



ROLE OF ZINC ($ZnSO_4$) IN MITIGATING SALT STRESS EFFECTS IN *ARACHIS HYPOGAEA L.*: A PHYSIOLOGICAL AND BIOCHEMICAL STUDY

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Abstract

Salinity is a major abiotic stress that significantly limits agricultural productivity, particularly in oilseed crops such as *Arachis hypogaea L.* The present study aimed to evaluate the physiological and biochemical responses of groundnut plants to individual and combined stresses from sodium chloride (NaCl) and zinc sulphate ($ZnSO_4$). The experiment was conducted under controlled pot conditions with four treatments: control, $ZnSO_4$ stress, NaCl stress, and combined NaCl + $ZnSO_4$ stress. After 90 days of treatment, growth parameters, physiological attributes, and biochemical markers were assessed. Salinity stress significantly reduced plant growth, biomass, relative water content, and chlorophyll concentration due to osmotic imbalance and impaired photosynthetic efficiency. However, $ZnSO_4$ treatment enhanced biomass accumulation and chlorophyll content. Notably, combined treatment improved relative water content and maintained higher chlorophyll levels compared to NaCl stress alone, indicating a protective role of zinc. Proline accumulation further suggested improved osmotic adjustment under stress conditions. These findings demonstrate that zinc supplementation can effectively mitigate salinity-induced damage and enhance stress tolerance in groundnut.

Keywords: *Arachis hypogaea L.*, salt stress, zinc toxicity, abiotic stress, relative water content, chlorophyll, proline.

1. Introduction

Salinity stress is one of the most critical environmental constraints affecting global agricultural productivity. It is estimated that nearly 20% of cultivated land and approximately 50% of irrigated land are adversely affected by salinity. Elevated salt concentrations disrupt plant water uptake, ion balance, and metabolic activities, ultimately reducing plant growth and yield. Excess accumulation of sodium ions (Na^+) interferes with essential cellular processes, including photosynthesis, enzyme activity, and nutrient uptake.[1] In addition to ionic imbalance, salinity stress induces oxidative stress through the excessive production of reactive oxygen species (ROS), which damage cellular components such as lipids, proteins, and nucleic acids. To counteract these effects, plants have evolved various physiological and biochemical mechanisms, including osmotic adjustment, ion compartmentalisation, and activation of antioxidant defence systems.[2] Salinity stress produces reactive oxygen species (ROS) like hydroxyl radicals (OH^\cdot) and superoxide radicals ($O_2^{\cdot-}$).[2] Salinity-induced hyperosmotic stress and ion toxicity trigger a complex signal transduction network that culminates in the differential

expression of stress-responsive genes. This genomic reprogramming is essential for maintaining cellular integrity and physiological function under adverse conditions.

Groundnut (*Arachis hypogaea L.*) is an economically important oilseed crop widely cultivated in tropical and subtropical regions. However, it is highly sensitive to salinity stress, which significantly limits its productivity. The application of micronutrients has emerged as a potential strategy to enhance plant tolerance to abiotic stress.[3]

Zinc (Zn) is an essential micronutrient involved in numerous physiological processes, including enzyme activation, protein synthesis, and maintenance of membrane integrity. Although excessive zinc can be toxic, optimal concentrations have been reported to enhance antioxidant defence and improve stress tolerance in plants. The application of zinc has been demonstrated to trigger defence mechanisms in plants, making them resilient to stress factors. Zinc toxicity reduced the length of roots and shoots, as well as the leaf area in tomato (*Solanum lycopersicum L.*).[4] Salt stress greatly reduces growth, but earlier research indicates that zinc may serve as a vegetative stimulant, possibly enhancing biomass and maintaining chlorophyll levels in stressed plants. The present study was designed to investigate the role of zinc sulphate ($ZnSO_4$) in alleviating salinity stress in groundnut by evaluating key physiological and biochemical parameters, including relative water content (RWC), transpiration rate, stomatal density, chlorophyll content, and proline accumulation. [5,6]

2. Materials and Methods

2.1 Test materials

Healthy seeds of *Arachis hypogaea L.* were sown in plastic pots containing a 2:1:1 mixture of sterilized red soil, sand, and farmyard manure.

In each instance, two or three seeds were sown at a depth of three centimetres in each pot. Following seven to ten days of germination, thinning was done to maintain one healthy, homogenous seedling per pot to avoid nutritional competition. The plants were regularly irrigated with deionised water to maintain the soil at field capacity throughout the first ten to fourteen days of establishment. No external fertilisers were used during this phase to ensure that the subsequent Zinc and NaCl treatments would be the only source of chemical stress.

2.2 Design of the experiment

The experiment followed a randomised design with four groups: (1) Control (distilled water), (2) Zinc stress (12 mg $ZnSO_4$ in 50 mL), (3) Salt stress (15 g NaCl in 50 mL), and (4) Combined stress (12 mg $ZnSO_4$ + 15 g NaCl in 50 mL). Stress induction commenced 15 days after germination and was maintained for 90 days at 3-day intervals.

2.3 Sample Collection

Physiological parameters, including relative water content (RWC), transpiration rate (Cobalt Chloride method), and stomatal density (leaf impression method), were measured at the end of the treatment period. A part of the samples was put in resealable bags so that the relative water content and chlorophyll content of the leaves could be measured.

2.4 Relative Water Content (RWC)

The leaf relative water content (LRWC) was determined using the drying method. Fresh leaves were collected, and their weight was measured. Next, leaves were immersed in distilled water for 24 hours to measure saturated fresh weight. The leaves were then put in an oven at 80°C for 8 hours to determine their dry weight. The calculation formula for

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$

2.5 Transpiration Rate and Stomatal Density

We employed the cobalt chloride ($CoCl_2$) paper method, which is a traditional way to measure stomatal transpiration, to determine the relative rate of transpiration. After being immersed in a 3% $CoCl_2$ solution, filter paper discs were dried in an oven until they turned blue, which meant they were anhydrous. The glass slides were clipped to the lower surface of the leaf to keep the dried discs from

getting wet from the air. The time required for the colour change from blue to pink was recorded. The leaf's transpiration released water vapour, which resulted in a colour change from blue to pink. [7]

Transpiration rate = 1/time taken for colour change

Stomatal characteristics were assessed using the leaf impression technique on fully expanded leaves. A thin layer of acrylic (synthetic nail polish) was applied to both the adaxial and abaxial surfaces of the leaf. After drying for a few seconds, the film was carefully peeled off and observed under a microscope.

Stomatal density (number of stomata per mm²) and the percentage of closed stomata were determined microscopically. Three observations were taken from randomly selected areas of each impression, and the mean value was calculated to represent the stomatal number for each leaf.[8]

2.6 Growth and Biomass: Determination of Fresh and Dry Weight

Plant growth parameters were recorded before the initiation of stress and after stress treatment. Plant height and ground diameter were measured to evaluate changes in height under different treatment conditions.

Biomass was determined using the whole-plant harvesting method at the end of the stress period and after rewatering. The potting soil was gently loosened by washing with running water, and the seedlings were carefully removed. Adhering soil particles were washed off, and excess surface moisture was removed. The fresh weights of aboveground parts (stems, branches, and leaves) and belowground parts (roots) were measured separately. Total fresh weight per plant and the root-to-shoot ratio (ratio of root fresh weight to shoot fresh weight) were then calculated.[9]

2.7 Osmotic potential

Osmotic potential of groundnut leaves was determined using the method described by Lanfermeijer et al., with slight modifications. Fresh leaf samples were collected from the plants at the end of the stress treatment. Approximately 100 mg of fresh leaf tissue was placed in a 1.5 mL plastic microcentrifuge tube and manually crushed with a glass rod to extract the cell sap. From the extracted sap, 20 µL of solution was carefully placed on a filter paper disc, which was then put into the chamber of the Vapour Pressure Osmometer. The osmometer measured the osmotic concentration of the sap and reported the values as millimoles per kilogram (mmol kg⁻¹).[6]

2.8 Determination of Chlorophyll Content

Chlorophyll content was calculated using Arnon's method. 10 ml of 80% acetone was used in the dark to extract fresh leaves, each comprising 0.5 grams of plant tissue. After centrifuging the test mixture for five minutes at 10,000 RPM, the supernatant was extracted, combined with a suitable proportion of 85% aqueous acetone solution, and the absorbance was measured at 663 and 645 nm. The formulae provided by Arnon were used to compute the chlorophyll content.[9]

$$\text{mg chlorophyll a/g tissue} = \frac{(12.7 A_{663} - 2.69 A_{645}) \times V}{1000 \times w}$$

$$\text{mg chlorophyll b/g tissue} = \frac{(22.9 A_{645} - 4.69 A_{663}) \times V}{1000 \times w}$$

$$\text{mg total chlorophyll /g tissue} = \frac{(20.2 A_{645} + 8.02 A_{663}) \times V}{1000 \times w}$$

Where A = absorbance at a specific wavelength

V = final volume of chlorophyll extract in 80% acetone

w = weight of tissue extracted.

2.9. Estimation of proline

An ethanol-based extraction technique was used to extract proline, total amino acids, pigments, and soluble carbohydrates. According to Cross et al. (2006), plant material was cooked twice with 80% ethanol and once with 50% ethanol, yielding a final ethanol: water ratio of 70:30 (v/v). The reaction mixture was produced by adding 1000 μL to 1.5 mL screw-cap tubes. Next, either 500 μL of ethanolic extract or 100 μL of proline standard (0.2–5 mM) was added, and the final volume was adjusted using 400 μL of ethanol: water (40:60, v/v). After sealing and properly mixing the tubes, a block heater was used to incubate them at 95°C for 20 minutes. Following incubation, the samples were centrifuged for one minute at 10,000 rpm. A spectrophotometer was used to measure absorbance at 520 nm after the supernatant was transferred to a 1.5 mL cuvette.[10]

3. Results

3.1. Effects of salt, zinc and combined stress on the Relative Water Content (RWC) of *Arachis hypogaea L.*

Salinity and zinc stress significantly impacted cellular turgidity. The lowest RWC was observed in the salt-only group (10%), while the combined treatment showed a recovery to 18% (Table 1).

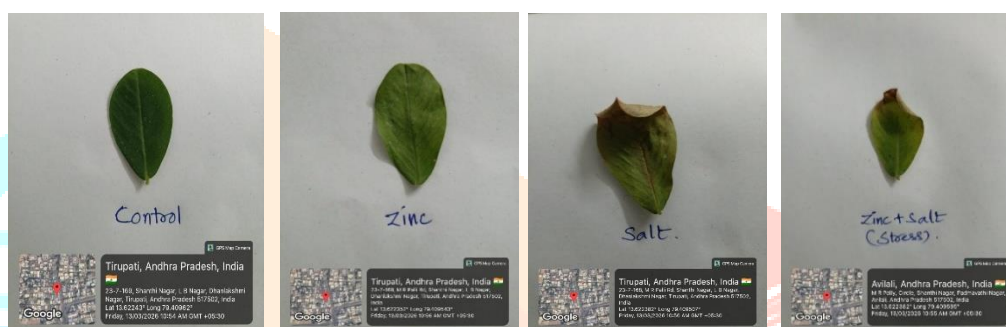


Fig 1.: (a) (b) (c) (d)

Fig 5. Effects of salt and zinc stress on leaf phenotypic characteristics of *Arachis hypogaea*. (a)-control, (b)-zinc treatment, (c)- salt treatment, (d)-zinc & salt treatments.

Table 1: Effects of salt, zinc, and combined stress on relative water content. (RWC)

Treatment	Fresh weight(g)	Dry weight (g)	Turgid weight(g)	RWC (%)
Control	0.17 g	0.05 g	0.35 g	40%
Zinc stress	0.08 g	0.06 g	0.16 g	20%
Salt stress	0.06 g	0.03 g	0.32 g	10%
Zinc +Salt	0.14 g	0.10 g	0.32 g	18%

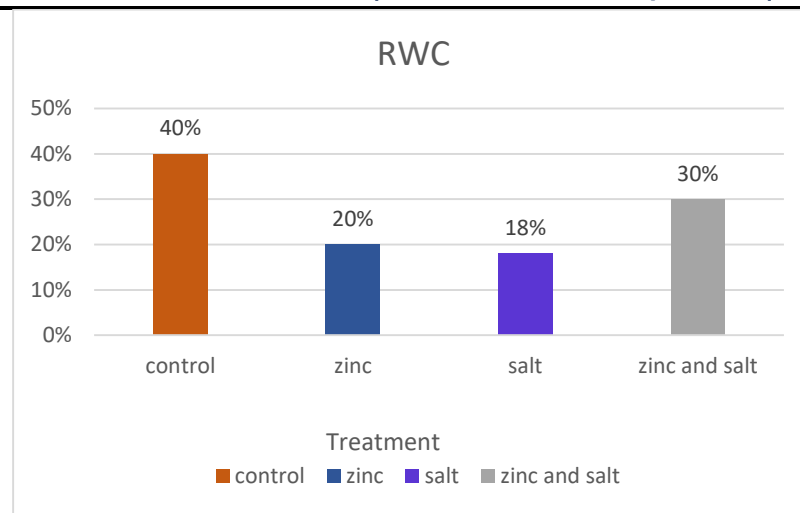


Figure 1. Effects of salt, zinc and combined stress on the relative water content of *Arachis hypogaea L.*

3.2. Effects of salt, zinc and combined stress on Transpiration Rate and Stomatal Density of *Arachis hypogaea L.*

Transpiration rates decreased under stress, with the salt-only group requiring 14 minutes for colour change compared to 6 minutes in the control. Stomatal density was reduced by 51.42% under salt stress, while the combined group maintained a density of 278.52 mm⁻² (Table 2).

Table.2: Effects of salt, zinc and combined stress on the transpiration rate and stomatal density of *Arachis hypogaea L.*

Treatment	Duration (min)	Stomatal Density (mm ⁻²)
Control	6	524.19 mm ⁻²
Zinc stress	10	318.31 mm ⁻²
Salt stress	14	254.65 mm ⁻²
Zinc +Salt	16	278.52 mm ⁻²

3.3. Growth and Biomass

Zinc acted as a growth stimulant for aerial parts, increasing plant weight to 21 g (a 50% increase over the control). Salinity reduced biomass to 11 g, but the addition of zinc in the combined group restored biomass to control levels (14 g) (Table 3)

Table.3: Effects of salt, zinc and combined stress on growth and biomass of *Arachis hypogaea L.*

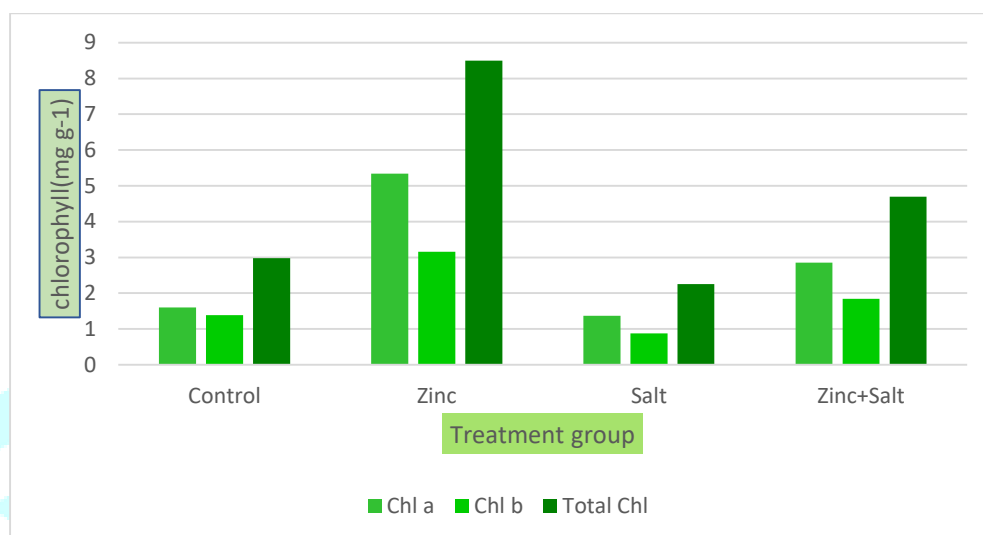
Treatment	Root Length (cm)	Shoot Length (cm)	Leaf Length (cm)	Plant Weight (g)
Control	8.0 cm	15.0 cm	2.45 cm	14 g
Zinc	6.2 cm	16.5 cm	3.0 cm	21 g
Salt	5.5 cm	12.0 cm	2.1 cm	11 g
Zinc +Salt	7.5 cm	14.5 cm	2.3 cm	14 g

3.4. Effects of Salt, Zinc and Combined Stress on Photosynthetic Pigment Content in Leaves of *Arachis hypogaea L.*

Chlorophyll levels were highest in the zinc-only group (8.496 mg/g). Combined-stress plants maintained a total chlorophyll content of 4.687 mg/g, significantly higher than that of the salt-only group (2.243 mg/g). (Table 4).

Table 4. Effects of salt, zinc, and combined stress on chlorophyll a, chlorophyll b, and total chlorophyll contents of *Arachis hypogaea*

Treatment	OD value (663 nm)	OD value (645 nm)	Chl a mg·g ⁻¹ /FW	Chl b mg·g ⁻¹ /FW	Total chlorophyll mg·g ⁻¹ /FW
Control	0.145	0.090	1.599 mg·g ⁻¹	1.382 mg·g ⁻¹	2.980 mg·g ⁻¹
zinc	0.470	0.234	5.339 mg·g ⁻¹	3.159 mg·g ⁻¹	8.4962 mg·g ⁻¹
Salt	0.121	0.063	1.367 mg·g ⁻¹	0.876 mg·g ⁻¹	2.2430 mg·g ⁻¹
Zinc +Salt	0.252	0.132	2.845 mg·g ⁻¹	1.843 mg·g ⁻¹	4.6874 mg·g ⁻¹

**Figure.2** Effects of salt, zinc, and combined stress on chlorophyll a, chlorophyll b, and total chlorophyll contents of *Arachis hypogaea L.*

3.5 Effects of Salt, Zinc and Combined Stress on Proline Content in Leaves of *Arachis hypogaea L.*

Proline content, an indicator of osmotic defence, was restored to control levels (1.51 $\mu\text{mol g}^{-1}$ FW) in the combined group after a drop in the salt-only group (Table 5).

Table .5 Effects of Salt, Zinc and Combined Stress on Proline Content in Leaves of *Arachis hypogaea L.*

Treatment	OD value (520 nm)	Estimated Proline (μmolg^{-1} FW)
Control	0.135	1.46 μmolg^{-1} FW
Zinc	0.155	1.68 μmolg^{-1} FW
Salt	0.0962	1.04 μmolg^{-1} FW
Zinc +Salt	0.139	1.51 μmolg^{-1} FW

4. Discussion

The results of the present study demonstrate that salinity stress significantly impairs physiological and biochemical processes in *Arachis hypogaea L.* The reduction in relative water content and transpiration rate under NaCl treatment indicates osmotic stress and reduced water uptake capacity. Furthermore, decreased chlorophyll content under salinity suggests damage to the photosynthetic apparatus.[13]

Zinc supplementation showed a positive effect on plant growth and physiological performance. The observed increase in biomass and chlorophyll content under ZnSO₄ treatment highlights its role in enhancing metabolic activity and maintaining membrane stability.[17] Notably, the combined NaCl and

ZnSO₄ treatment mitigated the adverse effects of salinity, as evidenced by improved RWC, chlorophyll content, and proline accumulation.[14]

Proline acts as an osmoprotectant, stabilising cellular structures and protecting plants against osmotic stress. The increased proline levels observed in the combined treatment indicate improved stress tolerance mechanisms. These findings are consistent with previous studies reporting the protective role of zinc in alleviating oxidative and osmotic stress in plants. [15,16]

5. Conclusion

The present study confirms that zinc sulphate (ZnSO₄) plays a significant role in mitigating the adverse effects of salinity stress in *Arachis hypogaea* L. Zinc supplementation improved physiological stability, increased chlorophyll content, and promoted osmotic adjustment by increasing proline accumulation. The combined treatment demonstrated that zinc can partially restore plant performance under saline conditions. Therefore, the strategic application of zinc-based micronutrients may be an effective approach to improving groundnut productivity in salt-affected soils.

6. References

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