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Humic acid supplementation improves salt tolerance in chili by modulating morpho-physio-biochemical variables

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Abstract
As agricultural challenges related to soil salinity continue to grow, incorporation of humic acid into cultivation practices could contribute significantly to global food security and sustainable agriculture. This study assessed the effects of different doses of soil applied humic acid (0, 50, 100, and 200 mg/kg w/w soil) on the growth, photosynthetic pigments, and antioxidant potential of chili grown under varying concentrations of salt (0, 50 and 100 mM of NaCl). The results showed that a linear decrease was noticed in the growth, biomass, and photosynthetic pigment attributes of the chili plants by increasing salt level. More reduction was noticed at the higher levels of salt stress as compared to the control, primarily due to lowering of total chlorophyll concentrations and increased sodium accumulation in leaves. Dry biomass significantly increased with humic acid treatments, regardless of the stress level. The reduced enzymatic activities associated with ROS detoxification underscored the pivotal role of humic acid in maintaining redox homeostasis. Optimal use of humic acid (200 mg/kg) showed promising results in comparison with the rest of the treatments in minimizing Na^+ uptake in the chili plants. This study demonstrates that the application of humic acid activates physio-biochemical defense responses against salt stress in chili plants and provides a significant pavement for the cultivation of chili in soils containing moderate levels of salt.

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Introduction

Salt stress, a pervasive and escalating global issue, pertains to the detrimental impact of excessive soil salinity on plant growth, agricultural productivity, and ecosystems (Abeed et al., 2023). It arises primarily from human activities such as improper irrigation practices, excessive use of fertilizers, and climate change-induced sea level rise, resulting in the accumulation of salts in soils (Aroh, 2021). High concentrations of salts in the soil impede water absorption by plants, triggering a water deficit within their tissues (Souri et al., 2021). This water scarcity not only hampers cell expansion and growth, but also diminishes photosynthetic activity, ultimately stifling overall plant productivity (Jia et al., 2002). The enrichment of ions of sodium and chloride, prevalent in saline soils, disturbs ion homeostasis within plant cells. This disrupts vital functions such as nutrient uptake, enzyme activity, and protein synthesis (Wang et al., 2023). Excessive uptake of sodium ions, in particular, can replace essential potassium ions in

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cell membranes, leading to impaired membrane integrity and cell death (Nicolas-Espinosa et al., 2023).

Chili (*Capsicum annuum* L.) is an important crop in Pakistan both for consumption and export. It is commonly known as "red chili" in Pakistan and is a significant ingredient in the country's cuisine (Hamza et al., 2022). Chili pepper cultivation contributes to the livelihoods of many farmers in rural areas. It is often grown as a cash crop due to its demand in both domestic and international markets. Like many crops, chili pepper cultivation in Pakistan faces challenges such as pests, diseases, irrigation with wastewater, and changing climate patterns (Dawid and Grzegorz, 2021). Salt stress affects chili plants through a multifaceted mechanism that involves various physiological, biochemical, and molecular processes (Chakraborti et al., 2022). Excessive salts in the soil create an osmotic imbalance, reducing the availability of water for uptake by chili plant roots. This leads to a water deficit within plant cells, causing reduced cell expansion, growth inhibition, and wilting of leaves (Singh et al., 2022). Additionally, the presence of excess sodium can significantly increase the oxidative stress in plants leading to the generation of reactive oxygen species (ROS) that may cause harm to lipids, proteins, and DNA within cells. Salt stress reduces carbon dioxide assimilation, limiting the plant's ability to produce energy-rich compounds essential for growth and development (Meftahizadeh et al., 2023).

Addition of different types of minerals, nutrients, and organic substances has been extensively employed to enhance the salt tolerance of various crop plants (Farooq et al., 2019; Hussain et al., 2022). The organic amendments such as humic acid (HA) has proved to be a useful part of humic substances and has been shown to play a vital role in enhancing plant tolerance against environmental stresses such as drought and salinity (Ali et al., 2019). Hatami et al. (2018) reported that the application of humic acid is useful to plants as one of the promising priming factors in terms of improving plant development and tolerance to abiotic stresses. Humic acids are complex molecules that are derived from the chemical breakdown of the biological remnants of plant and animal matter in soils by micro-organisms (Khaled et al., 2011) and are soluble at high pH conditions.

There are several positive impacts of humic substances on plant-soil systems such as water retention, high cation exchange capacity, bioavailability of nutrients, and reduction in leaching of cations from the soil (Dinçsoy et al., 2019). Furthermore, humic acids have the potential to activate many essential metabolic processes in plants including respiration, photosynthesis, nutrient uptake, and transcriptional activation (Aydin et al., 2012).

Soil salinity poses a significant threat to chili cultivation, leading to reduced crop yields and compromised plant health. The accumulation of salts in the soil disrupts water and ion uptake, induces osmotic stress, and triggers oxidative damage in chili plants. Conventional mitigation strategies often fall short of providing effective solutions. Hence, there is a need to explore alternative approaches, such as the addition of humic acid, to alleviate salt stress in chili plants and enhance their tolerance to adverse soil conditions. So, keeping in mind the current scenario, this study aimed to 1) evaluate the physiological and morphological changes induced by salt stress in chili plants, including alterations in growth parameters, chlorophyll content, and antioxidative enzyme activities, and 2) analyze how various doses of soil applied humic acid mitigate the negative impacts of salt stress. Overall, this study would help to develop guidelines and recommendations for farmers and growers on the use of humic acid as a viable strategy for enhancing chili plant resilience to salt stress.

Materials and Methods

Study Site, Experimental Design and Treatments

This experimental approach aimed to evaluate the effects of soil-applied humic acid for mitigation of salt stress in chili at the Department of Environmental Sciences, the University of Lahore, Pakistan. For the experimental work, a factorial design was employed within a completely randomized arrangement, with each experimental condition in triplicates.

Total experimental units were: 3 × 4 × 3 = 36. The experimental treatments comprised two factors, salt stress (0, 50, and 100 mM of NaCl) and humic acid treatments viz., 0, 50, 100, and 200 mg/kg of soil *w/w*.

Crop management

The soil used in this experiment work was sampled to a depth of 15 cm from the nearby agricultural field. For humic acid treatments, humic acid was mixed with the soil as 0, 50, 100, and 200 mg kg^{-1} of soil and incubated for 3 weeks, and then filled in the plastic containers at the rate of 3 kg per pot having dimensions (23 cm height and 25 cm top diameter). Five seeds of chili were sown in each pot. After 15 days of sowing, salt stress was applied by dissolving the measured amount of NaCl in distilled water as per desired treatment. After the salt stress application, manual hoeing was done for three days for the complete homogenization of the salt. To meet the nutritional requirements of the plants, Hoagland solution (50 percent) was applied at a rate of 1 L per week per pot. During the experiment, the necessary agronomic practices like weeding and irrigation were carried out on regular basis in accordance with the physical observations and characteristics of plants in pots. A plant set without humic acid and salt treatments was considered as a mock. After harvesting, data on morphological, physiological and biochemical attributes were recorded.

Growth and biomass variables

After three weeks of salt stress application, one plant was randomly selected from each replication and harvested for further analysis. Once the leaf counting was complete, the plant samples were cleaned with distilled water, and the plant's height, as well as the lengths of its shoots, roots, leaf and leaf width were measured. The leaf area of a plant was determined by multiplying the length and width of the chili plants. The stem diameter was also measured. After harvesting, the fresh weight was recorded and then the dry biomass of roots, shoots, and plants was measured by keeping the plant material in an oven (~65 °C) until achieving the constant dry weight.

Photosynthetic pigments

For the purpose of extracting pigments, a crushed leaf sample (about 5 g) was taken in a test tube that contained 85% acetone (v/v) and then left for 24 hours. The sample then underwent a 10-minute centrifugation at (4000 × *g*) and 4 °C. The chlorophyll contents and the carotenoid contents of the supernatant were determined using a spectrophotometer (Halo DB-20/DB-20S; Dynamica Scientific, Newport Pagnell, UK) at wavelengths of 470, 647, and 664.5 nm, in accordance with the Lichtenthaler's protocols (1987). The total chlorophyll content was measured by adding the chlorophyll a and b.

Enzymatic antioxidants

Fresh chili leaves (1.0 g) were extracted in 50 mM phosphate buffer (pH 7.8), homogenized, and centrifuged at 15,000 *g* for 10 minutes to determine the enzyme activities. Measurements of the activities of peroxidase (POD), catalase (CAT), or superoxide dismutase (SOD) were done in accordance with the methods published by Velikova et al. (2000), Aebi (1974), and Beauchamp and Fridovich (1971), respectively.

Ionic status in plant leaves

Chili leaf samples were air-dried first and then oven-dried (65 °C), ground, and kept in flasks by adding 10 mL sulfuric acid in each flask. Subsequently, about 2 mL of hydrogen peroxide (H_2O_2) were added, and the mixture underwent digestion within the temperature range of 300 to 350 °C. This was followed by a dilution step using distilled water. After filtration, the concentration of sodium (Na^+) ion was measured using a flame photometer (Chapman et al. 1961; Wolf, 1982). Chloride in the leaf tissues was determined using a standard protocol.

Data analysis

Statistical analysis of data was performed by applying Fisher’s ANOVA. Means of all treatments were compared by the Tukey’s highest significant differences (HSD) test using Statistix 8.1 software. Correlation and principal component analysis (PCA) were worked out using the Minitab (Version 18.1) software (Minitab Inc., State College, US) and data was visualized using RStudio, an open-source software.

Results

Growth variables

Induced salt stress caused a significant reduction in plant height (21.15% and 54.08%), root length (26.67% and 56.15%), shoot length (21.32% and 55.03%), number of leaves (25.77% and 32.68%), leaf length (17.15% and 41.52%), leaf width (23.02% and 35.47%), leaf area (34.89% and 61.98%) and stem diameter (12.83% and 25.23%) at 50 and 100 mM NaCl, respectively, in chili plants. Whereas, soil applied humic acid enhanced growth attributes both in control and NaCl treatments (**Figure 1**). Maximum growth attributes were observed where 200 mg kg^{-1} of humic acid was applied both to control and salt stressed plants.

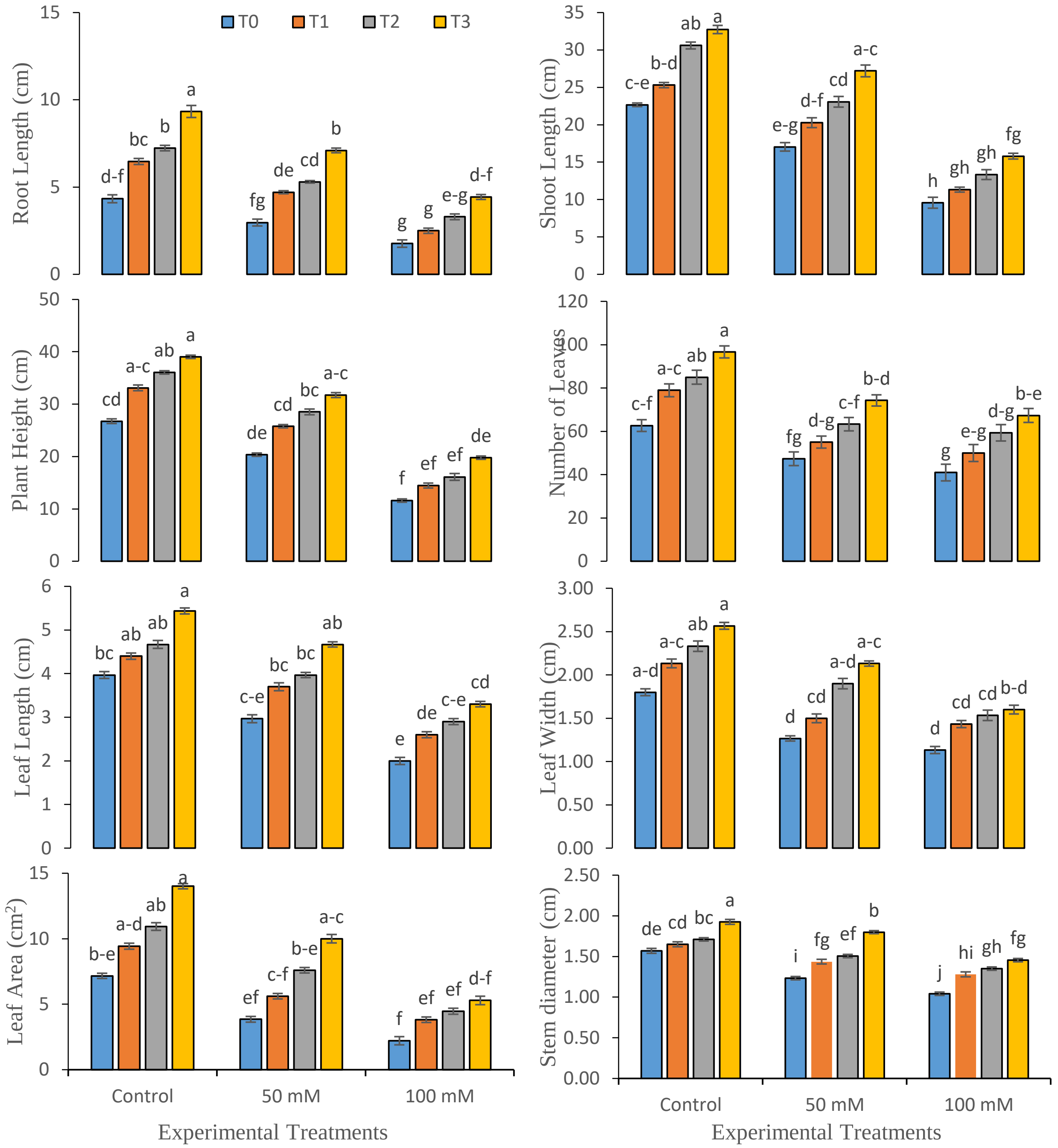


Figure 1. Effect of varying concentrations of soil applied humic acid on the growth variables of chili plants grown under salt stress. The lowercase letters in the bar graphs show significant variations across treatment means at *P* < 0.05. T_0 = Control; T_1 = Humic acid (50 mg kg^{-1} of soil *w/w*); T_2 = Humic acid (100 mg kg^{-1} of soil *w/w*); T_3 = Humic acid (200 mg kg^{-1} of soil *w/w*)

Fresh and dry biomass

Data analysis showed that the application of humic acid significantly enhanced the fresh and dry biomass of chili shoots and roots, and improvement in other plant parts (**Figure 2**). Escalation of humic acid dosage progressively enhanced the fresh and dry biomass of shoots and roots particularly under T_3 treatment both in salt regimes and under control conditions. However, T_2 treatment of humic acid slightly elevated the fresh and dry biomass as compared to that under T_1. The salt-affected plants exhibited decreased root fresh weight (16.06 and 23.45%), shoot fresh weight (14.32% and 41.42%), plant fresh weight (14.46% and 39.97%), root dry weight (41.60% and 60.63%), shoot dry weight (16.47% and 40.72%), and plant dry weight (18.77% and 42.54%) at 50 and 100 mM, respectively.

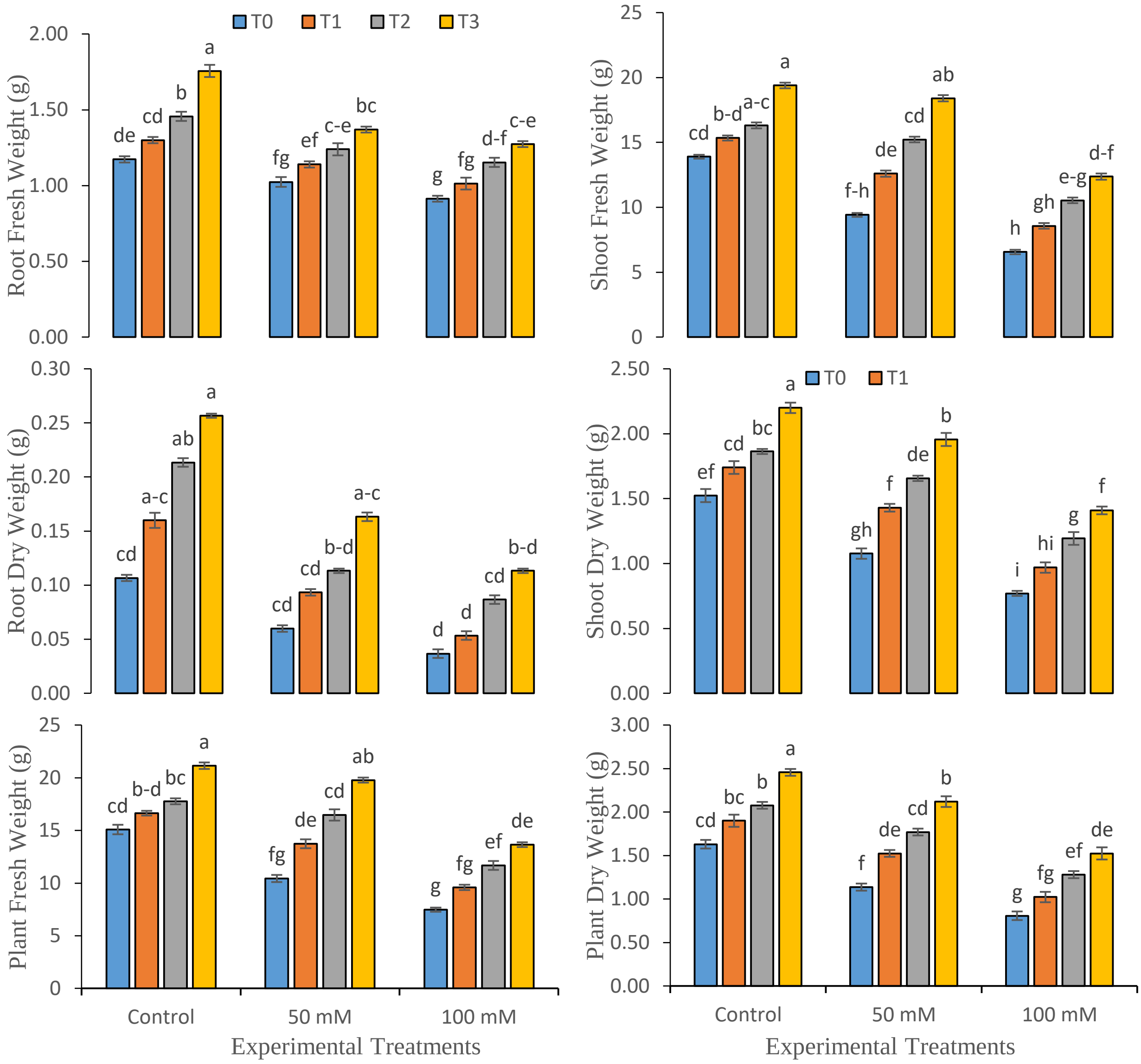


Figure 2. Effect of varying doses of soil applied humic acid on biomass (fresh and dry) of chili plants grown under salt stress. The lowercase letters in the bar graphs show significant variations across treatment means at *P* < 0.05. T_0 = Control; T_1 = Humic acid (50 mg kg^{-1} of soil *w/w*); T_2 = Humic acid (100 mg kg^{-1} of soil *w/w*); T_3 = Humic acid (200 mg kg^{-1} of soil *w/w*)

Photosynthetic pigments

The NaCl-induced toxicity reduced chlorophyll *a* (22.56% and 36.51%), chlorophyll *b* (12.84% and 39.83%), total chlorophyll contents (19.44% and 38.77%), and carotenoid contents (26.89% and 54.44%), at 50 and 100 mM, respectively as compared to the controls (no salt stress). A maximum of all the photosynthetic attributes was observed under T_3 where application of humic acid was done at the rate of 200 mg kg^{-1} of soil in control and salt stress conditions (**Figure 3**).

Enzymatic antioxidants

The activity of enzymatic antioxidants in chili plants was altered by the application of humic acid under induced salt stress (**Figure 4**). Increased NaCl stress from 50 to 100 mM caused increased activities of SOD (32.21% to 53.89%), POD (162.92% to 348.31%), and CAT (78.57% to 165.87%) significantly. In terms of the activities of antioxidant enzymes, salt stress was shown to have a decreasing pattern of 100 mM > 50 mM > control conditions, whereas humic acid treatments were observed to have a decreasing pattern of $T_0 > T_1 > T_2 > T_3$.

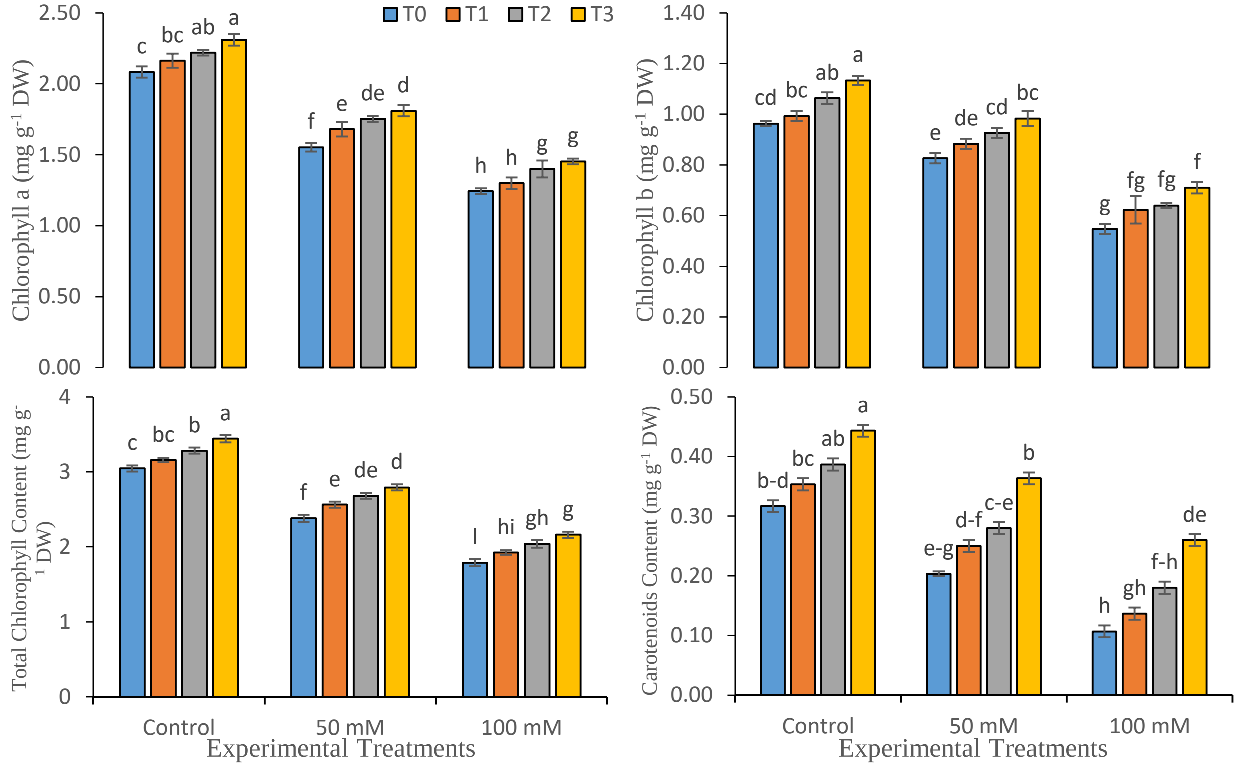
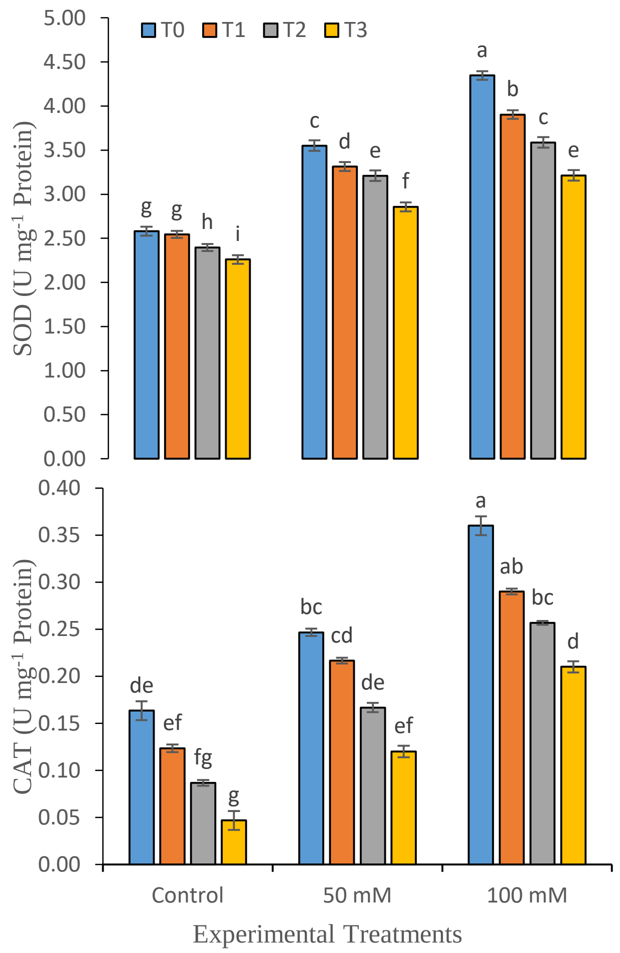


Figure 3. Effect of varying doses of soil applied humic acid on the photosynthetic pigments of chili plants grown under salt stress. The lowercase letters in the bar graphs show significant variations across treatment means at *P* < 0.05. T_0 = Control; T_1 = Humic acid (50 mg kg^{-1} of soil *w/w*); T_2 = Humic acid (100 mg kg^{-1} of soil *w/w*); T_3 = Humic acid (200 mg kg^{-1} of soil *w/w*)



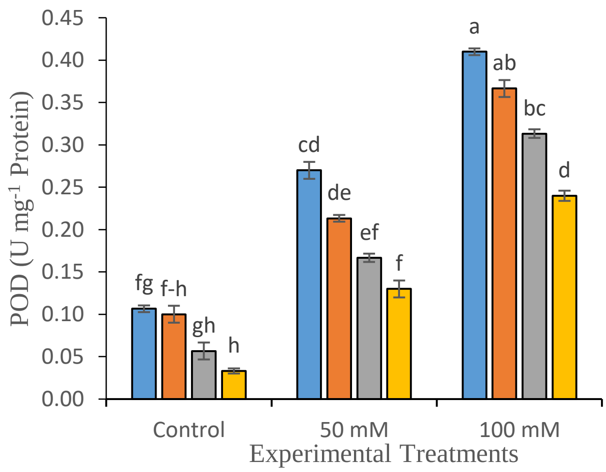


Figure 4. Effect of different doses of soil applied humic acid on the enzymatic antioxidants of chili plants grown under salt stress. The lowercase letters in the bar graphs show significant variations across treatment means at *P* < 0.05. T_0 = Control; T_1 = Humic acid (50 mg kg^{-1} of soil *w/w*); T_2 = Humic acid (100 mg kg^{-1} of soil *w/w*); T_3 = Humic acid (200 mg kg^{-1} of soil *w/w*)

Ionic status in plant leaves

Induced NaCl stress and varying treatments of soil applied humic acid significantly ($P \leq 0.01$) affected the leaf Na^+ and Cl^- accumulation in chili plants grown under salt stress as well as normal non-saline conditions. Salt stress increased the Na^+ contents as compared to that in the control (normal conditions). The maximum leaf Na^+ contents (0.97 and 1.66 mmol g^{-1}) were observed in plants receiving no addition of humic acid. Similarly, for Cl^- contents the opposite was observed (**Figure 5**).

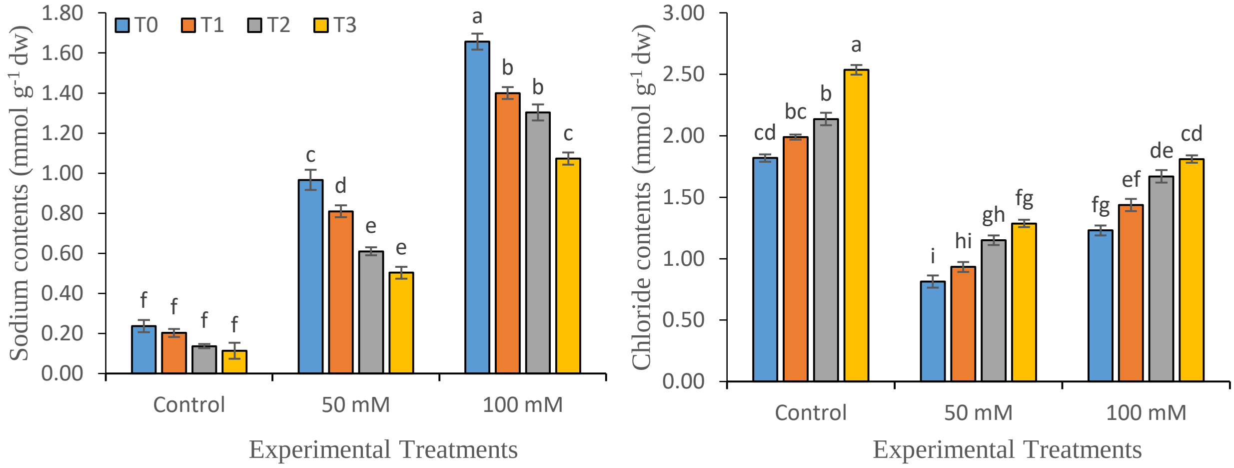


Figure 5. Effect of different doses of soil applied humic acid on the ionic status in the leaves of chili plants grown under salt stress. The lowercase letters in the bar graphs show significant variations across treatment means at $P < 0.05$. T_0 = Control; T_1 = Humic acid (50 mg kg^{-1} of soil *w/w*); T_2 = Humic acid (100 mg kg^{-1} of soil *w/w*); T_3 = Humic acid (200 mg kg^{-1} of soil *w/w*)

Pearson correlation matrix

Growth, enzymatic, biomass, and ion accumulation variables of chili plants were connected with one another in order to support the veracity of the conclusions drawn (**Figure 6**). All the enzymatic attributes (i.e., SOD, CAT, and POD) were correlated negatively to chlorophyll contents, growth, and biomass. However, a significant positive correlation was noticed for Na^+ and Cl^- contents. The POD, Na^+ and CAT showed a non-significant association with SOD.

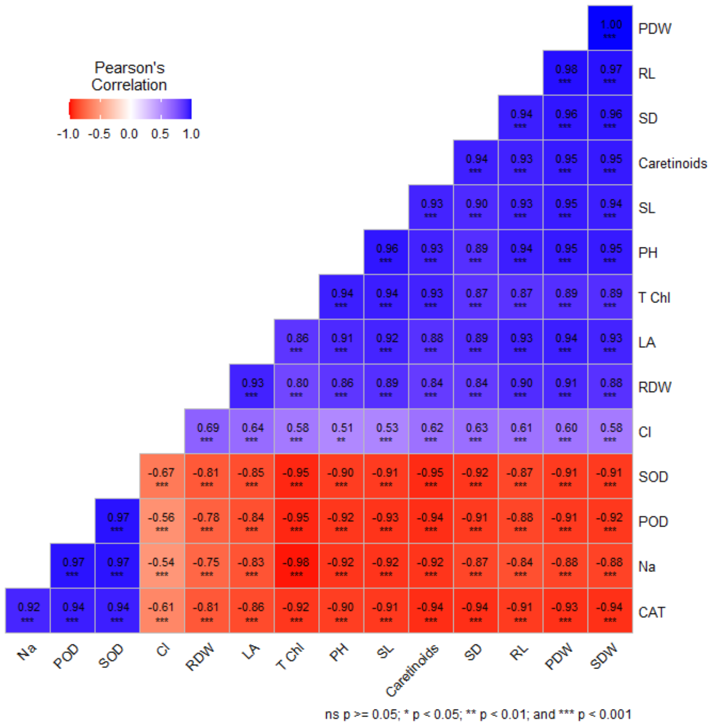


Figure 6. Correlation matrix of various attributes of chili plants by different doses (0, 50, 100, and 200 mg/kg of soil) of soil applied humic acid under salt stress. RL = Root length; SL = Shoot length; RDW = Root dry weight; SDW = Shoot dry weight; PDW = Plant dry weight; TCHL = Total chlorophyll contents; LA = Leaf area; SD = Stem diameter; SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; Na = Sodium contents; Cl = Chloride contents

Principal component analysis

Chili plants cultivated on NaCl-contaminated soil with soil applied humic acid treatments were shown to have a connection between ionic balance, growth, biomass, and photosynthetic variables using loading plots shown as straight lines in the principal component analysis (**Figure 7**). The first two principal components, PC1 and PC2, add up the largest proportion of all components and account for more than 88.3% of the measured data, whereas PC2 makes up 4.6% of it. The first set of variables shows that PC1 has a positive correlation with the growth, biomass, and photosynthetic attributes of chili plants. The placement of measured parameters in the quadrants helps identify the grouping and clusters based on associations among variables. The factors related to SOD, CAT, and POD are slightly negative with Na^+ contents. The factors related to growth parameters, leaf fresh weight, root fresh weight, and leaf dry weight were found to have a positive relationship with PC1 variables.

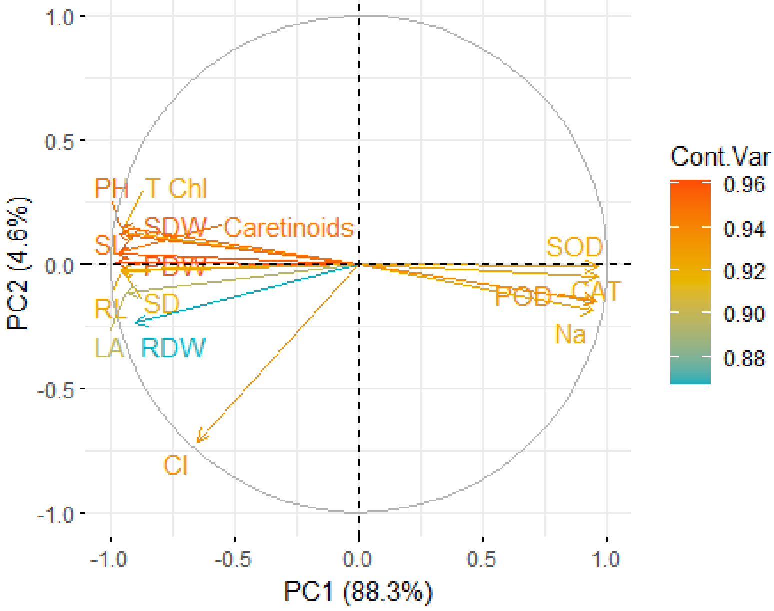


Figure 7. Principal component analysis (PCA) of different attributes of chili by varying doses (0, 50, 100, and 200 mg/kg of soil) of soil applied humic acid under salt stress. RL = Root length; SL = Shoot length; RDW = Root dry weight; SDW = Shoot dry weight; PDW = Plant dry weight; TCHL = Total chlorophyll contents; LA = Leaf area; SD = Stem diameter; SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; Na = Sodium levels; Cl = Chloride levels.

Discussion

In the context of chili plants subjected to salt stress, humic acid played a multifaceted and pivotal role in enhancing growth and overall physiological processes. When chili plants were exposed to high salinity levels, overall growth was hampered perhaps due to osmotic stress, ion toxicity, and disrupted nutrient uptake (Akladious and Mohamed, 2018). It has been observed that humic acid intervenes by promoting root development through its humic and fulvic acid components, which facilitate the formation of a more extensive root system (Kaya et al., 2018; Al-Falahi et al., 2022); this might have been the reason for the better root growth and biomass attributes of the chili plants under control and salt stressed conditions. This expanded root system in terms of growth and biomass not only aids in anchoring the plant securely in the soil but also enhances the plant's capacity to explore a larger soil volume for water and nutrients (Ali et al., 2019). Humic acid's chelation properties are particularly significant under salt stress conditions, since it forms complexes with metal ions, including sodium and chloride, thereby reducing their accumulation within the plant tissues (Van and Di, 2022). This chelation action mitigates ion toxicity, which is a common consequence of salt stress. Additionally, humic acid fosters better nutrient uptake by improving the cation exchange capacity of the soil, allowing plants to absorb essential nutrients like potassium, calcium, and magnesium more efficiently (Bacilio et al., 2016).

Salt stress often hampers chlorophyll synthesis and damages chloroplast structures, leading to reduced photosynthetic efficiency (El-Sarkassy et al., 2017). Similar, observations were also noticed in the current study that salt stress reduced the chlorophyll and carotenoid contents. The possible reason for the rise in chlorophyll levels in the chili plants is most likely the result of the enhancement of root growth and development. Similar observations were also noted by Akladious and Mohamed (2018) who stated that a well-developed root system facilitates better water and nutrient uptake, maintaining the necessary hydration and nutrient supply to the leaves. This sustained nutrient flow to the leaves, in turn, promotes chlorophyll synthesis and the overall green pigmentation of the plant (Elkhatib et al., 2021). By reducing the accumulation of excess sodium ions in the plant tissues, humic acid helps maintain the integrity of chloroplast membranes and thylakoid structures (Abou-Sreea et al., 2021). This prevents the disruption of the electron transport chain and the photosystem reactions, preserving the functionality of the chlorophyll molecules and ensuring efficient light absorption and energy conversion (Ahmed et al., 2013).

The application of humic acid showed a significant effect on the modulation of enzymatic antioxidants in the chili plants under salt stress (**Figure 4**). Humic acid can directly enhance the activity of enzymatic antioxidants. It may do so by interacting with enzyme molecules, stabilizing their structures, and promoting their proper folding (Jan et al., 2020). This interaction can increase the enzymes' catalytic efficiency, enabling them to more effectively neutralize ROS. For example, humic acid can enhance the binding affinity of SOD for superoxide radicals, accelerating their conversion into hydrogen peroxide (Salih et al., 2022). Similarly, enzymatic antioxidants often require metal ions as cofactors to function optimally (Akladious and Mohamed, 2018). Humic acid's chelation properties can help in delivering essential metals like copper, zinc, and manganese to the enzymes. These metal ions are crucial for the catalytic activity of SOD, POD, and CAT. By ensuring the availability of these cofactors, humic acid indirectly supports the enzymatic antioxidant systems under stressed conditions (Abdelkhalik et al., 2023).

Humic acid plays a vital role in limiting the uptake of sodium ions (Na^+) by plants under salt stress conditions. Excessive accumulation of Na^+ can disrupt cellular functions and hinder plant growth (**Figure 5**). Limiting the uptake of Na^+ under salt stress might have been due to the input of humic acid content in enhancing soil structure and water-holding capacity (Mishra et al., 2021). This enhanced water retention helps maintain a more favorable soil-water balance, which in turn affects ion movement in the soil environment and prevailing conditions (Barzegar et al., 2016; Rouphael et al., 2018). By optimizing soil moisture, humic acid indirectly reduces the mobility of sodium ions, making them less available for uptake by plant roots (Ondrasek et al., 2022). It can influence the expression of genes related to ion transport and ion channel activity (Forotaghe et al., 2021). This regulation can help maintain a balanced ratio of essential ions (like potassium) to sodium ions, preventing excessive sodium accumulation within plant tissues. High levels of Na^+ can lead to osmotic stress in plant cells (Jampílek and Kráľová, 2019; Amoanimaa-Dede et al., 2022). Optimal use of humic acid aids in osmotic adjustment by enhancing the synthesis and buildup of organic solutes such as proline and sugars (Akladious and Mohamed, 2018). These solutes help maintain turgor pressure and prevent water loss from cells, reducing the impact of osmotic stress caused by sodium ions (Rahbari et al., 2021). However, it is vital to acknowledge that the beneficial uses of humic acid may change depending on several factors such as application methods, concentrations, and environmental conditions. Further research is needed to fine-tune its application protocols and better understand its mechanisms at the molecular level.

Conclusion

Humic acid treated chili plants performed better than the control plants under salt stress in terms of enzymatic activity, photosynthetic pigment levels, and biomass (dry and fresh). In order to increase the activity of antioxidant enzymes in leaves and provide plant nutrition, it is advised to apply humic acid to chili plants under salt-stressed conditions. Overall, this study provides a compelling evidence that humic acid holds a significant strategic tool for alleviating salt stress in chili plants. Humic acid showed a significant role in promoting plant growth, limiting Na^+ uptake, and enhancing antioxidant defense mechanisms collectively contributing to an integrated approach for enhancing plant tolerance to salt stress. These findings have implications not only for sustainable agriculture, but also for the broader goals of environmental remediation and food safety in regions affected by salt contamination. Additionally, integration of the application of humic acid with various soil and irrigation strategies can amplify the tolerance of chili crops to withstand salt induced stress. However, before suggesting this as a viable commercial option, it is crucial to consider economic aspects and viability.

Author(s), Editor(s) and Publisher's declarations

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Conflict of interest

The authors declare no conflict of interest.

Source of funding
Declared none

Contribution of authors
Conceptualization and designing the study: QZ. Conduction of experiment: MZ and KF. Data collection: MZ, KF, KA, and AA. Visualization and interpretation: KS and KA. Proofreading and approval of the final version: QZ, NK and KS.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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Declaration of Generative AI and AI-assisted technologies in the writing process
It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

References

Abdelkhalik, A., El-Mageed, A., Taia, A., Mohamed, I.A., Semida, W.M., Al-Elwany, O.A. Gyushi, M.A. (2023). Soil application of effective microorganisms and nitrogen alleviates salt stress in hot pepper (*Capsicum annuum* L.) plants. *Frontiers in Plant Science* 13:1079260.

Abeed, A.H., AL-Huqail, A.A., Albalawi, S., Alghamdi, S.A., Ali, B., Alghanem, S.M., El-Mahdy, M.T. (2023). Calcium nanoparticles mitigate severe salt stress in *Solanum lycopersicum* by instigating the antioxidant defense system and renovating the protein profile. *South African Journal of Botany* 161:36-52.

Abou-Sreea, A.I., Azzam, C.R., Al-Taweel, S.K., Abdel-Aziz, R.M., Belal, H.E., Rady, M.M., Khaled, K.A. (2021). Natural biostimulant attenuates salinity stress effects in chili pepper by remodeling antioxidant, ion, and phytohormone balances, and augments gene expression. *Plants* 10(11):2316.

Aebi, H. (1974). Catalase. *In* "Methods of Enzymatic Analysis (Second Edition)" (H.U. Bergmeyer, ed.). 2:673-684. Academic press.

Ahanger, M.A., Tomar, N.S., Tittal, M., Argal, S., Agarwal, R. (2017). Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiology and Molecular Biology of Plants* 23:731-744.

Ahmed, A.H., Darwish, E.S.A.H., Hamoda, S.A.F., Alobaidy, M.G. (2013). Effect of putrescine and humic acid on growth, yield and chemical composition of cotton plants grown under saline soil conditions. *American-Eurasian Journal of Agriculture and Environmental Sciences* 13(4):479-497.

Akladious, S.A. Mohamed, H.I. (2018). Ameliorative effects of calcium nitrate and humic acid on the growth, yield component and biochemical attribute of pepper (*Capsicum annuum*) plants grown under salt stress. *Scientia Horticulturae* 236:244-250.

Al-Falahi, M.N., Al-Dulaimi, K.H., Ghani, E.T.A., Al-Taey, D.K., Farhan, K.J. (2022). Effect of humic acids and the amount of mineral fertilizer on some characteristics of saline soil, growth and yield of broccoli plant under salt stress conditions. *Journal of Agricultural Science* 1:11–20.

Ali A.Y.A., Ibrahim, M.E.H., Zhou, G., Nimir, N.E.A., Jiao, X., Zhu, G., Elsiddig, A.M.I., Zhi, W., Chen, X., Lu, H. (2019). Ameliorative effects of jasmonic acid and humic acid on antioxidant enzymes and salt tolerance of forage sorghum under salinity conditions. *Agronomy Journal* 111:3099–3108.

Amoanimaa-Dede, H., Su, C., Yeboah, A., Zhou, H., Zheng, D., Zhu, H. (2022). Growth regulators promote soybean productivity: a review. *Peer J* 10:e12556.

Aroh, J.E.K. (2021). Soil fertility status and sugarcane growth performance in the mangrove ecosystem of Nigeria. *In* "Sustainable Intensification for Agroecosystem Services and Management", (M.K. Jhariya, A. Banerjee, R.S. Meena, S. Kumar, A. Raj eds.). Springer, Singapore. https://doi.org/10.1007/978-981-16-3207-5_16

Aydin, A., Kant, C., Turan, M. (2012). Humic acid application alleviates salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane leakage. *African Journal of Agricultural Research* 7:1073–1086.

Bacilio, M., Moreno, M., Bashan, Y. (2016). Mitigation of negative effects of progressive soil salinity gradients by application of humic acids and inoculation with *Pseudomonas stutzeri* in a salt-tolerant and a salt-susceptible pepper. *Applied Soil Ecology* 107:394-404.

Barzegar, T., Moradi, P., Nikbakht, J., Ghahremani, Z. (2016). Physiological response of okra cv. Kano to foliar application of putrescine and humic acid under water deficit stress. *International Journal of Horticultural Science and Technology* 3(2):187-197.

Beauchamp, C., Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44(1):276-287.

Chakraborti, S., Bera, K., Sadhukhan, S., Dutta, P. (2022). Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical and molecular mechanisms. *Plant Stress* 3:100052.

Chapman, H.D., Pratt, P.F. (1961). "Method of Analysis for Soil, Plants and Waters", University of California, Division Agriculture Science: Berkeley, CA, USA.

Dawid, M., Grzegorz, K. (2021). Microwave-assisted hydrotropic pretreatment as a new and highly efficient way to cellulosic ethanol production from maize distillery stillage. *Applied Microbiology and Biotechnology* 105:3381-3392.

Dinçsoy, M., Sönmez, F. (2019). The effect of potassium and humic acid applications on yield and nutrient contents of wheat (*Triticum aestivum* L. var. Delfii) with the same soil properties. *Journal of Plant Nutrition* 33:2757–2772.

Elkhatib, H., Gabr, S.M., Elazomy, A.A. (2021). Salt stress relief and growth-promoting effect of sweet pepper plants (*Capsicum annuum* L.) by glutathione, selenium, and humic acid application. *Alexandria Science Exchange Journal* 42(3):583-608.

El-Sarkassy, N.M., Ibrahim, S.A., Desoky, E.M. (2017). Salinity stress amelioration using humic acid and mycorrhizae on pepper plants. *Zagazig Journal of Agricultural Research* 44(6):2515-2527.

Farooq M.A., Saqib Z.A., Akhtar J., Bakhat H.F., Pasala, R.K., Dietz, K.J. (2019). Protective role of silicon (Si) against combined stress of salinity and boron (B) toxicity by improving antioxidant enzymes activity in rice. *Silicon* 11:2193–2197.

Forotaghe, Z.A., Souri, M.K., Jahromi, M.G., Torkashvand, A.M. (2021). Physiological and biochemical responses of onion plants to deficit irrigation and humic acid application. *Open Agriculture* 6(1):728-737.

Hamza, M., Sehar, N., Khan, S.A.M.M., Saddozai, A.A., Mumtaz, A. (2022). Assessment of fungal flora and total aflatoxins in spices sold at retail outlets of two districts of Hazara division, KPK, Pakistan. *Pure and Applied Biology* 11(4):975-985.

Hatami, E., Shokouhian, A.A., Ghanbari, A.R., Naseri, L.A. (2018). Alleviating salt stress in almond rootstocks using of humic acid. *Scientia Horticulturae* 237:296–302.

Hussain, S., Shah, M.A., Khan, A., Ahmad, A.M., Hussain, F.M. (2020). Potassium enhanced grain zinc accumulation in wheat grown on a calcareous saline-sodic soil. *Pakistan Journal of Botany* 52:69–74.

Jampílek, J., Kráľová, K. (2019). Beneficial effects of metal- and metalloid-based nanoparticles on crop production. *In* "Nanotechnology for Agriculture", (D. Panpatte, Y. Jhala eds.). Springer, Singapore. https://doi.org/10.1007/978-981-32-9370-0_11

Jan, J.A., Nabi, G., Khan, M., Ahmad, S., Shah, P.S., Hussain, S. (2020). Foliar application of humic acid improves growth and yield of chilli (*Capsicum annuum* L.) varieties. *Pakistan Journal of Agricultural Research* 33(3):461.

Jia, W., Wang, Y., Zhang, S., Zhang, J. (2002). Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. *Journal of Experimental Botany* 53(378):2201-2206.

Kaya, C., Akram, N.A., Ashraf, M., Sonmez, O. (2018). Exogenous application of humic acid mitigates salinity stress in maize (*Zea mays* L.) plants by improving some key physico-biochemical attributes. *Cereal Research Communications* 46(1):67-78.

Khaled, H., Fawy, H.A. (2011). Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil Water Research* 6:21–29.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids pigments of photosynthetic biomembranes. *Methods in Enzymology* 148:350-382.

Meftahizadeh, H., Baath, G.S., Saini, R.K., Falakian, M., Hatami, M. (2023). Melatonin-mediated alleviation of soil salinity stress by modulation of redox reactions and phytochemical status in guar (*Cyamopsis tetragonoloba* L.). *Journal of Plant Growth Regulation* 42:4851-4869.

Mishra, P., Mishra, J., Arora, N.K. (2021). Plant growth promoting bacteria for combating salinity stress in plants–Recent developments and prospects: A review. *Microbiological Research* 252:126861.

Nicolas-Espinosa, J., Garcia-Ibañez, P., Lopez-Zaplana, A., Yepes-Molina, L., Albaladejo-Marico, L., Carvajal, M. (2023). Confronting secondary metabolites with water uptake and transport in plants under abiotic stress. *International Journal of Molecular Sciences* 24(3):2826.

Ondrasek, G., Rathod, S., Manohara, K.K., Gireesh, C., Anantha, M.S., Sakhare, A.S., Horvatinec, J. (2022). Salt stress in plants and mitigation approaches. *Plants* 11(6):717.

Rahbari, A., Sinaki, J.M., Damavandi, A., Rezvan, S. (2021). Castor bean (*Ricinus communis* L.) responses to drought stress and foliar application of Zn-nano fertilizer and humic acid: grain yield, oil content, antioxidant activity, and photosynthetic pigments. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 49(4):12003-12003.

Rouphael, Y., Kyriacou, M.C., Petropoulos, S.A., De Pascale, S., Colla, G. (2018). Improving vegetable quality in controlled environments. *Scientia Horticulturae* 234:275-289.

Salih, E.G.I., Wu, Q., Zhou, G., Zhu, G., Zhou, C., Muddathir, A.M., Ahmad, I. (2022). Ameliorative effects of seed treated with phytohormones on seedling growth, soluble protein content and antioxidant enzymes of hargel (*Solenostemma argel* Hayne) seedlings under salt stress. *Chilean Journal of Agricultural Research* 82(4):575-585.

Singh, V.K., Pandey, S., Verma, N., Singh, M., Pandey, J., Prasad, S.M. (2022). Cereals and phytohormones under salt stress. *In* “Sustainable Remedies for Abiotic Stress in Cereals” (A.A.H. Abdel Latef, ed.). pp. 291-311. Springer Nature, Singapore. doi.org/10.1007/978-981-19-5121-3_12

Souri, Z., Khanna, K., Karimi, N., Ahmad, P. (2021). Silicon and plants: current knowledge and future prospects. *Journal of Plant Growth Regulation* 40:906-925.

Van, T.T.H., Di, H.B. (2022). The effect of humic and salicylic acid on improving salt tolerance of yellow hot chili (*Capsicum annuum* L.). *JERAMI Indonesian Journal of Crop Science* 4(2):52-57.

Velikova, V., Yordanov, I., Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science* 151(1):59-66.

Wang, L., Qin, L., Sun, X., Zhao, S., Yu, L., Chen, S., Wang, M. (2023). Salt stress-induced changes in soil metabolites promote cadmium transport into wheat tissues. *Journal of Environmental Sciences* 127:577-588.

Wolf, B. (1982). A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Communication in Soil Science and Plant Analysis* 13:1035–1059.