



Assessing the Impact of Simulated Water and Salinity Stress on the Physiological and Biochemical Characteristics of [*Bacopa monnieri* (L.) Wettst.]

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The growth, development, and biosynthesis of secondary metabolites in the plant are strongly affected by environmental stress. The pharmaceutical values of medicinal herbs depend on the presence of secondary metabolites that are influenced by environmental stress. Drought and salinity stress are the two most significant abiotic factors that restrict plant growth and productivity. As a memory enhancer, anti-inflammatory, analgesic, antipyretic, sedative, and antiepileptic,

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Bacopa monnieri has been used for a long time. The aim of the present work was to study the impact of simulated abiotic stress on *Bacopa monnieri*.

Methodology: Potted plants of *Bacopa monnieri* were treated with various concentrations of NaCl and polyethylene glycol (PEG) solution to simulate salinity and drought stress. In order to evaluate the salinity and drought stress effect, several physiological parameters, such as cell membrane stability and relative water content (RWC), and biochemical parameters, such as proline, β -carotene, MDA, total phenolic, and total flavonoid content, were taken into consideration.

Results: In the present study, significant changes in the physiological and biochemical parameters have been observed in NaCl and PEG 6000-induced salt- and drought-stressed plants about control. Stress decreases the relative RWC and Membrane stability index of the *Bacopa* leaves. However, it increases the plant's proline, β -carotene, phenolic compounds, and total flavonoids content.

Conclusion: The data obtained from the current study indicated that both salinity and drought stress influence the physiological and biochemical traits of *Bacopa monnieri* L. By increasing the synthesis of proline, β -carotene, phenolic compounds, and total flavonoids, the *Bacopa monnieri* plant defends itself against oxidative stress.

Keywords: Salinity; drought; *bacopa monnieri*; RWC; proline; MDA; antioxidant.

1. INTRODUCTION

Abiotic factors like light, temperature, water, salt, and so on impact the development, growth, and geographic distribution of plants. These environmental factors reduce the productivity of plants to different extents, depending upon the severity of stress. Plants undergo several morphological, physiological, and biochemical changes to adapt to these abiotic stressors. The accumulation of specific low-molecular-weight natural metabolites, referred to as compatible solutes, aids in ensuring the survival and the development of the plant. It has also been reported that these substances are essential for cellular osmotic adjustment to salt and osmotic stressors (Gupta and Huang, 2014). Drought and salinity are the major environmental factors influencing plant growth and development and hindering crop yields. Thus, enhancing crop performance in salinity and water stress scenarios is a crucial study topic for plant scientists worldwide. Plants show a wide range of physiological and biochemical responses at the cell and whole-organism levels against existing water shortages, which makes it a complex phenomenon (Farooq et al., 2009; Seleiman et al., 2021). Plants that experience water deficit apply a variety of strategies, including osmotic and hormonal regulation, leaf rolling, root length increment, accumulation of compatible solutes etc. Although the effect of drought stress has been investigated extensively among various crop plants across the world, there is little evidence available on the effects of different water stresses on the growth, physiological, and biochemical characteristics of medicinal plants (Tátrai et al., 2016; Liu et al.,

2017). Similar to other abiotic stressors, salt stress has numerous detrimental effects on plants. Salt stress raises the cellular osmotic pressure and even can lead to an elevation of sodium to levels that are toxic. It causes oxidative and the cellular osmotic pressure and even can lead to elevation of sodium to toxic levels. It causes oxidative and osmotic stress, ion toxicity, nutritional and hormonal abnormalities, and a rise in plant disease susceptibility (Hasanuzzaman and Fujita, 2022; Xiao and Zhou, 2023). Developing salt-tolerant plant types, optimizing nutrient absorption and utilization, regulating plant hormones, and improving morpho-physiological features are essential strategies for reducing the negative impact of salt stress on plant (Ahmad et al., 2023). Abiotic stressors, such as salinity, are known to induce oxidative damage primarily by producing of excess reactive oxygen species (ROS), which can target proteins, carbohydrates, lipids, and DNA (Gill and Tuteja, 2010). Different plant species might have varying levels of tolerance to salt and water stress.

Since ancient times, medicinal herbs have been widely utilized to treat diseases and improve health among many indigenous groups worldwide. *Bacopa monnieri* (L.) Wettst., a popular medicinal plant from the Scrophulariaceae family, has been traditionally used for years as a memory booster and for its anti-inflammatory, analgesic, antipyretic, sedative, and anti-epileptic properties (Lal and Baraik, 2019). Both abiotic and biotic variables play a key role in promoting the synthesis of secondary metabolites in medicinal herbs (Ramakrishna and Ravishankar, 2011). In light of

the above fact, the present study was undertaken on the *Bacopa* plant to study the response of this plant to salinity and drought stress by examining the physiological and biochemical changes in plant leaves after subjecting to different levels of NaCl and PEG solution.

2. MATERIALS AND METHODS

2.1 Collection of the Plant

First the fresh plant of *Bacopa monnieri* was collected from the Hatma locality situated behind the DSPM University, Ranchi campus. The plant were then planted and maintained in the sandy soil of the garden for further experiment.

2.2 Establishment of Potted Plant and Stress Treatment

The *B. monnieri* runners of even length (6–8 cm) bearing at least 3–4 nodes were first kept in sterile water for the propagation of root. Once root growths were observed, plants of even length along with roots were transplanted to the experimental pots containing 0.5 kg of sandy soil consisting of sandy loam soil: vermicompost (3:1 w/w). The plants were further maintained in the University garden and watered daily for 15 days. After this period, they were treated with 0 mM (control), 50 mM, 100 mM, 150mM and 200 mM, and 250mM NaCl, as well as with PEG 6000 solutions at concentrations of 0% (control), 5%, 10%, 15%, and 20% in water for a week. All experiments were performed in triplicates.

2.3 Physiological and Biochemical Analysis

Following the treatments, the plants were used for further analysis of relative water content (RWC), Membrane Stability Index (MSI), proline, β -carotene, malondialdehyde (MDA), total phenolic content and flavonoid content.

2.3.1 Relative water content

Leaf relative water content (RWC) was determined according to Galmes et al. (2007). Fully expanded young leaves from each treatment were weighed to obtain fresh leaf weight. Immediately leaves were hydrated by floating on de-ionized water in a closed petri dish. After hydration turgid weights were taken and oven dried at 70°C for 48hr. Finally dry

weight of the leaves was measured. Leaf relative water content was calculated according to the equation:

$$\text{RWC (\%)} = (\text{Fresh weight} - \text{Dry weight} / \text{Turgid weight} - \text{Dry weight}) \times 100$$

2.3.2 MSI (Membrane stability index)

Leaves membrane stability index (MSI) has been calculated using the protocol of Premchandra et al. (1990) as modified by Sairam (1994). 100 mg of leaves were carefully cleaned under running water, then rinsed with double-distilled water. The leaves were heated for 30 minutes at 40°C in 10 ml of double-distilled water. The EC (Electrical Conductivity) meter was then used to measure electrical conductivity (C1). The same samples were then put in a boiling water bath (100°C) for 10 minutes, and the electrical conductivity (C2) was also noted. The following formula was used to determine the membrane stability index:

$$\text{Membrane stability index (MSI)} = [1 - (C_1/C_2)]100.$$

2.3.3 Estimation of proline content

The proline content was determined using the method described by Bates et al. (1973). 0.5g of plant material (leaf) was homogenized in 5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. 2 ml of the extract was thoroughly treated with 2 ml glacial acetic acid and 2 ml of Ninhydrin reagent (1.25 g Ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) at 100°C in a boiling water bath for 30 min. The reaction was then terminated instantly by dipping in an ice bath. The reaction mixture was extracted with 4 ml toluene. The chromophore containing toluene was warmed to room temperature and its optical density was measured at 520 nm in a spectrophotometer against a toluene blank.

Proline levels were calculated using a standard curve that ranged from 20-100 $\mu\text{g/ml}$.

2.3.4 Determination of leaf malondialdehyde (MDA)

The level of malondialdehyde (MDA) was determined using 2-thiobarbituric acid (TBA) based on the methods described by Hodges et al. (1999) and Sarkar and Oba (2018a). One gram of freshly ground *Bacopa* leaves was homogenized with 5 ml of 0.6% TBA in 10%

trichloroacetic acid (TCA) using a pestle and mortar. The mixture was then heated at 100°C for 15 minutes, cooled on ice, and centrifuged at 5000 rpm for 10 minutes. The absorptions were recorded at 450, 532, and 600 nm, and the MDA content was calculated using the formula provided.

$$\text{MDA } \mu\text{molg}^{-1}\text{FW} = 6.45(\text{OD}_{532} - \text{OD}_{600}) - 0.56 \text{OD}_{450}$$

2.3.5 β - Carotene

The β -carotene content was determined following the procedure described by Sarker and Oba (2019a). A fresh leaf sample weighing 500 mg was homogenized using a mortar and pestle with 10 ml of 80% acetone. The sample was centrifuged at a speed of 10,000 rpm for a duration of 3–4 minutes. After removing the supernatant, the volume was adjusted to 20 ml by using a volumetric flask. Absorbance readings were taken at 510 nm and 480 nm using a spectrophotometer. The β -carotene content was expressed as micrograms per gram of fresh weight ($\mu\text{g g}^{-1}$ FW) and calculated using the following formula

$$\beta\text{-carotene} = 7.6 (\text{Abs. at } 480) - 1.49 (\text{Abs. at } 510) \times \text{Final volume} / (1000 \times \text{fresh weight of leaf taken})$$

Samples extraction for total phenolic content (TPC) and total flavonoid content (TFC)

Bacopa plant leaves from each treatment were collected and dried in the shade before being subjected to chemical analysis. One gram of ground dry leaves from all treatments was extracted using 40 ml of 90% aqueous methanol in a 100 ml vial that was tightly closed and shaken repeatedly for one hour. The extract was subsequently filtered to detect polyphenols and flavonoids.

2.3.6 Estimation of total phenolic content (TPC)

Method of Sarker and Oba (2018b, 2019b) was followed to estimate the total phenolic content of the *Bacopa* plant using the folin-ciocalteu reagent with gallic acid as a standard phenolic compound. The results are expressed as μg gallic acid equivalent per gram dry weight ($\mu\text{gGAE g}^{-1}\text{DW}$).

2.3.7 Estimation of Total Flavonoid Content (TFC)

The AlCl_3 colorimetric method was used to estimate the total flavonoid content of *Bacopa* plant extract (Sarkar and Oba, 2019a, 2020a).

TFC is expressed as μg quercetin equivalent per gram dry weight ($\mu\text{gQE g}^{-1}\text{DW}$).

3. RESULTS

3.1 Relative Water Content (RWC)

The study showed that control plants not exposed to salinity or drought stress had the highest relative water content (RWC), which declined with increased stress levels. Under NaCl stress, control plants maintained an RWC of $95.3 \pm 0.62\%$, but it dropped to $92.5 \pm 0.28\%$ at 50mM NaCl. RWC decreased further to $85.3 \pm 1.2\%$ at 150mM, $76.36 \pm 0.52\%$ at 200mM, and $74.98 \pm 2.36\%$ at 250mM NaCl. Similarly, under PEG-induced drought stress, control plants had an RWC of $94.52 \pm 0.83\%$, declining slightly to $92.99 \pm 1.02\%$ at 5% PEG. Further drops were observed at 10% ($91.46 \pm 1.16\%$), 15% ($90.13 \pm 0.29\%$), and 20% PEG ($83.32 \pm 0.27\%$), indicating increasing water stress (Fig. 1).

3.2 Membrane Stability Index (MSI)

Under NaCl stress, MSI showed a slight decline with increasing salinity levels. With the application of 50mM NaCl, MSI slightly decreased to 94.83 ± 0.13 in compared to control plant (95.02 ± 0.33). Further increases in salinity led to progressive reductions in MSI, with values of 94.63 ± 0.24 at 100mM, 94.55 ± 0.34 at 150mM, and 94.01 ± 0.7 at 200mM NaCl. The most significant decline was observed at 250mM NaCl, where MSI dropped to 92.41 ± 0.59 . Similarly, in PEG-induced stress study the control plant maintained the highest MSI (95.23 ± 0.12), which gradually decreased with increasing stress level. At 5% PEG, MSI slightly decreased to 94.63 ± 0.08 , followed by a further reduction to 94.39 ± 0.33 at 10% PEG. A more noticeable decline was observed at 15% and 20% PEG, where MSI dropped to 91.42 ± 0.89 and 88.2 ± 0.51 respectively (Fig. 2).

3.3 Proline

The results of the present study demonstrated that the plants not exposed to any stress, exhibited the lowest proline, with values of $0.05 \pm 0.01 \mu\text{molg}^{-1}$ for NaCl stress and $0.04 \pm 0.01 \mu\text{molg}^{-1}$ for the PEG stress study. As NaCl concentration increases, there is a progressive rise in proline levels. At 100 mM and 150 mM, it increases to 0.07 ± 0.01 and $0.09 \pm 0.01 \mu\text{molg}^{-1}$, respectively. A significant increase in proline content was observed at 200 mM, where the proline level reaches $0.21 \pm 0.04 \mu\text{molg}^{-1}$, and it further increases to $0.43 \pm 0.02 \mu\text{molg}^{-1}$ at 250

mM. Similarly, PEG stress leads to an increase in proline content with rising concentrations. At 5%, the proline level is $0.05 \pm 0.01 \mu\text{mol g}^{-1}$, similar to the control for NaCl stress. This value

increases to 0.07 ± 0.01 at 10% PEG, then to $0.09 \pm 0.01 \mu\text{mol g}^{-1}$ at 15%. However, at 20% PEG, the proline level slightly declines to $0.08 \pm 0.01 \mu\text{mol g}^{-1}$ (Fig. 3).

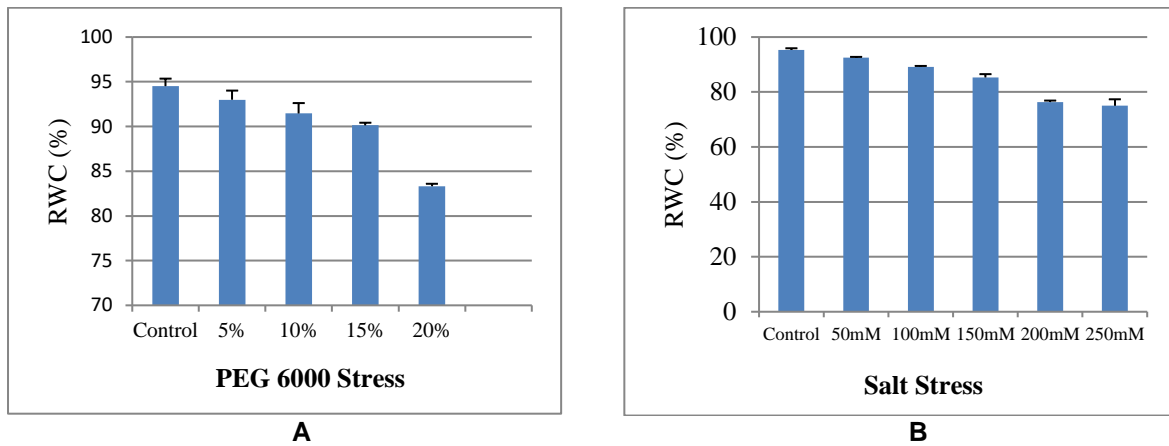


Fig. 1. RWC in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

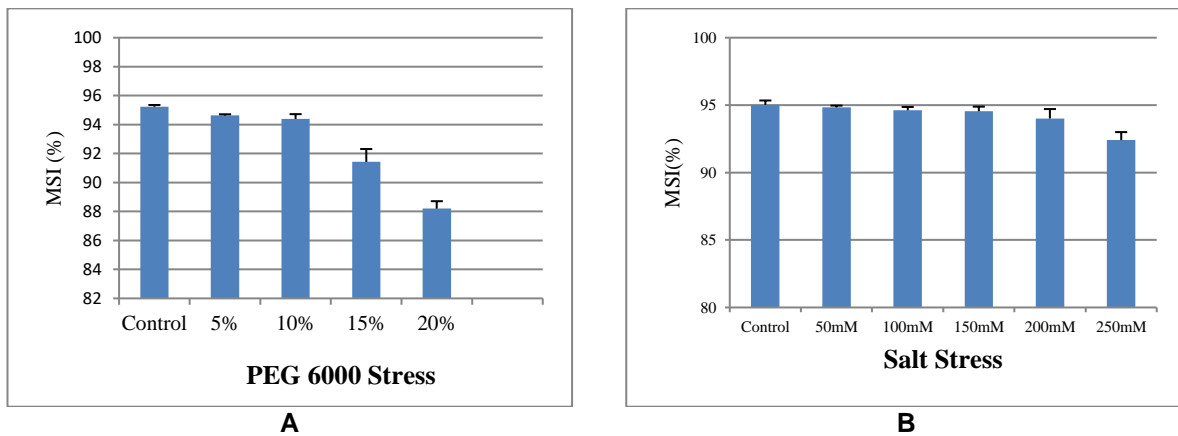


Fig. 2. MSI in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

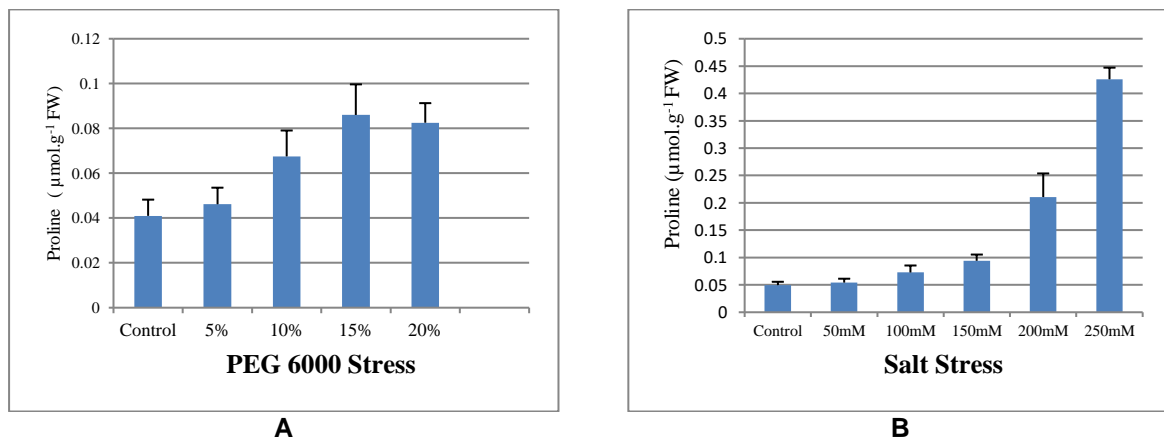


Fig. 3. Proline content in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

3.4 MDA

Under control conditions, the MDA levels are relatively low, measured at $1.61 \pm 0.17 \mu\text{mol g}^{-1}$ for NaCl stress and $1.46 \pm 0.31 \mu\text{mol g}^{-1}$ for PEG stress. With increasing NaCl concentrations, MDA levels rise progressively, reaching 1.97 ± 0.28 at 50 mM, 2.52 ± 0.1 at 100 mM, and peaking at $2.99 \pm 0.26 \mu\text{mol g}^{-1}$ at 200 mM. Interestingly, at 250 mM NaCl, the MDA level decreases slightly to $2.22 \pm 0.12 \mu\text{mol g}^{-1}$, possibly indicating a cellular adjustment to prolonged stress or a threshold effect. For PEG stress, MDA levels also increase with concentration initially, rising to 1.97 ± 0.13 at 5%, 2.69 ± 0.04 at 10%, and peaking at $2.81 \pm 0.13 \mu\text{mol g}^{-1}$ at 15%. However, at 20% PEG, a notable decline in MDA levels is observed, dropping to $1.83 \pm 0.09 \mu\text{mol g}^{-1}$, which may reflect a similar threshold or adaptive response as seen in NaCl stress (Fig. 4).

3.5 β -carotene

β -carotene levels under control conditions are measured at $0.17 \pm 0.02 \mu\text{g g}^{-1}$ for NaCl stress and $0.18 \pm 0.01 \mu\text{g g}^{-1}$ for PEG stress studies. With increasing NaCl concentrations, there is a gradual rise in β -carotene levels, reaching 0.19 ± 0.01 at 50 mM, 0.21 ± 0.01 at 100 mM, and $0.27 \pm 0.02 \mu\text{g g}^{-1}$ at 150 mM as compared to control plant (0.17 ± 0.02). The maximum β -carotene level of $0.28 \pm 0.05 \mu\text{g g}^{-1}$ is observed at 200 mM NaCl. However, at 250 mM, β -carotene levels decrease slightly to $0.24 \pm 0.02 \mu\text{g g}^{-1}$. For PEG stress, β -carotene levels also increase initially to 0.2 ± 0.02 at 5%, 0.26 ± 0.01 at 10%, and peaking at $0.29 \pm 0.02 \mu\text{g g}^{-1}$ at 15% in comparison to non stressed plant ($0.18 \pm 0.01 \mu\text{g g}^{-1}$). However, at 20% PEG, β -carotene levels decline slightly to $0.26 \pm 0.01 \mu\text{g g}^{-1}$ (Fig. 5).

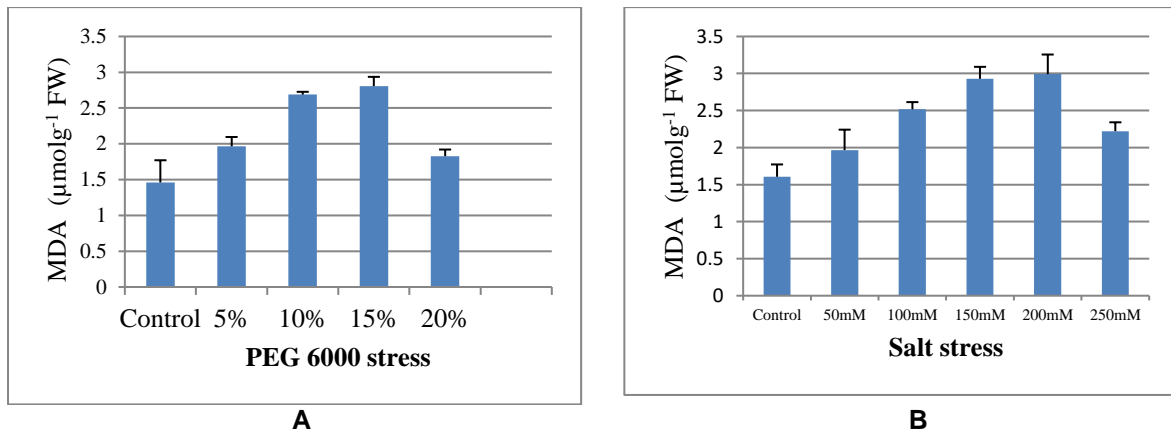


Fig. 4. MDA content in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

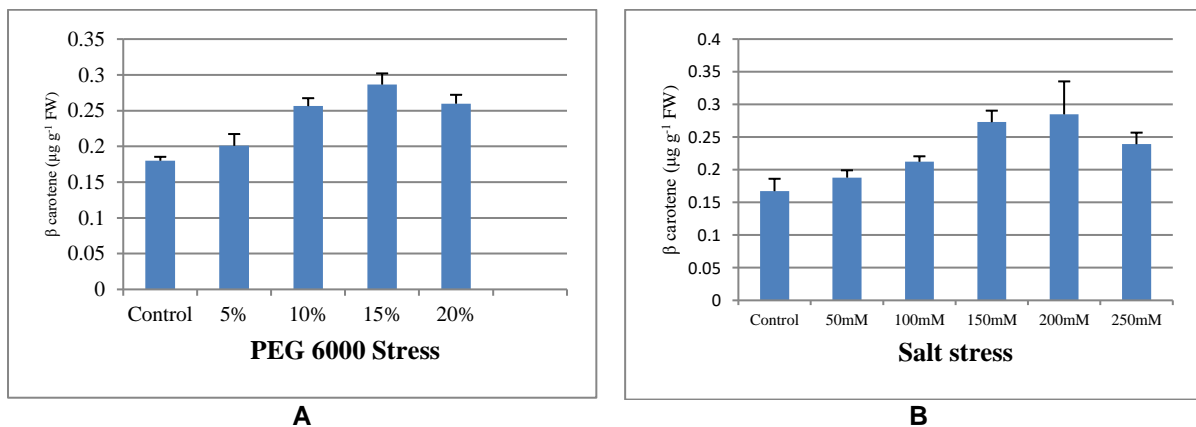


Fig. 5. β carotene in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

3.6 Phenol

Phenol levels increased significantly with rising salinity, indicating an enhanced antioxidant response to mitigate oxidative damage. Starting at $163.76 \pm 3.94 \mu\text{gGAE g}^{-1}$ in control plants, phenol content rose steadily to 256.92 ± 16.56 at 50mM and peaked at 150mM ($365.71 \pm 10.54 \mu\text{gGAE g}^{-1}$). However, at the highest concentration 200mM and 250mM NaCl, phenol levels decreased further to 326.09 ± 6.95 and $300.81 \pm 4.34 \mu\text{gGAE g}^{-1}$ respectively. Under PEG-induced drought stress, phenol levels also increased, with a similar trend to NaCl stress. Control plants recorded 161.63 ± 4.89 , rising to $270.5 \pm 25.06 \mu\text{gGAE g}^{-1}$ at 5% PEG. Phenol content peaked at 15% PEG ($347.95 \pm 15.22 \mu\text{gGAE g}^{-1}$), indicating a strong antioxidative response to severe drought conditions. However, at 20% PEG, phenol levels declined to $318.74 \pm 4.09 \mu\text{gGAE g}^{-1}$ (Fig. 6).

3.7 Flavonoid

Under NaCl stress, flavonoid levels increased progressively from the control group ($99.42 \pm 3.67 \mu\text{gQEg}^{-1}$) to a peak at 150mM NaCl, where the highest concentration of flavonoids was recorded at $160.63 \pm 4.5 \mu\text{gQEg}^{-1}$. However, at higher NaCl concentrations (200mM and 250mM) flavonoid content decreased to 143.49 ± 1.42 and $121.4 \pm 3.66 \mu\text{gQEg}^{-1}$, respectively. PEG-induced drought stress also led to a significant increase in flavonoid content. Starting from the control ($98.49 \pm 4.11 \mu\text{gQEg}^{-1}$), flavonoid levels increased to 138.9 ± 3.3 at 5% PEG and continued to rise to $141.4 \pm 8.85 \mu\text{gQEg}^{-1}$ at 10% PEG. The highest flavonoid content ($156.52 \pm 3.71 \mu\text{gQE g}^{-1}$) was observed at 15% PEG. However, at 20% PEG, flavonoid content decreased to $133.57 \pm 5.38 \mu\text{gQEg}^{-1}$ (Fig. 7).

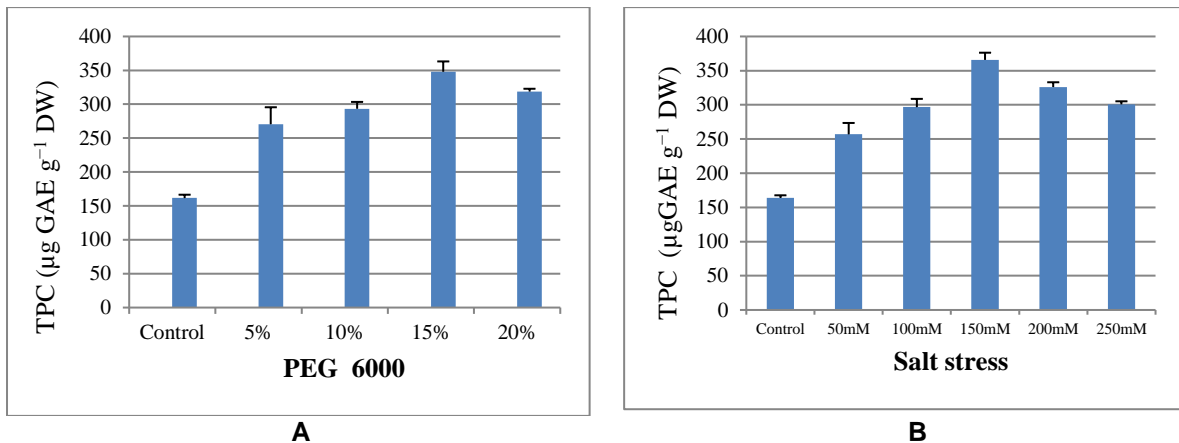


Fig. 6. Total phenolic content (TPC) in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

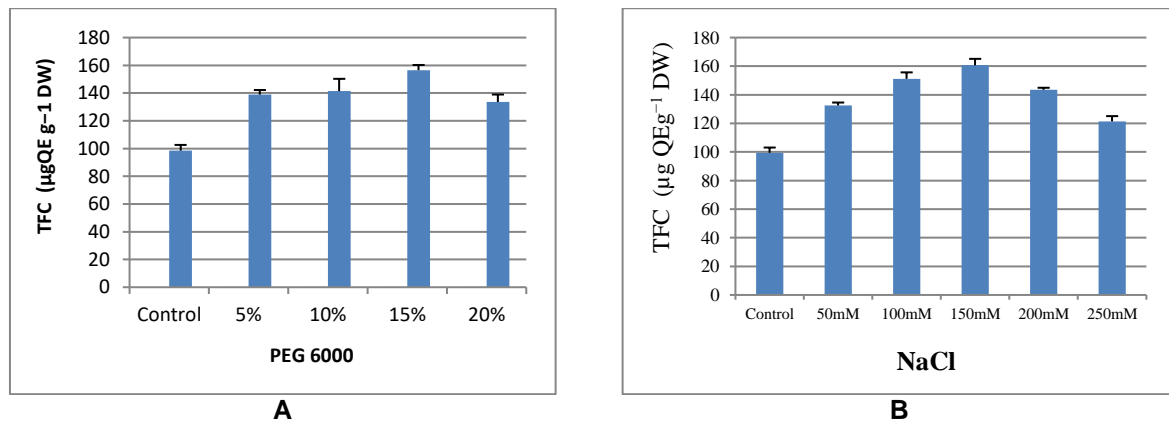


Fig. 7. Total Flavonoid content (TFC) in *Bacopa monnieri* simulated with (A) PEG 6000 and (B) NaCl induced stress

4. DISCUSSION

Relative water content is utilized as a marker of a plant's water status and its capacity to cope with osmotic stress. The present study clearly demonstrates that both NaCl and PEG-induced stress significantly reduce the RWC of the plants. The study suggests that under normal, non-stress environments, the plants maintained appropriate hydration and physiological performance and under salinity and dehydration stress, there is a consistent and significant reduction in RWC as the severity of stress increases. Plants under mild stress (50mM NaCl and 5% PEG) caused slight reductions in RWC, indicating the onset of osmotic stress, though the impact is still minimal. As the stress level increases, RWC decreases more noticeably, suggesting a stronger osmotic imbalance caused by the higher salt and PEG concentration. The finding of the present study is also supported by the findings of other authors in different plant species. RWC in the *Catharanthus roseus* leaves decreased under the treatment of PEG 6000 at different concentrations, especially, when treated with 35% PEG 6000, which caused a sharp decrease (Liu et al., 2017). Other plant species likewise showed a noticeable decrease in relative water content when facing water stress, such as *Thymus citriodorus* (Tátraí et al., 2016), Cotton Species (Ul-Hasan et al., 2018), peanut (Meher et al., 2018). Under salt stress, a significant decline in relative water content has also been noted (Kumar et al., 2021; Khan et al., 2022; Boussora et al., 2024). These findings indicate that both salinity and water stress disrupt the osmotic balance between the plant cells and their environment, which leads to reduced water uptake, loss of turgor, and compromised physiological processes. These results highlight the detrimental effects of salinity and drought on plant hydration, turgor, and physiological processes, which can ultimately impact growth, productivity and survival.

Environmental stress disrupts plant cellular membranes, reducing their stability and increasing ion leakage. Measuring electrolyte leakage from stressed leaf tissue in an aqueous medium is a common method to assess cell membrane stability (CMS) and evaluate stress resistance (Sairam, 1994; Lal et al., 2006). Membrane stability index under a given amount of stress has also been utilized as an indicator of resistance to stress by many researchers (Premchandra et al., 1990; Lal et al., 2006; Venkateswarlu & Ramesh, 1993; Ahmed et al.,

2013; Assaha et al., 2016). Additionally, the cell membrane stability test can be employed in vitro to distinguish between genotypes that are sensitive to drought and those that are resistant to it (Venkateswarlu and Ramesh, 1993). In the present study, NaCl and PEG-induced stresses significantly affected the Membrane Stability Index (MSI), with the highest MSI observed in control plants under normal conditions. NaCl stress caused a gradual MSI decline, most notably at 250mM, where MSI dropped to 92.41 ± 0.59 . Similarly, PEG-induced drought stress led to reduced MSI, with a pronounced decline at 20% PEG (88.2 ± 0.51). These results suggest that both salinity and drought-like conditions disrupt membrane stability, with more impacts at higher levels of stress. The data highlights the plants' susceptibility to environmental stresses, as the MSI values decrease as stress intensity increases. A significant decrease in MSI under salinity stress has been reported in mulberry (Lal et al., 2006). In barley, a notable decline in the cell membrane stability index was also noted in responses to the combined stress of saline and drought (Ahmed et al., 2013). A negative correlation between membrane stability as determined by electrolyte leakage and PEG concentration was found in groundnut cultivars (Venkateswarlu & Ramesh, 1993).

Plants are able to survive in harsh environments by synthesizing and accumulating osmolytes, or suitable solutes, in there. Because of their low molecular mass and solubility, these substances can sustain physiological and biochemical functions without interfering with them. Stress-induced accumulation of proline and soluble carbohydrates provides protection to the cell by preserving the cytosol's osmotic strength relative to the vacuole and surrounding environment. Proline not only protects against osmoprotection but also scavenges reactive oxygen species (ROS) and inhibits lipid peroxidation, hence reducing cellular damage (Szabados and Savouré, 2010), Hayat et al., 2012, Rahnesan et al., 2018, Shahid et al., 2020, Kishor et al., 2022). In the present study we observed increased proline content with increasing stress level under both salinity and drought stress. NaCl and PEG stress induce proline accumulation as a potential adaptive response, with NaCl stress showing a more pronounced increase at higher concentrations. The current study's findings corroborate previous reports on the accumulation of free proline under stress in *Bacopa monnieri*. (Debnath, 2008; Afroz et al., 2020). This trend

highlights the role of proline as a stress-responsive molecule in plants under salinity and drought conditions. However, the observed slight decrease in proline levels under severe PEG stress (20%) may be attributed to a decline in the plant's capacity to synthesize proline, a phenomenon likely associated with the detrimental effects of stress on overall plant growth. Accumulation of proline contents with increasing stress level indicates an active role in the osmotic regulation and adaptability to stress of *Bacopa*, as noted for other plant species like cotton (Ul-Hasan et al., 2018), *Oenanthе javanica* (Kumar et al., 2021), *Gossypium hirsutum* (Zhang et al., 2014) and *Moringa oleifera* (Azeem et al., 2023). An increase in proline accumulation in *Catharanthus roseus* under drought stress induced by PEG 6000 of different concentrations was also observed (Liu et al., 2017). *Mentha piperita* and *Catharanthus roseus* responded to stress by triggering the accumulation of secondary metabolites and osmolytes, which enhance plant water potential and boost ROS scavenging to sustain growth under stress (Alhaithloul et al., 2019). The increase of osmolytes that results from drought along with heat stress also validated the compounds' ability to mitigate the detrimental effects of drought and heat stress (Alhaithloul et al., 2019).

The measurement of malondialdehyde (MDA) content is a well-established marker for lipid peroxidation, widely used in studies investigating oxidative stress and redox signalling, particularly in the context of plant responses to abiotic and biotic stresses (Morales and Munné-Bosch, 2019; Sarker and Oba, 2020b). The findings from the present study indicate that both NaCl and PEG stresses induce oxidative damage in plants, which result into the elevated MDA levels. The finding of Azeem et al. (2023), which indicated that abiotic stresses increase the formation of reactive oxygen species (ROS), leading to peroxidation of lipids in *Moringa oleifera*, corroborate the pattern of rising MDA levels with both NaCl and PEG stress. In terms of MDA content, the salt stress markedly increased lipid peroxidation in the water dropwort cultivars' roots and leaves (Kumar et al., 2021). The MDA contents also increased in response to salinity in two cotton (*Gossypium hirsutum*) cultivars (Zhang et al., 2014). The patterns of MDA accumulation suggest that while oxidative stress intensifies with higher stress levels, plants may activate certain defense mechanisms to mitigate damage at extreme concentrations. This

response is slightly more pronounced in PEG-induced stress, where MDA levels decrease more significantly at higher stress conditions.

β -carotene, a non-enzymatic antioxidant, plays a vital role in quenching singlet oxygen and protecting photosynthetic machinery. Both NaCl and PEG stress stimulate β -carotene accumulation; likely as part of the plants defense mechanism against oxidative stress. The increasing trend in β -carotene levels under moderate NaCl and PEG stress aligns with the findings of other author, who showed enhanced β -carotene synthesis as part of the plant's defense mechanism. Numerous studies show that plants under environmental stress increase their β -carotene concentration, which mitigates the adverse impacts of stress. (Kim et al., 2008; Kim et al., 2012). Exogenous use of β -carotene also reduces the adverse effects of salinity stress on plants (Babaei et al., 2022). However, the observed decline in β -carotene levels at higher stress concentrations indicates a potential limitation in the plant's ability to sustain antioxidant production under extreme conditions. Therefore, the excessive generation of ROS that exceeds the antioxidant defense system may be the cause of this loss.

In the present study, both induced salinity and dehydration stress in the form of NaCl and PEG stresses significantly increased total phenolic and total flavonoid content, reflecting the plant's enhanced antioxidative mechanisms in response to stress. Highest total phenolic compound and total flavonoid production occurred at moderate stress levels (150mM NaCl and 15% PEG). These findings highlight the importance of phenolic compound and flavonoids as antioxidants in plant stress tolerance and their role in mitigating oxidative damage caused by salinity and drought. These natural antioxidants play a critical role in plant stress management and hold significant value for medicinal applications (Azeem et al., 2023). The decline in flavonoid and phenolic content at higher concentrations for both the NaCl and PEG stress indicates that extreme salinity and drought conditions can limit the plant's capacity to maintain high phenol and flavonoid production, suggesting a metabolic limitations or stress saturation. The increased synthesis of phenolic and flavonoid content has also been reported in other plant. In *Schizonepeta tenuifolia*, phenolic accumulation was stimulated at mild salinity levels (25 mM) but inhibited at severe salinity levels (75 and 100 mM) (Zhou et al., 2018). By

raising the amounts of antioxidant components, including glutathione, ascorbic acid, total flavonoids, and phenols, as well as their subsequent actions, salt stress also improved Moringa's therapeutic potential (Azeem et al., 2023). Total phenolic, total flavonoid, and antioxidant activity all increased in *Centella asiatica* as the level of salinity increased (Hoang and Rehman, 2022). The majority of phenolic acids kept building up as the stress duration increased; however, flavonoids in *Achillea pachycephala* drastically dropped on day 28 of drought stress (Gharibi et al., 2019). Alhaithloul et al. (2019) found that *Catharanthus roseus* and *Mentha piperita* responded to drought and/or heat stress by reducing their saponin, flavonoid, and phenol levels. On the other hand, the quantity of alkaloids, terpenoids, and tannins, enhanced during stress, with the highest level under the combination of heat and drought stress (Alhaithloul et al., 2019). By boosting the removal of ROS and enhancing plant water potential, the buildup of osmolytes and secondary metabolic compounds may help plants to maintain growth in adverse situations and minimize the negative impacts of stress (Alhaithloul et al., 2019). While the development of medicinal plants declines under adverse environments, the production of secondary metabolites (SM) may rise, this could have a substantial impact on the medicinal qualities of the plants (Miransari et al., 2021). Thus, applying abiotic stress could be a strategy to increase the amount of these plants' therapeutic secondary metabolites (Alhaithloul et al., 2019).

5. CONCLUSION

In the present study, significant changes in the physiological and biochemical parameters have been observed in NaCl- and PEG 6000-induced salt- and drought-stressed plants in relation to control. PEG and NaCl stress reduced RWC and MSI when compared to the control. Screening for drought-resistant genotypes that preserve cell turgor under water stress conditions can be done using parameters like relative water content and membrane stability index. By increasing the synthesis of proline, β -carotene, phenolic compounds, and total flavonoids, the *Bacopa monnieri* plant defends itself against oxidative stress. These compounds help plants to reduce cellular damage by scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation. In the current investigation, it was observed that proline content increased markedly when stress levels increased in both drought and salinity. As

an osmoprotectant, proline helps plants in maintaining the stability of their cellular structures under stress. In addition to being essential for managing plant stress, natural antioxidants have great potential for use in medicine. Plants that experience drought and salinity produce more bioactive molecules with antioxidant properties. The results also highlight the need for more investigation into how salinity and drought affect the various therapeutic benefits of *Bacopa monnieri* and other medicinal plants. In order to identify important pathways governing salinity and drought tolerance at the whole-plant level, it is necessary to combine data from genomic, transcriptomic, proteomic, and metabolomic studies. Additional research may also be conducted to test for the synthesis of additional active chemicals in plants under various stressors. If production rises under stressful conditions, the information gathered can be utilized to produce more active compounds at industrial scale.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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