Chitosan Nanoparticles as Combat Salinity Stress to Improve Biochemical Characteristics and Seedling Vigor in Maize (*Zea maize* L.)

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ABSTRACT:

Salt stress is classified as an abiotic stressor and is recognised as a significant contributor to the decline in agricultural output. Nanoparticles have emerged as a prominent material in contemporary agricultural research due to their distinctive physicochemical characteristics. The purpose of this research was to examine the impact of (ChNPs) on the morphological features and biochemical properties of *Zea maize* L. seedlings (Giza168) under salt stress using various concentrations of NaCl (0, 100, 200, 300, and 350 mM). The result showed that the proportion of seeds that germinated, the length of the shoots, the dry weight of the seedlings, and the amount of chlorophyll they contained all decreased significantly when increasing the concentration of NaCl. Seeds treated with ChNPs before planting had a higher germination rate, heavier seedlings, longer shoots, and more chlorophyll than those planted with untreated seeds. The activities of catalase (CAT), and peroxidase (POX) enzymes increased due to salinity stress, but the highest values of CAT were observed with ChNPs at 300 mM NaCl. Whereas the highest value of POX was found with ChNPs at 350 mM NaCl. Increasing activities of antioxidant enzymes may lead to an increase in tolerance to salinity stress. Furthermore, pre-treatment with ChNPs, led to the appearance of new protein bands others disappeared with different molecular weights under salinity in all seedlings. Finally, it could be concluded that pre-treatment of seeds with 0.3% ChNPs as soaking markedly reduced the harmful effects of salinity stress and also improved all the measured parameters.

Keywords: *Zea maize* L**;** salinity stress; chitosan nanoparticles; proline; antioxidant enzymes; protein electrophoresis.

INTRODUCTION

Nanotechnology represents a burgeoning field within the realm of bioengineering, capitalising on the distinctive attributes of nanoparticles with diameters below 100 nm. These particles exhibit exceptional properties that make them highly effective carriers for delivering diverse drugs and compounds. Notably, nanotechnology has been harnessed in an innovative approach to enhance plants' tolerance to elevated salinity levels. Salem (2023).

Nanoparticles in Sustainable Agriculture: New Opportunities Traditional agriculture often relies on large amounts of chemical fertilizers and pesticides, which negatively impact the environment Organisms and Ecosystems (Sharma et al., 2023). Nanoparticles increased growth, antioxidant status, and secondary metabolite synthesis in salt-stressed medically essential plants (Samynathan et al., 2023). Chitosan nanoparticles (ChNPs), a crustacean shell derivative, has been shown to safely encapsulate and sequester bioactive chemicals in several investigations. ChNPs carry slow-release and adsorption fertilisers, insecticides, herbicides, and plant growth regulators. Encapsulating and delivering bioactive chemicals in ChNPs protects plant cells against burst release (Hoang et al., 2022). *Zea maize* L**.**, a globally cultivated crop, has substantial importance as a staple food source and forage. Additionally, it is recognised as a salt-sensitive plant species. Egypt also cultivates this crop extensively, making it a key agricultural commodity in the region (Farooq et al., 2015). According to Soto and Pérez (2022), maize has ranked as the third most significant crop globally, behind wheat and rice. Furthermore, it is cultivated in a greater number of nations than any other crop. In addition to its use as a food source, maize serves as a fundamental raw material for several industrial applications.

In developed nations, there is a higher percentage of grain allocation towards animal feed and as an industrial resource for both food and non-food applications (Saeed et al., 2021). Maize production is impeded by several stressors, such as drought, floods, and heavy

metals, among others. However, it is worth noting that salinity stress is directly or indirectly associated with all of these stressors. According to Kumar et al., (2019), an elevated concentration of salt has been shown to result in a significant decrease in the germination rate of seeds and a delay in the growth of maize seedlings.

The development and production of agricultural crops are confronted by climate change as well as other biotic and abiotic pressures. Salinity is well recognised as a prominent abiotic stressor that significantly hampers the development and productivity of agricultural crops worldwide, particularly in arid and semiarid environments (Kaashyap et al., 2018). Salinity is a significant challenge that significantly impacts the development and physiological characteristics of crops, leading to a reduction in agricultural yield. According to the Food and Agriculture Organisation (FAO, 2009), salinity has already impacted around 6% of the global land surface and 20% of agricultural fields.

According to Zia et al., (2022), maize is among the grain species that exhibit sensitivity to salt stress. Maize is often regarded as a crop that exhibits sensitivity to salt. Salt stress has been found to have negative impacts on various aspects of plant growth and development. These include morphological performance, biochemical changes, and physiological mechanisms, all of which result in a decrease in seed germination (Rabie and Almadini, 2005; El-Khamissi and Ghaly, 2021), as well as reductions in fresh and dry biomass (Kamran et al., 2019) and photosynthesis (Naveed et al., 2020). The loss in productivity of Maize plants worldwide may be attributed to the significant issue of salinity stress (Sabagh et al., 2021). The degradation of chlorophyll is seen in salt stress conditions, which may be attributed to the heightened toxicity resulting from the presence of sodium chloride (Yang et al., 2011).

Osmotic stress mostly arises from low or moderate salt levels, which subsequently impact the plant's photosynthetic activity, leading to diminished growth and production (Masarmi et al., 2023). Plants use many enzymes, including peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD), to serve as antioxidants for the purpose of scavenging reactive oxygen species (ROS) (Sachdev et al., 2023). Previous research has shown that plants with a high tolerance to salt exhibit an increase in their antioxidant content as a response to salinity stress, while salt sensitive plants experience a decrease in their antioxidant levels (Ashraf and Harris, 2004; Ehab et al., 2011). Proline is a significant molecule that plays a crucial role in cellular protection via its involvement in protein stabilisation and the maintenance of cellular membranes.

The accumulation of proline has a significant role in enhancing salinity tolerance in plants and mitigating the negative effects of salt sensitivity in some plant species (Panuccio et al., 2022). El-hamamsy and El-Khamissi (2018) have suggested that the protein electrophoresis technique, namely SDS-PAGE, may be used to demonstrate the changes in protein composition during the developmental stages of plants subjected to salt stress.

In this regard, the objective of this study is to evaluate the impact of chitosan nanoparticles on several morphological characteristics and biochemical properties of maize. seedlings subjected to salt stress induced by NaCl.

MATERIALS AND METHODS

Plant material and treatments:

This study was conducted between laboratories of Biochemistry Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, and Plant Pathology Research Institute, Agricultural Research Center (ARC), Cairo, Egypt. The seeds of Maize (Zea maize L.) Giza168 were used in this study. Maize seeds were obtained from Field Crops Research Institute (FCRI) of the Agriculture Research Center (ARC), Ministry of Agriculture, Giza, Egypt. Homogeneous seeds were sterilized using 70% ethanol for 2 min. followed by 0.2% sodium hypochlorite (NaOCl) for 3 min. then rinsed for 3 times with distilled H_2O_2.

Synthesis of chitosan nanoparticles:

Chitosan nanoparticles were prepared using the ionic gelation method; approximately 2% of the polymer was dissolved in a 1.0% (v/v) acetic acid solution. A sodium TPP solution was also prepared in distilled water at a concentration of 5 mg/mL. The sodium TPP solution was added dropwise using a burette to the chitosan solution while stirring, followed by sonication for 20 min. The resulting suspension was subsequently centrifuged at 15,000 rpm for 10 min and then

dried at room temperature approximately 28°C (Ghadi *et al*., 2014).

Characterization of nanoparticles:

Transmission electron microscope (TEM):

The morphological and particles size of chitosan nanoparticles were demonstrated by using TEM model JEM-1230, Japan, operated at 120 kV, with maximum magnification of 600 × 10^3 and a resolution until 0.2 nm. A drop of an aqueous dispersion of the nanomaterial was placed on a carbon-coated copper grid and allowed to dry in air before characterization.

Dynamic Light Scattering (DLS):

Particle size and zeta potential were measured using a Zetasizer Nano-ZS-90 (Malvern Instruments, UK).

Application of chitosan nanoparticles in vitro:

Preparation of Zea maize L. Samples:

After washing the seeds of maize, they were soaked for 12 h at room temperature in distilled water or 0.3% each chitosan nanoparticles before sowing in NaCl solutions.

Germination and growth conditions:

Germination trials were carried out in 15 cm Petri dishes containing a layer of two filter paper whatman's two filter paper sterilized with distilled water or saline solution of sodium chloride (NaCl) treatment. Four salt stresses (0, 100, 200, 300, 350 mM Nacl) on Maize seeds. Three replications were sown in Petri dishes on two filter paper beds and each treatment contained 10 pure seeds, thenirrigated with 10 ml solution of respective treatment and incubated in growth chamber at 20±2 °C for 21 days.

Seed germination was observed daily with fresh salt solution added to the Petri dishes as necessary to maintain moisture levels. Germination percentage was calculated using the formula outlined by Krishnasamy and Seshu (1990). Seedling shoots and roots lengths of ten randomly selected seedlings were measured after 21 days of germination (ISTA, 1999). The dry weights of that seedling were determined after drying to aconstant weight in a hot air oven at 85° C for 12 h (Krishnasamy and Seshu, 1990).

Biochemical analysis:

Determination of chlorophyll:

The chlorophyll content of the seedlings was measured using the spectrophotometric method described by (Lichtentaler and Wellburn, 1985). Total chlorophyll was calculated using the formula:

$$\text{Chlorophyll}\left(\frac{\mu g}{mL}\right) = 25.8 \times \text{A650} + 4.0 \times \text{A665}$$

and then converted to mg $\frac{\text{chlorophyll}}{g}$ plant tissue.

Extraction of antioxidant enzymes and Enzymes activity assay:

Enzymes were extracted from 0.5 g leaf samples homogenized in a pre-chilled pestle and mortar containing ice cold 0.1 M phosphate buffer (pH 7.5) and 0.5 mM EDTA. Each homogenate was transferred to centrifuge tubes and centrifuged at 4°C in a Sorval model T21 (Thermo Scientific, Waltham, MA) refrigerated centrifuge for 15 min at 15000 x g. The supernatant was decanted and used for measuring enzyme activity assays (Esfandiari et al., 2007).

Catalase activity:

Catalase activity was determined according to the method used by Aebi (1984) in which the disappearance of H_2O_2 in a reaction mixture containing 0.3 mL 3% H_2O_2, 2.5 mL of 0.05 M phosphate buffer (pH 7), and 2.5 mL of plant extract is monitored by the decrease in absorbance at 240 nm.

Peroxidase activity:

Peroxidase was assayed spectrophotochemically according to (Amako et al., 1994) the assay was carried out at 25 °C in 1.0 cm light path cuvette and the reaction mixture consisted of 1500 µL phosphate buffer, 1000 µL pyrogallol and 480 µL H_2O_2 solution. After mixing, the reaction was initiated by adding the enzyme extract (20 µL) and the increase in optical density at 430 nm against blank (without extract) was continuously recorded every minute (for 1 min).

Determination of proline:

Proline content of shoot was determined according to a modification of the method of Bates *et al* (1973). The content of proline was calculated from a standard curve and calculated on a fresh weight basis as follows:

$$\text{The content of proline} = \{((\mu g \text{ proline/ml} \times \text{ml toluene}))/\ 115.5\ \mu g/(\mu mole)\}/\ \{(g \text{ sample})/5\}$$

, where the content of proline ware measure as μmoles proline/g of fresh weight material.

The SDS-protein electrophoresis:

Protein extracts of seeds of various genotypes were identified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970).

Statistical analysis:

The statistical differences among means were conducted in triplicates. SPSS software (V 24.0, SPSS IBM Inc. Chicago, IL, USA) was used to analyze the data. Duncan's test (at 5% level of probability, $p^* < 0.05$) was used to evaluate the statistical differences among means.

RESULTS AND DISCUSSION

Synthesis and Characterization of ChNPs:

In this study, we successfully synthesized a high yield of Chitosan Nanoparticles by using the ionic gelation method.

Transmission electron microscope (TEM):

The surface morphological structure was further investigated using TEM examination. The TEM images of ChNPs are shown in Figure 1, with a magnification scale decreased to 16.3 nm. The morphological structure of ChNPs was shown to possess a significant particle size, exhibiting various shapes. The particles were found to be in an agglomerated state, characterised by non-aggregation. This observation was then contrasted to the results acquired using Dynamic Light Scattering (DLS).

Dynamic light scattering (DLS:)

DLS was used to quantify the particle size (PS), polydispersity index (PDI), and zeta potential (ζ-potential) of ChNPs. The mean values obtained for all the systems exhibited a probability density function in nanometers, as seen in Figure 1. The ChNPs exhibited a mean size of about 70 nm with a polydispersity index (PDI) of 0.4, as seen in Figure 2A. Moreover, a ζ-potential value over 48 mV is regarded as stable owing to the maintenance of electrostatic equilibrium.

The increased positive ζ-potential charge observed in ChNPs can be attributed to the ionisation of the amino functional groups ($-NH_2$) present in the capping moieties under acidic pH conditions (Alshallash *et al.,* 2022). This high positive charge creates a repulsive barrier, which effectively prevents aggregation and enhances the colloidal stability of chitosan NPs, as shown in Figure 2B.

The effect of ChNPs on germination and growth parameters of maize seed under salinity:

The effect of ChNPs on germination and growth parameters of maize seedlings under salinity is presented in Table (1). Salinity stress by NaCl caused a significant reduction in germination percentage, shoot length, Seedling dry weight compared to control. Maximum reduction was observed in Giza168 (63.67 %) at 350 mM NaCl compared to control (97%) at 0 mM NaCl. Pretreatment of seeds with ChNPs as soaking caused significant increase in germination percentage in all.

Application of 0.3% of ChNPs caused an increase of germination percentage to 99.73% under normal conditions. In addition to the significant increase in other levels of salt stress. Germination percentage increased from (63.67%) at 350 mM NaCl to (90.67 %) with 0.3% of ChNPs at the same level of salt stress. On the other hand, the shoot and root length and seedling dry weight of maize were reduced by salt stress. The results in Table (1) it is observed that shoot length significantly decreased to (4.30 cm) at 350 mM NaCI compared to control. Root length significantly decreased with the increase of salinity stress in all maize. The lowest value of root length was observed (2.67 cm) under 350 mM NaCl.

Salinity also decreased seedling dry weight. ChNPs application led to significant increase in shoot, root length and seedling dry weight under salinity stress. The shoot length significantly increased under normal conditions and all level of salinity with 0.3% of ChNPs. Shoot length increased from (17.10 to 18.33 cm) under control and from (4.30 to 8.67 cm) at 350 mM NaCl with application of ChNPs. Also, root length significantly increased with application of 0.3% of ChNPs. Root length increased at 350 mM NaCI from (2.67 to 7.57 cm) with 0.3% of ChNPs.

Application of ChNPs as seed soaking led to significant increase in seedling dry weight under saline and no saline conditions. Seedling dry weight increased from 0.148 to 0.164 (g) with

ChNPs under control and increased from 0.0490 to 0.085 g at 350 mM NaCI with 0.3% of ChNPs (Table 1).

Based on the findings presented in Table 1, it is evident that the imposition of salt stress has a detrimental effect on the plant development characteristics of Giza 168. Nevertheless, the administration of exogenous ChNPs at a concentration of 0.3% resulted in significant improvements in all growth indices. There is a suggestion that the stimulation of growth by ChNPs may have a positive effect on crop yield.

Salt stress has been observed to impede plant growth by negatively impacting a range of physiological and biochemical mechanisms, such as photosynthesis, antioxidant capacity, and ion homeostasis. Additionally, this stressor induces an elevation in reactive oxygen species (ROS) levels, thereby causing oxidative stress. Consequently, plants experience detrimental effects at both the cellular and metabolic levels. The presence of such imbalances has a negative impact on several physiological and biochemical systems that are associated with the growth and development of plants (Azeem *et al.,* 2023).

Therefore, it is postulated that the observed promotion of plant development under salt stress conditions by ChNPs may be attributed to, therefore, it is postulated that the observed promotion of plant growth under salt stress conditions by ChNPs may be attributed to alterations generated by ChNPs in these biochemical or physiological mechanisms.

The alterations in certain biochemical or physiological processes that are brought about by a particular stimulus. The findings are consistent with the study conducted by Attaran *et al.,* (2022), which showed that the external administration of ChNPs mitigated the detrimental impacts of salt-induced stress on plant development. In a study conducted by Shams and Farzami (2018), it was evident that the use of ChNPs resulted in a significant enhancement in the biological yield under stressful conditions.

The effect of ChNPs on photosynthetic pigments in shoots of maize under salinity:

The findings illustrated in Table 2 demonstrate a negative correlation between salinity levels and the concentrations of Chlorophyll a, Chlorophyll b, and carotenoids. Maize plants exhibited the greatest concentrations of chlorophyll a, chlorophyll b, and carotenoids under normal conditions, but the lowest concentrations of these pigments were detected with a salt level of 350 mM NaCl. The immersion of seeds with ChNPs resulted in a mitigation of the detrimental impact of salt on the levels of chlorophyll a, chlorophyll b, and carotenoids in the shoots of maize plants.

The utilisation of 0.3% ChNPs resulted in a significant enhancement in the levels of chlorophyll a, b, and carotenoids. Specifically, the concentrations rose from 0.778, 0.706, and 0.112 mg/g to 1.058, 1.010, and 0.131 mg/g, respectively, when exposed to 350 mM NaCl, maintaining the same degree of salt stress. The findings demonstrate that the soaking of seeds with a solution containing 0.3% ChNPs effectively mitigated the detrimental effects of salt stress on the levels of chlorophyll a, chlorophyll b, and carotenoids.

According to a study conducted by Zangani et al., (2023), it has been shown that exposure to NaCl stress leads to an elevation in the activity of the enzyme cholorophyllase, which is responsible for the degradation of chlorophyll. This increase in enzyme activity subsequently results in the destabilisation of pigment protein complexes, as described by Stefanov et al., (2023). Furthermore, the overall consequence of this phenomenon is a decrease in the total content of chlorophyll. In a study conducted by Farooq et al., (2023), it was observed that the application of salt treatment resulted in a considerable decrease in the levels of leaf chlorophyll and carotenoids in plants.

The application of ChNPs to plants subjected to salt stress, as shown by Ingle et al., (2022), was seen to effectively reinstate chlorophyll levels to their normal state. The findings align with the results published by Mitra et al., (2023) and Balusamy et al., (2022), which indicate that the introduction of chitosan nanoparticles at a low concentration may boost the photosynthetic rate by enhancing the levels of leaf Chl a, b, and carotenoids.

Furthermore, Soni et al., (2023) reported a significant augmentation in chlorophyll levels in both saline and non-saline circumstances subsequent to the administration of ChNPs.

The impact of ChNPs on proline in shoots of maize under salinity:

The amounts of proline in plants under stress may contribute to the preservation of membrane integrity, mitigation of lipid membrane oxidation, safeguarding and stabilisation of enzymes involved in ROS scavenging, as well as involvement in the stabilisation of subcellular structures, scavenging of free radicals, and buffering of redox potential. Maize plants subjected to saline conditions exhibited a significant elevation in overall proline content in comparison to plants cultivated under normal conditions.

The salinity stress enhancement leads to a steady rise in the proline concentration in maize shoots. The shoots exhibited the maximum proline value of 24.25 millimoles per gramme at a NaCl concentration of 350 millimolar and a ChNPs concentration of 0.3%. The minimum recorded value was 8.25 millimoles per gramme in the absence of 0.3% ChNPs. The use of nanoparticles often leads to an augmented buildup of free amino acids, such as proline, in plants under stress. The treatment of ChNPs resulted in a considerable increase in proline content, both in the presence and absence of saline conditions, as shown in Table 3.

The adaptive response of plants to salt stress involves the buildup of proline in plant tissue. However, there is conflicting evidence about the involvement of proline in enhancing the stress tolerance of plants, as shown by Luo *et al.*, (2018). The application of ChNPs therapy led to a notable increase in the accumulation of free amino acids, namely proline, in plants subjected to stress. Under conditions of salt stress, maize seedlings exhibited a substantial increase in proline accumulation.

This rise was further enhanced when subjected to ChNPs treatment, resulting in a larger accumulation of free amino acids, including proline, in the stressed plants. According to Hidangmayum *et al.*, (2019), maize seedlings exhibited a significant increase in proline accumulation when subjected to salt stress. Furthermore, the application of ChNPs from an external source resulted in a further enhancement of proline accumulation. This, in turn, contributed to the mitigation of the detrimental impacts caused by salt stress.

This finding is consistent with the study conducted by Attia *et al.*, (2021), which demonstrated that the application of ChNPs resulted in an increase in proline accumulation in maize seedlings under normal or NaCl-induced stress conditions. This exogenous application of chitosan nanoparticles effectively mitigated the adverse impacts of salt, as reported by Nkuna (2022). The findings of this study are consistent with the research conducted by Abdelhamid *et al.*, (2019), which shown that the application of chitosan nanoparticles enhanced the accumulation of proline in maize seedlings subjected to NaCl-induced stress.

The impact of ChNPs on enzymes activity in shoots of maize under salinity:

Figure 3A showed that the impact of NaCl salinity and ChNPs on enzyme activity. The level of catalase (CAT) activity shown a positive correlation with the rise in salt levels. In maize, the maximum observed value of CAT activity was 35.38 U/mg fresh weight (F.W) when exposed to 350 mM NaCl, while the minimum value recorded was 32.99 U/mg F.W under standard circumstances. The use of ChNPs at a concentration of 0.3% for seed soaking resulted in a substantial enhancement in CAT activity. Under normal conditions, the activity of CAT exhibited a notable rise from 32.99 to 38.71 U/mg F.W when subjected to a concentration of 0.3% of ChNPs.

Furthermore, Figure 3B showed that a positive correlation between salt stress levels and the observed increase in peroxidase (POX) activity. The POX activity exhibited its greatest value of 31.59 U/mg in circumstances of salinity, specifically at a concentration of 300 mM NaCl. Conversely, the lowest reported value of 29.88 U/mg was recorded under normal conditions. The use of ChNPs as a pretreatment for seeds resulted in a notable enhancement in POX activity. The activity of POX exhibited a notable rise in the shoots, rising from 31.50 to 39.82 U/mg fresh weight (F.W) when exposed to a concentration of 350 mM NaCl together with 0.3% ChNPs.

Indeed, when administered exogenously at appropriate doses, ChNPs were shown to augment the efficacy of the antioxidant system in plants (Kaur et al., 2021). According to Divya et al., (2018), the enzyme activity was seen to decrease when subjected to treatment with greater doses of ChNPs. The findings presented

here align with those reported by Kumar et al., (2017).

The discovery of the enhancement of catalase activity under the salinity of Zea mays by the use of chitosan nanoparticles was attributed to a specific researcher or research team. In a recent study conducted by Balusamy et al., (2022), it was shown that the utilisation of ChNPs resulted in the augmentation of antioxidant enzyme activities, namely catalase (CAT) and peroxidase (POX), when administered exogenously to salinity-stressed zea mayze plants. In a study conducted by Pramanik et al., (2023), it was shown that the inhibition of peroxidase by the use of larger concentrations of ChNPs hinders the degradation route of H_2O_2 inside plant cells.

This inhibition subsequently results in elevated amounts of endogenous H_2O_2. Emerging research suggests that hydrogen peroxide (H_2O_2) has the capacity to facilitate the advancement of the cell cycle via the oxidation of certain thiol proteins.

The impact of salinity on protein banding pattern extracted from the shoots of maize using SDS-PAGE technique:

Table 4 shows protein patterns for shoot of maize after exposure to salinity levels for 21 days at the germination stage. The electrophoretic patterns have a total of 17 protein bands. Molecular weights were detected from 209.16 to 16.84 KDa. Electrophoretic analysis of protein patterns of maize showed that the polypeptides with molecular weights of 209.16, 169.91, 123.36, 97.63, 69.88, 40.27 and 23.37 KDa were the most prominent in the control. The effect of the applied salinity treatments resulted in the induction of new bands with molecular weights of 191.72, 81.66 and 32.47 KDa in all treatments with NaCl and 141.52 KDa at 300 and 350 mM. Also, protein bands are weights of 169.91, 69.88 and 40.27 kDa disappeared at 350 mM NaCl salinity. The results presented in Table (4) clearly show that, at the highest salinity level (350mM NaCl) gave of the number bands (9 bands). Some protein bands with molecular weights of 209.16, 123.36, and 97.63 KDa were observed under saline treatments and non-saline conditions. NaCl treatments enhanced protein synthesis to counteract the effects of salinity.

It is plausible to propose that these novel proteins may have a significant influence on the activation of a specialised mechanism that enhances the overall plant's ability to withstand NaCl-induced stress. According to the findings of Budran et al., (2023), it was seen that the presence of salinity led to the disappearance of some protein bands. This observation was made based on the conclusion that the disappearance of polypeptides under salt stress serves as a compensatory mechanism for the production of other proteins.

The difference in molecular weights of such distinct protein bands in shoots might reflect the difference in gene expression. In addition, they created distinct protein bands within the tissues, to a great extent, for the tolerance or the susceptibility of such maize to salinity stress. These results are in harmony with those obtained by Warsame et al., (2022).

The impact of ChNPs on protein banding pattern extracted from the shoots of maize using SDS-PAGE technique:

The results of SDS - PAGE electrophoretic patterns of proteins extracted from the shoots of maize after treatment of seeds with 0.3% chitosan nanoparticles and germinated under salinity conditions are shown in (Table 5). The electrophoretic patterns gave a total of 21 protein bands. Molecular weights were detected from 241 to 14.28 KDa. There is a clear variation in the number of bands at 0, 100, 200, 300, and 350 mM NaCl between mazie ranging from 12 in control to 11 bands at 100 mM, 12 bands at 200 mM, 13 bands at 300 mM, and 15 bands at 350 mM.

The effect of the application of 0.3% chitosan nanoparticles resulted in the induction of new bands with molecular weights of 241, 225.51, 15.19, and 14.28 KDa in maize (Table 6) which was not present in treatments without chitosan nanoparticles (Table 5). Electrophoretic analysis of protein patterns of maize showed that the polypeptides (12 bands) with molecular weights of 241.00, 225.51, 209.16, 183.63, 169.91, 123.36, 118.13, 97.63, 69.88, 40.27, 21, and 15.19 KDa were the most prominent in the control (0 salinity / 0.3% chitosan nanoparticles).

It is obvious that treatment with (350 mM NaCl / 0.3% chitosan nanoparticles) stimulated the appearance of 7 protein bands with molecular of 191.72, 155.56, 73.81, 53.12, 32.47, 16.84 and 14.28 KDa, and the disappearance of 4 protein bands with molecular weights of 225.51, 123.36, 97.63, and 21 KDa as compared with

control. The results presented in Table (5) clearly show that, at the highest salinity level (350mM NaCl/ 0.3% chitosan nanoparticles) maize gave the highest number of bands (15 bands) as compared with control (12 bands).

The analysis of maize shoot protein profile reveals that ChNPs have the potential to modulate the expression of proteins that are induced by salt stress. Additionally, they may stimulate the production of particular polypeptides, which are expected to contribute significantly to salt resistance mechanisms. The development of alterations in protein patterns has been related to the participation of growth regulators. This may be explained by their function in governing cell division in the apical meristems via the regulation of certain genes, such as proliferate or cyclins (Takeuchi *et al.,* 1995).

According to Brabazon *et al.,* (2017), proteins that are down-regulated are often responsible for regulating cell division and proliferation, whereas proteins that are up-regulated are typically associated with antioxidant activity and stress tolerance. The potential of nanoparticles to attach to proteins in their natural or denatured form is influenced by several factors, including the protein surface charge, hydrophobicity, intrinsic stability, and physical features of the nanoparticles (Cabaleiro *et al.,* 2010).

Numerous investigations have shown that the presence of nanoparticles may impede protein synthesis via two mechanisms: the promotion of protein aggregation and the interference with protein assembly during the folding process (Salahuddin *et al.,* 2021). This effect is more pronounced when nanoparticles are administered at elevated concentrations (Sauvage *et al.,* 2020). One of the hypothesised processes is that ChNPs has the ability to engage with histone protein, hence potentially influencing the differential expression of several cellular proteins (Bouyahya *et al.,* 2022).

Furthermore, it has been shown that chitosan has the ability to form protein-chitosan conjugates, as highlighted in the study conducted by Stasińska and Hawrylak (2022). The findings presented here align with the results reported by Mahdi *et al.,* (2022).

CONCLUSION

In summary, the findings of this research indicate that exposure to salt stress induced by NaCl resulted in a decrease in germination percentages, seedling development, and biochemical characteristics. The application of chitosan nanoparticles at a concentration of 0.3% on seeds, followed by a soaking period of 12 hours before to exposure to salt stress, resulted in a significant mitigation of the adverse impacts caused by salinity stress.

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Table 1: The effect of salinity NaCl and ChNPs on germination and growth parameters of maize

Salinity mM	Germination and growth parameters							
	Germination %		Shoot length (cm)		Root length (cm)		Dry weight (g)	
	Control	ChNPs 0.3%	Control	ChNPs 0.3%	Control	ChNPs 0.3%	Control	ChNPs 0.3%
0	97.00	99.73	17.10	18.33	15.23	16.70	0.1483	0.164
100	91.00	97.33	14.27	15.03	12.23	15.93	0.0953	0.102
200	82.33	96.33	11.43	12.37	9.27	14.43	0.0883	0.096
300	72.67	93.33	7.73	9.70	6.17	9.53	0.0683	0.092
350	63.67	90.67	4.30	8.67	2.67	7.57	0.0490	0.085
L.S.D 5%	1.86		0.61		0.83		0.07	

Table 2: The effect of ChNPs on photosynthetic pigments in shoots of maize under salinity

Salinity mM	Photosynthetic pigments									
	Chl a (mg/g F. Wt)		Chl b (mg/g F.Wt)		Chl (a+b)		Chl a/b		Total carotene (mg/g F.Wt)	
	Control	ChNPs 0.3%	Control	ChNPs 0.3%	Control	ChNPs 0.3%	Control	ChNPs 0.3%	Control	ChNPs 0.3%
0	1.510	1.581	1.366	1.396	2.875	2.977	1.105	1.132	0.308	0.317
100	1.358	1.414	1.057	1.167	2.415	2.581	1.285	1.212	0.208	0.219
200	1.086	1.258	0.937	1.120	2.024	2.377	1.159	1.123	0.171	0.191
300	0.978	1.207	0.884	1.092	1.862	2.299	1.106	1.106	0.127	0.137
350	0.778	1.058	0.706	1.010	1.484	2.068	1.101	1.047	0.112	0.131
L.S.D 5%	0.36		0.04		0.07		0.03		0.006	

Table 3: The impact of ChNPs on proline in shoots of maize under salinity.

Salinity mM	Proline (µmoles/g F.W)	
	Control	ChNPs 0.3%
0	8.25	18.19
100	14.45	20.08
200	16.48	21.51
300	17.99	22.20
350	19.93	24.25
L.S.D 0.05	0.97	

Table 4: The impact of salinity on protein banding pattern extracted from the shoots of maize using SDS-PAGE technique

Hybrid Molecular Weight			Maize 168 SC				
No .of bands	MW	RF	0 mM	100 mM	200 mM	300 mM	350 mM
1	209.16	0.094	+	+	+	+	+
2	191.72	0.187	-	+	+	+	+
3	183.63	0.189	-	-	-	+	-
4	169.91	0.192	+	+	+	-	-
5	155.56	0.195	-	-	+	-	-
6	141.52	0.198	-	-	-	+	+
7	123.36	0.201	+	+	+	+	+
8	118.13	0.217	-	-	-	+	-
9	97.63	0.236	+	+	+	+	+
10	81.66	0.259	-	+	+	+	+
11	73.81	0.293	-	+	+	-	-
12	69.88	0.357	+	+	-	-	-
13	53.12	0.458	-	+	+	-	+
14	40.27	0.491	+	-	-	-	-
15	32.47	0.579	-	+	+	+	+
16	23.37	0.658	+	+	-	-	+
17	16.84	0.731	-	-	-	+	-
Total number of bands			7	11	10	10	9

Table 5: The impact of ChNPs on protein banding pattern extracted from the shoots of maize using SDS-PAGE technique

Hybrid Molecular Weight			Maize 168 SC				
No .of bands	MW	RF	0 mM	100 mM	200 mM	300 mM	350 mM
1	241.00	0.068	+	+	+	+	+
2	225.51	0.077	+	-	+	+	-
3	209.16	0.094	+	+	-	+	+
4	191.72	0.187	-	-	+	+	+
5	183.63	0.189	+	-	+	+	+
6	169.91	0.192	+	+	-	-	+
7	155.56	0.195	-	-	+	-	+
8	141.52	0.198	-	-	-	+	-
9	123.36	0.201	+	+	+	+	-
10	118.13	0.217	+	-	-	+	+
11	97.63	0.236	+	+	+	-	-
12	73.81	0.293	-	+	+	+	+
13	69.88	0.357	+	+	-	+	+
14	53.12	0.458	-	+	+	-	+
15	40.27	0.491	+	-	-	-	+
16	32.47	0.579	-	+	+	+	+
17	23.37	0.658	-	+	-	-	-
18	21	0.699	+	-	-	+	-
19	16.84	0.731	-	-	+	-	+
20	15.19	0.769	+	-	+	-	+
21	14.28	0.791	-	+	-	+	+
Total number of bands			12	11	12	13	15

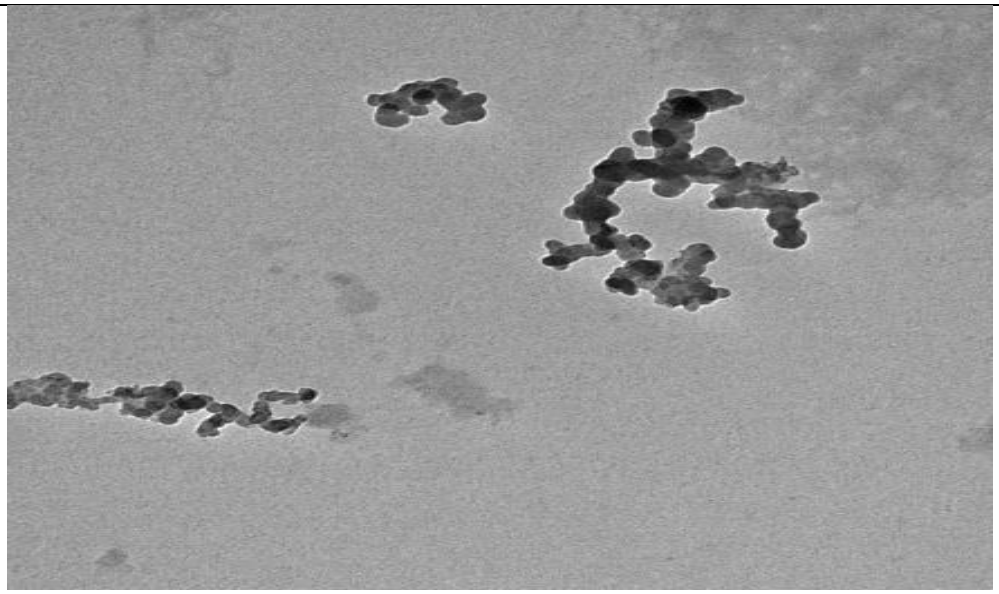
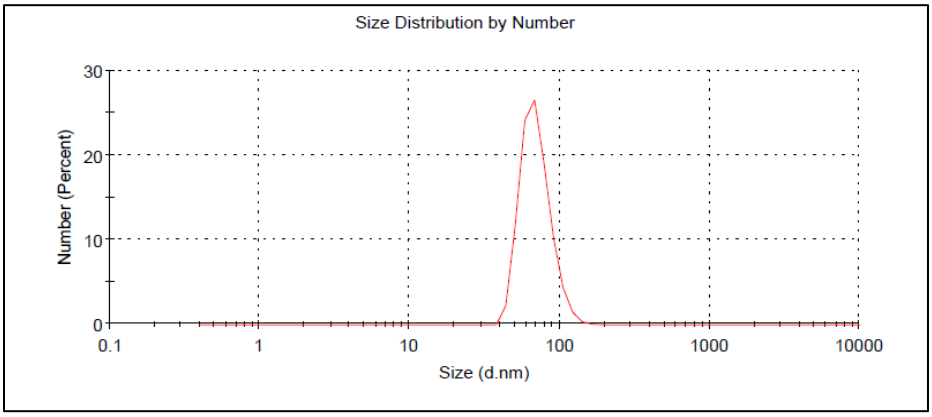
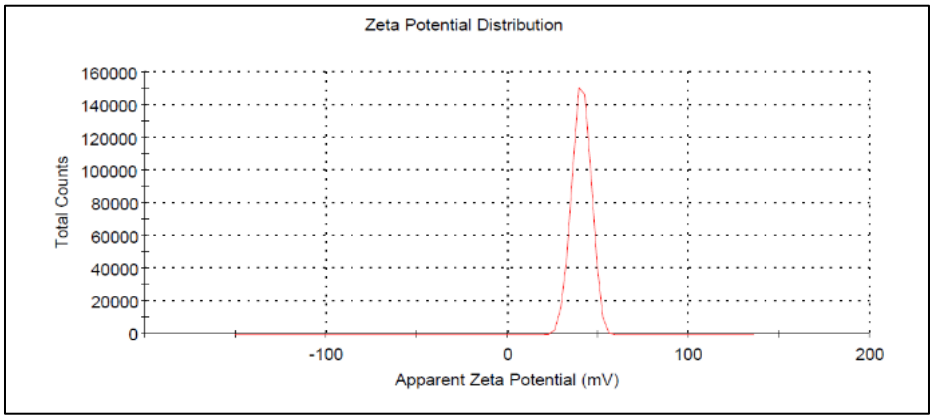


Figure 1: TEM chitosan nanoparticles



A



B

Figure 2: Particle size distribution (A) and zeta potential (B) of chitosan nanoparticles

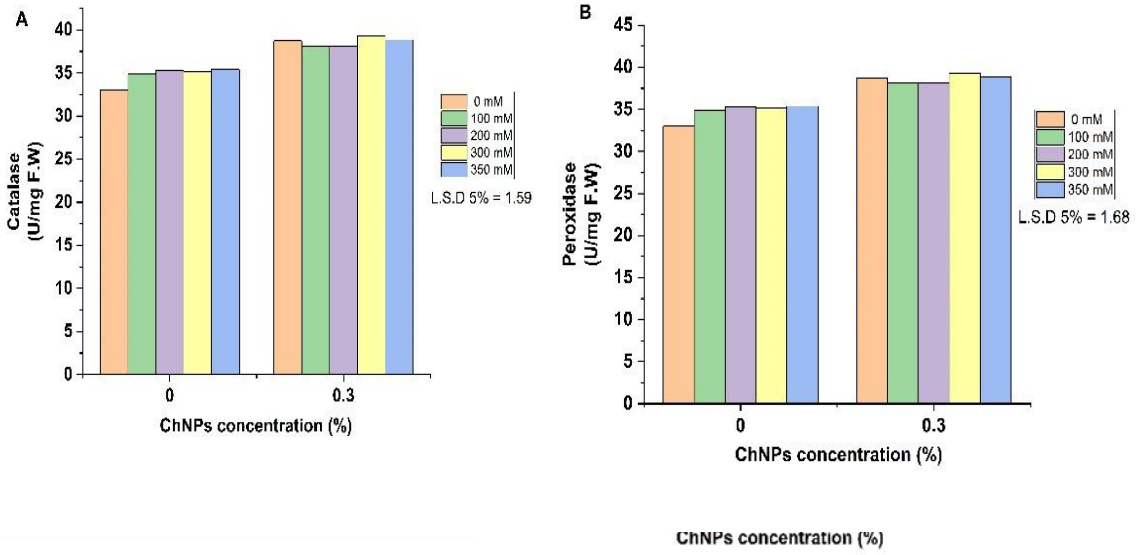


Figure 3: Impact of ChNPs on enzymes activity in shoots of maize under salinity

إستخدام جزيئات الكيتوزان النانوية لمكافحة الإجهاد الملحي وتحسين الخصائص البيوكيميائية وحيوية الشتلات في الذرة الشامية

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الملخص العربي

الإجهاد الملحي هو أحد الضغوط اللاأحيائية وإنه عامل رئيسي يقلل من إنتاجية المحاصيل، أصبحت الجسيمات النانوية مادة رائدة في البحوث الزراعية في الوقت الحاضر لأنها تتمتع بخصائص فيزيائية وكيميائية فريدة. يهدف هذا البحث إلى معرفة تأثير جزيئات الشيتوزان النانويةعلى بعض الصفات المورفولوجية والمحتوى الكلي للكلوروفيل والبرولين والنشاط الإنزيمي لبعض مضادات الأكسدة والتفريد الكهربي للبروتين تحت ظروف الإجهاد الملحي لبادرات الذرة الشامية (جيزة 168) بإستخدام تركيزات مختلفة من ملح كلوريد الصوديوم (0، 100، 200، 300، 350 ملي مول) لتقليل تأثير الإجهاد الملحي الضار خلال مرحلة الإنبات. أظهرت النتائج إنخفاضًا معنويًا في نسبة الإنبات، طول الريشة، الوزن الجاف للبادرة والمحتوى الكلي للكلوروفيل مع زيادة تركيز كلوريد الصوديوم. أدت المعالجة المسبقة للبذور بجزيئات الشيتوزان النانوية إلى زيادة في نسبة الإنبات والوزن الجاف للبادرات وطول الريشة وحيوية البادرات والمحتوى الكلي للكلوروفيل مقارنةً بالكنترول بدون أي معاملة. أدت المعالجة المسبقة للبذور من خلال النقع بجزيئتات الشيتوزان النانوية إلى الزيادة في نسبة الإنبات والوزن الجاف للبادرات وطول البادرة وحيوية البادرة والمحتوى الكلي للكلوروفيل بالمقارنة بالكنترول بدون معاملة. إزداد النشاط الإنزيمي للكتاليز (CAT) والبيروكسيديز(POX) بشكل كبير بسبب إجهاد الملوحة. ولكن لوحظت أعلى قيم للكاتليز مع المعالجة بجزيئات الشيتوزان النانوية عند300 ملي مولار كلوريد الصوديوم. قد تؤدي زيادة أنشطة إنزيمات مضادات الأكسدة إلى زيادة تحمل إجهاد الملوحة. علاوة على ذلك، أدت المعالجة المسبقة للبذور بجسيمات الشيتوزان النانوية إلى ظهور حزم بروتينية جديدة واختفت أخرى بأوزان جزيئية مختلفة تحت الملوحة في جميعالبادرات. التي قد تكون مؤشراً جيداً لآلية تحمل الملوحة. وأخيراً يمكن الإستنتاج أن المعالجة المسبقة للبذور بتركيز 0.3 ٪ من جزيئات الشيتوزان النانوية مثل النقع قللت بشكل ملحوظ الآثار الضارة للإجهاد الملحي وكذلك حسنت جميع المعاملات المقاسة.

الكلمات الاسترشادية: الذرة، الإجهاد الملحي، جزيئات الشيتوزان النانوية، البرولين، مضادات الأكسدة الإنزيمية، التفريد الكهربي للبروتين.