Mitigating Salinity Stress in *Stevia rebaudiana* Using Different Nanoparticles; Preparation, investigation and application in vitro

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ABSTRACT:

Stevia rebaudiana Bertoni is a valuable origin of natural sweeteners with potential health and economic benefits, making it a desirable alternative to traditional sugar. Nonetheless, salinity stress can significantly impact stevia plant growth, development, and steviol glycoside production. This study aimed to examine the efficacy of different nanoparticles (NPs) including chitosan, Fe_3O_4, Fe_3O_4/humic acid, and chitosan/Cu, in mitigating salinity stress and improving stevia growth and germination. The optimal concentrations were determined to be 2 mM, 1.4 mM, 0.1 mM, and 0.2 mM for chitosan, chitosan/Cu, and Fe_3O_4, and for Fe_3O_4/humic acid, respectively. For 30 days, Stevia plants were exposed to different concentrations and subjected to various levels of salt stress (0, 50, 100, 150, and 175 mM NaCl). Physiological parameters (growth, germination %), biochemical parameters (enzyme activities, proline content, photosynthetic pigments), and total stevioside content (TS) were evaluated. The results demonstrated that chitosan NPs (2 mM) effectively alleviated salinity stress and improved overall plant health, significantly increasing total stevioside content. Fe_3O_4/humic acid (0.2 mM) significantly enhanced chlorophyll a, chlorophyll b, total chlorophyll (a+b), a ratio of chlorophyll a/b, and total carotenoid. These findings suggest that these nano-biofertilizers hold great potential as a sustainable and environmentally friendly approach to improving stevia productivity in saline conditions, potentially contributing to the development of smart agricultural practices.

Keywords: salinity stress; chitosan nanoparticles; proline; antioxidant enzymes; steviol glycosides.

INTRODUCTION:

The perennial shrub *Stevia rebaudiana* is indigenous to South America, especially Brazil and Paraguay. Out of the 230 species in the Asteraceae family's genus Stevia, only *Stevia rebaudiana Bertoni* yields sweet steviol glycosides, commonly known as "Honey Leaf" (Sharma et al., 2023).

The cultivation of perennial crops offers numerous environmental benefits. These include reduced soil erosion, minimized nutrient leaching, increased carbon sequestration in soils, improved water resource protection, enhanced pest tolerance, and the provision of continuous habitat for wildlife (Sarwar et al., 2023). Furthermore, perennial species typically require less reliance on farm equipment, fertilizers, and herbicides compared to annual crops (Soto-Gómez and Pérez-Rodríguez, 2022)." Stevia preparations owe their sweetness primarily to steviol diterpene glycosides, notably stevioside and rebaudiosides, which are widely used as sweeteners in the food industry (Ranjbar et al., 2020).

Soil salinization, a serious risk to agricultural output, especially in dry and semi-arid areas, poses a serious challenge to the cultivation of Stevia plants, which are sensitive to salt stress (Abbas, 2021). Globally, around 953 million hec. (mha) of arable land (8percent of the total land area) are affected by varying degrees of soil salinization (Prakash, 2021).

Under increasing salt concentrations, a decline in protein content is often observed alongside a rise in proline accumulation. Studies have shown that elevated salt levels lead to a decrease in chlorophyll content relative to sugars, proline, and phenols in Stevia plants. Furthermore, research findings indicate that osmotic stress significantly impacts Stevia rebaudiana's growth and yield components (Guru and Dwivedi, 2023). Nanotechnology offers significant potential to enhance plant growth and development. Applications include the use of nanomaterials as herbicides, pesticides, and fertilizers, allowing for controlled release of active ingredients to target specific cellular organelles within plants (Sarraf et al., 2022).

Employing nanoparticles to improve plant stress tolerance is considered a promising approach due to its potential for profitability, cost-effectiveness, and sustainability (Tan et al., 2017). Research has demonstrated that abiotic disturbances effects on plants can be minimized via nanoparticles by influencing plant growth and development in a

concentration-dependent manner (Dimkpa et al., 2017).

Nanoparticles (NPs) enhance plant salinity tolerance through various mechanisms. They maintain photosynthesis, facilitate the removal of reactive oxygen species (ROS), and alleviate both osmotic and ionic stress (Maity, 2018). By influencing the activity of genetic material, transcription factors, proteins, and metabolites, NPs offer a promising approach to improving crop salt tolerance. In agriculture, chitosan NPs act as both biostimulants and elicitors. This non-toxic, biodegradable, and biocompatible polymer mitigates the negative impacts of abiotic stress by activating stress-responsive pathways and enhancing the expression of defense genes, including those involved in disease resistance, such as glucanase and chitinase (Mujtaba et al., 2020). Chitosan NPs help plants cope with salinity by regulating cellular osmotic pressure and improving the intake of vital nutrients and water.

Iron, a vital element for plant development, plays a crucial role in numerous biochemical processes, including respiration, nitrogen fixation, electron transport, chlorophyll synthesis, and protein synthesis. Iron deficiency, often prevalent in alkaline soils, can significantly impact plant health. As a cofactor for over 140 enzymes, iron is indispensable for plant metabolism (Nemati Lafmejani et al., 2018).

Among various metal nanoparticles, copper nanoparticles have been extensively studied in plant systems. Chitosan-coated copper NPs (Chitosan + Cu NPs) have demonstrated lower toxicity to plants compared to both copper sulfate and uncoated copper NPs (Aruna et al., 2015 Juárez-Maldonado et al., 2015).

The combination of iron oxide NPs (Fe_3O_4 NPs) with humic acid has been shown to reduce toxicity and enhance biocompatibility in plants. Studies have confirmed the safety of Fe_3O_4/humic acid (Fe_3O_4/HA NPs) (El-Ganainy et al., 2022).

This research attempted to determine the potential of NPs to mitigate salinity stress and improve stevia growth and germination rates.

MATERIALS AND METHODS

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), Giza, Egypt.

Preparation of *Stevia rebudiana* samples

Stevia rebudiana samples collection

Stevia seeds were gained from the Sugar Crops Research Institute, ARC, Egypt.

Sterilization of stevia seeds

Firstly, the seeds were sterilized by immersing them in a 10% sodium hypochlorite (NaOCl) solution for five minutes. Then, any remaining NaOCl was removed by washing them with fresh water followed by distilled water (dH_2O). In sterile Petri dishes with two layers of filter paper and either distilled water or saline solution, germination tests were conducted (De Jesus et al., 2016).

Germination and irrigation

For germination, the holes were made in seedling boxes or trays 0.5 cm deep and spaced 5 cm apart. Then, plant two seeds in each hole. The rice hulls or fine compost were used to cover the seeds. For Irrigation**,** by spray sprinkler water was used daily during the hot dry season or every two days during the cooler months.

Salt stress application

To alleviate stress, 500 ppm stock solutions of NaCl were made in distilled water. Media supplemented with several concentrations of NaCl (0, 50, 100, 150, and 175 mM) was made. 1.0 N of NaOH and 1.0 N of HCl solutions were used to maintain the pH between 5.7 and 5.8, and the mixture was autoclaved for 15 minutes at 121° C /15 pressures to sterilize it. Finaly, the medium was carefully wrapped after being placed into sterile culture tubes to avoid contamination (Jesus et al., 2016).

Preparation of NPs treatments

Preparation of Chitosan NPs

Chitosan NPs were prepared using the ionic gelation method (Ghadi *et al.,* 2014).

Preparation of Cu-chitosan NPs

Copper sulfate was added drop wise to the chitosan solution to create the NPs; the production of the chitosan-copper complex was indicated by the formation of a blue color and reduced to nano copper by the addition of hydrazine hydrate, as seen by the color shifting from blue to brick red (Dang et al., 2011).

Preparation of Fe_3O_4 NPs

Briefly, Fe_3O_4 NPs were synthesized by adding a base to an aqueous mixture of ferric chloride (Fecl $_3$) and ferrous sulphate ($Feso_4 \cdot H_2O$) in a molar ratio (1:2w/v), resulting in a black color (Xu et al., 2014).

Preparation of HA coated Fe_3O_4 NPs

The co-precipitation techniques with some modifications were used to coat 1.0 g of HA with 12.5 mL of Fe_3O_4 NPs. Then, the precipitate was neutralized with distilled water several times to remove the uncoated HA from the solutions (Koesnarpadi et al., 2019).

Characterization of NPs sampels

The size and morphology of NPs were determined using transmission electron microscope (TEM, JEOL JEM-1010, Japane), at 80 kV at The Regional Center for Mycology and Biotechnology (RCMB), Al- Azhar University (Amin et al, 2021).

Experimental design of NPs samples treatments of various concentrations at which NPs were used in the present study:

Chitosan NPs (2,1 and 0.5 mM), Chitosan/Cu NPs (1.2, 1.4, and 2 mM), Fe_3O_4 NPs (2, 1 and 0.1 mM) and Fe_3O_4/ humic acid NPs (2, 1 and 0.2 mM).

Effects of preparation NPs treatments on S. rebudiana growth parameters

Seeds were soaking on different concentrations of NPs (Chitosan, Ch. /Cu, Fe_3O_4 and Fe_3O_4/ humic acid NPs) for 2h.Then, sowing in trays, as mentioned before. On the 14th day of sowing, irrigation was carried out using different NPs which were being applied. Simultaneously 30th day, growth parameters (shoot and root length) and dry weight (Siddique et al., 1990) were estimated to choose the optimal concentration of each applied NPs

Estimation of chemical composition of S. rebudiana applied with various concentrations of NPs:

To choose the optimize concentration from NPs samples treatments for using in further studies estimation the values of the chemical composition (moisture, fat, protein, ash, fibers, and total carbohydrates) of the collected leaves of *S. rebaudiana* plants . *S. rebaudiana* plant were achieved with the electromagnetic spectrum using a near-infrared (NIR) spectrometer. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated for each quantitative trait using the statistical calculator developed by (Dhakre & Bhattachary, 2018).

Determination of total stevioside content (TS)

Stevioside content (TS) was calculated by using the equation reported by (Nishiyama et al., 1991) as follow

$$TS = TC - 7.56/0.96.$$

Salinity stress:

Effects of preparation NPs treatments on S. rebudiana under salinity stress

Firstly, different concentrations of NaCl concentrations (0, 50, 100, 150, and 175 mM) were prepared set up as outlined by Michel and Kaufmann (1973). The seeds were sterilized .Then; 20 ml of each NaCl solution was added to every box whole 5 cm deep. In a growth chamber, all of the germination boxes were kept at a temperature of 25 ± 3° C and a humidity of 80–90%. Dry weight, growth characteristics, and germination percentage were assessed 30 days after planting.

Biochemical studies on S. rebudiana under salinity stress 2.8.1. Determination of photosynthetic pigments

Using following equations of (Lichtentaler and Wellburn (1985).

$$Chlorophyll\ a\ (\mu g/ml) = 11.75\ A662 - 2.350\ A645$$

$$Chlorophyll\ b\ (\mu g/ml)$$

$$= 18.61\ A645 - 3.960\ A662 Carotenoids\ \left(\mu \frac{g}{ml}\right)$$

$$= 1000\ A470 - 2.270\ Chl\ a - 81.4\ Chl\ b/227$$

Determination of proline content

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:

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

μmoles proline/g of fresh weight material.

Enzymes activity assay

Catalase activity

Catalase activity was determined according to the method used by (Aebi, 1984)

Peroxidase activity

Peroxidase was assayed spectro photochemically according to (Amako *et al*., 1994) .

Ascorbic oxidase activity

Assay of ascorbic acid oxidase activity was carried out according to the procedure of (Oberbacher and vines, 1963).

Polyphenol oxidase activity

Polyphenol oxidase activity was determined according to the method used by Putter and Becker (1983).

Data Analysis:

DSAASTAT Version 1.1 software was used to do analysis of variance on the data (Snedecor and Cochran, 1980). Fisher's protected least significant difference (LSD) at 5% significance was used to infer mean differences.

RESULTS AND DISCUSSION

Characterization of preparation NPs treatments

The size and morphology of NPs were determined by TEM, the results are shown in (Fig.1A-D). Fig., 1A shows spherical shapes and some agglomerated of Fe_3O_4 NPs with an average size was around 14.9–18.5 nm. The obtained results are in closer with (El-Bahr et al., 2021).

Furthermore, the TEM image confirmed that the Fe_3O_4/HA NPs were quasi-triangular in Shape had nearly uniform distribution, with particle sizes ranging from 12 to 20 nm these results are agreement with (El-Ganainy et al., 2022).

Fig., 1C shows that chitosan NPs with variable shapes, non-aggregation and most of them oval show in irregular shapes. Chitosan NPs was shown to possess a significant particle size magnification scale decreased to 6.47 nm. The obtained results were proved by (El-Gazzar et al., 2018).

Fig.1D showed that Chitosan NPs with copper particles was variable shape, most of them present in spindle in nature with some others having occasionally oval shape. The size of the particle ranged from 13.5 to 33.9 nm these results are agreement with (Mott et al., 2007).

Effect of various concentrations of preparation NPs treatments on germination percent and growth parameters of S. rebaudiana

In order to the optimize concentrations for Fe_3O_4, Fe_3O_4/HA, chitosan and chitosan /Cu NPs. Healthy plants were sprayed using three concentrations of each treatment, previously prepared in the laboratory. After 14 days estimated shoot, root length and dry weight under greenhouse conditions.

In general, the results in (Table 1), showed that, higher concentration of treatments had a positive effect on *S. rebaudiana,* except Ch/ Cu which had a stabilizing effect on the percent of germination and growth parameters. Fe_3O_4 at 2, 1 and 0.1 mM and Fe_3O_4/ HA NPs at 2, 1 and 0.1 mM were achieved a high percentage of germination and improved the shoot, root length and dry weight. Furthhermore, chitosan NPs at 2 mM showed the best effect on germination, to 14. 8 and 10 .03 cm in shoot and root length respectively, and the dry weight 1.186 gm., the germination rate reached 97%. Results are in harmony with those obtained by (Chouhan and Mandal, 2021), who indicated that chitosan NPs promote plant and induces local and systemic defense responses in plants. (Geries et al., 2020 and Allam et al., 2024), reported that chitosan NPs increased the maize seedlings and shoot length. Also, fresh and dry weights were enhanced.

Effect of various concentrations of preparation NPs treatments on chemical composition of S. rebaudiana

Estimation the values of the chemical composition (moisture, fat, protein, ash, fibers, and total carbohydrates) of the collected leave from of the studied Plant. The results are shown in (Table2) indicated that, a decrease in the percentage of fat and fiber with the chitosan NPs at a concentration of (2mM) to (5.05 - 15.81%), respectively, compared to the control group, while the nanoparticles of the iron capsule with humic at a concentration of (0.2mM) achieved a noticeable increase in the percentage of protein to (12.08%). The moisture percentage increased to (8.94%) with Fe_3O_4 NPs at a concentration of (0.1 mM) compared to the control. Application of chitosan nanoparticles led to an increase in the, steviol glycosides content (50.47%), the obtained results are in a harmony with (Gerami et al., 2020). The application chitosan multiple increases in malondialdehyde content, steviol glycosides (stevioside and rebaudioside); this is in agreement with (Balusamy et al., 2022).

Salinity stress:

Effect of preparation NPs treatments on S. rebaudiana under salinity stress:

The salinity levels were increased in steps as (0, 50, 100, 150 and 175 mM NaCl). All germination boxes were stored in a growth chamber. Germination was measured at 30 days of sowing.

The impact of nanomaterials on germination ratio and growth parameters of stevia seedlings under salinity is presented in Table3 and 4.The best treatment was chitosan NPs (2mM) achieved 88.23% in the percentage of germination at 175 mM of NaCl. Followed by Fe_3O_4 (0.1mM). Finally, the last applied treatments were chitosan /Cu and Fe_3O_4/ HA, there no significant differences between then. (Zayed et al., 2017 and Gad-Allah et al., 2023) indicated that, using of chitosan. NPs resulted in a significant enhancement in the biological yield under salinity stress. On the other hand, the morphological features and biochemical properties of *Zea maize* L. seedlings (Giza168) under salt stress using decreased significantly when increasing the concentration of NaCl. Also, (Attaran et al., 2022) showed that the external administration of chitosanNPs mitigated the detrimental impacts of salt-induced stress on plant development.

Determination of photosynthetic pigments

In the context of knowing the effect of the different concentrations of applied nanomaterial's treatments on the photosynthetic pigments (Cha, Chb, Ch(a+b), Cha/b and total carotene) under pathological stress and salinity stress (50, 100, 150 and 175 mM) (Table, 5 and 6). Table 4 and 5 showed that all applied nanomaterial's treatments had a clear positive effect on the stevia plants compared to the control (without any treatments), there was a significant difference among all applied NPs in the effect of photosynthetic pigments. The best effect of all was Fe_3O_4/HA compared to control, under the influence of salinity stress, followed by Fe_3O_4NPs, and the least effect was chitosan at a concentration of 2mM. The increasing in salinity concentrations is inversely proportional to the photosynthetic pigment, the higher salinity percentage, the lower the photosynthetic pigment. Therefore, 175 mM was the most hazardous to the stevia plants.

Additionally, Fe_3O_4 NPs increased photosynthetic pigments and carotenoids. This result was agreement with (Khanizadeh et al., 2024), who explained thatApplication of Fe_2O_3 NPs positively impacted chlorophyll and carotenoid levels in *Dracocephalum kotschyi* Boiss plants. Also, significantly boosted pigment levels thereby increasing chlorophyll a, chlorophyll b, total chlorophyll and carotenoids compared to the control. Korani et al., 2023 explained that Fe_3O_4 NPs with humic acid led to improving plant productivity and resistance to salt stress, because humic acid works to chelate nutrients.

Determination of proline content

Fig, 2 showed the effect of NPs treatments under salt stress on the proline level in Stevia plants. The different NPs treatments have varying effects on proline accumulation.

The base treatment (chitosan NPs) and its combination with copper (chitosan/Cu) show higher proline levels, they achieved 27.27 μmoles/g F.W and 27.11 μmoles/g F.W, respectively at 175 mM of NaCl conc. compared to other treatments, indicating a potentially higher stress response. Treatments involving iron oxide nanoparticles (Fe_3O_4) and their combination with humic acid (Fe_3O_4/HA) generally induce lower proline accumulation, suggesting a low pronounced stress response compared to control treatment (withouttreated), which achieved 24.25 μmoles/g F.W at highly conc. of NaCl.

Proline can act as a signaling molecule, triggering various stress responses in plants. The observed increase in proline content suggests that the NPs might be activating signaling pathways related to stress tolerance. These results agreement with (Hidangmayum et al., 2019, (Attia et al., 2021 andAlenaz et al., 2024).

Enzymes activity assay

The results in (Table 7) illustrated that, activity of catalase and peroxidase enzymes in stevia plants under salinity. The results demonstrate that the application of nanomaterial's, particularly chitosan. (2mM) and Fe_3O_4/ HA, significantly enhanced the enzymatic activity compared to the control at 175 mM of NaCl concentrations. These findings suggest that nanomaterial's can effectively mitigate the adverse effects of salinity by improving the antioxidant defense system of stevia plants. The observed increase in catalase and peroxidase activity is consistent with previous studies reporting the protective role of these enzymes against oxidative damage. Moreover, the synergistic effect of nanomaterial's and salinity stress on enzyme activity highlights the complex interactions between these factors. Overall, the results of this study provide compelling evidence for the potential of nanomaterials as a promising

strategy for enhancing the stress tolerance of stevia plants.

The provided (Table 8) presents a comprehensive analysis of the effects of NPs treatments on the activity of ascorbate oxidase and polyphenol oxidase enzymes in *S. rebaudiana* under salinity and disease stress. The results reveal several significant findings between treatments and control without treated, as well as among treatments.

The application of various NPs particularly chitosan NPs and Fe_3O_4 NPs, resulted in a significant increase in the activity of both ascorbate oxidase and polyphenol oxidase enzymes. This suggests that nanomaterial's can effectively stimulate the plant's antioxidant defense system.

Both enzymes exhibited higher activity levels under increasing salinity stress, indicating that plants up regulate their antioxidant defense mechanisms to cope with oxidative stress induced by salinity. While all nanomaterials tested showed an enhancing effect on enzyme activity, chitosan-based treatments demonstrated the most pronounced impact. This suggests that chitosan may possess unique properties that make it particularly effective in stimulating antioxidant enzyme activity.

The observed increase in enzyme activity implies that nanomaterial's can enhance the stress tolerance of *S. rebaudiana*, particularly under salinity conditions. By stimulating the production of antioxidant enzymes, nanomaterial's can help mitigate oxidative damage caused by various environmental stresses. These findings suggest that nanomaterials could be used as bio stimulants to improve crop productivity and quality, especially in saline environments.

The exogenous chitosan NPs treatment greatly reduced oxidative stress in maize by increasing the activity of antioxidant enzymes (Chandra et al., 2015 and Dey et al., 2019). Therefore, by triggering the antioxidant defense mechanism, which aids in scavenging reactive oxygen species, chitosan NPs reduced oxidative stress and helped *C. roseus* overcome the negative effects of salt (Hassan et al., 2021).

CONCLUSIONS

This study demonstrates the potential of nanofertilizers to revolutionize agriculture, particularly through the development of smart agricultural practices. Our findings provide valuable insights into the effects of various nano-biofertilizers, including chitosan, Fe_3O_4, Fe_3O_4/humic acid, and chitosan/Cu nanoparticles, on mitigating salinity stress in *S. rebaudiana*. Optimal concentrations were determined to be 2 mM for chitosan, 1.4 mM for chitosan/Cu, 0.1 mM for Fe_3O_4, and 0.2 mM for Fe_3O_4/humic acid. Under salinity stress (0, 50, 100, 150, and 175 mM NaCl), chitosan and Fe_3O_4/humic acid NPs exhibited significant efficacy in alleviating stress, leading to improvements in plant physiological and biochemical parameters. Notably, these treatments also resulted in increased total stevioside content. The application of chitosan/Cu NPs demonstrated some limitations at higher concentrations (2 mM). This highlights the need for further research to optimize nanoparticle application, including the exploration of alternative coating materials and surface stabilization strategies to minimize toxicity and enhance biocompatibility.

REFERENCES:

Abbas, G., Amjad, M., Saqib, M., Murtaza, B., Asif Naeem, M., Shabbir, A., Murtaza, G. 2021: Soil sodicity is more detrimental than salinity for quinoa (Chenopodium quinoa Willd.): A multivariate comparison of physiological, biochemical and nutritional quality attributes. J. Agron. Crop. Sci ;207:59–73. doi: 10.1111/jac.12451

Aebi, H. 1984: Catalase in vitro. Methods of Enzymology, 105:121-126.

Alenazi, M.M., El-Ebidy, A.M., El-Shehaby, O.A., Seleiman, M.F., Aldhuwaib, K.J., Abdel-Aziz, H.M. 2024: Chitosan and chitosan nanoparticles differentially alleviate salinity stress in Phaseolus vulgaris L. plants. *Plants*, *13*(3), 398.

Allam, E., El-Darier, S., Ghattass, Z., Fakhry, A., Elghobashy, R.M. 2024: Application of chitosan nanopriming on plant growth and secondary metabolites of Pancratium maritimum L. *BMC Plant Biology*, *24*(1), 466.

Amako, A., Chen, K., Asada, K. 1994: Separate assays specific for ascorbiate peroxidase and gauiacol peroxidase and for the chloroplastic and cytosilic isoenxymes of ascorbate peroxidase in plants. Plant cell physiol., 35:497-504.

Amin, B.H., Ahmed, H.Y., El Gazzar, E.M., Badawy, M.M. 2021: Enhancement the mycosynthesis of selenium nanoparticles by using gamma radiation. Dose-Response, 19(4.(

Aruna, A., Namasivayam, S.K.R., Roy, E.A. 2015: Comparative evaluation of non-target toxic effect of free and polymer coated chemogenic metallic nanoparticles against Vigna mungo. Int. J. Pharm. Biol. Sci. 6(1): B461-B467.

Attaran Dowom, S., Karimian, Z., Mostafaei Dehnavi, M., Samiei, L. 2022: Chitosan nanoparticles improve physiological and biochemical responses of Salvia abrotanoides (Kar.) under drought stress. *BMC Plant Biology*, *22*(1), 364-377.

Attia, M.S., Osman, M.S., Mohamed, A.S., Mahgoub, H.A., Garada, M.O., Abdelmouty, E.S., Abdel Latef, A.A.H. 2021: Impact of foliar application of chitosan dissolved in different organic acids on isozymes, protein patterns and physio-biochemical characteristics of tomato grown under salinity stress. *Plants*, *10*(2), 388-396.

Balusamy, S.R., Rahimi, S., Sukweenadhi, J., Sunderraj, S., Shanmugam, R., Thangavelu, L., Perumalsamy, H. 2022: Chitosan, chitosan nanoparticles and modified chitosan biomaterials, a potentia tool to combat salinity stress in plants. *Carbohydrate Polymers*, *284*, 119189.

Bates, L.S., Waldren, R.P., Teare, I.D. 1973: Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207.

Chandra, S., Chakraborty, N., Dasgupta, A., Sarkar, J., Panda, K., Acharya, K. 2015: Chitosan nanoparticles: a positive modulator of innate immune responses in plants. *Scientific reports*, *5*(1), 15195.

Chouhan, D., Mandal, P. 2021: Applications of chitosan and chitosan based metallic nanoparticles in agrosciences-A review. *International journal of biological macromolecules*, *166*, 1554-1569.

Dang, T.M.D., Le, T.T.T., Fribourg-Blanc, E., Dang, M.C. 2011: The influence of solvents and surfactants on the preparation of copper nanoparticles by a chemical reduction method. *Adv. Nat. Sci.*

Dey, S., Kundu, R., Gopal, G., Mukherjee, A., Nag, A., Paul, S. 2019: Enhancement of nitrogen assimilation and photosynthetic efficiency by novel iron pulsing technique in *Oryza sativa* L. var Pankaj. *Plant Physiology and Biochemistry*, *144*, 207-221.

Dhakre, D.S., Bhattacharya, D. 2018: Statistical analysis step-by-step using statistical calculator. *International Journal of Current Microbiology and Applied Sciences*, *7*(11), 901-921.

Dimkpa, C.O., Bindraban, P.S., Fugice, J., Agyin-Birikorang, S., Singh, U., Hellums, D. 2017: Composite micronutrient nanoparticles and salts decrease drought stress in soybean. Agron Sustain Dev.; 37:1–13.

El-Bahr, S.M., Elbakery, A.M., El-Gazzar, N., Amin, A.A., Al-Sultan, S., Alfattah, M.A., .Hamouda, A.F. 2021: Biosynthesized iron oxide nanoparticles from Petroselinum crispum leaf extract mitigate lead-acetate-induced anemia in male albino rats: hematological, biochemical and histopathological features. Toxics, 9(6), 123.

El-Ganainy, S.M., El-Bakery, A.M., Hafez, H.M., Ismail, A.M., El-Abdeen, A.Z., Ata, A.A.E., Iqbal, Z. 2022: Humic acid-coated Fe3O4 nanoparticles confer resistance to Acremonium wilt disease and improve physiological and morphological attributes of grain Sorghum. Polymers. , 14(15), 3099.

El-Gazzar, N., El-Bakery, A.M., Ata, A.A. 2018: Influence of some bioagents and chitosan nanoparticles on controlling maize late wilt and improving plants characteristics. Egyptian Journal of Phytopathology, 46(2), 243-264.

Gad-allah, M.M, Yousef, H.Y.M., El-khamissi, H.A.Z., Elbakery, A.M. 2023: Chitosan nanoparticles as combat salinity stress to improve biochemical characteristics and seedling vigor in maize (Zea maize L.). Al-Azhar Journal of Agricultural Research, 48(3),), 401-415.

Gerami,.M., Majidian, P., Ghorbanpour, A., lipour, Z. 2020: Stevia rebaudiana Bertoni responses to salt stress and chitosan elicitor. Physiology and molecular biology of plants, 26(5), 965 974.0(2), 3584-3591.

Geries, L.S.M., Omnia, H.S., Marey, R.A. 2020: Soaking and foliar application with chitosan and nano chitosan to enhancing growth, productivity and quality of onion crop. Plant Arch, 20(2)3284-3591

Ghadi, A., Mahjoub, S., Tabandeh, F., Talebnia, F. 2014: Synthesis and optimization of chitosan nanoparticles: Potential applications in nanomedicine and biomedical engineering. Caspian journal of internal medicine, 5(3.(

Guru, A., Dwivedi, P. 2023: Optimizing Growth through Systematic Evaluation of Salinity, Putrescine, and Sodium Nitroprusside Levels in Stevia rebaudiana. International Journal of Environment and Climate Change, 13(11), 406-414.

Hassan, F.A.S., Ali, E., Gaber, A., Fetouh, M.I., Mazrou, R. 2021: Chitosan nanoparticles effectively combat salinity stress by enhancing antioxidant activity and alkaloid biosynthesis in Catharanthus roseus (L.) G. Don. Plant Physiology and Biochemistry, 162, 291-300.

Hidangmayum, A., Dwivedi, P., Katiyar, D., Hemantaranjan, A. 2019: Application of chitosan on plant responses with special reference to abiotic stress. Physiol. Mol. Biol. Plants 25, 313–326. doi: 10.1007/s12298-018-0633-1.

Jesus, V.A.M.D., Araújo, E.F., Neves, A.A., Santos, F.L., Dias, L.A.D.S., Silva, R.F.D.2016: Ratio of seeds and sodium hypochlorite solution on the

germination process of papaya seeds. Journal of Seed Science, 38(01), 57-61.

Juárez-Maldonado, A., Ortega-Ortíz, H., Pérez-Labrada, F., Cadenas-Pliego, G., Benavides-Mendoza, A. 2016: Cu Nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. J. Appl. Bot. Food Qual. 2016, 89, 183–189.

Khanizadeh, P., Mumivand, H., Morshedloo, M.R., Maggi, F. 2024: Application of Fe_2O_3 nanoparticles improves the growth, antioxidant power, flavonoid content, and essential oil yield and composition of Dracocephalum kotschyi Boiss. Frontiers in Plant Science, 15, 1475284.

Koesnarpadi, S., Santosa, S.J., Siswanta, D., Rudiarso, B. 2019: July: Modifying humic acids on magnetite nanoparticles to sorption of p-chlorophenol. In Journal of Physics: Conference Series (Vol. 1277, No. 1, p. 012007). IOP Publishing.

Korani, M., Ilkaee, M.N., Paknejad, F. 2023: Foliar application of humic acid and iron and zinc nano chelates alleviate the salinity damage on chicory (Cichorium intybus L.) in hydroponic culture. Journal of Organic Farming of Medicinal Plants, 2(1), 1-8.

Li, R., He, J., Xie, H., Wang, W., Bose, S.K., Sun, Y., Yin, H. 2019: Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (Triticum aestivum L.). *International journal of biological macromolecules, 126*, 91-100.

Lichtenthaler, H.K., Wellburn, A.R. 1985: Determination of total cartionoids and chlorophyll A and B of leaf in different solvents. Biochem. Soc. Trans, 11, 59-592.

Maity, A., Natarajan, N., Pastor, M., Vijay, D., Gupta, C.K., Wasnik, V.K. 2018: Nanoparticles influence seed germination traits and seed pathogen infection rate in forage sorghum (Sorghum bicolour) and cowpea (Vigna unguiculata). Indian J Exp Biol.;56:363–72.

Michel, B.E., Kaufmann, M.R. 1973: The osmoticpotential of polyethylene glycol 6000. Plant physiology, 51(5), 914-916.

Mott, D., Galkowski, J., Wang, L.Y., Luo, J., Zhong, C.J. 2007: Synthesis of size-controlled and shaped copper nanoparticles. Langmuir 2007, 23, 5740–5745

Mujtaba, M., Khawar, K.M., Camara, M.C., Carvalho, L.B., Fraceto, L.F., Morsi, R.E., Wang, D. 2020: Chitosan-based delivery systems for plants: A brief overview of recent advances and future directions. International journal of biological macromolecules, 154, 683-697.

Nemati Lafmejani, Z., Jafari, A.A., Moradi, P., Ladan Moghadam, A. 2018: Impact of foliar application of iron-chelate and iron nano particles on some morpho-physiological traits and rssential oil composition of peppermint (Mentha piperita L.). Journal of Essential Oil Bearing Plants, 21(5), 1374-1384

Nishiyama, P. 1991: correlation between total carbohydrate content and stevioside content in Stevia rebaudiana leaves. Arquivos de Biologia e Tecnologia,. 34: p. 3-4.

Oberbacher, M.F., Vines, H.M. 1963: Spectrophotometric assay of Putter, J., Becker, R. 1983: Qxidoreductases acting on a group other than CHOH. In. Methods of Enzymatic Analysis, 3, 286-293.ascorbic acid oxidase. Nature, 197(4873), 1203-1204.

Prakash J., Arora, N.K. 2021: Novel metabolites from Bacillus safensis and their antifungal property against Alternaria alternata. Antonie Van Leeuwenhoek. 114(8),1245-1258.

Putter, J., Becker, R. 1983: Qxidoreductases acting on a group other than CHOH. In. Methods of Enzymatic Analysis, 3, 286-293.

Ranjbar, T., Nekooeian, A.A., Tanideh, N., Koohi-Hosseinabadi, O., Masoumi, S.J., Amanat, S. 2020: comparison of the effects of Stevia extract and metformin on metabolic syndrome indices in rats fed with a high-fat, high-sucrose diet. J Food Biochem.;44(8):e13242. doi: 10.1111/jfbc.13242.

Sarraf, M., Vishwakarma, K., Kumar, V., Arif, N., Das, S., Johnson, R., 2022: Metal/metalloid-based nanomaterials for plant abiotic stress tolerance: an overview of the mechanisms. Plants.;11:316.

Sarwar, M., Saleem, M.F., Ullah, N., Maqsood, H., Ahmad, H. 2023: Physiological Ecology of Medicinal Plants: Implications for Phytochemical Constituents. In Herbal Medicine Phytochemistry: Applications and Trends (pp. 1-33). Cham: Springer International Publishing.

Sharma, S., Gupta, S., Kumari, D., Kothari, S.L., Jain, R., Kachhwaha, S. 2023: Exploring Plant Tissue Culture and Steviol Glycosides Production in Stevia rebaudiana (Bert.) Bertoni: A Review. Agriculture, 13(2), 475.

Siddique, K.H.M., Belford, R.K., Tennant, D. 1990: Root: shoot ratios of old and modern, tall and semi-dwarf wheats in a Mediterranean environment. Plant and soil, 121, 89-98.

Soto-Gómez, D., Pérez-Rodríguez, P. 2022: Sustainable agriculture through perennial grains: Wheat, rice, maize, and other species. A review. Agriculture, Ecosystems & Environment, 325, 107747.

Tan, W., Du, W., Barrios, A.C., Armendariz, R., Zuverza-Mena, N., Ji, Z. 2017: Surface coating changes the physiological and biochemical

impacts of nano-TiO2 in basil (Ocimum basilicum) plants. Environ Pollut.;222:64–72 .

Xu, J., Sun, J., Wang, Y., Sheng, J., Wang, F., Sun, M. 2014: Application of iron magnetic nanoparticles in protein immobilization. Molecules, 19(8), 11465-11486.

Zayed, M.M., Elkafafi, S.H., Zedan, A.M., Dawoud, S.F. 2017: Effect of nano chitosan on growth, physiological and biochemical parameters of Phaseolus vulgaris under salt stress. Journal of Plant Production, 8(5), 577-585.

Table 1: Effect of various concentrations of applied nanoparticles on Germination% and growth parameters on *S. rebaudiana* plants under laboratory conditions after 30 days of sowings.

Germination % and growth parameters					
Treatments NPs	Conc. mM.	Germination %	Shoot length (cm)	Root length (cm)	Dry weight (g)
Chitosan	2	97.0a	14.8a	10.03a	1.186a
	1	95.67c	14.2b	9.73b	1.171b
	0.5	94.93e	13.83d	8.66g	1.168c
Ch/Cu	2	82.41j	9.66k	5.03l	0.987i
	1.4	95.4d	13.93c	9.26d	1.161e
	1.2	94.76cfg	12.8h	7.23j	1.155g
Fe_3O_4	2	95.58c	13.13e	8.93e	1.161e
	1	94.69gh	12.83h	8.77f	1.158f
	0.1	94.58c	12.76i	7.67i	1.155g
Fe_3O_4/HA	2	95.92b	13.07f	9.39c	1.163d
	0.2	94.88ef	12.93g	8.82f	1.159f
	1	94.76fg	12.84h	7.77h	1.158f
Con.	-----	94.33i	12.67j	7.03k	1.153h
LSD at 0.05		0.161	0.054	0.161	0.002

Means significant 5% at level of probability

Table 2: Effect of various concentrations of applied nanoparticles (NPs) in chemical composition of *S. rebaudiana* plants in laboratory after 30 days of sowings.

Treatments NPs	Conc. mM	Fat	Moist.	Prot.	Ash	Fib.	T.carbo.	TS.
Chitosan	2	5.5d	7.72e	11.36g	4.84b	16.85c	56.02b	50.47b
	1	5.66c	6.10j	11.56e	4.54f	15.28k	56.86a	51.35a
	0.5	5.05k	6.91i	11.77c	4.44g	15.81j	53.68h	48.04h
Ch/CU	2	5.23gh	8.14c	11.01j	4.55f	16.53e	54.54e	48.93e
	1.4	5.33e	7.72e	11.78c	4.68d	16.47f	54.02g	48.39g
	1.2	5.11j	8.08c	11.32h	4.84b	16.11h	54.54e	48.93e
Fe_3O_4	2	5.15j	8.94a	11.45f	4.73c	16.04i	54.20f	48.58f
	1	5.00i	8.14c	11.76c	4.00j	16.91b	54.19f	48.57f
	0.1	5.30f	7.55f	11.88b	4.62e	16.45f	53.69h	48.05h
Fe_3O_4/HA	2	5.87a	8.37b	12.08a	4.29h	16.63d	55.06d	49.47d
	1	5.21h	6.99h	11.20i	4.87a	16.15h	55.58c	50.02c
	0.2	5.24g	7.93d	11.21i	4.43g	16.33g	52.76i	47.08i
Con		5.80b	7.32g	11.60d	4.18i	17.48a	53.62h	47.97h
LSD at 0.05		0.024	0.062	0.026	0.023	0.046	0.094	0.098

Means significant 5% at level of probability

Table 3: The effect of various applied nanoparticles on germination seedling % and dry weight of *S. rebaudiana* plants under salinity stress.

Treatments NPs	Conc. mM	Germination and Growth parameters									
		Germination %					Dry wt. gm.				
		Salinity Stress (mM)					Salinity Stress (mM)				
		0	50	100	150	175	0	50	100	150	175
Chitosan	2	97.00a	94.37a	91.90a	90.00a	88.23a	1.186a	1.099a	1.094a	1.090a	1.081a
Ch/Cu	1.4	95.40b	93.33b	90.63c	88.67d	86.38c	1.161b	1.077d	1.093b	1.081b	1.066b
Fe_3O_4	0.1	94.58d	92.88d	89.94d	89.39b	87.38b	1.155d	1.097b	1.093b	1.078c	1.062b
Fe_3O_4\HA	0.2	94.76c	93.17c	90.95b	89.08c	86.25c	1.158c	1.099a	1.094a	1.081b	0.982c
Con.		94.33e	91.67e	88.00e	84.83e	77.53d	1.153e	1.095c	1.088c	1.066d	1.066b
LSD at 0.05		0.077	0.062	0.095	0.134	0.283	0.001	0.001	0.000	0.001	0.004

Means significant 5% at level of probability

Table 4: The effect of various applied nanoparticles on growth parameters of *S. rebaudiana* plants under salinity stress.

Treatment NPs	Conc. mM	Germination and Growth parameters									
		Shoot length (cm)					Root length (cm)				
		Salinity Stress (mM)					Salinity Stress (mM)				
		0	50	100	150	175	0	50	100	150	175
Chitosan	2	14.80a	12.20c	11.47c	8.80a	6.67b	10.03a	8.60a	8.88a	7.20a	5.23a
Ch/Cu	1.4	13.93b	11.88b	11.64b	8.53b	5.31d	9.26c	7.58c	6.33c	4.82d	3.60c
Fe_3O_4	0.1	12.76c	12.70d	11.29d	8.80a	5.47c	8.67d	7.97b	6.50b	5.47c	3.97b
Fe_3O_4\HA	0.2	12.84c	12.57a	11.95a	8.37e	6.80a	9.77b	8.01b	6.51b	5.86b	3.68c
Con.		12.67d	10.93e	9.73e	6.80d	4.60e	7.03e	6.59d	5.87d	4.40e	2.77d
LSD at 0.05		0.110	0.083	0.102	0.099	0.111	0.115	0.073	0.116	0.106	0.403

Means significant 5% at level of probability

Table 5: Effect of various applied nanoparticles on Photosynthetic pigments in *S. rebaudiana* plants under salinity stress.

Treatments NPs	Conc. mM	Photosynthetic pigments									
		Chl a (mg/g F. Wt)					Chl b (mg/g F.Wt)				
		Salinity Stress (mM)					Salinity Stress (mM)				
		0	50	100	150	175	0	50	100	150	175
Chitosan	2	1.503c	1.417d	1.309c	1.015c	0.962c	1.195d	1.106d	0.988d	0.968d	0.975b
Ch/Cu	1.4	1.513c	1.451c	1.363b	1.173a	0.991b	1.263c	1.160c	1.113a	1.094 a	0.980ab
Fe_3O_4	0.1	1.640b	1.494b	1.364b	1.046b	0.989b	1.276b	1.190b	1.006c	0.993c	0.907c
Fe_3O_4\HA	0.2	1.742a	1.516a	1.415a	1.044b	0.998a	1.329a	1.199a	1.027b	1.005b	0.982a
Con.		1.407d	1.374e	1.220d	1.011c	0.867d	1.183e	1.102e	0.978e	0.943e	0.831d
LSD at 0.05		0.011	0.005	0.006	0.006	0.005	0.005	0.004	0.005	0.005	0.006

Means significant 5% at level of probability

Treatments NPs	Conc. mM	Photosynthetic pigments														
		Chl (a+b)					Chl a/b					Total carotene (mg/g F.Wt)				
		Salinity Stress (mM)					Salinity Stress (mM)					Salinity Stress (mM)				
		0	50	100	150	175	0	50	100	150	175	0	50	100	150	175
Chitosan	2	2.698d	2.523d	2.297d	1.983c	1.937b	1.258c	1.281a	1.325c	1.049c	0.987e	0.450c	0.342c	0.258c	0.221c	0.154d
Ch/Cu	1.4	2.776c	2.611c	2.476a	2.267a	1.971a	1.197d	1.251d	1.225e	1.072a	1.012d	0.447c	0.334d	0.261b	0.224b	0.157c
Fe_3O_4	0.1	2.916b	2.684b	2.370c	2.039b	1.896c	1.285b	1.255c	1.356b	1.053b	1.091a	0.640b	0.450b	0.237d	0.211d	0.195b
Fe_3O_4\HA	0.2	3.071a	2.715a	2.442b	2.048b	1.980a	1.311a	1.264b	1.377a	1.039d	1.017c	0.673a	0.500a	0.322a	0.250a	0.197a
Con.		2.590e	2.476e	2.198e	1.954d	1.698d	1.190e	1.247e	1.247d	1.072a	1.043b	0.358d	0.312e	0.225e	0.174e	0.136e
LSD at 0.05		0.016	0.009	0.014	0.011	0.010	0.004	0.001	0.005	0.001	0.003	0.012	0.007	0.003	0.002	0.002

Table 6: Effect of various applied nanoparticles on Photosynthetic pigments in *S. rebaudiana* plants under salinity stress.

Means significant 5% at level of probability

Table 7: Effect of various applied nanoparticles on Enzymes activity in *S. rebaudiana* plants under salinity stress

Treatments NPs	Conc. mM	Enzymes activity									
		Catalase (U/mg F.W)					Peroxidase (U/mg F.W)				
		Salinity Stress (mM)					Salinity Stress (mM)				
		0	50	100	150	175	0	50	100	150	175
Chitosan	2	43.813b	44.990c	47.980a	48.877a	48.947a	33.733d	36.077	37.837a	39.033a	41.360a
Ch/Cu	1.4	45.527a	46.937a	46.093d	45.947c	47.210d	35.973b	36.599	35.560d	37.600c	39.000c
Fe_3O_4	0.1	42.527c	43.690d	46.797c	46.057c	47.343c	34.357c	37.107	36.500c	38.197b	40.310b
Fe_3O_4\HA	0.2	45.387a	45.480b	47.370b	46.893b	47.453b	36.797a	37.333	36.837a	38.260b	41.247a
Con.		40.690d	42.837e	45.767e	44.897d	45.797e	32.397e	34.390	33.830e	34.930d	35.013d
LSD at 0.05		1.196	0.156	0.089	0.146	0.109	0.207	0.138	0.176	0.186	0.308

Means significant 5% at level of probability

Table 8: Effect of various applied nanoparticles on Enzymes activity in *S. rebaudiana* plants under salinity stress

Treatments NPs	Conc. mM	Enzymes activity									
		Ascorbate oxidase (U/mg F.W)					Polyphenol oxidase (U/mg F.W)				
		Salinity Stress (mM)					Salinity Stress (mM)				
		0	50	100	150	175	0	50	100	150	175
Chitosan	2	62.567d	70.017c	76.810a	77.497a	79.640a	39.487d	42.700c	46.677a	47.817a	50.940a
Ch/Cu	1.4	66.440a	70.560b	75.650c	74.233d	77.870d	40.703c	43.410b	43.5100d	46.237d	48.767d
Fe_3O_4	0.1	63.193c	71.073a	75.983b	74.870c	79.120b	41.300b	44.307a	45.387c	47.333c	49.503c
Fe_3O_4\HA	0.2	65.873b	63.220e	76.057b	75.877b	78.333c	43.190a	44.347a	46.040b	47.667b	49.860b
Con.		60.450e	68.587d	74.393d	71.543e	76.780e	37.553e	39.733d	42.367e	44.317e	46.390e
LSD at 0.05		0.282	0.367	0.101	0.252	0.127	0.181	0.164	0.155	0.126	0.147

Means significant 5% at level of probability

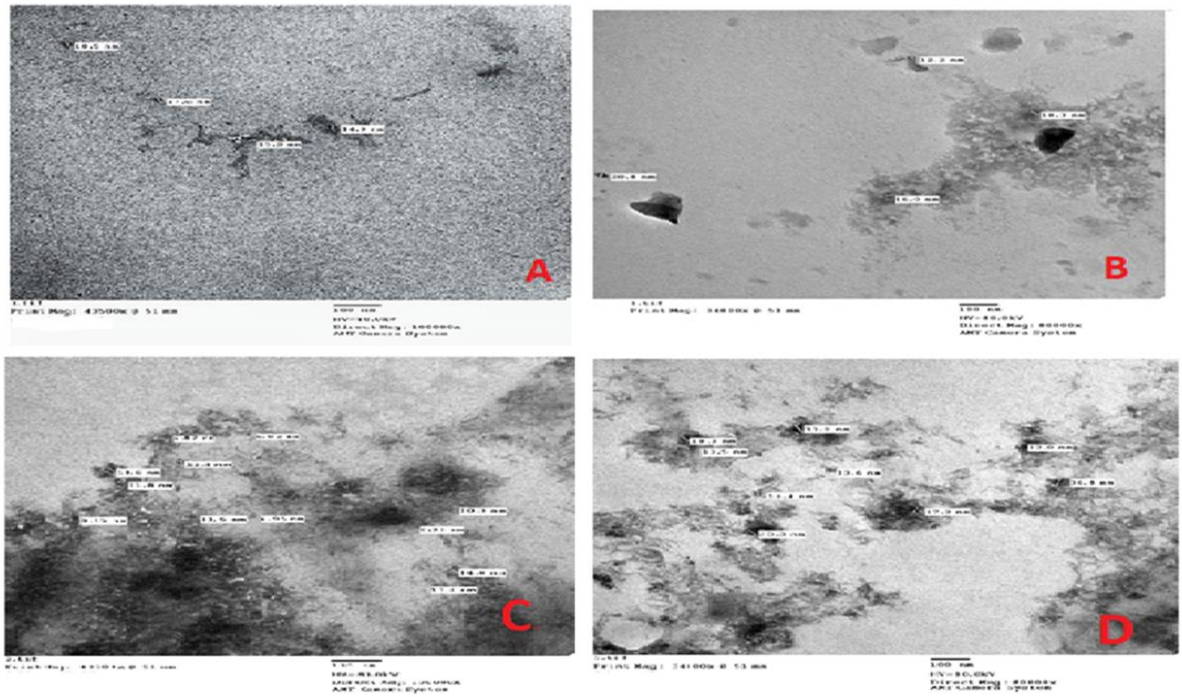

Figure 1: TEM image: (A), Fe_3O_4 NPs; (B) Fe_3O_4/ humic acid NPs; (C), Chitosan NPs and (D), Chitosan\Cu NPs.

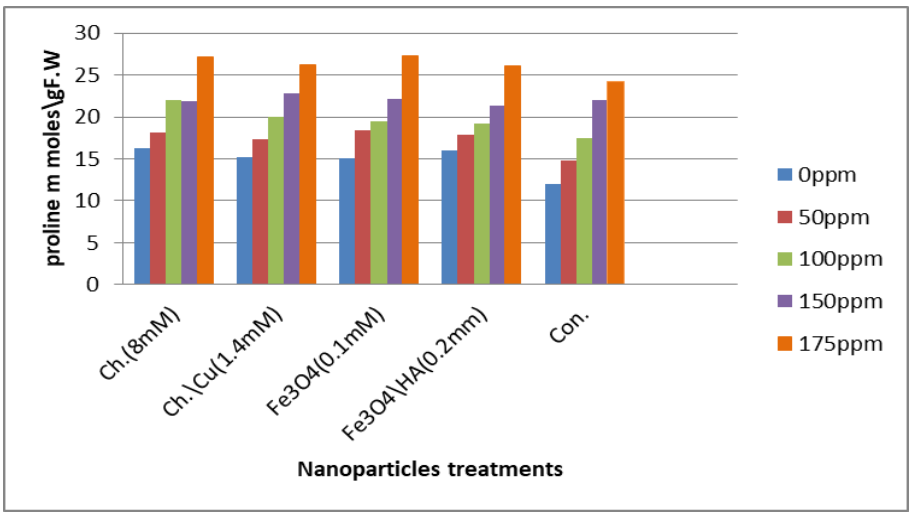

Figure 2: Effect of various applied nanoparticles on Proline in *S. rebaudiana* plants under salinity stress:

تخفيف إجهاد الملوحة في نبات ستيفيا ريبوديانا بإستخدام جسيمات نانوية مختلفة التحضير والتحقيق والتطبيق في المختبر

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الملخص العربي:

ستيفيا ريبوديانا بيرتوني هي مصدر قيم للمحليات الطبيعية ذات الفوائد الصحية والاقتصادية المحتملة، مما يجعلها بديلاً مرغوبًا للسكر التقليدي. ومع ذلك، يمكن أن يؤثر الإجهاد الملحي بشكل كبير على نمو نبات ستيفيا وتطوره وإنتاج جليكوسيد ستيفيول. تهدف هذه الدراسة إلى فحص فعالية الجسيمات النانوية المختلفة (NPs) بما في ذلك الكيتوزان، وFe_3O_4، وFe_3O_4/حمض الهيوميك، والكيتوزان/نحاس، لتخفيف الإجهاد الملحي وتحسين نمو ستيفيا وإنباتها. تم تحديد التركيزات المثلى لتكون 2 ملليمول، و1.4 ملليمول، و0.1 ملليمول، و0.2 ملليمول للكيتوزان، والكيتوزان/نحاس، وFe_3O_4 وFe_3O_4/حمض الهيوميك، على التوالي. لمدة 30 يومًا، تعرضت نباتات ستيفيا لتركيزات مختلفة وتعرضت لمستويات مختلفة من الإجهاد الملحي (0، 50، 100، 150، و 175 ملليمولار كلوريد الصوديوم). تم تقييم المعلمات الفسيولوجية (النمو، الإنبات٪)، والمعلمات الكيميائية الحيوية (أنشطة الإنزيم، محتوى البرولين، الصبغات الضوئية)، ومحتوى ستيفيوسيد الكلي (TS). أظهرت النتائج أن جزيئات النانو الكيتوزان (2 ملليمولار) خففت بشكل فعال من الإجهاد الملوحة وحسنت صحة النبات بشكل عام، مما أدى إلى زيادة محتوى ستيفيوسيد الكلي بشكل كبير. عزز Fe_3O_4/حمض الهيوميك (0.2 ملليمولار) بشكل كبير الكلوروفيل أ، والكلوروفيل ب، والكلوروفيل الكلي (أ + ب)، ونسبة الكلوروفيل أ / ب، والكاروتينويد الكلي. تشير هذه النتائج إلى أن هذه الأسمدة الحيوية النانوية تتمتع بإمكانات كبيرة كنهج مستدام وصديق للبيئة لتحسين إنتاجية ستيفيا في الظروف المالحة، مما قد يساهم في تطوير الممارسات الزراعية الذكية.

الكلمات الاسترشادية : الإجهاد الملحي، جسيمات الكيتوزان النانوية، البرولين، الإنزيمات المضادة للأكسدة، جليكوسيدات ستيفيول.