon decomposition, enhancing its bioavailability and biological activity. NO is a critical signaling molecule in plants, playing a pivotal role in growth, development, and stress responses. As a small, highly diffusible, lipophilic, and reactive molecule, NO is involved in a wide range of processes, including photosynthesis, germination, leaf expansion, flowering, and senescence. It also modulates antioxidant defense mechanisms, mitigating oxidative stress induced by ROS (Zangani *et al.*, 2023; Corpas and Palma, 2023; Allagulova *et al.*, 2023b; Ali *et al.*, 2024). Furthermore, SNP treatment during drought induced an increase in NO buildup. Exogenous SNP enhances NO buildup in safflower (Chavoushi *et al.*, 2020) and maize (Majeed *et al.*, 2020) under water stress. However, the efficacy of NO in alleviating stress depends on factors such as concentration, tissue type, plant species, and developmental stage. Specifically, SNP has been shown to regulate the activity of antioxidant enzymes, reduce ROS accumulation, and improve water-use efficiency in drought-stressed plants (Ghadakchiasl *et al.*, 2017; Ragaey *et al.*, 2022;

Chamani *et al.*, 2025). NO can act as a signalling molecule at low concentrations, or it can be toxic at high concentrations and can provoke nitro-oxidative stress. Therefore, practical use requires scrupulous study regarding the mechanisms of SNP action and those of its active derivatives—reactive nitrogen species (RNS). For example, when NO interacts with $O_2^{\cdot-}$, peroxynitrite ($NOOO^-$) is formed, which is characterized by less toxic properties than $O_2^{\cdot-}$. Thus, $NOOO^-$ formation can be recognized as a direct antioxidant effect of NO (Roy, 2021; Lubyanova and Allagulova, 2024).

While NO's role as a signaling molecule in enhancing plant tolerance to various abiotic stresses has been well documented (Gupta and Seth 2023; Mariyam *et al.* 2023; Prajapati *et al.* 2023), its specific effect on under drought stress in *D. moldavica* plants remains less explored. This study aimed to evaluate the potential of SNP to mitigate PEG-induced drought stress in *D. moldavica* under in vitro conditions. By examining various morphological and physiological parameters, the research provides insights into the mechanisms through which SNP enhances drought tolerance in this medicinally important plant. The findings contribute to understanding the role of NO as a stress-alleviating agent and highlight the practical applications of SNP in promoting the cultivation of drought-tolerant crops in arid and semi-arid regions.

MATERIALS AND METHODS

Plant material

The experiment was conducted as a bi-factorial study in a completely randomized design with three replications in 2023 at Islamic Azad University, Khorasgan (Isfahan), Iran. Seeds of *Dracocephalum moldavica* L. were procured from Pakan Bazr Company, Isfahan, Iran. Seeds were first treated with 70% ethanol for one minute, followed by immersion in a 2% sodium hypochlorite solution for 10 minutes. After sterilization, seeds were thoroughly rinsed several times with sterile distilled water to eliminate any residual sterilizing agents.

In vitro drought induction

The seeds were sown on Petri dishes containing filter paper in half-strength MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 15 minutes and then they were grown under illumination of 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 16 h photoperiod and ambient temperature of 22–24 °C for 4 days. The 6-day-old seedlings were SNP-pretreated through the roots for 24 h via supplementation with SNP (0, 100, and 200 μM) into growth medium. The 6-day-old plant samples were subjected to osmotic stress through treatment with polyethylene glycol 6000 (PEG 6000) (0%, 10%, 15%, and 20%), were added to the medium. PEG-induced drought conditions were evaluated based on their effects on several morpho-physiological parameters, including the number and length of shoots, number of leaves, number and length of roots, and survival percentage. Observations were recorded 30 days after induction, with specific emphasis on identifying the PEG concentration that imposed optimal drought stress without excessive damage.

Determination of hydrogen peroxide (H_2O_2)

H_2O_2 content was measured following the protocol by Alexieva *et al.* (2001). Briefly, fresh leaf tissues (0.2 g) were homogenized in 1.5 mL of 0.1% (v/v) trichloroacetic acid (TCA) at 4°C. The homogenate was centrifuged at 15,000× g for 15 minutes at 4°C. The supernatant (500 µL) was mixed with 500 µL phosphate buffer (10 mM, pH 7.0) and 1 mL of 1 M potassium iodide (KI). The absorbance of the reaction mixture was measured at 390 nm using a spectrophotometer to estimate H_2O_2 content.

Hydroxyl radical ($\cdot OH$) scavenging assay

The scavenging capacity of hydroxyl radicals ($\cdot OH$) was determined following the method by Manda *et al.* (2010). Briefly, a reaction mixture containing 1 mL of $FeSO_4$ (1.5 mM), 0.7 mL of H_2O_2 (6 mM), 0.3 mL sodium salicylate (20 mM), and 100 µL of plant tissue extract was incubated at 37°C for one hour. The absorbance of the mixture was measured at 562 nm using a UV–visible spectrophotometer to determine $\cdot OH$ scavenging activity.

Antioxidant enzyme activities

The activities of key antioxidant enzymes were evaluated using fresh leaf tissues (0.3 g), homogenized in 3 mL of serine borate buffer (100 mM Tris-HCl, 10 mM borate, 5 mM serine, and 1 mM diethylenetriaminepentaacetic acid, pH 7.0). The homogenate was centrifuged at 5,000× g for 10 minutes at 4°C, and the supernatant was used as the enzyme source.

- **Superoxide dismutase (SOD, EC 1.12.1.1):** SOD activity was measured based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), following the method by Beauchamp and Fridovich (1971). The absorbance was recorded at 560 nm.
- **Catalase (CAT, EC 1.11.1.6):** CAT activity was determined by measuring the decrease in absorbance at 240 nm due to the degradation of H_2O_2 , as described by Patra *et al.* (1978).
- **Peroxidase (POD, EC 1.11.1.7):** POD activity was assessed using the method of Nickel and Cunningham (1969), with absorbance measured at 470 nm.

Total soluble protein content

Total protein content was quantified using the Bradford (1976) method. Fresh leaf samples (0.1 g) were ground in liquid nitrogen and homogenized in 4 mL sodium phosphate buffer (pH 7.2). During grinding, 50 mg of polyvinylpyrrolidone (PVP) and 1.5 mL potassium phosphate buffer containing sodium metabisulfite (0.01 g/100 mL) were added. The homogenate was centrifuged at 15,000 × g for 20 minutes at 4°C. A mixture of 20 µL of the extract and 980 µL of Bradford reagent was prepared, and absorbance was measured at 595 nm using a spectrophotometer. The protein content was calculated using bovine serum albumin (BSA) as a standard.

Statistical analysis

All experimental data were analyzed using analysis of variance (ANOVA) in SAS software version 9.4. The least significant difference (LSD) test was applied to determine significant differences among mean values at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of in vitro drought stress induced by polyethylene glycol (PEG) on morphological parameters in D. moldavica

The results demonstrated that increasing concentrations of PEG significantly reduced the morphological parameters of *D. moldavica*, including the number of shoots per explant, shoot length, number of leaves, root number, root length, and survival percentage (Table 1). The results demonstrated that reduced its level in the growth medium by 25.8%, 41.5% and 81.4% for the number of shoots, 38.5%, 63.2% and 87.7% for shoot length, 26.1%, 50.9% and 82.7% for number of leaves, 38.4%, 46.5% and 72.9% for root number, 36.6%, 57.9% and 92.7% for root length, and 39%, 50% and 86% for survival percentage, in concentrations 10%, 15% and 20% PEG respectively, relative to the control. Among the tested PEG concentrations, 10% PEG induced the least reduction in these parameters compared to higher concentrations of 15% and 20%. These results indicate that 10% PEG imposed moderate drought stress that was optimal for further experiments, as it provided measurable stress effects while maintaining sufficient survival. In contrast, severe drought stress induced by 20% PEG drastically decreased plant growth, with survival rates dropping to only 14%, accompanied by significant reductions in all other morphological traits. These observations suggest that higher PEG concentrations impose excessive osmotic stress, leading to poor plant viability and limited growth.

Table 1. Effect of drought stress induced *in vitro* by polyethylene glycol (PEG) on different morphological parameters in *D. moldavica*.

PEG	Number of shoots	Length of shoots (cm)	Number of leaves	Number of roots	Length of root (cm)	Survival (%)
0	2.48 ± 0.27 ^a	2.85 ± 0.03 ^a	12.35 ± 0.27 ^a	10.15 ± 0.36 ^a	5.76 ± 0.12 ^a	100.00 ± 0.00 ^a
10	1.84 ± 0.22 ^{ab}	1.75 ± 0.01 ^b	9.12 ± 0.31 ^b	6.25 ± 0.21 ^b	3.65 ± 0.04 ^b	61.00 ± 0.03 ^b
15	1.45 ± 0.00 ^{ab}	1.05 ± 0.02 ^c	6.06 ± 0.34 ^c	5.43 ± 0.16 ^b	2.42 ± 0.12 ^c	50.00 ± 0.12 ^c
20	0.46 ± 0.24 ^b	0.35 ± 0.02 ^d	2.13 ± 0.23 ^d	2.75 ± 0.31 ^c	0.42 ± 0.16 ^d	14.00 ± 1.25 ^d

where, PEG = Polyethylene glycol. All the parameters have been recorded after 30 days of transfer in rooting media. Data are in the form of mean ± SEM, and means followed by the same letters within the columns are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

Environmental stress, particularly drought, poses a significant challenge to agricultural productivity by adversely affecting plant growth, physiology, and metabolism. Drought stress inhibits cellular division and elongation, leading to reduced leaf, stem, and root growth. This stress also disrupts photosynthetic efficiency by decreasing stomatal conductance and increasing stomatal resistance, ultimately lowering transpiration and photosynthetic rates (Golparvar *et al.*, 2015; Yang *et al.*, 2021). In this study, polyethylene glycol (PEG) was used to induce *in vitro* drought stress in *Dracocephalum moldavica*, and the alleviating effects of

sodium nitroprusside (SNP) were investigated. Drought stress significantly reduced all measured morphological parameters, including shoot and root traits and survival rates. The highest stress levels induced by 20% PEG resulted in severe growth inhibition, with survival rates dropping to 14%. These findings align with prior research indicating that severe drought stress reduces turgor pressure, impairs mitosis, and accelerates leaf senescence due to reduced water availability and nutrient absorption (Mahmood *et al.*, 2019; Seleiman *et al.*, 2021; Yang *et al.*, 2021). Meanwhile, Ali *et al.* (2024), found that higher concentrations of PEG (20%) resulted in adverse effects, including reduced callus mass and regeneration potential, while moderate PEG (5%) stimulated callus induction and regeneration in rice.

Effect of sodium nitroprusside (SNP) on morphological parameters under in vitro drought stress

SNP application demonstrated significant potential to mitigate the adverse effects of PEG-induced drought stress in *D. moldavica*. Morphological parameters showed marked improvements with the application of 100 μ M SNP (T2 treatment) compared to untreated controls (T1) and plants treated with 200 μ M SNP (T3). Specifically, T2-treated plants recorded the highest values for the number of shoots (1.90 ± 0.21), shoot length (1.93 ± 0.01 cm), number of leaves (14.12 ± 1.04), root number (8.46 ± 0.43), root length (4.91 ± 0.41 cm), and survival percentage ($79.21 \pm 0.34\%$). In comparison, plants treated with 200 μ M SNP (T3) exhibited a decline in these parameters, with significantly reduced shoot and root growth and survival rates. This indicates that excessive SNP concentrations may lead to oxidative stress, negatively affecting plant growth. Thus, 100 μ M SNP emerged as the optimal concentration for enhancing morphological traits under drought conditions (Table 2).

Table 2. Effect of sodium nitroprusside and polyethylene glycol on different morphological parameters in *D. moldavica* under *in vitro* drought condition.

PEG	Number of shoots	Length of shoots (cm)	Number of leaves	Number of roots	Length of root (cm)	Survival (%)
T0 (0% PEG) (control without stress)	1.95 ± 0.02^a	2.25 ± 0.12^a	13.40 ± 1.21^a	10.32 ± 0.16^a	6.12 ± 0.23^a	100.0 ± 0.0^a
T1 (10% PEG) (control with stress)	1.62 ± 0.17^{ab}	1.42 ± 0.14^{ab}	7.23 ± 0.14^{ab}	5.68 ± 0.36^b	2.89 ± 0.25^b	48.34 ± 0.31^b
T2 (10% PEG + 100 μ M SNP)	1.90 ± 0.21^a	1.93 ± 0.01^a	14.12 ± 1.04^a	8.46 ± 0.43^{ab}	4.91 ± 0.41^{ab}	79.21 ± 0.34^{ab}
T3 (10% PEG + 200 μ M SNP)	1.45 ± 0.29^b	0.35 ± 0.21^b	5.63 ± 0.31^b	5.78 ± 0.48^b	2.13 ± 0.15^b	47.43 ± 0.03^b

where, PEG = Polyethylene glycol, SNP = Sodium nitroprusside. Data are in the form of mean $\pm$ SEM, and means followed by the same letters within the columns are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

In the present investigation, T2 treatment showed best response with respect to number of shoots (1.90 ± 0.21) and length of shoots (1.93 ± 0.01 cm), as compared to control with stress (T1) under *in vitro* drought stress (Table 2). It was noted from the data that drought stress induced by 10 % PEG + 200 μ M SNP (T3) significantly reduced number of leaves by (5.63 ± 0.31) as compared to control without stress (T0) (Table 2). Also, these data are consistent with the previously obtained data on root growth stimulation in different plant species through treatment with SNP in optimal concentrations (Kolbert *et al.*, 2019; Pradhan *et al.*, 2020; Allagulova *et al.*, 2023a).

Moderate stress levels (10% PEG), however, allowed for partial growth, making this concentration ideal for further experimentation. SNP treatment demonstrated substantial

potential to counteract the effects of drought stress, particularly at 100 μM . Plants treated with 100 μM SNP exhibited significantly improved growth indices, including higher shoot and root numbers, increased length, and enhanced survival percentages. These results indicate the effectiveness of SNP as a nitric oxide (NO) donor in mitigating drought-induced growth inhibition. By promoting cytokinin signaling, NO likely enhances cell division and elongation, leading to improved shoot and root development (Ghadakchiasl *et al.*, 2017; Ullah *et al.*, 2025). NO plays a pivotal role in the formation of root architecture, modulating the growth of the primary roots, lateral and adventitious roots, and root hair development (Lubyanova and Allagulova, 2024). There is considerable evidence demonstrating the alleviation of the negative effects of different stresses via the exogenous treatment of plants with NO donors in proper concentrations, indicating their potential practical application to improve crop growth and productivity (Lubyanova *et al.*, 2022).

Shehzad *et al.* (2023) reported pretreatment SNP under drought caused a remarkable increase in growth traits like shoot length, root length, shoot fresh weight, shoot dry weight, root dry weight in sunflower.

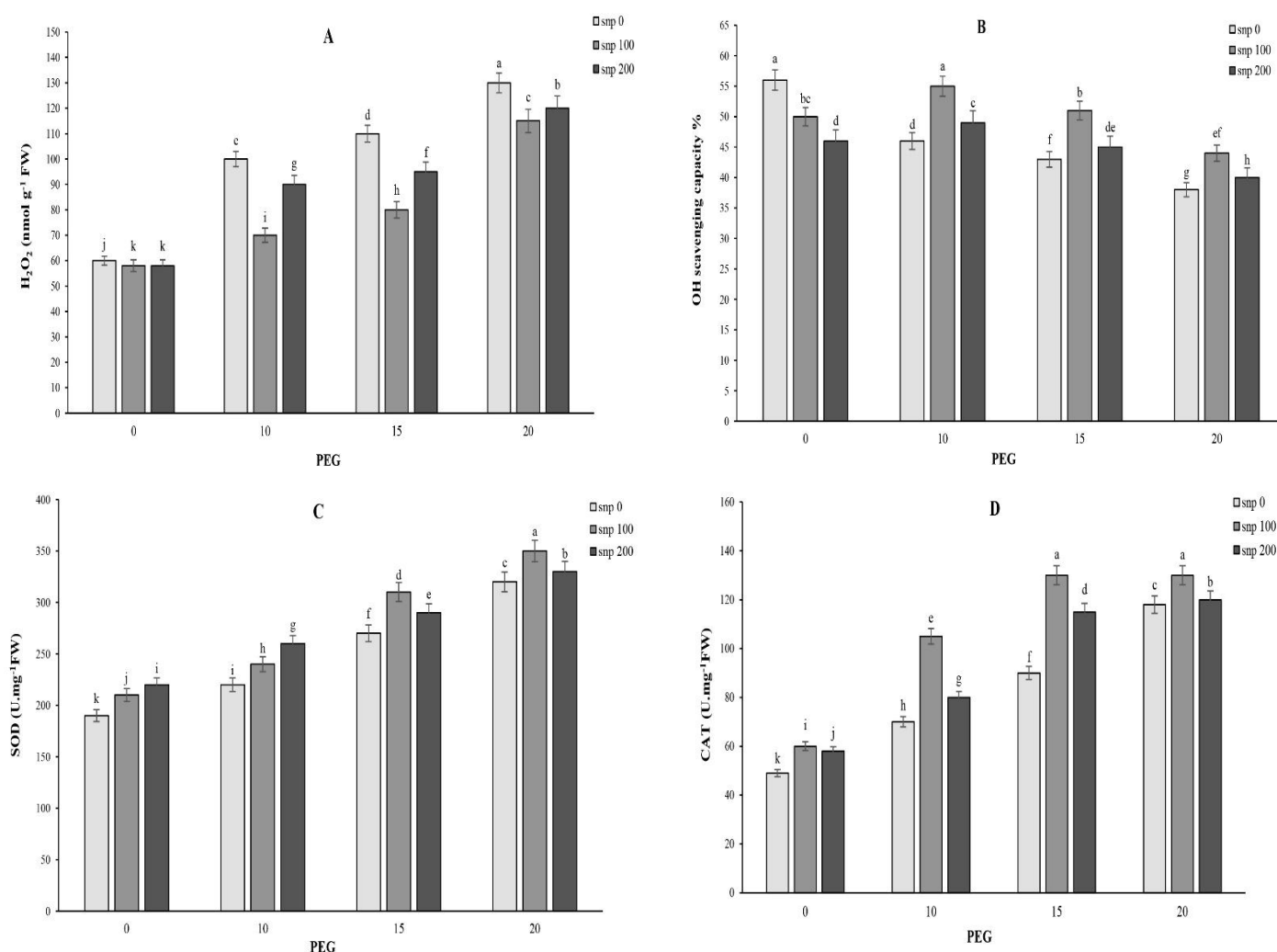
In research by Jafari and Shahsavar, (2022) showed that drought stress by using polyethylene glycol (PEG 6000) led to a reduction in shoot number, shoot length, leaf number, and fresh and dry weight, while the application of 25 μM SNP led to a drought stress tolerance of *Citrus aurantifolia* under in vitro conditions. Also Sundararajan *et al.* (2022) reported pretreatment with SNP in the concentrations of 150 μM resulted in effective mitigation of drought stress in *Solanum lycopersicum* seedlings. Also, in research by Allagulova *et al.* (2023a) showed that SNP pretreatment has stimulatory and protective effects on the growth of shoots and roots of wheat seedlings subjected to salinity or PEG-induced dehydration. Maslennikova *et al.* (2017) reported pretreatment with SNP or its presence in the germination medium at the concentrations of 50–200 μM promoted the subsequent increase in the linear sizes of the shoots and roots of 4–7-day-old wheat seedlings. On the other hand, it has been reported that NO is able to suppress root growth, the exogenous NO treatment of tomato plant inhibited the growth of the primary roots, indicating its concentration dependent role in the regulation of root growth (Correa-Aragunde *et al.*, 2006).

Effect of SNP on physiological parameters under in vitro drought stress

Our previous studies suggested that high concentrations of polyethylene glycol (PEG) increased the production of H_2O_2 , superoxide dismutase activity, catalase activity, peroxidase activity and a significant drop in protein content (Figure 1). Pretreatment with SNP decreased PEG-induced root and leaves damages by differently regulating the antioxidant enzymes under stress conditions (Table 2). PEG-induced osmotic stress caused a rapid and reversible increase H_2O_2 production, plants exposed to drought stress without SNP treatment (20% PEG + 0 SNP) exhibited the highest H_2O_2 levels, indicating severe oxidative stress. SNP application, particularly at 100 μM , significantly reduced H_2O_2 accumulation compared to untreated controls, demonstrating its role in mitigating oxidative damage (Figure 1A).

Drought stress reduced the antioxidant capacity of plant tissues, reflected by decreased $\cdot\text{OH}$ scavenging activity. However, SNP-treated plants showed significant improvements in

antioxidant capacity, with 100 μM SNP restoring $\cdot\text{OH}$ scavenging activity to near-optimal levels (Fig. 1B). SNP treatments significantly enhanced the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Plants treated with 100 μM SNP exhibited the highest enzyme activities compared to other treatments, underscoring the role of SNP in activating enzymatic defense mechanisms (Figure 1C, D, E). Drought stress (20% PEG) significantly reduced protein content in untreated plants. SNP application restored protein levels, with the highest content observed in plants treated with 100 μM SNP. This indicates the role of SNP in maintaining cellular homeostasis and metabolic activity under stress conditions (Figure 1F).



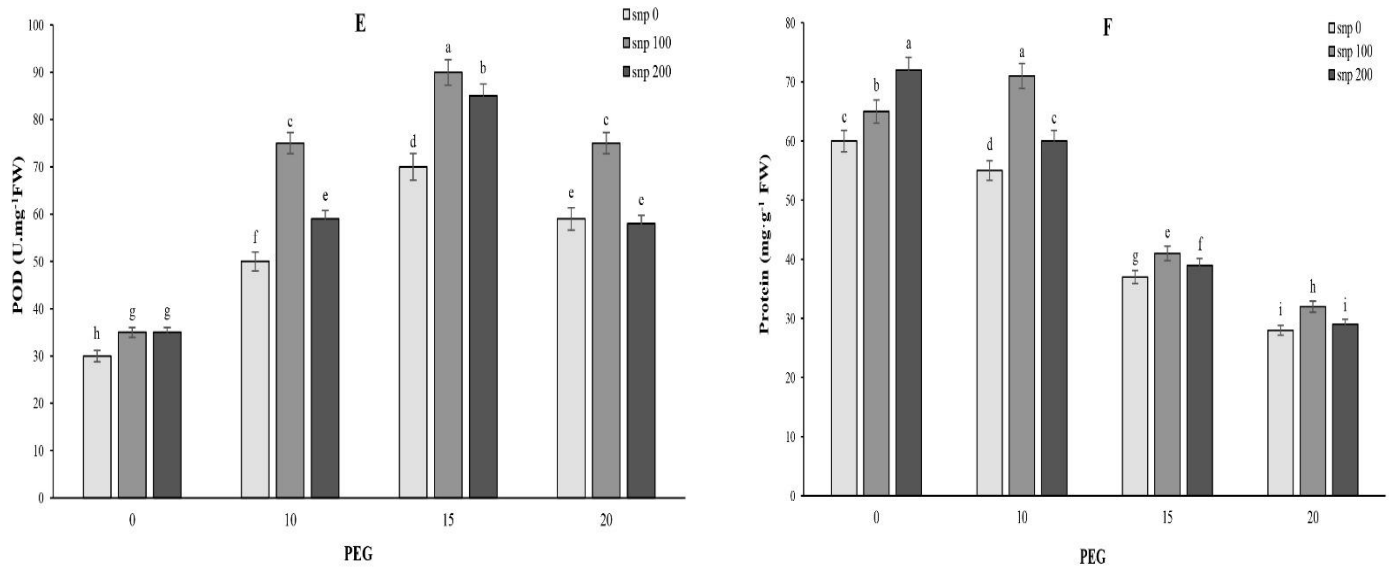


Figure 1. Effect of sodium nitroprusside (SNP) and polyethylene glycol (PEG) on H₂O₂ content (A), OH scavenging (B), superoxide dismutase (SOD) activity (S), catalase (CAT) activity (D), peroxidase (POD) activity (E) and protein content (F) in *D. moldavica* grown under *in vitro* drought condition. Different letters above the bars indicate statistically significant differences ($P < 0.01$).

Drought stress elevates reactive oxygen species (ROS) levels, including free radicals (superoxide anion, O₂^{•-}, hydroproxyl radical HO₂[•], alkoxy radical RO[•] and hydroxyl radical, •OH) and nonradical molecules (hydrogen peroxide, H₂O₂, and singlet oxygen ¹O₂), which cause oxidative damage to lipids, proteins, and nucleic acids (Hasanuzzaman *et al.*, 2020; Farooq *et al.*, 2020), the present outcomes are in agreement with Farooq *et al.* (2020) and Farouk and Al-Huqail, (2020). This study found that untreated plants exposed to high PEG concentrations exhibited elevated H₂O₂ levels, indicating heightened oxidative stress. SNP application, particularly at 100 μM, significantly reduced H₂O₂ accumulation, highlighting its role in alleviating oxidative damage.

The improved oxidative stress response in SNP-treated plants can be attributed to enhanced antioxidant defense systems. Plants keep ROS under control by an efficient and versatile scavenging system; including antioxidant enzymes (catalase, CAT; superoxide dismutase, SOD; peroxidase, POD; ascorbate peroxidase, APX; etc.) and non-enzymatic (ascorbate, ASC; glutathione, GSH; carotenoids; phenolic acids; flavonoids; etc.). These substances can react directly with ROS or appear as substrates of enzymes in the ROS scavenging mechanism (Young and Lowe, 2018; Laxa *et al.*, 2019). SNP-treated plants exhibited higher activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which are key enzymes in detoxifying ROS.

SOD activity acts to alleviate these effects by promoting the transformation of O₂^{•-} into less harmful H₂O₂ and O₂ (Kaya *et al.*, 2024). The study observed an enhancement in SOD activity due to drought, but SNP treatment markedly increased SOD activity in *D. moldavica*, indicating that SNP does promote SOD activity to counteract ROS generation in the presence of drought. Additionally, CAT and POD enzyme activity further degrade H₂O₂ into H₂O and O₂, thereby maintaining cellular redox balance (Farouk and Al-Huqail 2020), increased in

response to drought and was further boosted by SNP treatment in *D. moldavica*. This suggests that SNP treatment enhances CAT activity, potentially contributing to the plant's competence to tolerate oxidative stress induced by drought. Moreover, the recorded increased in POD activity with SNP treatment may help reduce H₂O₂ accumulation, further enhancing the *D. moldavica* plant's resilience to oxidative damage. These findings corroborate previous studies indicating the role of SNP in enhancing enzymatic antioxidant activity under abiotic stress (Beligni *et al.*, 2002; Verma *et al.*, 2014).

SNP treatment increased the activity of antioxidant enzymes and upregulated the expression of APX genes in wheat plants under heat stress (Iqbal *et al.*, 2022). Spraying the soybean plants with 100 µM SNP reduced the levels of H₂O₂ accumulation and contributed to the additional activation of SOD, CAT, and APX in leaves under PEG-induced drought (Rezayian *et al.*, 2020), which is consistent with the results of this study. The decrease in PEG-induced H₂O₂ production by seedling under the influence of SNP during stress may be due to direct ROS interaction with the NO, leading to NOOO⁻ formation, which in turn may affect the catalytic activity of antioxidant enzymes (Lubyanova and Allagulova, 2024). Drought stress significantly reduced protein content in untreated plants, reflecting impaired metabolic activity. SNP application restored protein levels, with the highest content observed in plants treated with 100 µM SNP. This suggests that SNP mitigates drought-induced metabolic disruptions, enabling the maintenance of essential cellular processes. The protective effect of SNP likely involves stabilizing proteins and enzymes, enhancing stress tolerance (Farouk and Al-Huqail, 2020).

Interestingly, the study revealed a concentration-dependent effect of SNP, with 100 µM proving more effective than 200 µM in alleviating drought stress. While low concentrations of SNP promote growth and antioxidant activity, excessive concentrations may induce oxidative stress, impairing cellular function. High SNP levels may lead to ion imbalance, membrane degradation, and increased ROS production (Ghadakchiasl *et al.*, 2017), as evidenced by reduced growth and survival rates in plants treated with 200 µM SNP. It seems promising that the exogenous application of NO donors, including SNP, could increase plant stress resistance and crop productivity (Wimalasekera and Scherer, 2022). However, SNP is an unstable compound decomposing with the release of iron and cyanides, which can have a toxic effect on the plants, negating the positive effects of NO (Keisham *et al.*, 2019).

This finding supports the hypothesis that SNP, as a source of NO, improves plant resilience to drought by regulating ROS and enzyme activities. By enhancing morpho-physiological traits, antioxidant activity, and protein content, SNP application could improve the cultivation of this medicinally important plant in arid and semi-arid regions. The use of SNP offers a promising strategy for enhancing drought tolerance and optimizing essential oil production. Future research should focus on understanding the molecular mechanisms underlying SNP-induced stress tolerance. Studies exploring different SNP concentrations, application methods, and interactions with other signaling molecules could provide deeper insights into its role in mitigating abiotic stress. Additionally, field experiments are needed to validate the efficacy of SNP under natural drought conditions, paving the way for its practical application in sustainable agriculture.

CONCLUSION

This study demonstrates the significant impact of polyethylene glycol (PEG)-induced drought stress on the growth and physiology of *Dracocephalum moldavica* L., as well as the mitigating effects of SNP, a nitric oxide (NO) donor. Drought stress severely reduced the morphological traits of *D. moldavica*, including shoot and root growth, number of leaves, and survival rates, with the most pronounced effects observed at higher PEG concentrations. SNP application significantly alleviated the adverse effects of drought stress, with 100 μ M SNP emerging as the most effective concentration for improving plant performance. The application of 100 μ M SNP not only enhanced growth parameters but also bolstered the plant's physiological defense mechanisms by reducing H_2O_2 levels, increasing ($\cdot OH$) scavenging activity, and boosting the activities of key antioxidant enzymes such as (SOD), (CAT), and (POD). Moreover, SNP-treated plants exhibited higher protein content, reflecting improved metabolic activity and cellular homeostasis under drought stress. The results underscore the potential of SNP as an effective growth regulator for improving drought tolerance in *D. moldavica*. This finding holds practical significance for the cultivation of drought-tolerant medicinal plants, particularly in arid and semi-arid regions, where water scarcity is a major limiting factor. The enhanced stress resilience provided by SNP can contribute to increased yields of essential oils and other bioactive compounds, thereby supporting the pharmaceutical and aromatic industries. Our results indicate that SNP is able to mitigate the destructive effects of osmotic stress on *D. moldavica* seedlings. However, the mechanisms of SNP protective action may be different at certain periods of stress exposure. Additionally, field trials are necessary to validate these findings under natural drought conditions, enabling the development of practical applications for sustainable agriculture. Investigations into the synergistic effects of SNP with other plant growth regulators and biostimulants could also provide insights into optimizing stress management strategies.

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