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# Potential Of Micronutrient-Based Intervention To Improve Pennisetum Glaucum Resilience Under Salt And Drought Stress

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**Abstract**
*Abiotic stress poses a significant challenge to agricultural productivity, particularly in arid and semi-arid regions where Pennisetum glaucum (pearl millet) is a staple crop. Despite its natural resilience, stresses like drought, salinity, and extreme temperatures adversely affect its growth and yield. This study aimed to assess the effects of abiotic stresses on pearl millet and explore the role of zinc (Zn) and iron (Fe) supplementation in mitigating these impacts. Under controlled conditions, plants were subjected to salt and drought stress, followed by foliar treatments with zinc sulfate and iron sulfate. Physiological and biochemical parameters, including chlorophyll content, protein levels, phenolic compounds, soluble sugars, and proline, were analysed. Results showed that abiotic stresses reduced chlorophyll and protein content while increasing phenolic and soluble sugar levels. However, Zn and Fe supplementation mitigated these adverse effects, with zinc demonstrating a stronger influence on chlorophyll biosynthesis and protein content. Combined Zn-Fe treatments exhibited a synergistic effect, particularly under moderate stress, by enhancing pigment retention and stabilizing biochemical parameters. These findings highlight the potential of micronutrient-based interventions to improve pearl millet resilience under stress conditions, providing a sustainable strategy for enhancing crop performance and ensuring food security in vulnerable regions.*
**Keywords:** *Pennisetum glaucum, abiotic stress, zinc, iron, stress tolerance, sustainable agriculture.*

## INTRODUCTION

Abiotic stress represents a significant challenge in agriculture, severely impacting crop productivity and quality. Pennisetum glaucum, commonly known as pearl millet, is a crucial cereal crop cultivated primarily in arid and semi-arid regions (Yasir et al., 2021). Its resilience to harsh climatic conditions and ability to thrive in nutrient-deficient soils make it a vital source of food and fodder for millions of people across the globe. Despite its inherent adaptability, pearl millet remains vulnerable to various abiotic stresses such as drought, salinity, extreme temperatures, and nutrient imbalances. These stressors can profoundly affect the plant's growth, physiology, and overall yield potential (Dhawi, 2023). Understanding the mechanisms through which abiotic stresses influence pearl millet and exploring strategies to mitigate their impact is essential for sustainable agricultural practices, especially in the context of climate change and food security challenges.

Drought is among the most critical abiotic stresses affecting pearl millet. Pearl millet often experiences water scarcity during its growth cycle (Yadav et al., 1999; Kholoya et al., 2010). Drought stress not only reduces water availability but also disrupts physiological processes such as photosynthesis, nutrient uptake, and cellular homeostasis. The plant's ability to cope with drought is primarily governed by its inherent traits, such as deep root systems and osmotic adjustment. However, prolonged periods of drought can overwhelm these mechanisms, leading to reduced biomass accumulation and lower grain yield. Addressing drought stress in pearl millet requires a comprehensive understanding of its physiological and molecular responses, as well as the integration of agronomic and biotechnological approaches to enhance its drought tolerance (Govindaraj et al., 2013).

Salinity is another abiotic stressor that poses a significant threat to pearl millet cultivation, particularly in regions with saline irrigation water or degraded soils. High salinity levels in the soil can lead to osmotic stress and ion toxicity, disrupting essential metabolic processes and impairing plant growth (Sharma et al., 2021). Pearl millet, although relatively tolerant to saline conditions compared to other cereals, experiences a decline in germination rates, shoot and root development, and photosynthetic efficiency under high salinity. Research has shown that certain genotypes of pearl millet exhibit better salinity tolerance due to their ability to regulate ion homeostasis and maintain cellular integrity. These insights underscore the importance of identifying and promoting salinity-tolerant cultivars as part of a broader strategy to address this challenge.

Extreme temperatures, whether in the form of heat or cold stress, also impact pearl millet's growth and productivity (Varshney et al., 2017). Excessive heat can accelerate the rate of transpiration, disrupt enzymatic activities, and lead to oxidative stress, all of which contribute to reduced grain filling and lower yield. Conversely, cold stress, cold-tolerant genotypes through breeding programs and advanced genetic techniques is a critical area of research to safeguard pearl millet against temperature extremes.

Micronutrients play an indispensable role in alleviating abiotic stress and enhancing the resilience of pearl millet. Elements such as zinc, iron, manganese, and boron are vital for various physiological and biochemical processes, including enzyme activation, chlorophyll synthesis, and stress signalling. Under abiotic stress conditions, the demand for these micronutrients often increases, as they contribute to the plant's ability to mitigate oxidative damage and maintain metabolic balance (Kumar et al., 2016). For instance, zinc has been shown to enhance drought tolerance by stabilizing cell membranes and promoting the synthesis of osmoprotectants. Similarly, iron and manganese are crucial for maintaining photosynthetic efficiency and reducing the accumulation of reactive oxygen species during stress conditions.

The application of micronutrients, either through soil amendments or foliar sprays, has emerged as an effective strategy to improve the stress tolerance of pearl millet. Several studies have demonstrated the positive impact of micronutrient supplementation on the crop's growth and yield under stress conditions (Moumouni et al., 2015). For example, zinc-enriched fertilizers have been found to improve drought resilience, while boron application has shown promising results in mitigating salinity stress. However, the effectiveness of these interventions depends on factors such as soil type, crop stage, and the severity of stress. A balanced approach that integrates micronutrient management with other agronomic practices is therefore essential for optimizing the benefits. Aim of the study is to investigate the impact of abiotic stresses on the growth and productivity of Pennisetum glaucum and evaluate the role of micronutrient supplementation in enhancing stress tolerance and improving yield outcomes.

## METHODOLOGY

### Plant Material and Experimental Setup

The seeds were sourced from ICAR-Indian Institute of Wheat and Barley Research in Karnal, Haryana. Experiments were carried out in pots and subjected to salt stress through irrigation and reduction in water for drought stress. The experimental setup included multiple treatment groups to assess the effects of abiotic stresses such as salinity and drought. Each group consisted of three replicates, and stress conditions were introduced 15 days after seedling establishment.

## STRESS INDUCTION AND TREATMENT GROUPS

### Salt Stress

Salt stress was induced by applying sodium chloride (NaCl) solutions at increasing concentrations (S1 to S4). Control plants (C) were maintained without salt stress. Additional treatment groups included zinc-treated (ZnS1 to ZnS4), iron-treated (FeS1 to FeS4), and combined zinc and iron-treated salt-stressed plants (ZnFeS1 to ZnFeS4).

### Drought Stress

Drought stress was imposed by withholding water and monitoring soil moisture levels, categorized as mild (D1), moderate (D2), and severe (D3). Control plants (C) received normal watering. Treatment groups included zinc-treated (ZnD1 to ZnD3), iron-treated (FeD1 to FeD3), and combined zinc and iron-treated drought-stressed plants (ZnFeD1 to ZnFeD3).

### Nutrient Supplementation

Zinc and iron were supplied as zinc sulfate ($ZnSO_4$) and iron sulfate ($FeSO_4$), respectively, at standardized concentrations. The supplementation was administered through weekly foliar sprays throughout the experimental period.

## PHYSIOLOGICAL PARAMETERS

### Chlorophyll Content

The contents of chlorophyll a (CHLa), chlorophyll b (CHLb), and total chlorophyll (TCHL) were quantified using Arnon's method (1949). Absorbance was measured at 663 nm and 645 nm for CHLa and CHLb, respectively, and chlorophyll content was calculated using standard equations. Chlorophyll content was expressed as mg/g.

## BIOCHEMICAL PARAMETERS

### Protein Content

Protein content was estimated using the Lowry method (1951). Fresh leaf tissues (500 mg) were homogenized in phosphate buffer, centrifuged, and the supernatant was analysed using bovine serum albumin (BSA) as the standard.

### Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu reagent(1999). Leaf extracts were prepared by homogenizing fresh tissue in 80% ethanol, followed by centrifugation. Results were expressed as gallic acid equivalents (GAE).

### Total Soluble Sugar Content

The anthrone method(1944) was used to quantify soluble sugars. Fresh leaf tissues were homogenized in 80% ethanol, and the supernatant was analysed at 620 nm using a spectrophotometer.

### Statistical Analysis

All experimental data were subjected to one-way analysis of variance (ANOVA) to assess the significance of treatment effects. Tukey's HSD post-hoc tests were performed to identify pairwise differences among treatment groups, with statistical significance set at $p < 0.05$.

## RESULTS

### Physiological Parameters

### Effect of Salt stress on Chlorophyll a, b and total chlorophyll content of Pennisetum glaucum

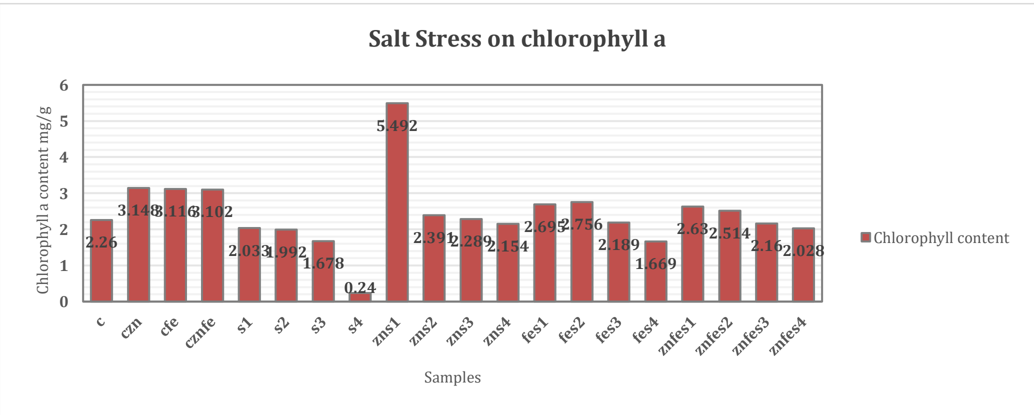
The data on chlorophyll content measured at different wavelengths (CA663nm), alongside chlorophyll a (CHLa), chlorophyll b (CHLb), and total chlorophyll (TCHL) across various treatment groups, reflecting the physiological responses of plants to different nutrient supplements and salt stress conditions. The control group (c) exhibits moderate levels of chlorophyll, with CHLa at 5.119 (mg/g) and TCHL at 7.379, providing a baseline measure of pigment concentration under unstressed conditions. In comparison, plants treated with zinc (czn) demonstrate higher chlorophyll levels (CHLa: 6.174, TCHL: 9.322), suggesting that zinc supplementation may enhance chlorophyll biosynthesis, potentially by stabilizing chlorophyll molecules or promoting enzymatic pathways involved in pigment production. A similar trend is observed in the iron-treated group (cfe), where CHLa reaches 6.059 and TCHL 9.175, indicating a comparable enhancement in photosynthetic pigment levels. The combination of zinc and iron (cznfe) further amplifies chlorophyll synthesis, with CHLa at 6.387 and TCHL peaking at 9.489, reflecting a possible synergistic effect that enhances the plants' photosynthetic capacity.

In stark contrast, plants subjected to salt stress (s1 to s4) exhibit a clear decline in chlorophyll content, with the severity of reduction increasing progressively from s1 to s4. This pattern is most pronounced in s4, where CHLa drops to 0.453 and TCHL to 0.693, indicative of severe physiological disruption likely caused by pigment degradation or inhibited chlorophyll biosynthesis under high salinity. Such results align with established knowledge on the detrimental impact of salinity stress on photosynthetic efficiency and pigment stability. Interestingly, zinc supplementation in salt-stressed plants (zns1 to zns4) yields variable but generally improved outcomes, with zns1 showing the highest total chlorophyll content at 13.403, suggesting that zinc plays a protective role in mitigating the adverse effects of salinity. This protective effect may stem from zinc's involvement in maintaining membrane integrity, reducing oxidative damage, and enhancing antioxidant enzyme activity.

The data also reveals that iron supplementation under salt stress (fes1 to fes4) helps to retain chlorophyll to a moderate extent, with fes1 displaying relatively higher CHLa (5.557) and TCHL (8.252). However, the progressive decline observed from fes2 to fes4 suggests that while iron may offer some degree of protection, its effectiveness diminishes under increasing levels of salt stress. The combination of zinc and iron (znfes1 to znfes4) under salt stress results in intermediate chlorophyll levels, implying that while the cumulative effect of both micronutrients can alleviate some stress-induced pigment loss, the extent of protection varies depending on the severity of salinity (Figure 1).

Statistically, the variance in chlorophyll levels across treatments highlights significant differences between unstressed control groups and salt-stressed plants, reinforcing the notion that salt stress negatively impacts pigment synthesis and stability. The higher mean chlorophyll values in zinc- and iron-supplemented groups suggest that these treatments significantly improve photosynthetic pigment content compared to the control. Overall, the data underscores the complex interplay between nutrient supplementation and salinity stress, revealing that while zinc and iron can mitigate some of the detrimental effects of salt stress, their effectiveness is contingent upon the severity of the stress and the specific combination of nutrients applied.

The ANOVA results for chlorophyll content (Chlorophyll a, Chlorophyll b, and Total Chlorophyll) under salt stress and drought stress show significant treatment effects with p-values of 0.001. Under salt stress, chlorophyll content (both individual and total) significantly decreases compared to the control group. However, zinc and iron supplementation significantly enhance chlorophyll levels, with zinc demonstrating a particularly strong effect, as indicated by p-values of 0.001. This suggests that zinc supplementation plays a critical role in mitigating the negative impact of salt stress on chlorophyll synthesis. The combined treatment of zinc and iron also yields higher chlorophyll levels than individual treatments, indicating a synergistic effect between the two micronutrients. Similarly, under drought stress, chlorophyll levels decline as the severity of stress increases. Zinc and iron supplementation help maintain higher chlorophyll levels, with zinc showing a more pronounced effect. The combined treatment of zinc and iron further enhances chlorophyll retention, with p-values of 0.001, suggesting that these micronutrients work together to support photosynthetic efficiency and chloroplast stability during drought stress.

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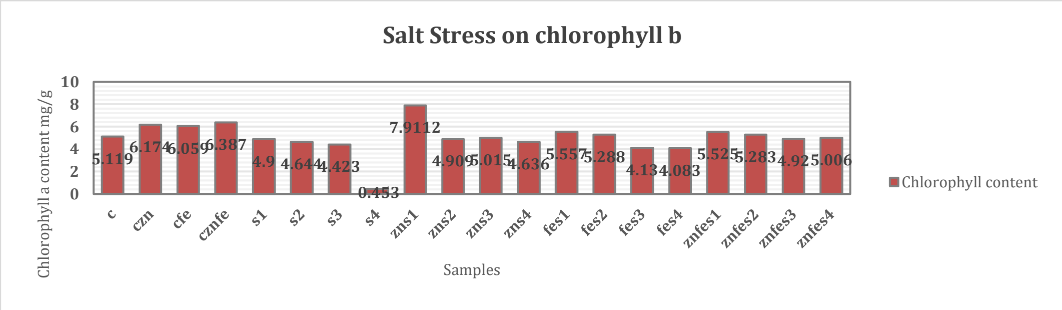


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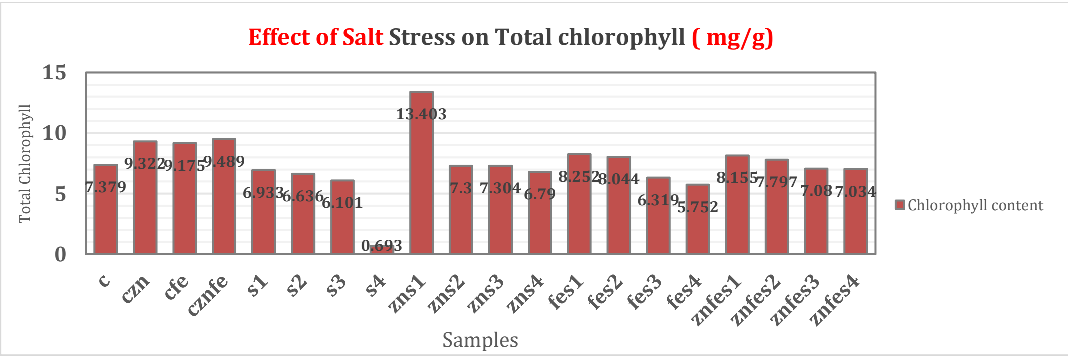



(c)

**Figure 1.** Effect of Salt stress on, a) Chlorophyll a; b) Chlorophyll b, and; c) total chlorophyll content of Pennisetum glaucum

**Effect of Drought stress on Chlorophyll a, b and total chlorophyll content of Pennisetum glaucum**

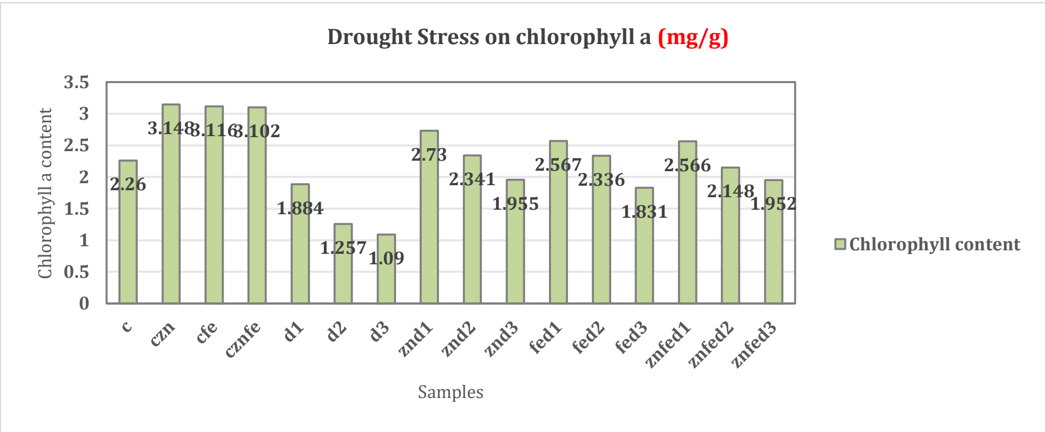
The Fig 2 data reflects the effect of drought stress on the chlorophyll content of Pennisetum glaucum, highlighting the variations in chlorophyll a (CHLa), chlorophyll b (CHLb), and total chlorophyll (TCHL) across different treatment groups. The control (c) plants, representing non-stressed conditions, exhibit CHLa at 2.26 and TCHL at 7.379, serving as a benchmark for comparison. In plants supplemented with zinc (czn), there is a noticeable increase in chlorophyll levels (CHLa: 3.148, TCHL: 9.322), suggesting that zinc enhances chlorophyll synthesis, possibly through its role in stabilizing chloroplast membranes and supporting enzymatic functions involved in photosynthetic pigment production. Similarly, iron-treated plants (cfe) show elevated chlorophyll levels (CHLa: 3.116, TCHL: 9.175), reinforcing the significance of iron in photosynthetic processes, likely due to its involvement in chlorophyll biosynthesis and electron transport chains. The combined treatment of zinc and iron (cznfe) yields the highest chlorophyll values (CHLa: 3.102, TCHL: 9.489), indicating a synergistic effect that may optimize photosynthetic efficiency by enhancing the synthesis and stability of chlorophyll pigments.

Under drought stress conditions, plants exhibit a clear reduction in chlorophyll content, with the severity of decline intensifying from d1 to d3. In the d1 group, CHLa is recorded at 1.884, while TCHL decreases to 6.189, reflecting mild drought-induced physiological disruption. This trend continues in d2 (CHLa: 1.257, TCHL: 4.355) and becomes most pronounced in d3, where CHLa drops to 1.09 and TCHL to 4.275, indicating severe stress that significantly compromises chlorophyll biosynthesis or accelerates pigment degradation. The progressive decline in chlorophyll levels across the drought-stressed samples highlights the detrimental impact of water deficiency on photosynthetic machinery, consistent with known drought-induced limitations on nutrient uptake, impaired enzymatic activity, and structural damage to chloroplasts.

However, the introduction of zinc and iron in drought-stressed plants mitigates the negative effects to varying extents. Zinc supplementation in drought-stressed plants (znd1 to znd3) improves chlorophyll content, with znd1 showing CHLa at 2.73 and TCHL at 8.616, suggesting that zinc helps alleviate drought-induced pigment loss, possibly by enhancing osmotic adjustment and reducing oxidative stress. As drought severity increases (znd2 and znd3), chlorophyll levels decline but remain higher than in untreated drought-stressed plants, reinforcing zinc's protective role in maintaining chlorophyll stability. A similar pattern emerges in iron-treated drought-stressed plants (fed1 to fed3), where CHLa values range from 2.567 in fed1 to 1.831 in fed3, with corresponding decreases in TCHL. Although iron appears to mitigate chlorophyll loss, the magnitude of protection diminishes with increasing drought severity, suggesting that while iron supports pigment retention, its efficacy is limited under severe stress.

The combined zinc and iron treatments under drought stress (znfed1 to znfed3) produce intermediate results, with znfed1 exhibiting CHLa at 2.566 and TCHL at 8.414, indicating that the dual supplementation enhances chlorophyll levels beyond those observed in single treatments. As drought severity progresses, chlorophyll values decrease (znfed2 and znfed3), yet the retention of higher chlorophyll content compared to the d groups alone suggests that the combination of zinc and iron offers a cumulative protective effect. This may result from their complementary roles in enhancing antioxidant defenses, stabilizing cellular structures, and promoting enzymatic activities essential for chlorophyll biosynthesis (Figure 2.).

Statistically, the variance across treatment groups signifies significant differences between the control, drought-stressed, and nutrient-supplemented plants. The higher mean chlorophyll values in zinc- and iron-treated groups suggest that these micronutrients play a critical role in sustaining photosynthetic pigment levels under stress conditions. Overall, the data underscores the detrimental effects of drought stress on chlorophyll content while highlighting the beneficial role of zinc and iron supplementation in partially alleviating pigment loss and supporting plant resilience under water-deficit conditions.

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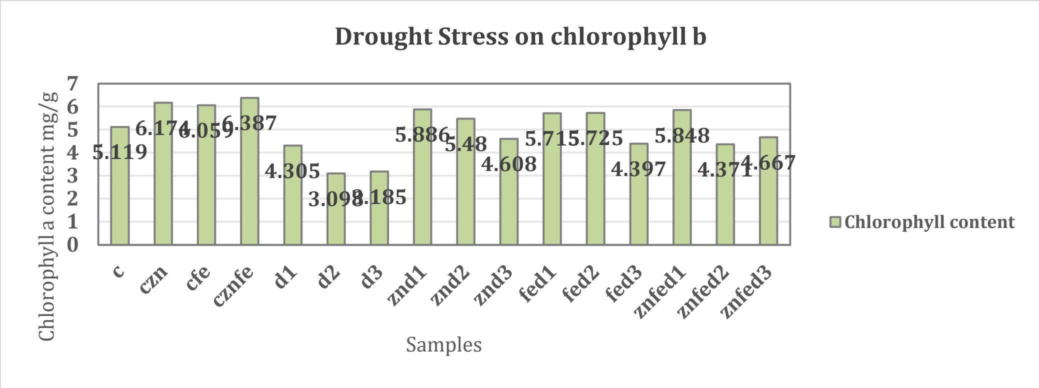


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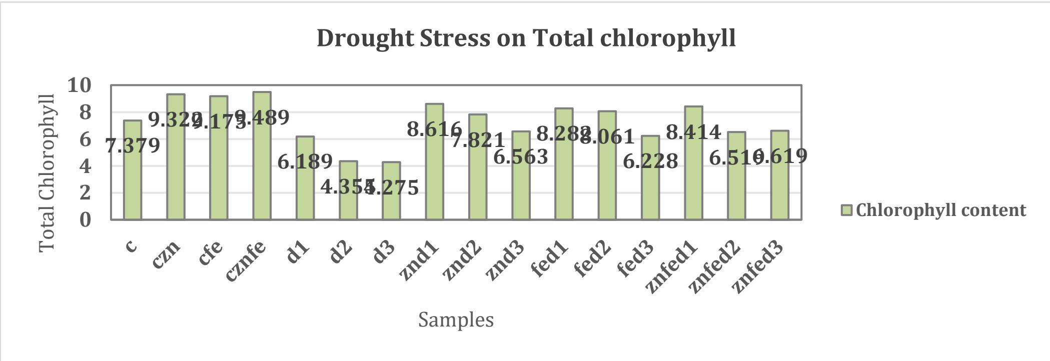



c)

**Figure 2.** Effect of Drought stress on, a) Chlorophyll a; b) Chlorophyll b, and; c) total chlorophyll content of Pennisetum glaucum

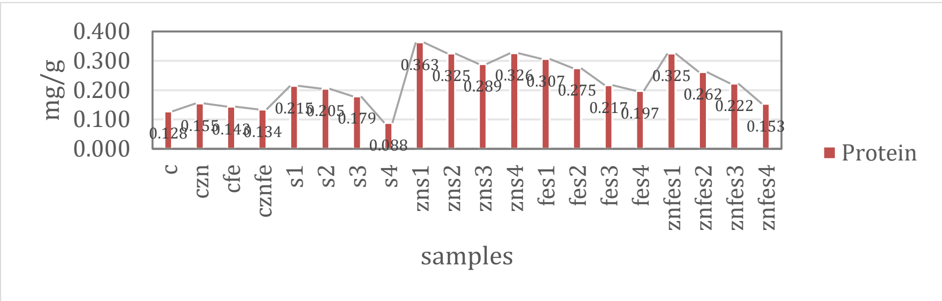
## BIOCHEMICAL PARAMETERS

### Effect of zinc sulphate and iron sulphate on protein content of water and salt stressed Pennisetum glaucum

The data presented in Figures 3a and 3b highlight the effect of salt and drought stress on the protein content of Pennisetum glaucum samples and the subsequent influence of zinc sulphate ($ZnSO_4$) and iron sulphate ($FeSO_4$) treatments. In the control (c) and treatment groups (czn, cfe, and cznfe), protein content varied between 0.128(mg/g) and 0.155, indicating a slight increase in protein levels with the addition of zinc and/or iron. Under salt stress (s1, s2, s3, and s4), protein content showed a notable increase compared to the control, with values ranging from 0.088 to 0.215, peaking at s1 (0.215) and decreasing as the stress intensity increased (s4: 0.088). In plants treated with zinc under salt stress (zns1, zns2, zns3, zns4), protein content was significantly higher (ranging from 0.289 to 0.363) than the salt-stressed groups without treatment, suggesting a positive effect of zinc supplementation on mitigating salt-induced protein loss. Similarly, the iron-treated salt-stressed plants (fes1, fes2, fes3, fes4) exhibited an increase in protein content (ranging from 0.197 to 0.307), though the effect was less pronounced compared to zinc. When both zinc and iron were applied (znfes1, znfes2, znfes3, znfes4), the protein content in salt-stressed plants was slightly improved, ranging from 0.153 to 0.325, with the highest value observed in znfes1 (0.325). In the case of drought stress (d1, d2, d3), protein content in untreated samples drastically reduced, with values decreasing from 0.089 in d1 to 0.013 in d3. Zinc treatment under drought stress (znd1, znd2, znd3) resulted in improved protein content (ranging from 0.109 to 0.230), while iron treatment (fed1, fed2, fed3) showed a more consistent, though less pronounced, increase in protein content (ranging from 0.208

to 0.310) (Figure 3.). Combining zinc and iron under drought stress (znfed1, znfed2, znfed3) had a marginal effect on protein content, ranging from 0.102 to 0.207. These results demonstrate that zinc and iron sulphate treatments have a differential impact on protein content under various stress conditions, with zinc generally showing more substantial benefits, particularly under salt stress conditions. The findings suggest that zinc supplementation could be a promising strategy for enhancing plant resilience to abiotic stresses such as salt and drought.

The ANOVA for protein content under both salt stress and drought stress shows significant differences (p-value = 0.001) between the treatment groups. Under salt stress, protein content significantly declines, but the application of zinc and iron supplementation leads to significantly higher protein levels. Zinc shows the most pronounced effect, with a p-value of 0.001, indicating its importance in mitigating the protein loss caused by salt stress. Similarly, under drought stress, protein content declines in untreated plants, but both zinc and iron supplementation help maintain protein levels. Zinc has a more substantial effect than iron (p-value = 0.001), further confirming zinc's role in supporting protein synthesis under water-deficit conditions. These findings highlight that micronutrient supplementation improves protein synthesis and stability, enhancing plant resilience to both salt and drought stress.

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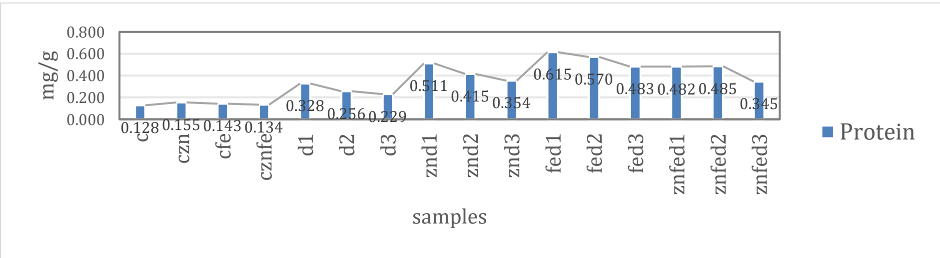


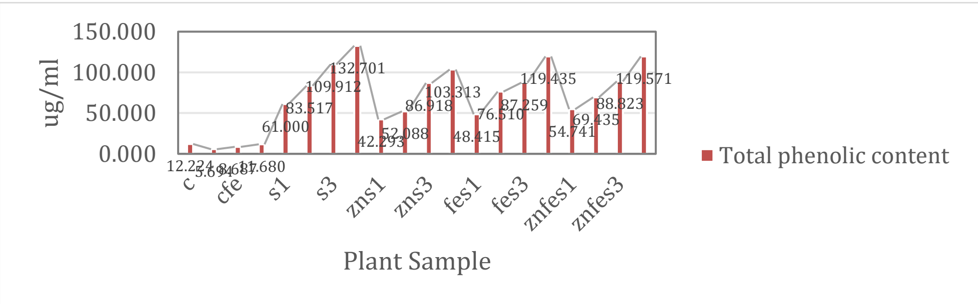

b)

**Figure 3.** Effect of, a) salt stress (s1, s2 and s3), and; b) drought stress (d1, d2 and d3) on protein content of plant and effect of zinc sulphate and iron sulphate on protein content of water stressed plants (znd1, znd2, znd3, fed1, fed2, fed3, znfed1, znfed2 and znfed3) and salt stressed plants (zns1, zns2, zns3, fes1, fes2, fes3, znfes1, znfes2 and znfes3).

**Effect of zinc sulphate and iron sulphate on total phenolic content of water and salt stressed Pennisetum Glaucum**

The data presented in Figures 4a and 4b provide insights into the effect of salt and drought stress on the total phenolic content of Pennisetum glaucum samples, as well as the influence of zinc sulphate ($ZnSO_4$) and iron sulphate ($FeSO_4$) treatments under these stresses. In the control group (c), total phenolic content was 12.22(ug/ml), and treatments with zinc (czn), iron (cfe), and both zinc and iron (cznfe) led to a decrease in phenolic content, with values of 5.69, 8.69, and 11.68, respectively. Under drought stress (d1, d2, d3), phenolic content increased dramatically, with values escalating from 44.74 in d1 to 169.50 in d3, indicating a significant accumulation of phenolic compounds in response to drought severity. Zinc treatment under drought stress (znd1, znd2, znd3) resulted in a substantial increase in phenolic content, with values ranging from 14.40 to 101.34, though these values were still lower than those observed under drought stress alone. Iron treatment (fed1, fed2, fed3) also enhanced phenolic content, ranging from 21.88 to 106.10, with an increasing trend corresponding to higher stress levels. The combination of zinc and iron (znfed1, znfed2, znfed3) showed the most notable effect, with phenolic content increasing from 32.29 in znfed1 to 130.12 in znfed3, suggesting a synergistic effect of both treatments under drought stress. In contrast, under salt stress (s1, s2, s3, s4), total phenolic content also increased with stress severity, ranging from 61.00 in s1 to 132.70 in s4. Zinc treatment under salt stress (zns1, zns2, zns3, zns4) moderately enhanced phenolic content, with values ranging from 42.29 to 103.31. Iron treatment (fes1, fes2, fes3, fes4) also caused a similar increase in phenolic content (48.41 to 119.44), although the effect was generally less pronounced than zinc treatment. When both zinc and iron were combined (znfes1, znfes2, znfes3, znfes4), phenolic content ranged from 54.74 to 119.57, further suggesting a beneficial role of the combined treatments, though not as substantial as under drought stress (Figure 4.). These findings indicate that both drought and salt stresses lead to a significant increase in phenolic content, a known adaptive response in plants, and that zinc and iron supplementation, particularly in combination, can influence phenolic accumulation under these stresses. Zinc showed a stronger effect on enhancing phenolic content compared to iron, especially under drought stress conditions.

The ANOVA for total phenolic content indicates significant treatment effects under both salt stress and drought stress (p-value = 0.001). Salt stress leads to a significant increase in phenolic accumulation, as a protective response to oxidative stress. However, zinc and iron supplementation help modulate this increase, with the combined treatment yielding the most controlled phenolic accumulation. The p-value for the combined treatment is 0.001, highlighting the role of micronutrients in optimizing phenolic defence mechanisms. Under drought stress, phenolic content increases significantly as stress intensifies, with zinc and iron supplementation further enhancing this accumulation. The combined treatment of zinc and iron again shows the highest increase in phenolic content, with a p-value of 0.001, confirming the synergistic effect of these micronutrients in enhancing antioxidant defenses during drought conditions

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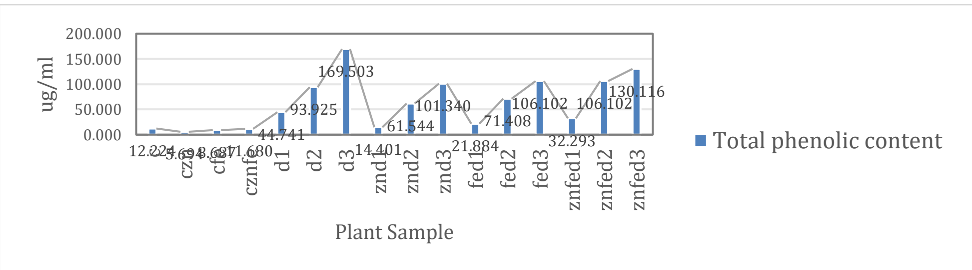


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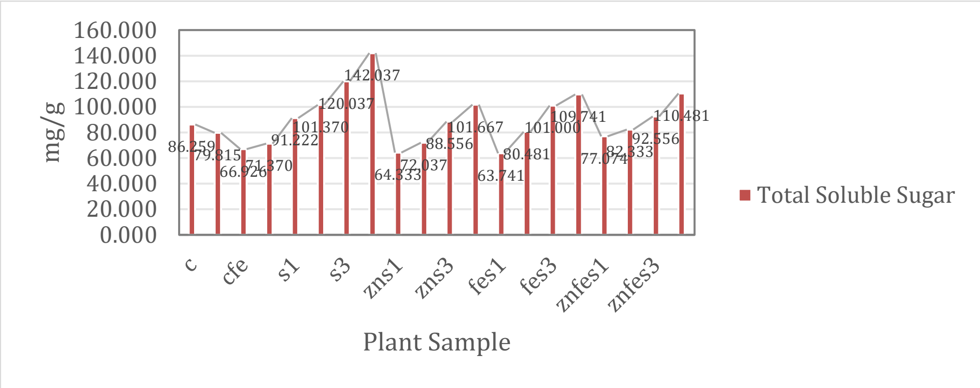
**Figure 4.** Effect of, a) salt stress (s1, s2 and s3), and; b) drought stress (d1, d2 and d3) on total phenolic content of plant and effect of zinc sulphate and iron iron sulphate on total phenolic content of water stressed plants (znd1, znd2, znd3, fed1, fed2, fed3, znfed1, znfed2 and znfed3) and salt stressed plants (zns1, zns2, zns3, fes1, fes2, fes3, znfes1, znfes2 and znfes3)

**Effect of zinc sulphate and iron sulphate on total soluble sugar in water and salt stressed Pennisetum glaucum**

The data from the effect of zinc sulphate ($ZnSO_4$) and iron sulphate ($FeSO_4$) on total soluble sugar content in Pennisetum glaucum under drought and salt stress reveal distinct patterns in the response of the plant to these treatments (Fig 5.). In the control group (c), the total soluble sugar content was 86.26(mg/g), and the application of zinc (czn), iron (cfe), and both zinc and iron (cznfe) led to a decrease in sugar levels, with values of 79.81, 66.93, and 71.37, respectively. Under drought stress (d1, d2, d3), soluble sugar content increased progressively, with values rising from 104.63 in d1 to 145.96 in d3, indicating a higher accumulation of soluble sugars as the stress intensity increased. Zinc treatment under drought stress (znd1, znd2, znd3) resulted in relatively lower increases in sugar content, with values ranging from 83.15 to 101.74. Iron treatment (fed1, fed2, fed3) also led to increases in soluble sugars (88.26 to 109.96), with the highest values seen under the most severe drought stress (fed3). The combination of zinc and iron (znfed1, znfed2, znfed3) showed a consistent increase in soluble sugars, ranging from 96.04 to 115.30, demonstrating a synergistic effect in enhancing sugar accumulation under drought conditions. In the case of salt stress (s1, s2, s3, s4), soluble sugar content increased in a similar fashion, from 91.22 in s1 to 142.04 in s4, reflecting an adaptive response to salt stress. Zinc treatment under salt stress (zns1, zns2, zns3, zns4) resulted in lower sugar content compared to the salt-stressed group without treatment, with values ranging from 64.33 to 101.67, suggesting that zinc might slightly inhibit sugar accumulation in salt-stressed plants. Iron treatment (fes1, fes2, fes3, fes4) showed an increase in sugar levels (63.74 to 109.74), with the highest value observed in fes4. The combination of zinc and iron under salt stress (znfes1, znfes2, znfes3, znfes4) led to a moderate increase in soluble sugars, ranging from 77.07 to 110.48, indicating that both treatments, while beneficial, did not significantly alter the sugar accumulation when compared to individual treatments. These results suggest that both drought and salt stresses enhance soluble sugar content in Pennisetum glaucum, with zinc and iron treatments having variable effects on sugar accumulation under these stresses. Zinc seems to have a more pronounced inhibitory effect on soluble sugars under salt stress, while its impact under drought stress is less inhibitory and may contribute to maintaining sugar levels in the plant. Iron, on the other hand, tends to promote soluble sugar accumulation under both stress conditions, especially in combination with zinc (Figure5.).

The ANOVA for total soluble sugar content shows significant differences between treatments under both salt stress and drought stress (p-value = 0.001). Salt stress induces a marked increase in soluble sugar levels, a typical osmotic adjustment response. However, both zinc and iron supplementation significantly reduce this accumulation, with p-values of 0.001, indicating that these micronutrients help prevent excessive sugar buildup under salt stress. In drought stress, soluble sugar content also increases with stress severity, but zinc and iron supplementation reduces this accumulation, showing a significant effect (p-value = 0.001). The combined treatment of zinc and iron shows the most beneficial effect, with a p-value of 0.001, suggesting that both micronutrients work together to help maintain osmotic balance and mitigate the negative impact of drought stress on sugar metabolism.

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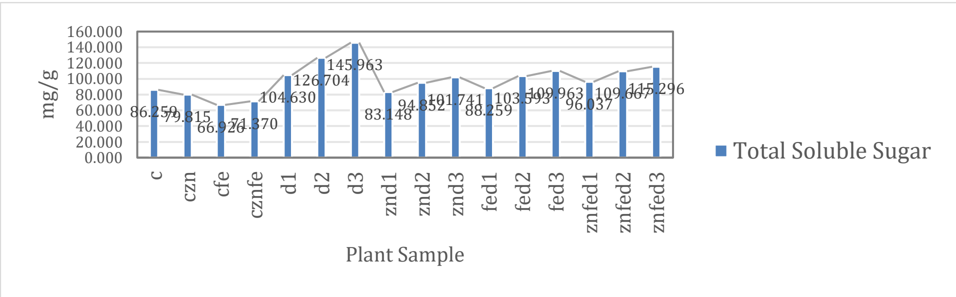


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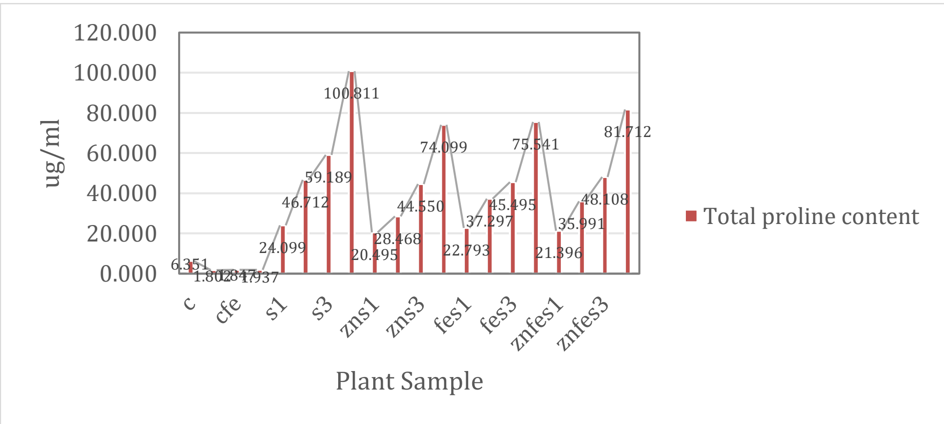
**Figure 5.** Effect of, a) salt stress (s1, s2 and s3), and; b) drought stress (d1, d2 and d3) on total soluble sugar in plant and effect of zinc sulphate and iron iron sulphate on total soluble sugar in water stressed plants (znd1, znd2, znd3, fed1, fed2, fed3, znfed1, znfed2 and znfed3) and salt stressed plants (zns1, zns2, zns3, fes1, fes2, fes3, znfes1, znfes2 and znfes3).

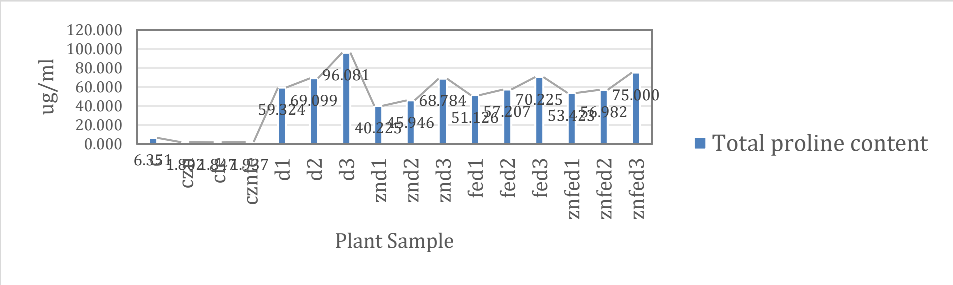
**Effect of zinc sulphate and iron sulphate on total proline content of water and salt stressed Pennisetum glaucum**

The data presented on the total proline content in Pennisetum glaucum under salt and drought stress, along with the effect of zinc sulphate ($ZnSO_4$) and iron sulphate ($FeSO_4$) treatments, show a clear trend of proline accumulation as a response to abiotic stress, with variations depending on the treatment applied,(Fig 6). In the control group (c), proline content was 6.35(ug/ml), and treatments with zinc (czn), iron (cfe), and both zinc and iron (cznfe) resulted in significant reductions in proline levels, with values of 1.80, 1.85, and 1.94, respectively, indicating that these treatments may mitigate the accumulation of proline under normal conditions. Under salt stress (s1, s2, s3, s4), proline content increased significantly, reflecting the plant's adaptive response to osmotic stress, with values rising from 24.10 in s1 to 100.81 in s4. Zinc treatment under salt stress (zns1, zns2, zns3, zns4) resulted in lower proline accumulation compared to the salt-stressed group without treatment, with values ranging from 20.50 to 74.10, suggesting that zinc may help mitigate the proline accumulation in salt-stressed plants. Iron treatment (fes1, fes2, fes3, fes4) also caused a decrease in proline content (22.79 to 75.54), though the effect was somewhat less pronounced than zinc treatment. When both zinc and iron were combined (znfes1, znfes2, znfes3, znfes4), proline content ranged from 21.40 to 81.71, with a trend similar to the individual treatments but with a slight synergistic effect in reducing proline accumulation under salt stress (Figure 6).

In the case of drought stress (d1, d2, d3), proline content also increased, with values rising from 59.32 in d1 to 96.08 in d3, indicating a significant accumulation of proline as the stress intensity increased. Zinc treatment under drought stress (znd1, znd2, znd3) resulted in a reduction in proline content, with values ranging from 40.23 to 68.78, showing that zinc treatment mitigates proline accumulation under drought stress, though the proline levels were still elevated compared to the control. Iron treatment (fed1, fed2, fed3) also reduced proline accumulation, with values ranging from 51.13 to 70.23, and similarly to zinc, iron treatment alleviated some of the stress-induced proline accumulation. The combination of zinc and iron (znfed1, znfed2, znfed3) further reduced proline content, with values ranging from 53.42 to 75.00, indicating a beneficial effect of combined treatments on proline accumulation under drought stress. These results suggest that both zinc and iron treatments are effective in reducing proline accumulation under both salt and drought stress, with zinc showing a stronger mitigating effect compared to iron, especially under salt stress. The combination of both nutrients further enhances this effect, helping the plant manage osmotic stress more effectively. The ANOVA results for proline content under both salt and drought stress conditions show statistically significant differences between treatment groups (p-value = 0.001). Salt stress leads to a marked increase in proline content, which is a known adaptive response to osmotic stress. However, zinc supplementation significantly reduces proline accumulation, suggesting that zinc helps alleviate the osmotic stress caused by salt stress by regulating proline levels. Similarly, under drought stress, proline content increases as the severity of stress rises. Zinc and iron supplementation both help reduce proline accumulation, with zinc demonstrating a stronger mitigating effect. The combined zinc and iron treatment further enhances this reduction, indicating that the dual supplementation offers a synergistic benefit in mitigating proline accumulation during drought stress.The ANOVA for total proline content under salt and drought stress indicates significant treatment effects with p-values of 0.001 for both conditions, confirming that salt stress and the applied treatments (zinc and iron) significantly affect proline accumulation. Under salt stress, proline content significantly increases compared to the control group. However, zinc supplementation significantly reduces proline accumulation, with p-values of 0.001, indicating that zinc helps alleviate osmotic stress caused by salt. Similarly, under drought stress, proline content increases with stress severity, but both zinc and iron supplementation show significant reduction in proline levels. Zinc demonstrates the most pronounced effect, with p = 0.001. The combined treatment of zinc and iron further reduces proline accumulation, with a p-value = 0.001, showing a synergistic effect in alleviating proline accumulation under drought stress




a)




b)

**Figure 6.** Effect of, a) salt stress (s1, s2 and s3), and; b) drought stress (d1, d2 and d3) on total proline content in plant and effect of zinc sulphate and iron sulphate on total proline content in water stressed plants (znd1, znd2, znd3, fed1, fed2, fed3, znfed1, znfed2 and znfed3) and salt stressed plants (zns1, zns2, zns3, fes1, fes2, fes3, znfes1, znfes2 and znfes3).

## DISCUSSION

### Effect of Salt Stress on Chlorophyll Content

The observed reduction in chlorophyll content under salt stress in Pennisetum glaucum is consistent with previous findings that salinity adversely affects photosynthetic pigment stability and biosynthesis. The progressive decline in chlorophyll a (CHLa), chlorophyll b (CHLb), and total chlorophyll (TCHL) from s1 to s4 highlights the detrimental impact of increased salinity. Such results are supported by the studies of (Parida and Das, 2005), which demonstrated that high salinity disrupts chloroplast structure and impairs the enzymatic pathways involved in chlorophyll synthesis. Similarly, Zhao et al. (2007) reported that salt stress induces oxidative damage, leading to pigment degradation.

Interestingly, zinc supplementation (zns1 to zns4) mitigated the adverse effects of salinity, as evidenced by higher CHLa and TCHL levels, particularly in zns1. This aligns with by (Cakmak, 2000), who showed that zinc plays a critical role in maintaining chloroplast integrity and activating antioxidant enzymes. Iron supplementation (fes1 to fes4) also showed moderate protective effects, although its efficacy was lower compared to zinc. The combined treatment (znfes1 to znfes4) demonstrated intermediate results, suggesting a cumulative yet suboptimal interaction between zinc and iron under high salinity stress.

### Effect of Drought Stress on Chlorophyll Content

Under drought stress, a similar trend of declining chlorophyll levels was observed, with CHLa and TCHL decreasing significantly from d1 to d3. Drought-induced reductions in chlorophyll content have been widely reported, with (Farooq et al., 2009) highlighting that water deficiency limits nutrient uptake, disrupts photosynthetic machinery, and accelerates chlorophyll degradation. Zinc treatment (znd1 to znd3) effectively alleviated drought-induced pigment loss, with znd1 showing the highest retention of chlorophyll. This finding is consistent with (Hussain et al., 2024), who reported that zinc enhances osmotic adjustment and reduces oxidative damage under drought conditions. Iron supplementation (fed1 to fed3) also showed a positive effect, albeit less pronounced than zinc. The combined zinc and iron treatment (znfed1 to znfed3) further improved chlorophyll levels, reflecting the complementary roles of these micronutrients in promoting pigment stability and photosynthetic efficiency.

### Effect of Zinc Sulphate and Iron Sulphate on Protein Content

The increase in protein content under salt stress (s1 to s4) suggests an adaptive response, potentially involving stress-induced protein synthesis. Similar observations were made by (Saleem et al., 2022), who noted that plants under salinity stress accumulate stress proteins to mitigate cellular damage. Zinc-treated plants (zns1 to zns4) showed a significant enhancement in protein content, likely due to zinc's role in stabilizing ribosomes and facilitating protein synthesis (Wahid and Close, 2007). Iron-treated plants (fes1

to fes4) also exhibited increased protein levels, though to a lesser extent. The combined treatment (znfes1 to znfes4) showed synergistic effects, particularly under mild to moderate salt stress.
Under drought stress, protein content declined sharply in untreated plants (d1 to d3), reflecting severe physiological disruption. However, zinc and iron treatments (znd1 to znd3 and fed1 to fed3) significantly mitigated protein loss, with zinc showing a more pronounced effect. These findings align with studies by (Kabir et al., 2024), which demonstrated that zinc and iron supplementation under drought conditions enhances the synthesis of stress-related proteins, improving plant resilience.

**Effect of Zinc Sulphate and Iron Sulphate on Total Phenolic Content**
The marked increase in total phenolic content under both salt and drought stress is consistent with the role of phenolics as antioxidant compounds that mitigate oxidative damage **(Reginato et al., 2015)**. Under drought stress, phenolic accumulation was particularly pronounced in d3, indicating a robust protective response. Zinc and iron treatments further influenced phenolic content, with combined treatment (znfed1 to znfed3) showing the highest enhancement. Similar findings were reported by **(Alghamdi et al., 2022)**, who noted that micronutrient supplementation boosts phenolic biosynthesis, enhancing antioxidant capacity under abiotic stress.

**Effect of Zinc Sulphate and Iron Sulphate on Total Soluble Sugar Content**
The increase in total soluble sugar content under salt and drought stress reflects an adaptive mechanism to maintain osmotic balance and protect cellular structures. These results are consistent with those of **(Kazemi, 2014)** [20], who demonstrated that soluble sugars act as osmoprotectants under abiotic stress. Zinc and iron supplementation under both stresses reduced the accumulation of soluble sugars, suggesting that these treatments alleviate stress severity and reduce the need for osmotic adjustment. The combined treatment (znfes1 to znfes4 and znfed1 to znfed3) showed intermediate effects, highlighting the importance of micronutrient synergy in modulating carbohydrate metabolism under stress conditions.

## CONCLUSION

The findings highlight the significant impact of abiotic stresses, such as salt and drought, on Pennisetum glaucum, with marked reductions in chlorophyll and protein content alongside increases in phenolic and soluble sugar levels under stress conditions. Salt stress led to a severe decline in chlorophyll levels, particularly at higher stress intensities, while drought stress similarly diminished chlorophyll content and protein synthesis. However, the application of zinc sulphate ($ZnSO_4$) and iron sulphate ($FeSO_4$) mitigated these adverse effects to varying extents, with zinc showing a more pronounced role in enhancing chlorophyll biosynthesis, protein content, and phenolic accumulation. Combined treatments of zinc and iron demonstrated a synergistic effect, particularly in alleviating pigment loss and supporting physiological stability under moderate stress conditions. These results underscore the critical role of micronutrients in enhancing the resilience of Pennisetum glaucum to abiotic stresses, with implications for sustainable agricultural practices in stress-prone environments.

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