

IMPACT OF EPSOM SALT APPLICATION ON BIOCHEMICAL CHANGES IN BEETROOT (*Beta vulgaris* L.)

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ABSTRACT

Epsom salt, also known as magnesium sulfate, is widely used in agriculture, horticulture and medicine. Despite its importance in plant growth and development, excessive use of Epsom salt can induce stress and negatively impact various plant components. Here, an attempt was made to investigate whether and to what extent the application of Epsom salt confers drought stress tolerance to beetroot plants exposed to drought stress. The present study used various concentrations of Epsom salt (ECe 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 mS m⁻¹) to investigate the effect of its application on chlorophyll content, titratable acidity, protein, and proline. Among all these treatments, a significant increase in chlorophyll and proline was recorded up to ECe 12.5 mS m⁻¹; leaf and root protein also increased significantly at 7.5 mS m⁻¹. However, the concentrations above ECe 12.5 mS m⁻¹ showed a gradual decrease in biochemical contents. The treatments below 7.5 mS m⁻¹ can be considered as possible concentrations, which lead to increased photosynthetic activity and overall plant biomass and thus productivity.

Keywords: Chlorophyll, Electrical conductivity, Epsom salt, Proline, Titratable acidity.

The beet is a herbaceous plant whose roots are dark red, white, or yellow (Ceclu and Nistor, 2020). It is primarily utilized in the manufacturing of food, sugar and as a food coloring. Also, it is high in carbohydrates, proteins and is a good source of vitamins, iron, and other minerals (Bangar *et al.*, 2022). According to recent research, there is a growing interest in the biological activity of beetroot and its potential application as a health-promoting and disease-prevention functional food (Bangar *et al.*, 2022). It has anti-inflammatory and antioxidant properties and is used in both food and medicine (Thiruvengadam *et al.*, 2022). It is grown worldwide, native to the Mediterranean, Europe's Atlantic coast, the Near East, and India (Wascher *et al.*, 2022). In Maharashtra, Ahmednagar district, and the nearby region, a dark red cultivar of beetroot is grown. The majority of cultivable land is monsoon-dependent, i.e., rainfed (Department of Agriculture, Government of Maharashtra). Nevertheless, plant growth gets affected that may affect its yield due to the hot summer and general dryness during a significant part of the year in this area (Pinjarla *et al.*, 2019). Indian soils have become deficient in primary plant nutrients such as phosphorus, nitrogen, and potash and secondary nutrients such as magnesium and sulfur (Department of Agriculture, 2012). A naturally occurring mineral consisting of magnesium and sulfur is radially available as magnesium sulfate (MgSO₄), also known as Epsom salt, which can be effective in deficient soils. A large

quantity of minerals such as Magnesium (Mg) are required in large quantities by plants, which contribute to photosynthetic activity and the subsequent long-distance transport of photoassimilates. Magnesium is a plant nutrient essential for chlorophyll synthesis, whereas, sulfur is a component of proteins. If magnesium is not present in sufficient quantities in the photosynthetic tissues, complex interactions of anatomical, physiological, and biochemical responses reduce photosynthetic carbon assimilation (Tränkner *et al.*, 2018). Low magnesium levels can cause magnesium deficiency in the soil, fertilizer misuse or overuse, and nutrient imbalance/interference with other nutrients in the soil (Bir *et al.*, 1987; Finér, 1992; Fuksman *et al.*, 1998). Magnesium deficiency is typically encountered in sandy, acidic soils (Mikkelsen, 2010). Farhat *et al.* (2016) found that magnesium deficiency negatively affects photosynthesis and carbohydrate partitioning in plants. Several methods were used to remediate the problem for decades, like magnesium sulfate soil application, foliar spray, and even injection into the branches. Supplementation with elemental sulfur can create soil acidity by forming sulfuric acid with the reaction with water. In acid conditions, a progressive reduction of phosphorus levels occurs, and available nitrogen increases aluminum, manganese, and iron (Edwards and Bell, 1989). However, in the soil, the sulfate ion in Epsom salt does not form sulfuric acid or affect soil pH. In this article, we look at the role of magnesium in processes directly or indirectly related to photosynthesis. Focus is given on contents like

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chlorophyll, titratable acidity, protein, and proline.

MATERIALS AND METHODS

To investigate the effects of different concentrations of Epsom salt treatments on beet, the seed of Detroit dark red variety was obtained from a Kalash seed company in Jalna, Maharashtra. The pilot experiments were carried out in order to establish the ideal concentration to use in further investigations.

The salt treatment includes a series of increasing ECe (Electrical Conductivity of Soil Saturation Extract) 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 mS cm^{-1} of Epsom salt maintained throughout the experiment with the help of a Hanna, Portable High Range EC/TDS Meter - HI99301P pH meter. All of the experiments were carried out with triplicates. The influence of different magnesium sulfate levels on some morphological characteristics were evaluated after 30 days, while biochemical changes recorded were chlorophyll, proline, protein, and titratable acidity before maturity.

Chlorophyll (Chl *a*, Chl *b*, and total chlorophylls), titratable acidity, proline, and protein content were measured at the time of harvesting using the fully expanded leaf. Three mature leaves were selected from each pot.

Chlorophyll estimation

In 10 ml of acetone (80%), a 0.5 g leaf sample from each treatment was crushed, filtered, and the final volume was made using acetone (80%). Absorbance was measured at 645nm and 663nm against a solvent (acetone) blank using a spectrophotometer (Shimadzu UV-1900). The chlorophyll content was calculated using equations from Arnon's method of chlorophyll estimation (1949).

Chlorophyll *a* (mg g $^{-1}$ F.W.): $12.7(A_{663}) - 2.69(A_{645}) \times V/1000 \times W$

Chlorophyll *b* (mg g $^{-1}$ F.W.): $22.9(A_{645}) - 4.68(A_{663}) \times V/1000 \times W$

Total Chlorophyll (mg g $^{-1}$ F.W.): $20.2(A_{645}) + 8.02(A_{663}) \times V/1000 \times W$

Titratable acidity

For all Epsom salt treatments and control, the titratable acidity was measured every 6 hours for a 24-hour cycle in the morning, afternoon, evening, and night using the method of Garner *et al.* (2003) for measuring pH and titratable acidity.

The titratable acidity was calculated using the following formula:

$$\% \text{ acid} = \frac{[\text{vol of NaOH used}] \times [0.1 \text{ N NaOH}] \times [\text{milliequivalent factor}] \times [100]}{\text{grams of sample}}$$

Protein estimation

0.5 g of treated beet leaf and root samples were ground in phosphate buffer; the supernatant was used for protein estimation after centrifugation. The blue color was developed by reducing the phosphomolybdic-phosphotungstic components in the Folin-Ciocalteu reagent by the amino acids tyrosine and tryptophan present in the protein. The color developed by the biuret reaction of the protein with the alkaline cupric tartrate was measured by Lowry's method (Lowry *et al.*, 1951). The absorbance was measured at 660 nm using a spectrophotometer (Shimadzu UV-1900).

Proline estimation

The estimation of proline was done spectrophotometrically using the ninhydrin method of Bates *et al.* (1973). 3 mL of 3% sulphosalicylic acid was used to homogenize leaf and root samples of treated beet (1g from each treatment). The homogenate filtrate was reacted for 1 hour with 1 mL acid ninhydrin and glacial acetic acid in a test tube placed in a water bath at 100 °C and later extracted with toluene. Using L-proline as a standard, the absorbance was measured at 520 nm using a spectrophotometer (Shimadzu UV-1900).

Software SPSS 16.0 was used to analyze the data to calculate the mean value, standard deviation, and least significant difference (LSD).

RESULTS AND DISCUSSION

After the application of Epsom salt on beet, its effects were evaluated in the form of organic metabolism like chlorophyll content, titratable acidity, protein content, proline content and carbohydrates.

Effect of epsom salt on chlorophyll

Chlorophylls are the essential components for photosynthesis, it can be affected by various abiotic/biotic stresses. In the present study, observations were recorded for the various concentrations of Epsom salt on chlorophyll content (Chl-*a*, Chl-*b* and total Chlorophyll) (Fig.1.)

Under the application of the Epsom salt, a significant increase ($P < 0.05$ level) in chlorophyll *a* was recorded, i.e., 2.89 ± 0.17 , 3.49 ± 0.12 , 3.19 ± 0.06 , 3.58 ± 0.41 and 2.85 ± 0.07 mg/g in the treatments of EC of 2.5, 5.0, 7.5, 10.0 and 12.5 mS cm^{-1} of Epsom salt over the control (1.95 ± 0.31 mg/g) respectively.

In the case of chlorophyll *b*, at ECe 2.5, 7.5 and 10.0 mS cm^{-1} ES, there was a significant ($P < 0.05$ level) increase in chlorophyll *b*, i.e., 1.39 ± 0.17 , 1.44 ± 0.16 and 1.36 ± 0.07 mg/g over the control (1.09 ± 0.04 mg/g). Chlorophyll *b*, i.e., 0.83 ± 0.03 and 0.76 ± 0.07 mg/g, decreased significantly at 17.5 and 20.0 mS cm^{-1} ES.

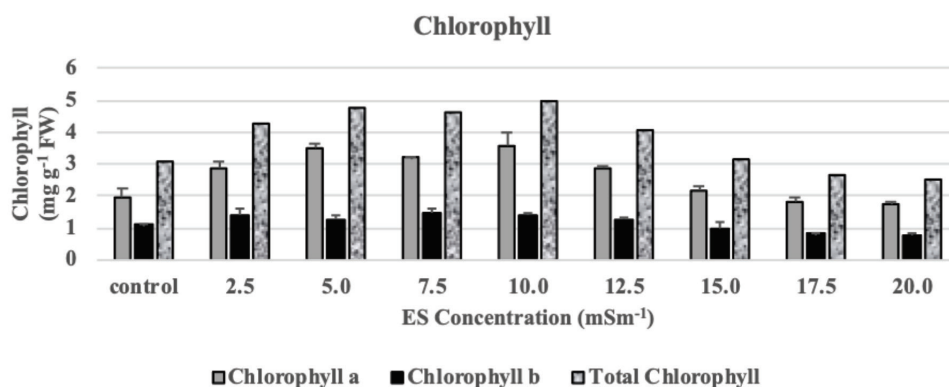


Fig .1. Study on a) Chlorophyll in *Beta vulgaris* L. under increasing concentrations of epsom salt. (Values given in mean $\pm$ S.D. Bars in each group show significant difference at $p < 0.05$.)

The increase in chlorophyll *a* was observed up to 12.5 mSm⁻¹ ES; however, there was no significant decrease in chlorophyll *a* at the higher levels of epsom salt treatments. Application of 8 and 16 kg Mg ha⁻¹ magnesium sulfate showed higher chlorophyll *a*, chlorophyll *b*, and total chlorophyll content in maize (Ahmed, 2020). Magnesium plays a role as the only mineral in the constituent of chlorophyll because each chlorophyll molecule contains one of magnesium atom (Sreedhara and Cowan, 2002; Encyclopædia Britannica, 2020). The increase in chlorophyll content with increasing doses of magnesium sulfate is related to the role of magnesium in chlorophyll formation (Hasanah *et al.*, 2020). Magnesium's metabolic process is linked to chlorophyll formation, CO₂ fixation, and photosynthate loading (Lemoine *et al.*, 2013). Magnesium deficiency reduced photosynthetic pigment content, chloroplast light absorption capacity, apparent quantum yield, and carboxylation efficiency while increasing the light and CO₂ compensation point, as well as the light and CO₂ saturation point in prior studies (Yan, 2001). Total

chlorophylls decreased with increasing concentration of Epsom salt above 10.0 mSm⁻¹ (Fig.1.). Oliveira (2000) found that MgSO₄ more than 10 kg ha⁻¹ lowered Mg, Ca, and P contents in the fully opened trifoliate leaf in common bean (*Phaseolus vulgaris* L.). Lower Mg in the leaf may have resulted in lower total chlorophyll in the beet. Thus, the study revealed that only a small amount of magnesium is required to boost bean yield in irrigated areas; greater levels were found to be harmful.

Effect of epsom salt on titratable acidity

For all Epsom salt-treated plants along with the control, the titratable acidity was measured every six hours for a 24-hour cycle in the morning, afternoon, evening and night (Fig., 2.). From the results, it is clear that with the application of epsom salt ECe 2.5, 5.0, 7.5 and 10.0 mSm⁻¹, the significant increase ($P < 0.05$ level) in titratable acidity i.e., 0.166 ± 0.01 , 0.201 ± 0.01 , 0.174 ± 0.01 and 0.161 ± 0.02 was recorded over the control (0.113 ± 0.01) respectively in the morning followed by the significant decrease in titratable acidity, i.e., $0.094 \pm$

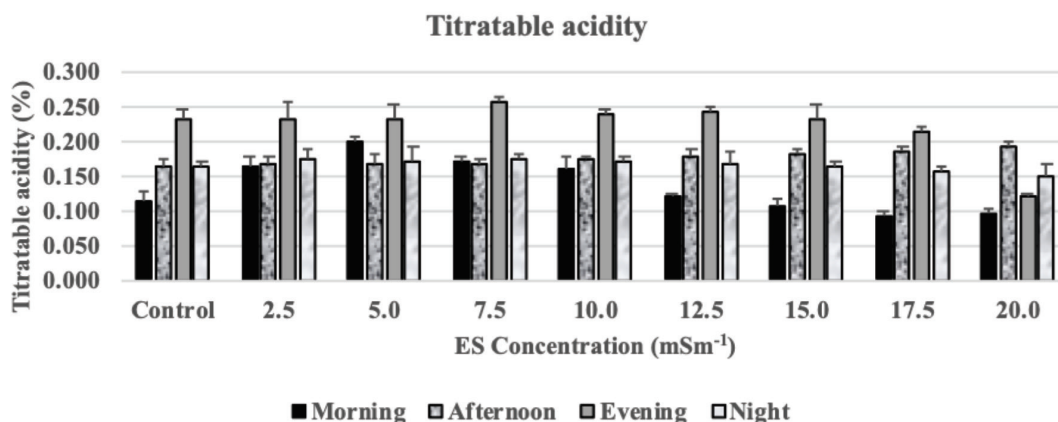


Fig. 2. Study on titratable acidity in *Beta vulgaris* L. under increasing concentrations of epsom salt. (Values given in mean $\pm$ S.D. bars in each group show significant difference at $p < 0.05$.)

0.01 and 0.096 ± 0.01 under ECe 17.5 and 20.0 mSm^{-1} ES treatment over the control. In the afternoon samples, the titratable acidity increased significantly 0.184 ± 0.01 , 0.186 ± 0.01 and 0.195 ± 0.01 with the concentration of ECe 15.0, 17.5 and 20.0 mSm^{-1} ES over the control i.e., 0.165 ± 0.01 . In the evening samples, titratable acidity i.e., 0.259 ± 0.01 increased significantly with ECe 7.5 and 10.0 mSm^{-1} ES as compared to control (0.232 ± 0.02). This was followed by a significant decrease in titratable acidity, i.e., 0.121 ± 0.01 at the higher treatment of Epsom salt at ECe 20.0 mSm^{-1} ES. There was no significant difference in the titratable acidity of plants at night for all Epsom salt treatments. According to Kwon (2019), with increasing extremely high magnesium stress (higher than 3.0 dSm^{-1}), the fresh fruit weight, total soluble solids, and titratable acidity (TA) of tomato fruit samples decreased. According to Bahar (2011), under abiotic stress conditions (extreme water stress), grapevine cultivars and berries became smaller and fully exposed to sunlight; anthocyanin increased with pH and increased total acidity values at harvest time.

According to Strogonov (1964), enhancing organic acids is one of the salt tolerance mechanisms in plants. Higher magnesium concentrations reduced the titratable acidity of tomato juice samples along with fruit's fresh weight and total soluble solids (Kwon, 2019). Organic acids play a crucial role in cell metabolism. Exudation of organic acids at the root-soil interface is involved in many plant stress responses, which can improve soil mineral acquisition and toxic metal tolerance (Panchal, 2021). In particular, succinic, malic, and galacturonic acids improve the plant's response to long-term drought stress (Khan *et al.*, 2020). According to Dondoni (2006), amino acids are used as precursors for proteins and other organic compounds, such as nucleic acids, which play an essential role in plant stress responses; they

can act as regulatory and signalling molecules. The current study shows that one of the adaptations for beet's survival under salinity is its ability to synthesize higher amounts of organic acids (Fig., 2.). However, the increased organic acid content does not necessarily aid beet productivity.

Effect of epsom salt on protein content

Proteins perform a variety of enzymatic, structural and functional functions such as photosynthesis, biosynthesis, transport and immunity. Changes in protein synthesis in the roots and leaves caused by stress may protect plants from further damage (Mohammadkhani and Heidari, 2008). The effect of different Epsom salt concentrations on root and leaf protein content was evaluated in treated plants.

With the application of epsom salt treatment ECe 7.5 mSm^{-1} ES, a significant increase ($P < 0.05$ level) in root protein, i.e., $18.19 \pm 0.8 \text{ g.kg}^{-1}$, was recorded as compared to control ($16.42 \pm 1.0 \text{ g.kg}^{-1}$) followed by a significant decrease in root protein, i.e., 12.77 ± 0.9 , 12.11 ± 1.2 , and 12.23 ± 1.1 with the treatment of ECe 15.0, 17.5, and 20.0 mSm^{-1} ES as compared to the control. In the case of leaf protein i.e., 38.11 ± 1.1 and 38.08 ± 1.1 increased significantly at the concentration of ECe 5.0 and 7.5 mSm^{-1} ES over the control, i.e., $34.95 \pm 1.4 \text{ g.kg}^{-1}$ at $P < 0.05$ level (Fig., 3.). Followed by a significant decrease was observed in leaf protein, i.e., 29.36 ± 1.7 , 28.40 ± 2.1 , and $28.43 \pm 1.9 \text{ g.kg}^{-1}$ at the higher Epsom salt treatment at ECe 15.0, 17.5, and 20.0 mSm^{-1} ES. Our findings imply that the plant can sustain higher productivity and protein metabolism at lower (5.0 and 7.5 mSm^{-1}) levels of magnesium sulfate salinization. A significant influence of Epsom salt foliar fertilization on protein contents was observed and resulted in an increase of 0.67% as compared to the

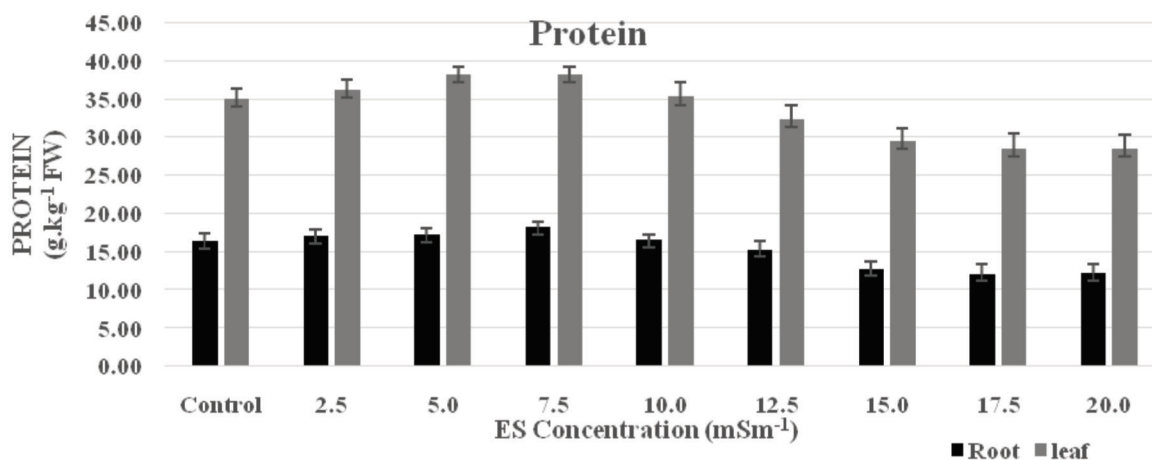


Fig. 3. Study on root and leaf protein in *Beta vulgaris* L. under increasing concentrations of epsom salt. Values given in mean $\pm$ S.D. bars in each group show significant difference at $p < 0.05$

control (Vrataric, 2006).

In many crop plants, salinity reduces protein synthesis and increases its hydrolysis. Under NaCl salinity, Klyshev and Rakova (1964) reported decreased protein synthesis and increased hydrolysis in pea roots. The availability of nitrogen substrate for growth is reflected in the amount of leaf soluble protein. Mg is necessary for nitrogen uptake and utilization (Grzebisz, 2013). Lasa *et al.* (2000) also supported this hypothesis that increasing Mg supply decreased leaf soluble protein content and shoot-root ratio in ammonium-fed sunflower. Salinity changes the balance between soluble amino acids and proteins. Strogonov (1962) suggested that amino acids and amides accumulate in salt-stressed plant tissues, indicating disturbed protein metabolism and the accumulation of toxic diamines. However, our results suggest that the plant can maintain more productivity and protein metabolism under low levels of magnesium sulfate salinization. However, decreased protein content at all higher levels of salt appears to be one of the causative factors for reduced growth.

Effect of epsom salt on proline content

Proline, an amino acid, is essential in plants. It protects the plants from various stresses and aids in the faster recovery of stressed plants. The effect of different Epsom salt concentrations on root and leaf proline in treated plants was evaluated.

The effect of Epsom salt treatments on the proline content of the root and leaf of treated plants and the control are shown in Fig. 4. With the application of epsom salt ECe 5.0, 7.5, 10.0 and 12.5 mSm⁻¹ ES the significant increase ($P < 0.05$ level) in root proline, i.e., 4.2 ± 0.9 , 4.3 ± 0.9 , 4.3 ± 0.7 and 3.8 ± 0.8 $\mu\text{mol/g}$ was recorded as compared to the control (2.5 ± 0.5 $\mu\text{mol/g}$). No significant decrease in root proline was observed for all other treatments.

In the case of leaf proline, i.e., 9.9 ± 0.7 , 10.2 ± 0.5 , 11.1 ± 0.6 , 10.8 ± 0.9 and 10.5 ± 0.7 $\mu\text{mol/g}$ increased significantly at the concentration of ECe 2.5, 5.0, 7.5, 10.0 and 12.5 mSm⁻¹ ES respectively as compared to control, i.e., 7.6 ± 0.7 $\mu\text{mol/g}$ at $P < 0.05$ level. No significant decrease in leaf proline has been observed for all other treatments. At the highest (300 mM) magnesium chloride salinity stress, cultivars PhuleRevati and PhuleVasudha showed higher proline accumulation (Tushar *et al.*, 2012).

According to Ashraf and Harris, 2004; Tripathi *et al.*, 2007, Plants generally accumulate low-molecular-mass compatible solutes like proline. Proline accumulation is a common response to many biotic and abiotic stresses, such as salinity stress (Aghaei *et al.*, 2009). Proline functions as an osmolyte and antioxidant in plants, assisting cell survival (Huang *et al.*, 2000). According to Dawood *et al.* (2014), proline has been shown to improve plant growth, physiological, biochemical, and anatomical characteristics, and antioxidant system defense when exposed to salinity. However, the results show that plant productivity was maintained because of more proline content at 7.5 and 10.0 mSm⁻¹; a decrease in productivity was recorded at higher concentrations of magnesium sulfate salinization.

The application of Epsom salt has the potential to enhance the organic content of beets, resulting in increased productivity and yield, especially at lower concentrations. The findings indicate that up to ECe 12.5 mSm⁻¹, there is a significant increase in chlorophyll and proline, as well as leaf and root protein. However, concentrations exceeding ECe 12.5 mSm⁻¹ result in a gradual decrease in biochemical contents. The treatments mentioned in this study can stimulate photosynthetic activity and biomass, leading to increased productivity. These insights are valuable for improving fertilization strategies and highlighting the specific need for magnesium sulfate in various crops, including beets,

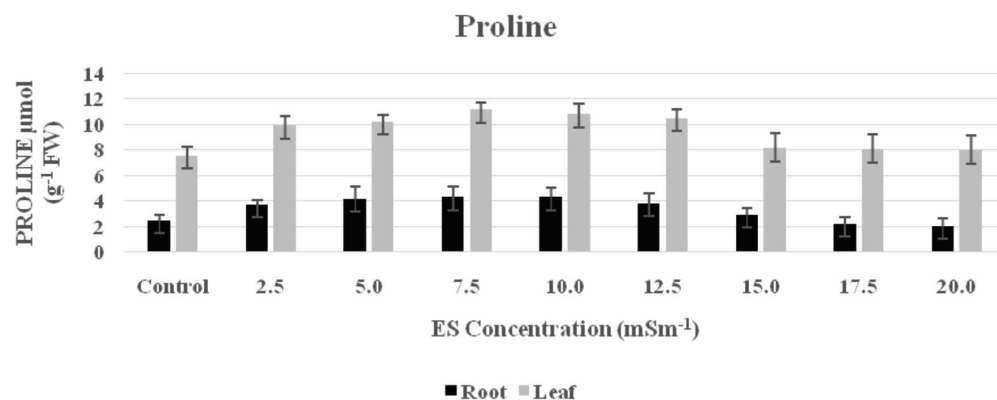


Fig. 4. Study on root and leaf proline in *Beta vulgaris* L. under increasing concentrations of epsom salt. Values given in mean $\pm$ S.D. bars in each group show significant difference at $p < 0.05$

to support essential physiological processes critical for optimal plant growth and productivity.

Authors' contribution

Conceptualization and designing of the research work (DDA, SBP); Execution of field/lab experiments and data collection (DDA, SBP); Analysis of data and interpretation (DDA, SBP, ADH); Preparation of manuscript (DDA, SBP, ADH)

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