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Mitigation Of Sodium Chloride Stress In Tomato (Solanum Lycopersicum L.) Through Foliar Application Of Chitosan Nanoparticles

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Abstract.
Salinity stress is a major problem in farming since it makes crops grow more slowly and yield less because of ion toxicity, osmotic imbalance. One of the most common horticultural crops, tomatoes (Solanum lycopersicum L.), are very vulnerable to salt, which reduces their levels of growth-and metabolism-supporting proteins and amino acids. This study evaluated the efficacy of foliar sprays containing chitosan nanoparticles (ChNPs) in preventing salt stress in tomato plants. As a consequence of exposure to salt, plants produced less soluble protein and more free amino acids. But when ChNPs were sprayed on the leaves of plants that were stressed by salt, the protein content and amino acid levels improved a lot, bringing them back to levels that were more like those of plants that weren't affected. Chitosan nanoparticles (ChNPs) have ability to enhance ROS removal and regulate stress-associated plant hormones. The work highlights the potential of Chitosan nanoparticles (ChNPs) as an eco-friendly and economical biostimulant to mitigate salt stress and enhance crop resilience, growth, and yield.

Keywords: *Stress from salt, Tomato (Solanum lycopersicum L.), Chitosan nanoparticles (ChNPs), Protein concentration, Free Amino acids content, Reducing salt stress, Reactive oxygen species (ROS).*

INTRODUCTION

Most crops don't like salinity, which is when there is too much salt in the soil. Salinity is one of the most harmful environmental factors for crop production since it affects more and more areas with time (Shrivastava and Kumar 2015).). In order to restore deteriorated and salty areas, saline agriculture was proposed (Nikalje et al. 2017). The complex interplay of morphological, physiological, and metabolic systems is what leads to salinity repercussions (Akbarimoghaddam et al. 2011). Salinity has a detrimental effect on several physiological processes and plant development, including enzymatic and metabolic activity, cell homeostasis, photosynthesis, respiration, and transpiration (Mahajan and Tuteja, 2005). According to many authors, including Sun et al. (2016), photosynthetic electron transport, stomatal conductance, and photosynthetic carbon assimilation were all shown to be less efficient when exposed to salt. The buildup of sodium and chloride ions in plant tissues is a particularly harmful consequence of salt stress. Major ion imbalance results from both ions entering the cells, and excessive absorption may lead to serious physiological malfunctions (James et al. 2011).

Tomato (Solanum lycopersicum L.) is a horticultural crop grown in almost all latitudes. It is also one of the most popular vegetables since it is beneficial for you (FAO). Tomatoes are usually grown in greenhouses for about five weeks before being moved to the field. This method helps the seeds germinate and the roots grow well. Bio stimulants and starter solutions are often used to help roots grow and form early (Maynard and Hochmuth, 2006). Improved early growth can increase the overall health of a plant and, in turn, increase agricultural output (Servin et al., 2015). Because employing the incorrect starting solutions could harm soil microbes and plants, it is crucial to carefully regulate their quantity and kind. There has been recent interest in particulate biomaterials derived from polymers with the goal of enhancing crop development, production, and nutritional value with minimal negative effects on the environment (Tilman et al., 2011; Tittonell, 2014). The merits of chitosan a polysaccharide derived from tomato chitin (Solanum lycopersicum) that is biocompatible, biodegradable, abundant, and cost-effective, have been recognized (Malerba and Cerana, 2018; Pirbalouti et al., 2017). Based on their research, Kashyap et al. (2015) found that chitosan is the polymer that drug delivery particles often employ. According to research conducted on several horticultural crops, the biocidal and plant-eliciting

characteristics of bulk Chitosan greatly aid in the reduction of plant stress (Yin et al., 2010; Iriti and Varoni, 2015; Mansilla et al., 2013). By controlling enzymes in the system that scavenges reactive oxygen species (ROS), such as peroxidases, superoxide dismutase (SOD), and catalase (CAT), CS-mediated plant defense against environmental stress is often associated with the production of reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Kumaraswamy et al., 2018).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) serve as essential signalling molecules, engaging with hormonal pathways to modulate plant growth and development (Turkan, 2018). Chitosan has a nuanced influence on the intricate regulation of plant development. Minor alterations in Chitosan levels may transition its role from facilitating plant development to inducing cytotoxic effects, including growth inhibition (Pichyangkura and Chadchawan, 2015), Asgari-Targhi et al. (2018), Lopez-Moya et al. (2017), and Plants increase their sensitivity to Chitosan treatment, according to research by Pichyangkura and Chadchawan (2015), when they rapidly and dramatically accumulate many phytohormones.

MATERIALS AND METHODS.

1.1.Seed Collection and Chemical Reagents.

Agricultural University in Coimbatore, Tamil Nadu, India, supplied us tomato seeds of the PKM1 variety (Solanum lycopersicum L.) and Sigma Aldrich Chemical Laboratories in Bangalore, India, supplied us chitosan nanoparticles (ChNPs), a growth regulator.

Experimental Design

The botanical garden of Annamalai University in Tamil Nadu was the site of the experimental investigation. Locations 11°23'23.1" N and 79°43'05.3" E were used for the investigation. After being externally sterilized with a 0.3% $HgCl_2$ solution for one-minute, healthy seeds were washed extensively with ddH_2O. The seeds were then separated into four groups and placed into ninety pots. Red dirt, sand, and farmyard manure were all proportionately distributed among the pots. Three different treatments were administered to the plants: a control group, one that contained (100 Mm) NaCl alone, one that included 120 mg/L (ChNPs) and a combination of the two. The soil samples in each container were tested for salt using an electrical conductivity meter. At 35,45, and 55-days post-sowing (DAS), plants were harvested for biochemical analysis. The effects of the treatments on the plants' development and biochemical characteristics may be evaluated using the specified collecting periods as a foundation.

1.2. Statistical Analysis

Using SPSS software (Version 21.0), the experimental data was statistically analysed. To check for statistically significant changes between the treatment groups, we used a one-way ANOVA. Data shown in bar charts represent mean values that were on a number of copies. The statistical significance was checked using Dunnett's Multiple Range Test (DMRT), with a significance criterion of $P < 0.05$.

1.3 Estimations

relative leaf water content (RLWC)

The relative water content (RWC) was determined in fresh leaf discs of $2cm^2$ diameter excluding midrib. Discs were weighed quickly and immediately floated on deionized distilled water (DDW) in Petri dishes to saturate them with water for the next 4h, in dark. The adhering water of the discs was blotted and turgor mass was noted. The dry mass of the discs was recorded after dehydrating them at 80°C for 24 h in a hot air oven (THE I.L.E. Co., Chennai). RWC was calculated according to the formula given by Barrs and Weatherley (1962).

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

Membrane stability index (MSI)

Membrane stability index (MSI) was estimated by adding 100 mg (0.1g) leaf material to 10 ml of DDW in two sets. One set was heated at 40°C for 30 min in a water bath and the electrical conductivity bridge C1 was measured with a conductivity meter (LABTRONICS -Model LT-23). The second set was boiled at 100°C in a water bath for 10min and the electrical conductivity bridge C2 was also measured with a conductivity meter. MSI was calculated using the formula (Sairam, 1994).

$$MSI = [1 - (C1/C2)] \times 100$$

Electrolyte leakage determination (EL)

The total inorganic ions leaked out in the leaves were estimated by the method of Sullivan and Ross (1979). 20 leaf discs (pre-weighted plant material was taken in a boiling test tube containing 10 ml of deionized water and electrical conductivity (ECa) was measured. The content was heated at 45°C - 55°C for 30 min each in a water bath and electrical conductivity (ECb) was measured. Later the content was again boiled at 100 °C for 10 min and electrical conductivity (ECc) recorded. The electrolyte leakage was calculated by using the formula:

$$\text{Electrolyte leakage (\%)} = \frac{EC_b - EC_a}{EC_C} \times 100$$

Protein Content.

To estimate the concentration of soluble proteins, the Bradford technique (1976) was used. One gram of fresh plant material was ground up using twenty cc of 20% trichloroacetic acid (TCA) in a mortar and pestle. A 15-minute spin at 800 rpm was applied to the homogenate, after which the liquid above was removed. In order to hydrolyze the proteins inside the pellet, five milliliters of 0.1 N sodium hydroxide (NaOH) were added. The next step was to spin the mixture once again for 20 minutes at 1000 rpm. Prior to protein measurement, the supernatant was collected and diluted with 0.1 N NaOH until it reached a final volume of 10 ml. We used 12×100 mm test tubes and added 0.1 ml of the protein solution, which contained 10 to 50 µg of protein, in order to conduct the test. The mixture was vortexed after being added to each tube with five milliliters of Bradford reagent. Two minutes later, a reagent blank was used to collect absorbance values at 595 nm. The blank was prepared using 5 milliliters of Bradford reagent, 0.1 milliliters of 0.1 N NaOH, and 0.1 milliliters of distilled water. . Bovine serum albumin (BSA) concentrations were used to create a standard curve that was then plotted against the absorbance readings from a Nanodrop spectrophotometer (Implen-Inkarp 380). Using the standard curve, we were able to ascertain the protein content of the unidentified samples. The product's unit of measurement was milligrams per gram solid weight.

Free Amino Acid content

Using a procedure developed by Moore and Stein (1948), the total free amino acids were extracted and quantified. To begin, we used a mortar and pestle to ground 500 mg of fresh plant material with 15 ml of 80% boiling ethanol. For 15 minutes, the homogenate was spun in a centrifuge at 800 × g. Next, 10 ml of the supernatant was added to it with 80% ethanol for further examination. For the purpose of measuring free amino acids, 1 milliliter of the ethanol extract was added to a 25-milliliter test tube and then 1 milliliter of 0.1 N sodium hydroxide (NaOH) was added to neutralize it. To determine the point of neutralization, a little amount of methyl red indicator was added. Following that, 1mL of ninhydrin reagent was added while vigorously stirring the mixture. The test tube was immersed in boiling water for a duration of 20 minutes. The mixture was then diluted with 5 cc of water and allowed to cool in the running tap. The absorbance was measured at 570 nm in comparison to a blank sample using a UV-visible spectrophotometer (Model-116, Systronics India Limited, Gujarat, India) with a volume of 30 ml of distilled water. A standard curve was constructed using leucine as the reference. Free amino acid content in milligrams per gram of dry weight was determined and reported on this curve.

2.0. RESULTS AND DISCUSSION.

Leaf relative water content (RWC).

The relative water content in Tomato plants significantly decreased after NaCl stress was applied on all sampling days compared to the control, showing a reduction of 90.64 percent over the control on day 55 (Fig. 4.1) (Raza et al., 2019). However, foliar applications of Chitosan Nanoparticles (ChNPs) to NaCl-stressed of Solanum Lycopersicum L. resulted in an increase in the relative water content of leaves, reaching 95.36% for NaCl + ChNPS on day 55 over the control. Although these values were higher than those under NaCl stress, they were still lower than those of the control Abdallah et al., 2018. Additionally, the foliar application of Chitosan Nanoparticles (ChNPs) to unstressed plants led to a slight increase in relative water content, with values of 102.51 percent over control respectively, compared to the control plants at 55 DAS. ; El Sayed et al., 2019).

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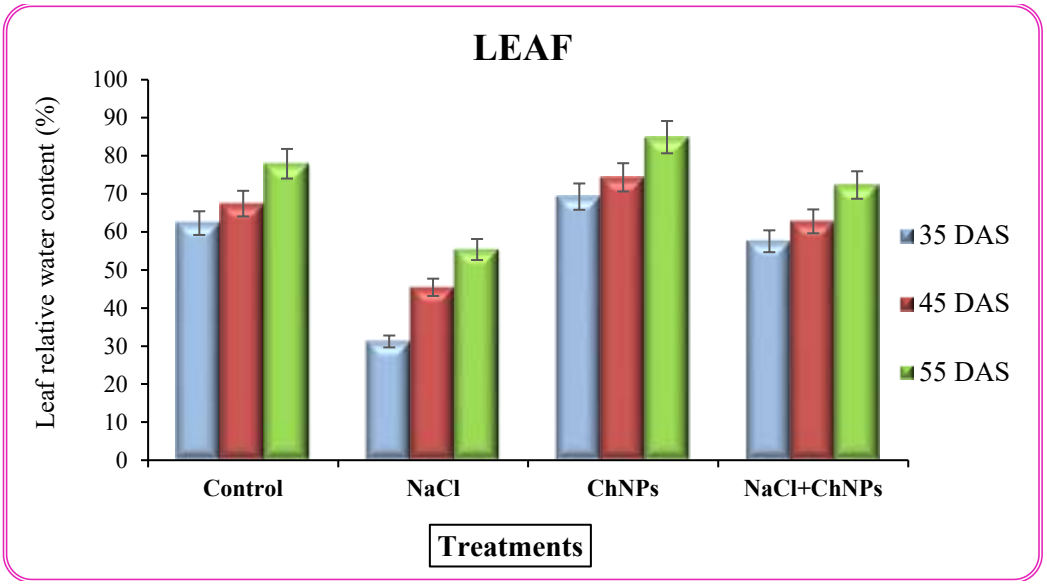
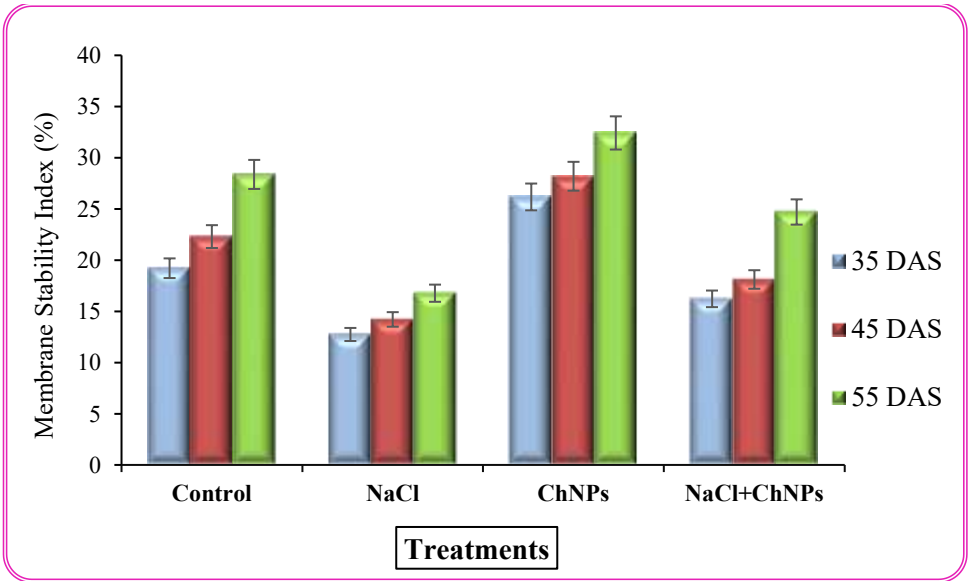


The values are the mean ± SE of seven replicates. Mean values differ significantly at P≤ 0.05 (DMRT). **NaCl**-Sodium Chloride; **ChNPs**- Chitosan Nano particles.

Membrane stability index (MSI).

After exposure to NaCl stress, the membrane stability index (MSI) in the leaves of Tomato plants significantly decreased on all sampling days, with the highest reduction of 69.27% observed at 55 days after sowing (DAS) compared to control Solanum Lycopersicum L. plants (Ru et al., 2025; Tanveer et al., 2020; Raza et al., 2017.However, NaCl-stressed plants treated with foliar applications of ChNPs) showed an increase in MSI, recorded at 89.26%, over control plants at 55 DAS(Alenazi et al., 2024. Additionally, in non-stressed plants, ChNPs increased the MSI by 119.100%, compared to control plants at 55 DAS, and these increases were higher than those observed in NaCl-stressed plants. Mohammadi et al., 2025.

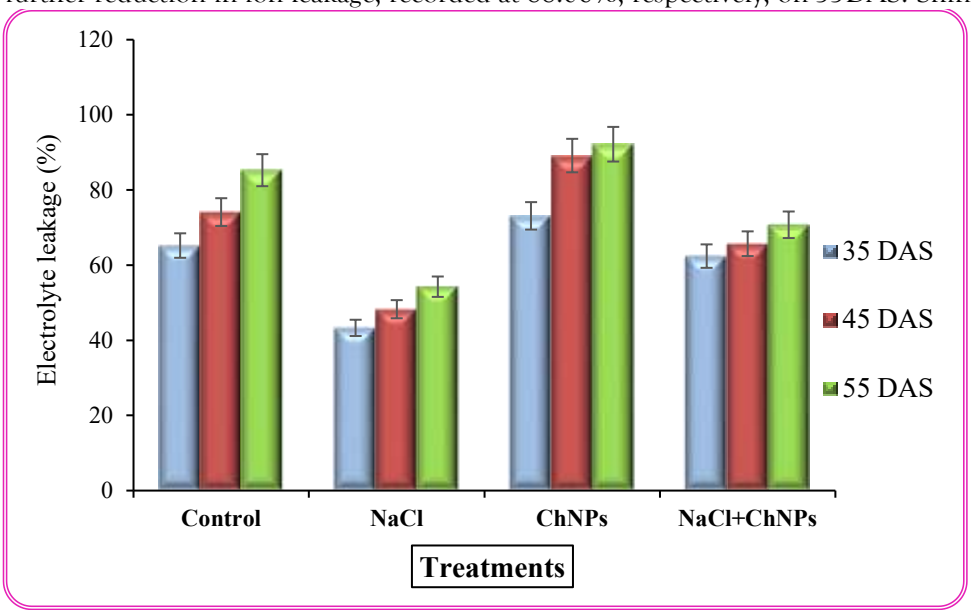


The values are the mean ± SE of five replicates. Mean values differ significantly at P≤ 0.05 (DMRT). **NaCl**-Sodium Chloride; **ChNPs**- Chitosan Nano particles.

Electrolyte Leakage (EL)

After exposure to NaCl salt stress, a significant increase in ion leakage was observed in Tomato plants, with greater increases noted as the salt stress period extended compared to the control. The percentage increase in electrolyte leakage (EL) in NaCl-stressed plants was recorded at 131.09%, 122.51%, and 106.48% on 35, 45, and 55 (DAS) (Tanveer et al., 2020, Raza et al., 2017 respectively. However, foliar application of ChNPs to NaCl-stressed Tomato plants resulted in a sharp decline in ion leakage percentage, with the highest reduction observed on 55 DAS at 109.81%, compared to the control;

Panahirad et al., 2025. Additionally, when non-stressed plants were treated WITH ChNPs, there was a further reduction in ion leakage, recorded at 88.06%, respectively, on 55DAS. Shinde et al., 2024).

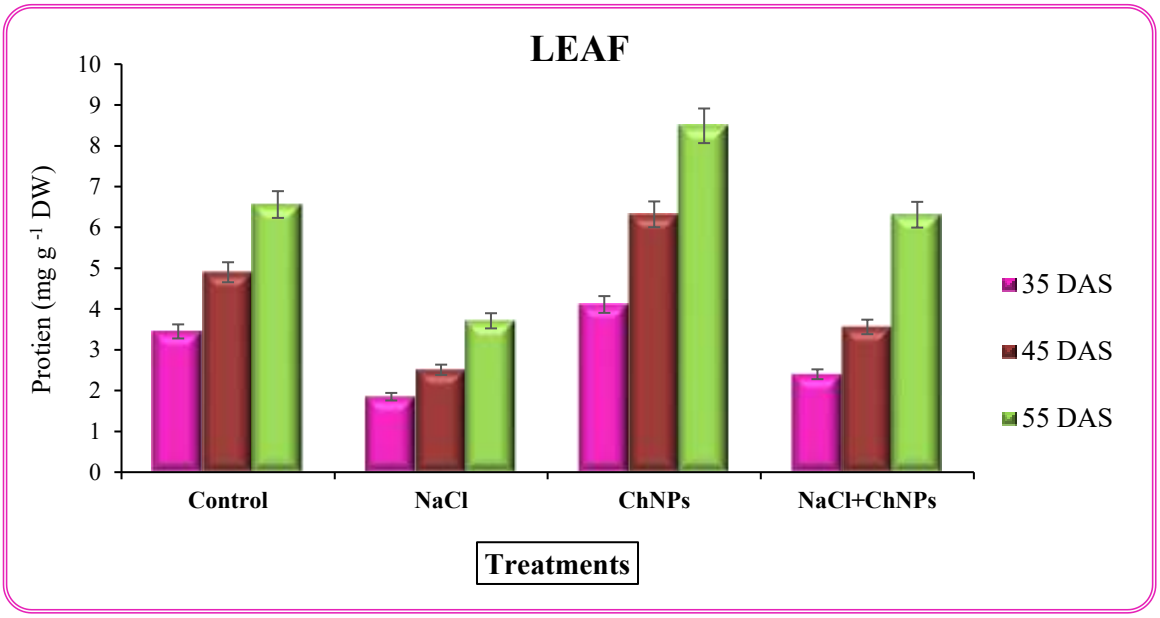


The values are the mean ± SE of five replicates. Mean values differ significantly at P≤ 0.05 (DMRT). **NaCl**- Sodium Chloride; **ChNPs**- Chitosan Nano particles

Protein Content analysis

Leaf.

Our research indicates When exposed to NaCl stress, Tomato (Solanum lycopersicum L.) plants showed a lower amount of leaf-soluble protein compared to the control group at all observation times. On the 35th, 45th, and 55th days after sowing (DAS), the reductions were 68.13%, 71.22%, and 81.95%, respectively, compared to the control. When chitosan nanoparticles were applied to tomato (Solanum lycopersicum L.) plants that were stressed by NaCl, the amount of protein in the leaves increased by

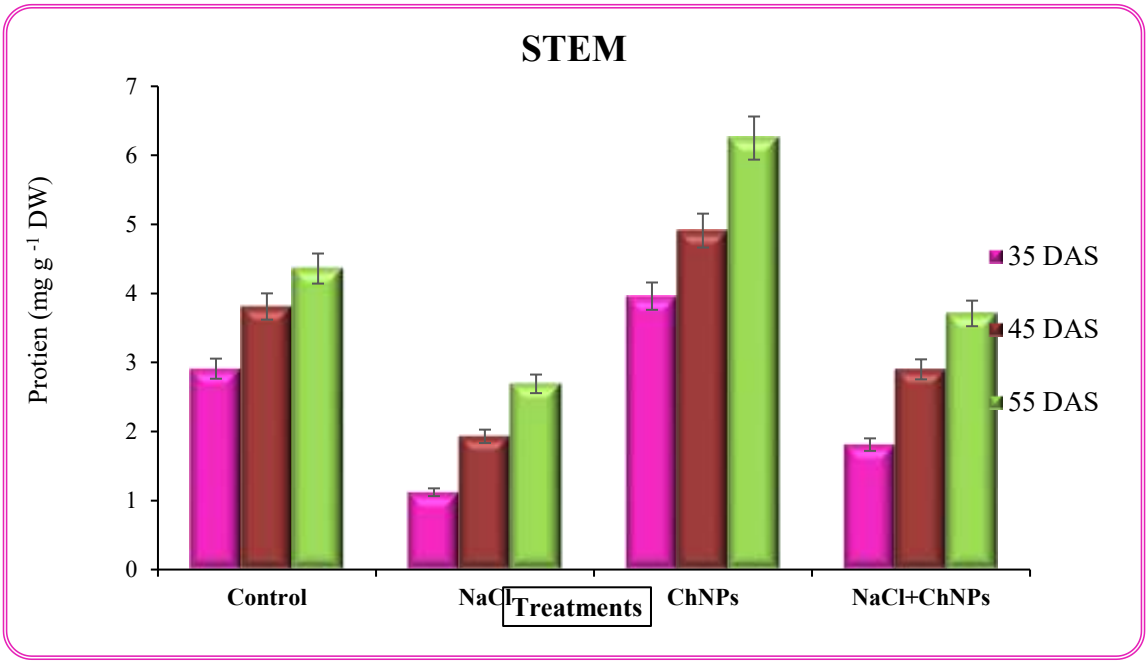


97.18% compared to plants that were stressed by NaCl but didn't receive these treatments. This effect was measured on the 55th day after sowing (DAS). Despite this improvement, the levels remained lower than those of the control group. Plants that were treated with chitosan nanoparticles and not exposed to salt stress had a protein content of 198.32, which was higher than that of the control group. Previous studies have demonstrated that salt stress diminishes the overall protein content in certain plants, such as Solanum lycopersicum L. (Sadiq et al.) and Vicia faba (Qados et al.). Phaseolus vulgaris has also been studied (Seemann et al. 24). Chitosan nanoparticles applied externally enhance protein content and mitigate salt stress in maize and Helianthus annuus (Sen, S.K., Chouhan, D). The ChNPs regulator plays a big role in helping plants endure salinity stress.

The values represent the mean ± standard error of five replicates. Mean values exhibit significant differences at P≤ 0.05 (DMRT). **NaCl** - Sodium Chloride; **ChNPs** - Chitosan Nanoparticles

Stem.

Stressed green gram plants showed decreased protein levels in their stems across the board when exposed to NaCl salt, as compared to the control group. Fifty days after planting, there was the biggest decline (DAS). When compared to the control, the protein content dropped by 37.78%, 53.67%, and 63.44% at 35-, 45-, and 55-days post-sowing, respectively. In contrast, green gram plants exposed to NaCl stress were shown to have a 98.85% increase in protein content in their stems when treated with chitosan nanoparticles (ChNPs) as opposed to when treated with simply NaCl. Day 55 after seeding (DAS) was the day this outcome was assessed. While these improvements were apparent, they were dwarfed by those of the control plants and other types that were not challenged. A larger quantity of soluble proteins was seen in the stems of stress-free plants after treatment with chitosan nanoparticles, as compared to the control group. At 55 DAS, the reported increase was 174.67% higher than the control group. Our results are consistent with previous research showing that other plants, such cowpea, may have their soluble protein concentration reduced by NaCl ((Chen, C. et al.2007). Hordeum vulgare and Catharanthus roseus are



the plants in concern. The plants in question are Catharanthus roseus (Cheruth et al.) and Hordeum vulgare. (Khosravinejad et al.2009)**.** Plant health and crude protein levels are improved by applying foliar chitosan nanoparticles (ChNPs) to Pelargonium graveolens L. at a concentration of 100 mg/L. That is, El-Mashad and colleagues. Protein content in stems is improved by foliar application of growth regulators ChNPs in this research. Researchers in the soybean species Leymus chinensis and others came to similar conclusions (AlKahtani et al.2020).

The values represent the mean ± standard error of five replicates. Mean values exhibit significant differences at P≤ 0.05 (DMRT). **NaCl** - Sodium Chloride; **ChNPs** - Chitosan Nanoparticles.

Root.

Under NaCl stress, the soluble protein level in the roots of tomato (Solanum lycopersicum L.) plants decreased on all sample days compared to the control group. The reductions were 56.87%, 65.48%, and 74.27% compared to the control on the 35th, 45th, and 55th days after sowing, respectively Chen et al. (2009),. The use of chitosan nanoparticles as a foliar therapy on plants that were stressed by NaCl led to a big rise in the amount of protein in their roots. On the 55th day after planting, the increase was 94.29% compared to the control. Even with these increases, the levels stayed below those of the control group Faizan et al. (2021) and Alenazi et al. (2024). Applying chitosan nanoparticles (ChNPs) as a foliar spray to plants without stress significantly boosted the protein content in the roots. Plant health and crude protein levels are improved by applying foliar chitosan nanoparticles (ChNPs) to Pelargonium graveolens L. at a concentration of 100 mg/L Hassan et al. (2021). Protein content in stems is improved by foliar application of growth regulators CHNPS in this research. Researchers in the soybean species Leymus chinensis and others came to similar conclusions Otie et al. (2021),

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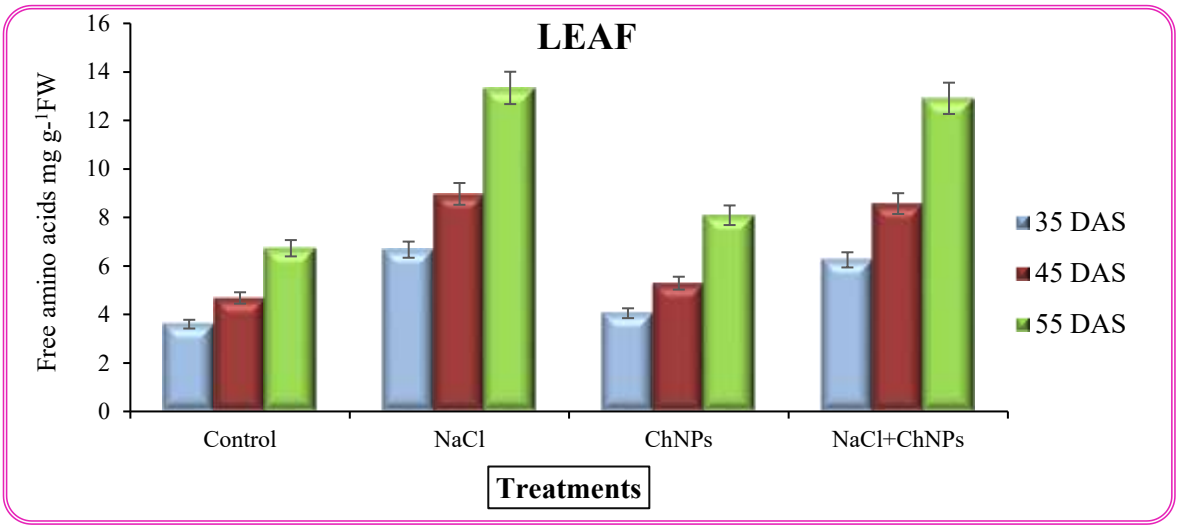
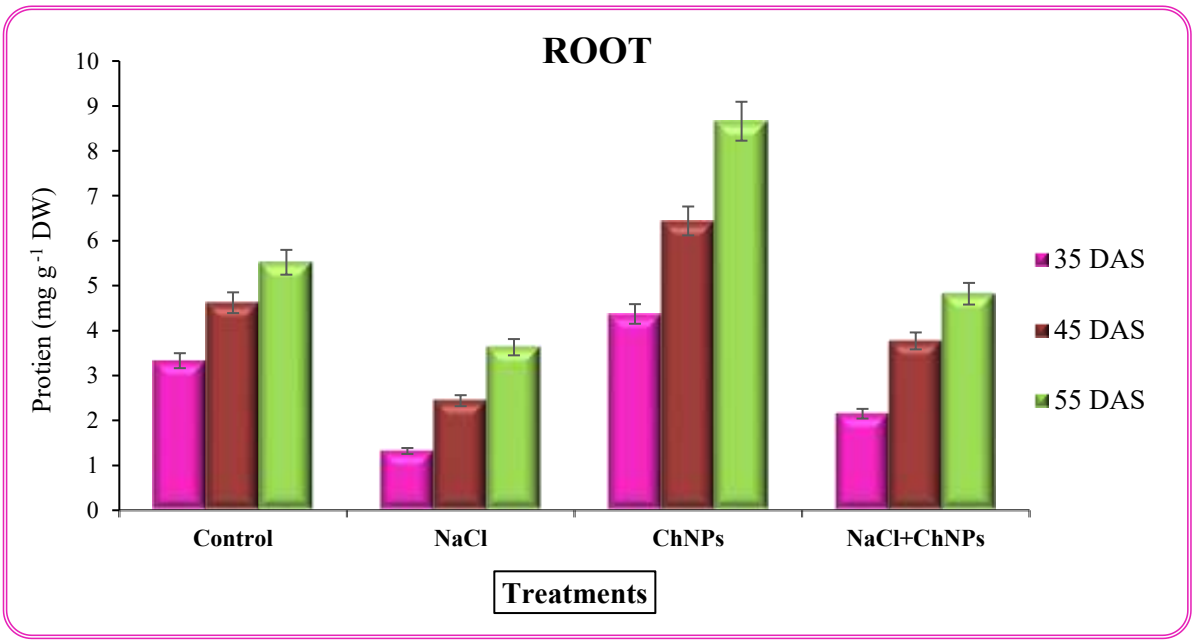


Free Amino Acid content analysis.

Leaf

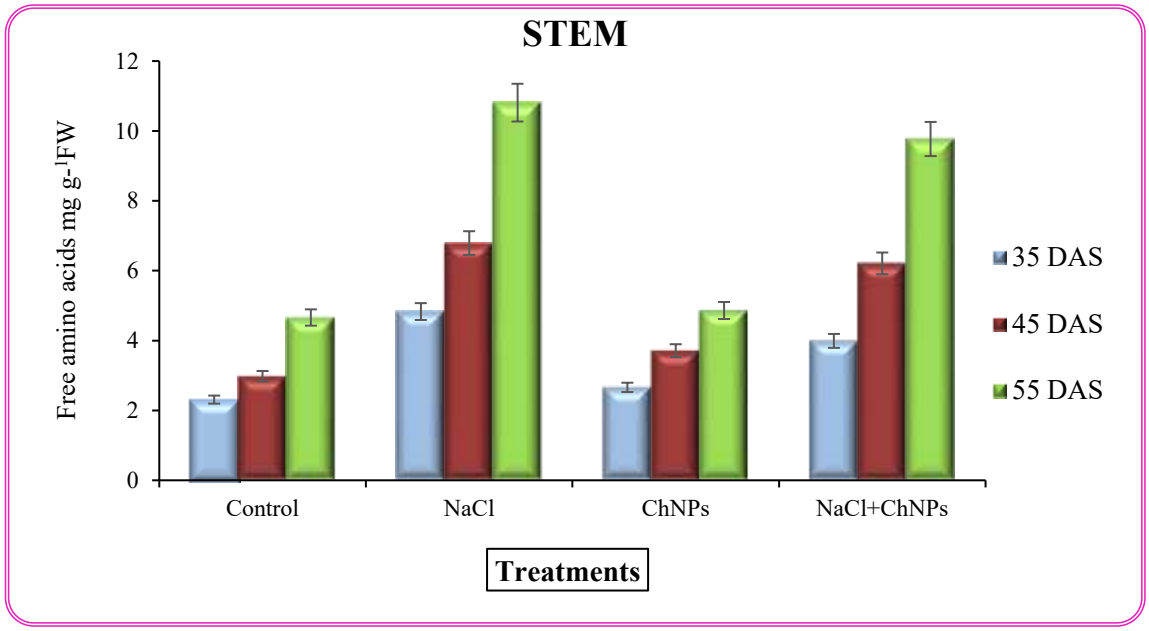
The content of free amino acids in the leaves of tomato plants subjected to NaCl salt stress was significantly higher than that of the control group. On the 35th day after sowing (DAS), this growth was 185.09%; on the 45th day after sowing (DAS), it was 196.22%; and on the 55th day after sowing (DAS), it was 213.08% Peña Calzada, K., et al. (2022). Plants exposed to salt stress and administered Chitosan Nanoparticles, in contrast to those treated with NaCl, exhibited a reduction in the amount of free amino acids in their leaves. On the 55th day after surgery (DAS), however, the decrease was even greater than in the control group, with a considerable decline of 193.80%. In addition, non-stressed green gram plants that were sprayed with chitosan nanoparticles showed a significant increase in leaf free-amino acid content compared to the control group, with a 138.29% jump on the 55th day after planting. Because their proteins degrade in salty soil, plants grow with elevated amino acid concentrations. Hussain and his colleagues carried out the research. Osman et al., Fougere et al., and Filiz et al. found that snap bean, Medicago sativa, and Solanum lycopersicum L. all showed an increase in amino acid content when subjected to salt stress Roşca, M., et al. (2023). In spite of this, chitosan nanoparticle (ChNPs) treated plants exhibited less salt stress and increased amino acid levels. Both okra plants and Vigna radiata L. (Sadiq et al 2016.) showed similar outcomes.



The values represent the mean ± standard error of five replicates. Mean values exhibit significant differences at P≤ 0.05 (DMRT). **NaCl** - Sodium Chloride; **ChNPs** - Chitosan Nanoparticles.

Stem

Our research shows that at all development stages and sampling days, the control group exhibited lower levels of free amino acids in the stems of green gram plants stressed with NaCl. At35,45, and 55 days after sowing (DAS), the increases were 228.51%, 249.36%, and 228.43%, respectively, in comparison to the control% (Peña Calzada et al., 2022). On the other hand, plants exposed to salt stress and sprayed foliar with chitosan nanoparticles (ChNPs) had a lower stem free amino acid content than plants untreated by NaCl. By the 55th day after planting (DAS), the values had surpassed the control by 218.55%. These levels were still far greater than the control groups, even after the drop. Additionally, the application of chitosan nanoparticles (ChNPs) via foliar spray on non-stressed plants led to a substantial increase in stem free amino acid content, exceeding the control group by 123.42% on the 55th day after sowing (DAS) (Roşca et al., 2023). Our experimental results demonstrated that the amino acid concentration was much higher in NaCl-treated plants compared to those treated with chitosan nanoparticles (ChNPs) under salt stress, supporting findings by several authors in sorghum bicolor ((Sadiq et al., 2016). Additionally, free amino acids function as osmoregulatory agents that protect plants from salt stress by decreasing membrane permeability and increasing membrane resilience.



The numbers are the average plus or minus the standard error of five replicates. The mean values are substantially different at P≤ 0.05 (DMRT)**. NaCl** is sodium chloride, and **ChNPs** are chitosan nanoparticles.

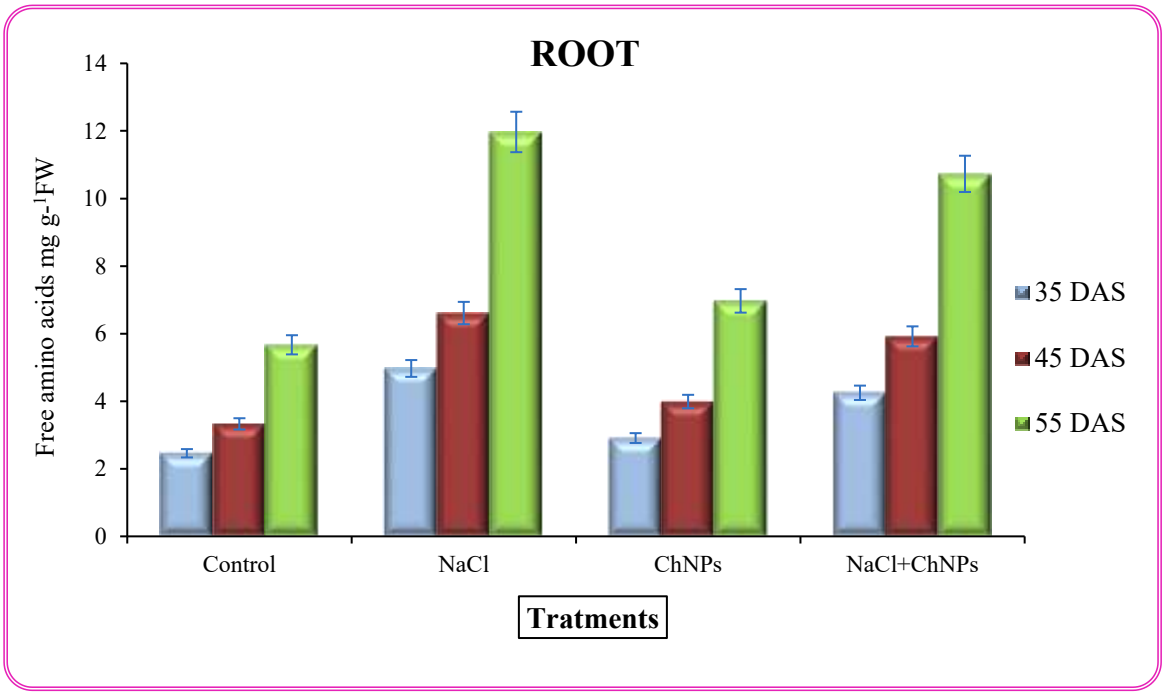
Root.

On every sample day, the experimental results revealed that the control group of tomato plants had the lowest concentration of free amino acids in their roots, whereas the NaCl stressed plants had the highest concentration. At 35 DAS, 212.04%, and 228.37%, compared to the control, respectively, the recorded percentages were 191.85%, 45 DAS, and 228.37% higher (Rosca et al., 2023). A significant decrease in the quantity of free amino acids in the roots was seen in plants that were stressed by both NaCl and chitosan nanoparticles (ChNPs) when applied as a foliar spray. Roots in the experimental group had a 194.46% greater concentration of free amino acids on the 55th day after seeding (DAS) compared to controls AlKahtani et al. Nonetheless, compared to the control group, these decreases were much larger. Root concentrations of free amino acids were significantly altered after foliar spraying non-stressed plants with chitosan nanoparticles. At 55 days post-sowing (DAS), there had been a 128.64% increase in comparison to the control group. When plants were subjected to salt treatment alone, their root amino acid content was greater than when they were treated with chitosan nanoparticles (ChNPs). Phragmites australis has also been the subject of comparable results from other studies Xie et al.2020). Glycine max and rice have both shown comparable results in research.

The values are the mean ± SE of five replicates. Mean values differ significantly at P≤ 0.05 (DMRT)**.** **NaCl**-Sodium Chloride**; ChNPs**- Chitosan Nanoparticles.

2.1 CONCLUSION

Fifth, salinity is a big problem for farmers since it lowers agricultural output and soil health. Plant growth and harvest are negatively impacted by ion toxicity and osmotic stress, which may result from salt buildup in water and soil. When plants are subjected to salt stress, their protein and amino acid levels fall. Protein deficiency retards plant development, while shifts in amino acid concentrations indicate stress. Plants that are stressed by salt show a big rise in protein and amino acid levels when they are treated with foliar applications of chitosan nanoparticles (ChNPs). The plants react well to the outside use of ChNPs.



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