

Efficiency of seed priming and co-treatment strategies in salt effect mitigation using *Nicotiana glauca* leaf extract on tomato seedlings (*Solanum lycopersicum* L.)

H. Maaroufi-Dguimi^{1,*}, S. Gamal Mohammed¹, A. Nasraoui Hajaji² and H. Abdalgadir¹

¹Al-Baha University, Faculty of Science, Department of Biology, SA 65779-7738 Alaqiq, Saudi Arabia

²University of Carthage, National Research Institute in Rural Engineering, Forest Ecology Laboratory, Water and Forestry Bp 10, TN 2080 Ariana, Tunisia

*Correspondence: houda_maaroufi@yahoo.fr

Received: December 18th, 2024; Accepted: March 31st, 2025; Published: April 3rd, 2025

Abstract. The Al-Baha region suffers from soil salinity, negatively impacting agriculture. Current study examined aqueous extracts from *Nicotiana glauca* as plant-based biostimulants to alleviate salinity's effects and reduce chemical fertilizer use. An aqueous extract of *N. glauca*, applied in ascending doses of 20%, 50%, and 100%, was being used. This biostimulant was applied using two methods: seed priming and co-treatment on the seeds of *Solanum lycopersicum* L. Results indicated that all tested doses, whether applied through seed priming or co-treatment, enhanced the final germination percentage (FGP) and reduced the mean germination time (MGT) under saline conditions. With priming, the 20% dose was most effective in reducing salt treatment effects on FGP, improving it by 5% compared to untreated salt-stressed seeds. In co-treatment, the 100% dose showed a 4.5% reduction in FGP decline referring to untreated and stressed seeds. For priming treatments, the 20% dose reduced MGT by 22%, while the 50% dose in co-treatment reduced it by 28% compared to untreated and stressed seeds. The priming strategy used in this study did not result in significant enhancements in growth parameters, particularly in the fresh weight (FW) and dry weight (DW) of the epicotyls. As priming, the 20% dose had the most significant mitigation effect on epicotyl FW, DW and chlorophyll (Chl) content by 15%, 10% and 30% referring to untreated and stressed seedlings, respectively. However, all tested biostimulant doses used as co-treatment proved effective in mitigating the negative effects of salt on epicotyl and root FW, DW, and Chl content. Seedlings treated with various strategies and doses showed a smaller increase in malondialdehyde (MDA) and proline (Pro) levels under salinity stress. As stress indicators, both Pro and MDA levels were significantly reduced when a co-treatment strategy was applied. Furthermore, the results indicated that the effectiveness of the *N. glauca* aqueous extract in alleviating salt stress could be attributed to its content of several phenolics, flavonoids, and tannins, which possess antioxidant properties that enhance the plant's tolerance against salt-induced oxidative stress.

Key words: antioxidant activity, biostimulant, flavonoids, germination, polyphenolics, tannins, tree tobacco, salinity.

INTRODUCTION

Climate change has affected enormous agriculture areas worldwide through soil salinization (Eswar et al., 2021). Farmers have implemented a variety of strategies to address agricultural production challenges in saline soils, particularly through the use of chemical fertilizers (Sharma & Chetani 2017). With the tremendous use of the chemical fertilizer, the soil physicochemical parameters have been seriously affected reducing soil fertility. To address this challenge, a range of natural substances has been employed as biostimulants (Pascual et al., 2021; Kumari et al., 2022; Chanthini et al., 2024; Sarov et al., 2024).

The use of plant-based biostimulants to mitigate salinity can be regarded as an environment friendly and sustainable way of fighting abiotic stress, as it contains no synthetic chemicals. Chernikova et al. (2021) reported that biostimulant effectiveness depend strongly on type of soils. Depending upon the plant species and plant part used to prepare biostimulants, it may contain various amounts of bioactive compounds such as flavonoids, phenolics, tannins, glycosides, phytohormones, mineral elements and photosynthetic pigments. Several bioactive components had a potential antioxidant capacity; therefore, it's deduced that they might also be effective in plants against salinity as it disrupts redox balance in plants. This has been demonstrated in previous studies that plant-based biostimulants contribute to a better growth, development, yield, disease and stress-resistance in plants given the presence of aforementioned compounds (Howladar, 2014; Drobek et al., 2019; Desoky et al., 2020; Zulfiqar et al., 2020). Polyphenols, flavonoids, and tannins are the largest groups of specialized metabolites found in plants, and they are widely recognized for their role in salt stress protection (Kiani et al., 2021). This study suggests that applying external *N. glauca* extract containing polyphenols and flavonoids may help alleviate the effects of salt stress on tomato plant. Nevertheless, sources of biostimulants, methods of application, doses and their implications against salinity should be investigated.

Tomato production is vital in Saudi Arabia, playing a crucial role in ensuring food security, supporting the agricultural sector, and contributing to the economy. However, tomato crops face significant challenges due to soil salinity (Alshami et al., 2023). On the other hand, *Nicotiana glauca*, commonly known as tree tobacco, is an invasive species in Saudi Arabia that presents both ecological and agricultural challenges (Alharthi et al., 2021). Despite its invasive nature, tree tobacco is rich in bioactive compounds, including polyphenolics, flavonoids, and tannins, which contribute to its antioxidant properties (Alghamdi et al., 2021). Recent studies have highlighted its potential as a source of natural antioxidants, showing that extracts from *N. glauca* possess significant free radical scavenging activity. Research by Khedr et al., 2024 indicated that the leaves of *N. glauca* have a high phenolic content, suggesting its potential for use in pharmaceutical and agricultural applications. Jmii et al. (2022) demonstrated that *N. glauca* is abundant in allelochemicals, which may be utilized to manage the invasive weed *Cynodon dactylon* and enhance tomato growth.

The objective of current study was to investigate the biochemical characteristics of the tree tobacco. Specifically, the analysis of the contents of polyphenols, flavonoids, and tannins in the aqueous extract of *N. glauca*, as well as to examine its effects on mitigating salt stress in tomato seedlings using two strategies of biostimulant application with ascending doses.

MATERIALS AND METHODS

Plant materials used as bio-stimulants

Collection of plant material Fresh *Nicotiana glauca* leaves (fronds) were picked at random from the Al-Baha area in the southeastern of Saudi Arabia. Fifteen middle leaves were collected in the morning (7:00–10:00 am) from thirty-five different shrub samples (1.7–2 m). The fresh collected leaves were cleaned, shade dried, then pulverized with a blender and kept in airtight bottles.

Aqueous extract preparation (used as biostimulant). Fifty gram of leaf powder was soaked in 100 mL of distilled water. The suspension was shaken for 24 hours at room temperature (25 °C). Then, it was centrifuged at 3,000 X g for 30 min at 4 °C. Then, the extract was filtered using double layers of Whatman filter paper No. 1. The leaf extract was kept in a refrigerator at 4 °C. The aqueous extract was diluted with distilled water to give 20, 50 and 100% solutions.

Determination of total phenolic compounds. The total phenolic content was determined using the Folin–Ciocalteu method as described by (Attard, 2013). Briefly, the procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1:10) in a 96-well microplate. Then, 80 µL of 1M Na₂CO₃ was added and incubated at room temperature (25 °C) for 20 min in the dark. At the end of incubation time, the resulting blue complex color was measured at 630 nm. A gallic acid stock solution of 2 mg mL⁻¹ in methanol was used as standard, and the following dilutions were prepared: 1,000, 500, 250, 125, 62.5, and 31.25 µg mL⁻¹. Data are represented as means ± SD.

Determination of total flavonoids. The total flavonoid content was determined using the aluminum chloride method as described by (Kiranmai et al., 2011), with minor modifications to be carried out in microplates. Briefly, 15 µL of sample/standard was placed in a 96-well microplate, then, 175 µL of methanol was added followed by 30 µL of 1.25% AlCl₃. Finally, 30 µL of 0.125 M C₂H₃NaO₂ was added and incubated for 5 min. At the end of incubation, the resulting yellow color was measured at 420 nm. A stock solution of standard Rutin was prepared at 2,000 µg mL⁻¹ in methanol, from which the following dilutions were prepared: 1,000, 500, 250, 125, and 62.5 µg mL⁻¹. Data are represented as means ± SD.

Determination of total tannins. The total tannin content was determined using the method of Ojha et al., 2018 with minor modifications to be carried out in microplates. Briefly, 20 µL of sample/standard was placed in a 96-well microplate, then, 20 µL of 1% FeCl₃ was added followed by 20 µL of 1% K₄[Fe (CN)₆]. Finally, the volume was completed to 200 µL with distilled water and the mixture was incubated for 5 min. At the end of incubation, the resulting yellow color was measured at 720 nm. A stock solution of standard tannic acid was prepared at 2,000 µg mL⁻¹ in distilled water, from which the following dilutions were prepared: 1.5625, 3.125, 6.25, 12.5, and 25 µg mL⁻¹. Data are represented as means ± SD.

Determination of antioxidant activity of *N. glauca* extract by DPPH assay. *N. glauca* leaf powder was dissolved in distilled water with final concentrations of 15.625, 31.25, 62.5, 125 and 250 µg mL⁻¹. A stock solution of 1,000 µg mL⁻¹ concentration of Trolox was prepared in methanol from which 5 concentrations were prepared

including 3.125, 6.25, 12.5, 18.75 and 25 $\mu\text{g mL}^{-1}$. DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) free radical assay was carried out according to the method of Boly et al., 2016 adopting the modifications of Elkholy et al., 2023. Briefly, 100 μL of freshly prepared DPPH reagent (0.1% in methanol) were added to 100 μL of the sample in 96 wells plate ($n = 3$), the reaction was incubated at room temperature for 30 min in the dark. At the end of incubation, the resulting reduction in DPPH color intensity was measured at 540 nm. Data are represented as means \pm SD according to the following equation: percentage inhibition = ((Average absorbance of blank-average absorbance of the test)/ (Average absorbance of blank)) *100). Data were normalized using Microsoft Excel and the IC50 value was calculated using Graph pad Prism 9., the concentrations were converted into logarithmic values, and a non-linear inhibitor regression equation: (log (inhibitor) vs. normalized response - variable slope equation) was selected.

Qualitative HPLC analysis of polyphenolics and flavonoids. A Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector was used for the qualitative analysis of polyphenolics and flavonoids. The mobile phase consisted of a gradient mixture of 0.1% orthophosphoric acid and methanol (gradient method). The column used was an Intersil C₁₈ (5 μm , 4.6 \times 250 mm) maintained at a temperature of 25 °C. The flow rate was set at 1 mL min^{-1} , and the detection wavelength was 280 nm. A volume was transferred from a stock solution containing 10 standards (Gallic acid, Rutin, Chlorogenic acid, Catechin, Ellagic acid, Quercetin, Kampeferol, Apigenin, Hesperidin, and Caffeic acid) with conc 1 mg mL^{-1} for each standard, to obtain solutions with conc. of 10 $\mu\text{g mL}^{-1}$ then inject 20 μL . About 20 mg from each extract sample were weighted individually, then they were dissolved in 1 mL water then the solution was vortex followed by sonicated for 20 min. The solution was filtrated through a 0.45 μm PTFE syringe filter and then injected into the HPLC system.

Plant material and experimental design

For the current study, the seeds used are GRObite Desi Tomato Vegetable Seeds, which are indian non-hybrid seeds with a 99% germination rate and drought tolerance. Seeds were first sterilized in a 0.25% sodium hypochlorite solution. After sterilization, an aqueous extract of *N. glauca*, was applied at various concentrations as part of the priming process. The primed seeds were divided into three groups based on the bio-stimulant concentrations: 20%, 50%, and 100%. Priming was carried out for 48 hours with gentle shaking (Moaaz Ali et al., 2020). Following this, the seeds were sown in greenhouse trays filled with a 1:1 mixture of commercial peat and vermiculite. They were subjected to two irrigation conditions: normal conditions using distilled water and salt-stressed conditions using a 100 mM NaCl solution.

For the co-treatment strategy, sterilized seeds directly were sown in the same conditions and irrigated with the corresponding biostimulant concentrations three times per week. For irrigation, 10 mL of distilled water and a salt solution were used for control and salt-treated primed seeds, respectively. For co-treatment, the same volume was used for each biostimulant concentration and a salt solution for control and salt-treated seeds, respectively. The co-treatment involved irrigating the seeds with the biostimulant three times a week. For both strategies, salt treatment was initiated since the beginning of seed sowing.

Two controls are designed: Negative control means no stressed and no primed seeds; positive control means no primed and salt stressed seeds.

Two seeds were sown in each cell of tray with dimension 54D × 28W × 8.7H centimeters (20 cells / tray). Plants were grown in a growth chamber with 26 °C/70% relative humidity and the photoperiod was 16 h daily. For each treatment, 80 seeds were used. The treatments were replicated three times.

After 3 weeks, seedlings were sorted by epicotyl and radicle for determination of fresh weight (FW), dry weight (DW), photosynthetic pigments, malondialdehyde (MDA) and proline content.

Germination parameters. Seeds were considered germinated when the radicle and epicotyl emerged.

According Wu et al., 2019, we determined Final germination percentage (FGP).

$$1. \text{FGP} = \text{NGS}/\text{NTS} \cdot 100$$

Also mean germination time (MGT)

$$2. \text{MGT} = \Sigma (\text{N}_1\text{T}_1 + \text{N}_2\text{T}_2 + \dots + \text{N}_i\text{T}_i) / \Sigma (\text{N}_1 + \text{N}_2 \dots + \text{N}_i)$$

Abbreviations used in above equations were:

NGS: number of final germinated seeds (in the end of germination: 6 days); NTS: number of total tested seeds; N_i : number of seeds germinated in the i^{th} time; T_i : time taken for seed germination at i^{th} .

Photosynthetic pigments. The method used to determine leaf chlorophyll content was that of Arnon (1949). UV spectrophotometer (PG instrument T60 UV V United Kingdom) was used to measure the absorbance of each sample at 663 nm and 645 nm. Using the formula provided by MacKinney (1941), the chlorophyll content was computed and expressed in mg per g FW.

$$\text{Total Chlorophylls (mg L}^{-1}\text{)} = 20.2 \cdot A_{645} + 8.02 \cdot A_{663}$$

A represents the extract's absorbance at the specified wavelength.

Proline content. The leaf proline content was determined using the method described by Bates et al., 1972. Sulpho-salicylic acid is used to extract proline from dry matter samples. A spectrophotometric measurement at 520 nm is performed based on the complexation of proline with Ninhydrin.

Malondialdehyde (MDA) content. The determination of leaf MDA content was carried out utilizing the Thiobarbituric Acid-Reacting Substances (TBARS) method described by Heath & Packer in 1968. MDA was extracted using trichloroacetic acid (TCA: 10%). The MDA concentration in epicotyl samples was measured as nmol g⁻¹ FW.

Statistical analysis. The data depicted in the figures are the average of six replicates per treatment with means ± confidence limits at alpha = 0.05 level. To compare and determine significant differences between means, at probability level ≤ 0.05, ANOVA analysis and Tukey's HSD tests were used. SPSS software version 20.1 (IBM version 20.0.2004) was used.

RESULTS AND DISCUSSION

Biostimulant effects on germination parameters

In the absence of *N. glauca*-based biostimulant, salinity reduced the final germination percentage (FGP) (Fig. 1, A). Under normal conditions, *N. glauca* extract used in seed priming or as co-treatment had no noticeable effect on FGP. Under saline conditions, all tested doses of the biostimulant improved the FGP. For seed priming, a

20% concentration was the most effective, reducing the FGP decline by 5% compared to untreated and stressed seeds. In the case of co-treated seeds, the 50% and 100% extract doses reduced the FGP decline by 3.6% and 4.5%, respectively, compared to untreated stressed seeds.

Fig. 1, B illustrates the effects of salinity and different doses of biostimulant on mean germination time (MGT). The results indicate that MGT increases with salinity level, suggesting that seed germination occurs at a slower rate. Under normal conditions, the *N. glauca* extract did not have a significant impact, whether used as a priming method or as a co-treatment. However, in saline conditions, all tested doses of the plant extract reduced MGT compared to untreated and stressed seeds, particularly when used as a co-treatment. In priming treatments, a 20% dose was most effective in reducing MGT when compared to untreated and stressed seeds (22%). In co-treatment, a 50% dose was most effective, showing a reduction of 28% relative to untreated and stressed seeds.

In the absence of biostimulant, salinity reduced the FGP and increased MGT (Fig. 1, A and Fig. 1, B). Salt stress adversely affects seed germination and seedling growth through osmotic stress, ion toxicity, and oxidative stress. High salinity decreased germination stimulants like gibberellins, increased abscisic acid level, and altered cell membrane permeability and water behavior within the seed, which can impede germination (Uçarlı, 2020). Moreover, *Rosmarinus officinalis* L. (rosemary) and *Artemisia* L. (wormwood) leaf extracts have been shown to improve seed germination in maize under salinity conditions. This improvement is attributed to the enhancement of the antioxidant system and the maintenance of higher photosynthetic efficiency during salt stress (Panuccio et al., 2018). Additionally, the use of moringa leaf extract for priming wheat seeds demonstrated positive effects on

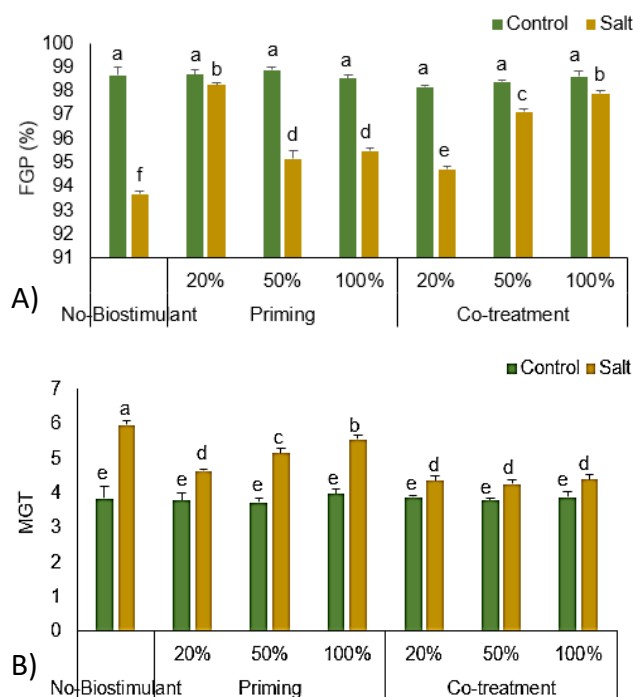


Figure 1. Effect of different concentration of *N. glauca* aqueous extract (20%, 50% and 100%) on Final germination percentage (FGP) (A) and mean germination time (MGT) (B) in normal and salt conditions.

Different treatments were applied: (No-Bio-stimulants: seeds sown without *N. glauca* extract in normal and salt conditions, priming: seeds were pre-treated with different concentrations of aqueous extract of *N. glauca* (20%, 50% and 100%) within 48 h, in normal and salt conditions. Co-treatment: seeds were irrigated with different concentrations of aqueous extract of *N. glauca* (20%, 50% and 100%) in normal and salt conditions. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The *Tukey test* reveals no significant difference between bars denoted by identical letters with a 5% probability.

germination and growth, by promoting efficient nutrient uptake while limiting the accumulation of toxic ions and reactive oxygen species (Ahmed et al., 2021).

Previous studies reported too that several seaweed biostimulants and microalgal extracts had an important germination enhancer effect (Supraja et al., 2020; Hussain et al., 2021; Mzibra et al., 2021). The current plant-based biostimulant showed similar effects to those reported with seaweed extract used for seed priming, which improves germination parameters in tomato plants (Di Stasio et al., 2020).

Bio-stimulant effects on growth parameters

In all tested treatments, salt reduced tomato seedling growth (Fig. 2, A; 2, B; 2, C). In normal conditions, all tested doses of plant extract applied as co-treatment had more improvement effect on seedling growth referring to priming treatment (Fig. 2, C).

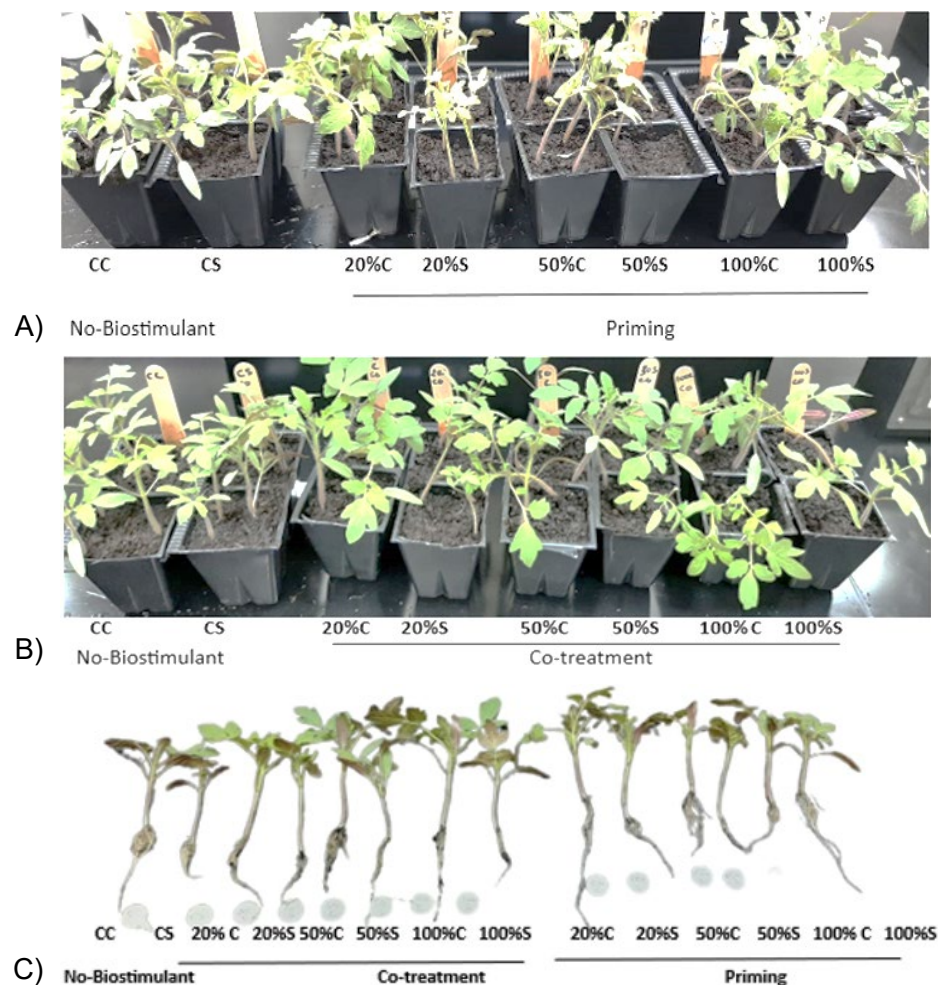


Figure 2. Effect of different concentrations of *N. glauca* extract (20%, 50% and 100%) applied as priming and co-treatment on tomato seedling growth in normal and salt conditions.

(CC: seedlings without extract in normal conditions, CS: seedlings without extract in salt conditions; 20% C and 20%S seedlings treated with 20% of aqueous extract in normal and salt condition as priming and co-treatment; 50% C and 50%S seedlings treated with 50% of aqueous extract in normal and salt condition as priming and co-treatment; 100% C and 100%S seedlings treated with 100% of aqueous extract in normal and salt condition as priming and co-treatment).

Without bio-stimulant, salinity led to a decrease in fresh weight (FW) in the epicotyls (Fig. 3, A). Under normal conditions, co-treatment significantly increased epicotyl FW compared to the control, particularly at the 100% dosage. Seed priming with 20% and 100% plant-based bio-stimulant also improved epicotyl FW, but the effect was less important than that observed with co-treatment.

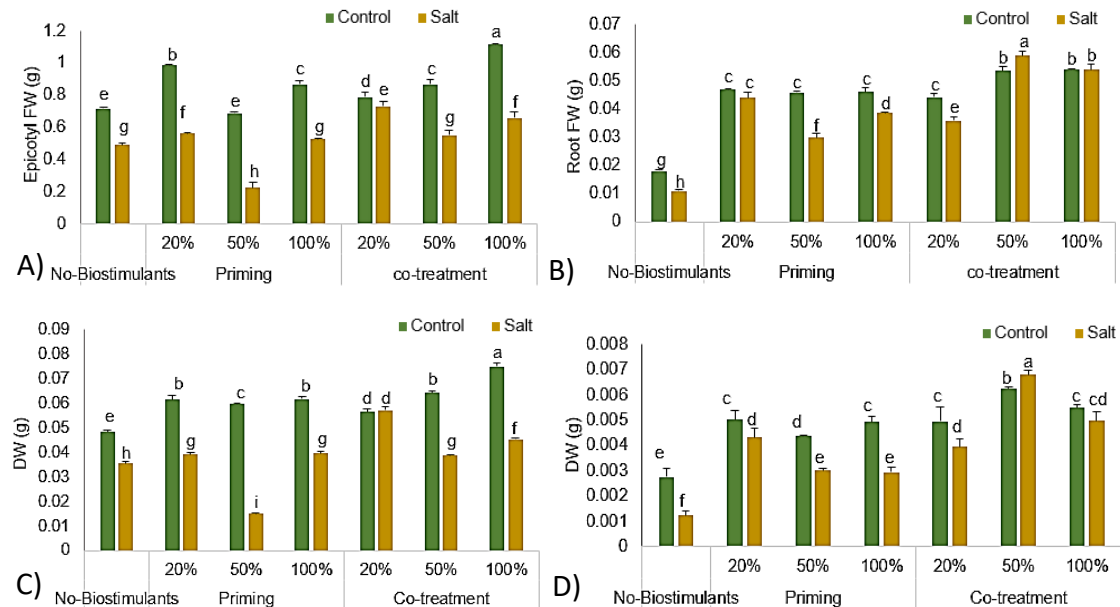


Figure 3. Effect of different concentration of *N. glauca* extract (20%, 50% and 100%) on epicotyl (A) and root (B) fresh weight (FW: g) and epicotyl (C) and root (D) dry weight (DW: g) in normal and salt conditions, as priming and co-treatment.

(CC: seedlings without extract in normal conditions; CS: seedlings without extract in salt conditions; 20% C and 20%S seedlings treated with 20% of aqueous extract in normal and salt condition as priming and co-treatment; 50% C and 50%S seedlings treated with 50% of aqueous extract in normal and salt condition as priming and co-treatment; 100% C and 100%S seedlings treated with 100% of aqueous extract in normal and salt condition as priming and co-treatment). Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The *Tukey test* reveals no significant difference between bars denoted by identical letters with a 5% probability.

Under stress conditions, 20% and 50% doses of plant extract used as a co-treatment significantly increased the fresh weight (FW) of epicotyls. Notably, the 20% dose mitigated the decrease in FW by 49% compared to untreated salt-stressed seedlings. In seedlings issued of primed-seeds, only the 20% dose alleviated the salt-induced reduction in FW (15%), but it was less effective than co-treatment.

In absence of biostimulant, salinity reduced root FW by approximately 40% compared to the control (Fig. 3, B). Under normal conditions, all tested doses used for seed priming or as co-treatments resulted in an increase in root FW, with the 50% and 100% doses as co-treatments showing the most significant effects. In seedlings that were seed-primed, salt stress decreased root FW, with the exception of the 20% dose. When using a co-treatment with 100% of the plant extract, salinity did not have a significant impact on root FW. However, with the 50% dose, salinity actually increased root FW (Fig. 3, B). Overall, under saline conditions, all tested doses helped alleviate the negative effects of salinity on root FW, when applied as co-treatments.

In all examined doses, salinity decreased the epicotyl DW (Fig. 3, C), particularly in untreated stressed seedlings and those derived from seeds that had been primed at 50% extract. Under normal conditions, plant extracts used for seed priming or as co-treatments increased epicotyl DW compared to the control, with the 50% and 100% doses for co-treatment and the 20% dose for seed priming showing the most significant effects. In seedlings that had been seed-primed, salt further reduced epicotyl DW, especially in those from 50% primed seeds (Fig. 3, C). The co-treatment of stressed seedlings was more effective in minimizing the salt decline of epicotyl DW, particularly at the 20% dose.

In roots, in normal conditions, all tested doses used for seed priming or as co-treatment increased DW (Fig. 3, D). Under salinity, seed priming with all tested doses mitigated the salt-induced decrease of root DW. The co-treatment of stressed seedlings with different extract doses alleviated too the salt-induced decrease in root DW but more effectively compared to seed priming effect.

Chlorophyll content generally correlates positively with photosynthetic capacity and biomass accumulation. A strong relationship has been established between the maximum carboxylation rate in leaves and chlorophyll content across various plant species (Qian et al., 2021). Among the factors influencing the net photosynthetic rate, chlorophyll (Chl) content (particularly Chl b) and stomatal conductance are crucial (Tang et al., 2022). An increase in Chl b content indicates a larger light-harvesting antenna size, thereby enhancing photosynthesis (Tanaka & Tanaka, 2011; Jia et al., 2016). Therefore, studying chlorophyll content is essential for assessing the effects of salinity and the biostimulant alleviation effects.

Under normal conditions, both the 50% and 100% extract doses used in co-treatment resulted in a slight increase in Chl content, with an increase of approximately 19% (Fig. 4). The seed priming method led to a 10% increase in Chl content when using a 20% dose. In the absence of plant extract, salt stress reduced Chl content by 45%. However, when comparing untreated seedlings under salt stress to those treated with various extract doses, all treatments, except for the 50% dose used in the priming method, effectively mitigated the negative impact of salt on Chl content. In co-treated, salt-stressed seedlings, Chl content increased by 96% for the 50% dose and by 76% for the 100% dose. These

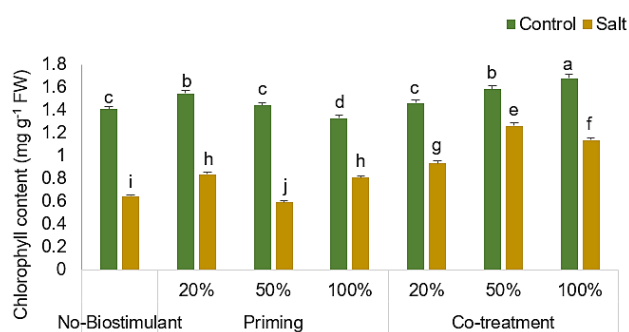


Figure 4. Effect of different concentration of *N. glauca* extract (20%, 50% and 100%) on leaf chlorophyll content (Chl: mg g⁻¹ FW) in normal and salt conditions as priming and co-treatment.

(CC: seedlings without extract in normal conditions, CS: seedlings without extract in salt conditions; 20% C and 20%S seedlings treated with 20% of aqueous extract in normal and salt condition as priming and co-treatment; 50% C and 50%S seedlings treated with 50% of aqueous extract in normal and salt condition as priming and co-treatment; 100% C and 100%S seedlings treated with 100% of aqueous extract in normal and salt condition as priming and co-treatment). Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The *Tukey test* reveals no significant difference between bars denoted by identical letters with a 5% probability.

results demonstrate that all tested doses in the co-treatment method have the potential to mitigate the salt effect on chlorophyll content, as well as on FW and DW.

Several studies reported that salinity affected tomato growth in all stages of plant development (Roşca et al., 2023). Studies done on tomatoes highlighted a negative influence of salinity stress on the physiological parameters such as photosynthetic rate, nitrogen assimilation, transpiration, stomatal conductance, chlorophyll content and mineral uptake (Maeda et al., 2020; Kapadia et al., 2021; Ors et al., 2021; Sassine et al., 2022; Ghorbani et al., 2023).

In literature, several biostimulant types with various doses and method applications were used. Seed priming with *Moringa oleifera* extract, *Garcinia mangostana* and *Cupressus macrocarpa* induced an important salt effect mitigation in *Helianthus annuus*, *Phaseolus vulgaris* and *Cucurbita pepo* (Taha, 2016; Suryaman et al., 2021; El-Sayed et al., 2022). Seed priming induced salt effect mitigation by improving growth parameters photosynthetic capacity, rubisco activities, antioxidant enzyme activities and proline level under salinity (Kapadia et al., 2021).

Current results demonstrated that co-treatment method have more important potential to mitigate the salt adverse effect on tomato growth parameters. Exogenous administration of plant extract boosted the content of photosynthetic pigments during salt stress due to it's abundant in chlorophyll and carotenoids (xanthin, beta-carotene, alpha-carotene, and lutein), which have antioxidant effects (Hasan & sultana, 2018). Sassine et al., reported too, that lithovit-urea and bioumik used as biostimulants, improved salt tolerance in tomato (Sassine et al., 2022).

Recently, Sheteiwy et al. (2021) reported that both seed priming method and foliar application with jasmonic acid showed efficiency in protect early seedlings and alleviate salt stress damage in seedlings in soybean. Similarly, other studies affirmed the effectiveness of both methods in salt stress alleviation (Akram et al., 2020; El-Hawary et al., 2023). Priming seeds enhanced seed imbibition capacity and pre-germinative metabolic processes, leading to improved seedling emergence, growth, vigor, productivity, and adaptability under saline conditions (Biswas et al., 2023). Additionally, seed priming has been shown to reduce the adverse effects of salt-induced oxidative stress on plant biomass and grain yield in wheat cultivars (Ali et al., 2017). The co-treatment helps protect plants against the harmful effects of salinity by enhancing growth, relative water content, membrane stability index, photosynthetic pigment content, soluble sugars, proline, and essential nutrients such as nitrogen (N), phosphorus (P), potassium (K⁺), calcium (Ca²⁺), as well as hormones like indole-3-acetic acid, gibberellic acid, and cytokinins. It also improves the K⁺/Na⁺ and Ca²⁺/Na⁺ ratios and increases the activities of antioxidant enzymes like superoxide dismutase, catalase, and peroxidase, along with contents of glutathione and ascorbate (Rady et al., 2019). Some studies suggest that co-treatment or foliar application is more effective in improving physiological and biochemical parameters, such as ion homeostasis, osmotic adjustment, and antioxidant defense. In contrast, others argue that seed priming is the superior method (Rady et al., 2018; Dustgeer et al., 2021). These conflicting findings indicate that the most effective application method may depend on plant extract type, plant extract concentration, salinity level, and the specific context (Vali et al., 2024). This highlighted the necessity for a more thorough understanding of the underlying mechanisms and their interactions with plant responses under different salinity conditions.

Bio-stimulant effect on plant oxidative status

Malondialdehyde (MDA) is frequently used as a marker for oxidative lipid injury, and its levels fluctuate in response to abiotic stresses (Davey et al., 2005). Under normal conditions, applying different doses of plant extract did not significantly impact the leaf MDA levels (Fig. 5, A). However, in the absence of biostimulant treatment, MDA content doubled under salinity stress. The rise in MDA concentration under salinity was reported in several species such as tomato, in wheat and tunisian squash (Alzahib et al., 2021; Tarchoun et al., 2022; Shahzadi et al., 2024). The increase of MDA content is attributed to increased lipid peroxidation rate caused by salt-induced oxidative stress (Hnilickova et al., 2021).

When exposed to salt stress, all tested doses of *N. glauca* extract effectively reduced the salt-induced increase in MDA levels. Specifically, co-treating seedlings with 50% and 100% doses under salinity conditions resulted in the most significant mitigation of MDA levels. Similar results were revealed when using Moringa leaf extract, Chitosan and seaweed extract to mitigate salt-stress effect in damask rose (Hassan et al., 2020; Elkarmout et al., 2022; Abd-Elkader et al., 2023).

Plants that face specific abiotic stress conditions often produce proline, which serves several important functions: it acts as an active osmolyte, metal chelator, antioxidant, and signaling molecule (Yaish, 2015). Under normal conditions, the application of various doses of biostimulant, whether through seed priming or co-treatment, did not significantly affect leaf proline levels (Fig. 5, B).

However, in the presence of salt stress, Pro levels increased in both control and biostimulant-treated seedlings, with the most significant rise noted in positive control seedlings, which experienced an increase of more than eight-fold. An activation of the defense and adaptation mechanisms associated with increased activity of the antioxidant system, limited the formation of ROS, and suppressed the level of lipid peroxidation.

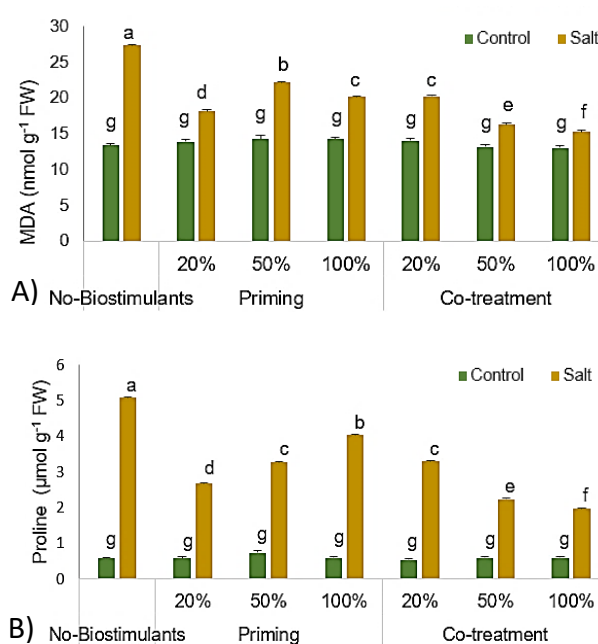


Figure 5. Effect of different concentration of *N. glauca* extract (20%, 50% and 100%) on malondialdehyde (MDA) content (A) and Proline content (B) in leaves of tomato seedlings in normal and salt as priming and co-treatment.

(CC: seedlings without extract in normal conditions, CS: seedlings without extract in salt conditions; 20% C and 20% S seedlings treated with 20% of aqueous extract in normal and salt condition as priming and co-treatment; 50% C and 50% S seedlings treated with 50% of aqueous extract in normal and salt condition as priming and co-treatment; 100% C and 100% S seedlings treated with 100% of aqueous extract in normal and salt condition as priming and co-treatment). Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The *Tukey test* reveals no significant difference between bars denoted by identical letters with a 5% probability.

These mechanisms are manifested by a rapid increase in proline content and osmotic adaptation, which are closely related to salinity tolerance and the ability of the antioxidant system to scavenge free radicals, suppress lipid peroxidation, and promote the accumulation of osmoprotective agents such as proline. Several previous studies reported that proline content was greatly increased in response to the saline environmental conditions (Shin et al., 2020).

Among the seedlings treated with biostimulant, Pro levels tripled in those that received co-treatments with 50% and 100% doses. Therefore, co-treating seedlings with 50% and 100% doses under saline conditions resulted in the most significant reduction of Pro levels referring to positive control.

Several plant-based biostimulants, such as Moringa leaf extract (Howladar, 2014; Rossi et al., 2016; Al-Taisan et al., 2022), have been shown to mitigate the effects of salt stress by reducing ROS damage, as evidenced by lower MDA levels. Previous studies on tomato plants demonstrated that biostimulants significantly reduced salt-induced proline accumulation compared to untreated plants (Zuzunaga-Rosas et al., 2023; Zuzunaga-Rosas et al., 2024). A similar effect was observed with a different amino acid-based biostimulant applied to tomatoes (Gil-Ortiz et al., 2023). These findings are consistent with other research (Cristofano et al., 2023) that utilized plant-based biostimulants on lettuce.

Proline is recognized as an excellent biomarker for stress in many crops (Arteaga et al., 2020; Alvarez et al., 2022). Therefore, it can be concluded that these biostimulants help alleviate stress in plants subjected to high salinity conditions. However, this conclusion is not universally applicable, as other studies have reported the opposite effect, with an increase in proline levels in biostimulant-treated plants compared to untreated controls at the same salt concentration. For instance, lettuce plants treated with various plant protein hydrolysates showed heightened proline contents (Zuluaga et al., 2023), and a seaweed extract applied to tomato plants produced similar results (Gil-Ortiz et al., 2023).

Alghamdi et al. (2021) reported varying amounts of alkaloids, steroids, tannins, flavonoid, and saponins were present in the leaf extract of *N. glauca*. These secondary metabolites had widely reported with potential effect on salt stress mitigation (Ishtiaq et al., 2021).

Phytochemical characterization of leaf aqueous extract of tested plants

The DPPH radical scavenging assay is a common spectrophotometric method used to measure the antioxidant capacity of beverages, foods, and herbal extracts (Gulcin & Alwasel, 2023). Trolox was used as standard for DPPH assay (Fig. 6). Table 1 illustrated the total phenolic, flavonoid, tannin contents and antioxidant activity. The extract of *N. glauca* demonstrated a significant phenolic content of 97.8 $\mu\text{g eq mg}^{-1}$. The aqueous extract also showed the presence of flavonoids and tannins, with concentrations of 21.27 $\mu\text{g RE mg}^{-1}$ and 23.25 $\mu\text{g eq mg}^{-1}$, respectively (Table 1). The results indicated that the *N. glauca* aqueous extract exhibited considerable antioxidant capacity, comparable to various species, such as mistletoe, *Guazuma ulmifolia*, and *Citrus limon* (Rafi et al., 2020; Ehiobu et al., 2021; Khalili et al., 2022). Certain polyphenolic compounds act as scavengers, removing reactive oxygen species (ROS) generated during oxidative bursts. Phenols (ArOH) are recognized for their ability to slow down the oxidation of organic matter by donating a hydrogen atom (from their hydroxyl groups) to

chain-carrying ROO• radicals. This process likely involves a coordinated transfer of hydrogen as a proton along with one electron between the two oxygen atoms, represented as O–H•••O (a proton-coupled electron transfer mechanism) (Foti, 2007).

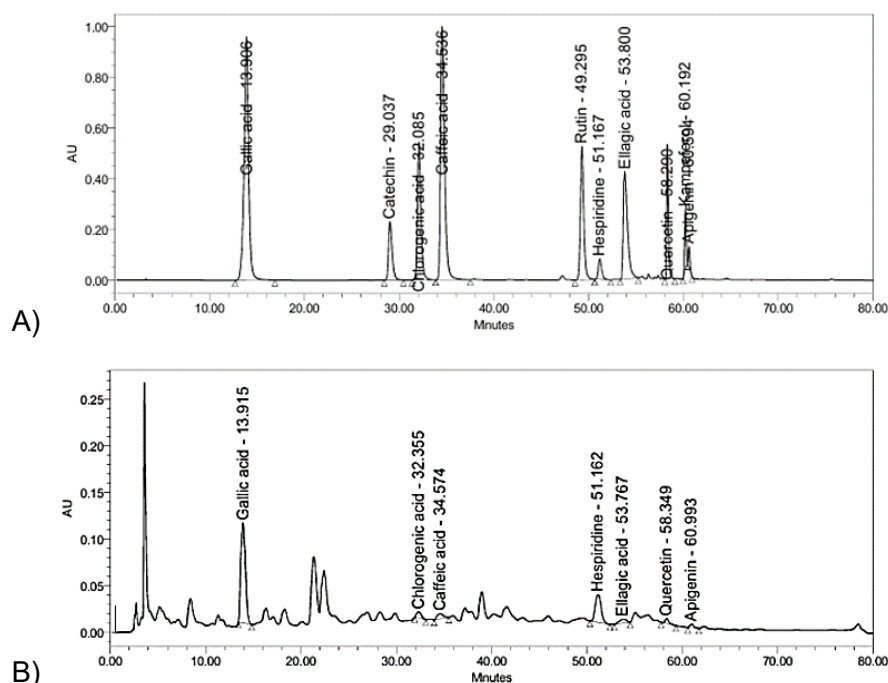


Figure 6. HPLC chromatogram of Mix-standard (A) and *N. glauca* extract (B).

Some of these compounds, such as flavonoids and anthocyanins, have protective properties (Kumar et al., 2023). Polyphenols and flavonoids have been shown to contribute to salt tolerance in various plant species. Kiani et al. (2021) reported that polyphenols play an antioxidant and protective role against oxidative stress induced by salt stress.

Table 1. Total phenolics, total flavonoids, total tannins and Antioxidant Activity (DPPH radical scavenging ($\mu\text{g mL}^{-1}$)) in *N. glauca* aqueous extract. Data are represented as means \pm SD

	Total phenolics ($\mu\text{g eq mg}^{-1}$)	Total flavonoids ($\mu\text{g R E mg}^{-1}$)	Total tannins ($\mu\text{g eq mg}^{-1}$)	DPPH free radical scavenging activity: ($\mu\text{g mL}^{-1}$)
<i>N. glauca</i> (Aqueous extract)	97.8 \pm 9.38	21.27 \pm 1.23	23.25 \pm 2.41	162.5 \pm 1.02

The significant antioxidant activity and the substantial accumulation of phenolic compounds in the leaves suggest the development of defense-oriented strategies to prevent the buildup of intracellular free radicals generated under salt stress (Kiani et al., 2021). Furthermore, the application of a biostimulant based on tannins affects root architecture and enhances salinity tolerance in tomato plants. The modification of root structure, along with the regulation of genes involved in root growth, abiotic stress responses, and nutrient uptake, indicates that the tannin-based biostimulant may play a

crucial role not only in plant nutrition but also in altering morphological traits to improve survival and adaptation to salt stress (Campobenedetto et al., 2021).

For HPLC analysis of aqueous extract, ten standard solutions of phenolics and flavonoids were used (gallic acid, catechin, chlorogenic acid, rutin, ellagic acid, hesperidin, quercetin, kaempferol, apigenin and caffeic acid). The qualitative HPLC analysis of these compounds is summarized in Table 2. The aqueous extract revealed seven components (gallic acid, chlorogenic acid, rutin, ellagic acid, quercetin, apigenin and caffeic acid).

Ozdenefe et al., 2023 reported the presence of antioxidant components in *N. glauca* leaf extract that could protect against the detrimental effects of free radicals. Current result of qualitative HPLC analysis of aqueous extract revealed the presence of several components with antioxidant capacity such as Gallic acid, Chlorogenic acid, Caffeic acid and Ellagic acid (Table 2). This propriety, along with others, have the potential to alleviate the negative effects of salt stress. Current study showed the presence of phenolics, flavonoids and tannins in aqueous extract of *N. glauca* (Table 1). These components play an important role in salt effect mitigation (Ishtiyag et al., 2023). The aqueous extract revealed seven components (Gallic acid, Chlorogenic acid, Rutin, Ellagic acid, Quercetin, Apigenin and Caffeic acid). Interestingly, these components were related to abiotic stress effect mitigation in several studies. Gallic acid, used as priming agent, is reported efficient in mitigating the adverse effects of salt stress on faba bean at the germination stage (Bouazzi et al., 2024). In rice, Gallic acid reversed salt-induced damage through improving osmotic and ionic homeostasis and upregulating the ROS and MG detoxification system (Rahman et al., 2022). Zhang et al., 2021 found that exogenous phenolics, especially chlorogenic acid, helped lettuce withstand

salt stress. Chlorogenic acid mitigate salt adverse effects by triggering significant metabolic changes, enhancing secondary metabolism and the production of small molecules like electron carriers and vitamins and effectively stimulating nitrogen-containing compounds, osmoprotectants, and polyamines. Chlorogenic acid, caffeic acid and apigenin could having a possible role in the cellular defense against oxidative stress (Kianai et al., 2021). Ramzan et al. (2024) reported that using Caffeic acid with had the potential to improve potato growth under salinity stres. Additionally, Muslu, 2024 reported the effectiveness of improving salt stress tolerance by modulating osmolytes accumulation and antioxidant capacity with Rutin. Rutin was also used with Quercetin to enhance antioxidant profile in *Medicago truncatula* (Gaonkaret al., 2024).

To summrize, while there is variability in the reported efficiency of biostimulant application methods in the literature, the current study emphasized the greater potential of co-treatment for alleviating salt stress. This effectiveness may be attributed to the

Table 2. Qualitative analysis of polyphenolics and flavonoids in aqueous extract of *N. glauca*

Analyte name	<i>N. glauca</i> (Aqueous extract)
Gallic Acid	+
Catechin	-
Chlorogenic acid	+
Rutin	+
Ellagic acid	+
Hesperidin	-
Quercetin	+
Kampeferol	-
Apigenin	+
Caffeic acid	+

Note: + present; - Absent.

continuous exposure to the biostimulant, which is not available to primed seeds. Co-treatment provides a sustained antioxidant effect along with essential nutrients, secondary metabolites, and phytohormones.

Several studies reported that hormonal interactions are essential for enhancing the effectiveness of plant-based biostimulants in salt stress mitigation. Biostimulants like seaweed extracts and humic substances influence hormones such as auxins, cytokinins, and abscisic acid, leading to improved root growth, water-use efficiency, and stress tolerance. For instance, auxin-like compounds in seaweed extracts promote root development for better nutrient absorption in saline conditions (Shukla et al., 2019). Cytokinins delay leaf aging and maintain photosynthesis, while ABA regulates stomatal closure to minimize water loss (Rouphael & Colla, 2018). Additionally, biostimulants reduced ethylene production, preventing premature leaf drop, and enhanced jasmonic and salicylic acid for antioxidant defense (Calvo et al., 2014). Collectively, these interactions boost plant resilience to salt stress, making biostimulants valuable for sustainable agriculture. Recently, Iqbal and Poor, 2024 reported that under stress, plant responses are regulated by defense-related phytohormones such as salicylic acid, jasmonic acid, ethylene and abscisic acid, which act as regulators of tannin production under adverse conditions.

The integration of plant-based biostimulants with conventional fertilization practices optimized their efficiency in mitigating salt stress by enhancing nutrient uptake and improving plant resilience. Biostimulants, such as seaweed extracts and humic acids, work synergistically with fertilizers to improve soil structure, increase nutrient availability, and modulate hormonal pathways, such as auxin and abscisic acid signaling, which are critical for stress adaptation (Rouphael & Colla, 2020). For example, combining biostimulants with nitrogen fertilizers has been shown to enhance root growth and nitrogen-use efficiency under saline conditions, reducing ion toxicity and osmotic stress (Colla et al., 2017). This integrated approach not only improves crop productivity but also reduces fertilizer dependency, promoting sustainable agriculture in salt-affected soils.

CONCLUSIONS

The current study conducted a comprehensive examination of *N. glauca* extract used as a biostimulant to alleviate salt stress effects. The study compared two different application strategies to test their mitigation efficiency at varying doses. Results suggested that co-treatment strategy had more significant salt-effect mitigation. Moreover, the success of these mitigation strategies was critically contingent on the specific doses of the biostimulants, highlighting the importance of precise application in agricultural practices. This result could be related to a continuous plant exposure to biostimulant (irrigation three time per week), while, only one time seed exposure was effected for priming strategy.

N. glauca aqueous extract showed promising results in alleviating salt stress. Its effectiveness in alleviating salinity effects could be attributed to its rich composition of bioactive compounds, including phenolics, flavonoids, and tannins. Chlorogenic acid, caffeic acid, rutin, and apigenin, detected through HPLC analysis of *N. glauca* extract, are recognized for their significant role in cellular defense against oxidative stress. These

components bolster the plant's defense mechanisms against salt-induced oxidative stress. Briefly, this study illustrated the potential advantages of *N. glauca* extract used as biostimulant and underscored the necessity for further research to refine dosage and application methods. Continued exploration of natural plant extracts like *N. glauca* could pave the way for sustainable agricultural practices that enhance crop performance in saline environments.

REFERENCES

- Abd-Elkader, A., Salama, M. & Oraby, S. 2023. Mitigation Negative Effects of Salt Stress on Common Bean (*Phaseolus vulgaris*) Using Seaweed Extracts. *Egyptian Journal of Chemistry* **66**(6), 49–60. doi 10.21608/ejchem.2022.143061.6254
- Ahmed, T., Abou Elezz, A. & Khalid, M.F. 2021. Hydropriming with moringa leaf extract mitigates salt stress in wheat seedlings. *Agriculture* **11**(12), 1254. <https://doi.org/10.3390/agriculture11121254>
- Akram, N.A., Hafeez, N., Farid-ul-Haq, M., Ahmad, A., Sadiq, M. & Ashraf, M. 2020. Foliage application and seed priming with nitric oxide causes mitigation of salinity-induced metabolic adversaries in broccoli (*Brassica oleracea* L.) plants. *Acta Physiologiae Plantarum* **42**, 1–9. <https://doi.org/10.1007/s11738-020-03140-x>
- Alghamdi, A.A. 2021. Phytoconstituents screening and antimicrobial activity of the invasive species *Nicotiana glauca* collected from Al-Baha region of Saudi Arabia. *Saudi Journal of Biological Sciences* **28**(3), 1544–1547. <https://doi.org/10.1016/j.sjbs.2020.12.034>
- Alharthi, A.S., Abd-ElGawad, A.M. & Assaeed, A.M. 2021. Influence of the invasive shrub *Nicotiana glauca* Graham on the plant seed bank in various locations in Taif region, western of Saudi Arabia. *Saudi Journal of Biological Sciences* **28**(1), 360–370.
- Ali, Q., Daud, M.K., Haider, M.Z., Ali, S., Rizwan, M., Aslam, N., ... & Zhu, S.J. 2017. Seed priming by sodium nitroprusside improves salt tolerance in wheat (*Triticum aestivum* L.) by enhancing physiological and biochemical parameters. *Plant physiology and biochemistry* **119**, 50–58. <https://doi.org/10.1016/j.plaphy.2017.08.010>
- Alshami, A.K., El-Shafei, A., Al-Omran, A.M., Alghamdi, A.G., Louki, I. & Alkhasha, A. 2023. Responses of Tomato Crop and Water Productivity to Deficit Irrigation Strategies and Salinity Stress in Greenhouse. *Agronomy* **13**(12), 3016. doi: 10.3390/agronomy13123016
- Alzahib, R.H., Migdadi, H.M., Al Ghamdi, A.A., Alwahibi, M.S., Ibrahim, A.A. & Al-Selwey, W.A. 2021. Assessment of morpho-physiological, biochemical and antioxidant responses of tomato landraces to salinity stress. *Plants* **10**(4), 696. doi: 10.3390/plants10040696
- Al-Taisan, W.A.A., Alabdallah, N.M. & Almuqadam, L. 2022. Moringa leaf extract and green algae improve the growth and physiological attributes of *Mentha* species under salt stress. *Scientific Reports* **12**(1), 14205. <https://doi.org/10.1038/s41598-022-18481-5>
- Alvarez, M.E., Savouré, A. & Szabados, L. 2022. Proline metabolism as regulatory hub. *Trends Plant Sci.* **27**, 39–55. doi: 10.1016/j.tplants.2021.07.009
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24**, 1–15. doi: 10.1104/pp.24.1.1
- Arteaga, S., Yabor, L., Diez, M.J., Prohens, J., Boscaiu, M. & Vicente, O. 2020. The use of proline in screening for tolerance to drought and salinity in common bean (*Phaseolus vulgaris* L.) genotypes. *Agron.* **10**(6), 817. doi: 10.3390/agronomy10060817
- Asoegwu, C.R., Awuchi, C.G., Nelson, K.C.T., Orji, C.G., Nwosu, O.U., Egbufor, U.C. & Awuchi, C.G. 2020. A review on the role of biofertilizers in reducing soil pollution and increasing soil nutrients. *Himalayan Journal of Agriculture* **1**(1), 34–38. <http://inlightpublisher.com/journal/hja>

- Attard, E. 2013. A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols. *Open Life Sciences* **8**(1), 48–53. <https://doi.org/10.2478/s11535-012-0107-3>
- Bates, L.S., Walden, R.P. & Teare, I.D. 1972. Rapid determination of free proline for water stress studies. *Plant Soil* **39**, 205–207. doi.org/10.1007/BF00018060
- Biswas, S., Seal, P., Majumder, B. & Biswas, A.K. 2023. Efficacy of seed priming strategies for enhancing salinity tolerance in plants: An overview of the progress and achievements. *Plant Stress* 100186. <https://doi.org/10.1016/j.stress.2023.100186>
- Boly, R., Lamkami, T., Lompo, M., Dubois, J. & Guissou, I. 2016. DPPH Free Radical Scavenging activity of two extracts from *Agelanthus dodoneifolius* (Loranthaceae) leaves. *Int J Toxicol Pharmacol Res* **8**(1), 29–34.
- Bouazzi, A., Bouallegue, A., Kharrat, M., Abbes, Z. & Horchani, F. 2024. Seed priming with gallic acid and hydrogen peroxide as a smart approach to mitigate salt stress in faba bean (*Vicia faba* L.) at the germination stage. *Russian Journal of Plant Physiology* **71**(4), 104. <https://doi.org/10.1134/S1021443724605354>
- Calvo, P., Nelson, L. & Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant and Soil* **383**(1–2), 3–41. <https://doi.org/10.1007/s11104-014-2131-8>
- Campobenedetto, C., Mannino, G., Beekwilder, J., Contartese, V., Karlova, R. & Berteà, C.M. 2021. The application of a biostimulant based on tannins affects root architecture and improves tolerance to salinity in tomato plants. *Scientific Reports* **11**(1), 354. <https://doi.org/10.1038/s41598-020-79770-5>
- Chanthini, K.M.P., Pavithra, G.S., Senthil-Nathan, S. & Malafaia, G. 2024. An in-depth review on the mechanistic insights of marine macroalgal compounds in enhancing plant tolerance to stress induced by saline soil conditions. *Toxin Reviews*, 1–19. <https://doi.org/10.1080/15569543.2024.2382989>
- Chernikova, O., Mazhaysky, Y., Buryak, S., Seregina, T. & Ampleeva, L. 2021. Comparative analysis of the use of biostimulants on the main types of soil. *Agronomy Research* **19**(S1), 711–720. <https://doi.org/10.15159/ar.21.075>
- Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R. & Roupael, Y. 2017. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Frontiers in plant science* **8**, 2202. doi: 10.3389/fpls.2017.02202
- Cristofano, F., El-Nakhel, C., Colla, G., Cardarelli, M., Pii, Y., Lucini, L. & Roupael, Y. 2023. Modulation of morpho-physiological and metabolic profiles of lettuce subjected to salt stress and treated with two vegetal-derived biostimulants. *Plants* **12**, 709. doi: 10.3390/plants12040709
- Davey, M.W., Stals, E., Panis, B., Keulemans, J. & Swennen, R.L. 2005. High-throughput determination of malondialdehyde in plant tissues. *Analytical biochemistry* **347**(2), 201–207. <https://doi.org/10.1016/j.ab.2005.09.041>
- Desoky, E.S.M., Saad, A.M., El-Saadony, M.T., Merwad, A.R.M. & Rady, M.M. 2020. Plant growth-promoting rhizobacteria: Potential improvement in antioxidant defense system and suppression of oxidative stress for alleviating salinity stress in *Triticum aestivum* (L.) plants. *Biocatalysis and Agricultural Biotechnology* **30**, 101878. doi: 10.1016/j.bcab.2020.101878
- Di Stasio, E., Cirillo, V., Raimondi, G., Giordano, M., Esposito, M. & Maggio, A. 2020. Osmo-priming with seaweed extracts enhances yield of salt-stressed tomato plants. *Agronomy* **10**(10), 1559. <https://doi.org/10.3390/agronomy10101559>
- Drobek, M., Frąc, M. & Cybulska, J. 2019. Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress – A review. *Agronomy* **9**(6), 335. <https://doi.org/10.3390/agronomy9060335>

- Dustgeer, Z., Seleiman, M.F., Imran, K.H.A.N., Chattha, M. U., Alhammad, B.A., Jalal, R.S., ... & Hassan, M.U. 2021. Glycine-betaine induced salinity tolerance in maize by regulating the physiological attributes, antioxidant defense system and ionic homeostasis. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **49**(1), 12248–12248. doi: 10.15835/nbha49112248
- Ehiobu, J.M., Idamokoro, M.E. & Afolayan, A.J. 2021. Phytochemical content and antioxidant potential of leaf extracts of Citrus limon (L.) Osbeck collected in the Eastern Cape Province, South Africa. *South African Journal of Botany* **141**, 480–486. doi: 10.1016/j.sajb.2021.06.001
- El-Hawary, M.M., Hashem, O.S. & Hasanuzzaman, M. 2023. Seed priming and foliar application with ascorbic acid and salicylic acid mitigate salt stress in wheat. *Agronomy* **13**(2), 493. <https://doi.org/10.3390/agronomy13020493>
- Elkarmout, A.F., Yang, M. & Hassan, F.A. 2022. Chitosan treatment effectively alleviates the adverse effects of salinity in Moringa oleifera Lam via enhancing antioxidant system and nutrient homeostasis. *Agronomy* **12**(10), 2513. <https://doi.org/10.3390/agronomy12102513>
- Elkholy, N.S., Hariri, M.L.M., Mohammed, H.S. & Shafaa, M.W. 2023. Lutein and β -carotene characterization in free and nanodispersion forms in terms of antioxidant activity and cytotoxicity. *Journal of Pharmaceutical Innovation* **18**(4), 1727–1744. doi: 10.1007/s12247-023-09745-2
- El-Sayed, A.I., Rafudeen, M.S., Ganie, S.A., Hossain, M.S. & Gomaa, A.M. 2022. Seed priming with cypress leaf extract enhances photosynthesis and antioxidative defense in zucchini seedlings under salt stress. *Scientia Horticulturae* **293**, 110707. doi: 10.1016/j.scienta.2021.110707
- Eswar, D., Karuppusamy, R. & Chellamuthu, S. 2021. Drivers of soil salinity and their correlation with climate change. *Current Opinion in Environmental Sustainability* **50**, 310–318. <https://doi.org/10.1016/j.cosust.2020.10.015>
- Foti, M.C. 2007. Antioxidant properties of phenols. *Journal of Pharmacy and Pharmacology* **59**(12), 1673–1685. doi: 10.1211/jpp.59.12.0010
- Gaonkar, S.S., Sincinelli, F., Balestrazzi, A. & Pagano, A. 2024. Quercetin and Rutin as Tools to Enhance Antioxidant Profiles and Post-Priming Seed Storability in Medicago truncatula. *Agriculture* **14**(5), 738. <https://doi.org/10.3390/agriculture14050738>
- Ghorbani, A., Pishkar, L., Saravi, K.V. & Chen, M. 2023. Melatonin-mediated endogenous nitric oxide coordinately boosts stability through proline and nitrogen metabolism, antioxidant capacity, and Na⁺/K⁺ transporters in tomato under NaCl stress. *Frontiers in Plant Science* **14**, 1135943. <https://doi.org/10.3389/fpls.2023.1135943>
- Gil-Ortiz, R., Naranjo, M.Á., Atares, S. & Vicente, O. 2023. Antioxidant responses of water-stressed cherry tomato plants to natural biostimulants. *Agronomy* **13**, 2314. doi: 10.3390/agronomy13092314
- Gulcin, İ. & Alwasel, S.H. 2023. DPPH radical scavenging assay. *Processes* **11**(8), 2248.
- Hasan, T. & Sultana, M. 2018. Antidiabetic potency of Bangladeshi medicinal plants. *J. Ayurvedic Herb. Med.* **4**(1), 35–42.
- Hassan, F., Al-Yasi, H., Ali, E., Alamer, K., Hessini, K., Attia, H. & El-Shazly, S. 2020. Mitigation of salt-stress effects by moringa leaf extract or salicylic acid through motivating antioxidant machinery in damask rose. *Canadian Journal of Plant Science* **101**(2), 157–165. <https://doi.org/10.1139/cjps-2020-0127>
- Heath, R.L. & Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* **125**, 180–198. doi: 10.1016/0003-9861(68)90654-1
- Hnilickova, H., Kraus, K., Vachova, P. & Hnilicka, F. 2021. Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in Portulaca oleracea L. *Plants* **10**(5), 845. <https://doi.org/10.3390/plants10050845>

- Howladar, S. M. 2014. A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. *Ecotoxicology and Environmental Safety* **100**, 69–75. <https://doi.org/10.1016/j.ecoenv.2013.11.022>
- Hussain, H.I., Kasinadhuni, N. & Arioli, T. 2021. The effect of seaweed extract on tomato plant growth, productivity and soil. *Journal of Applied Phycology* **33**(2), 1305–1314. <https://doi.org/10.1007/s10811-021-02387-2>
- Ishtiyag, S., Kumar, H., Varun, M., Ogunkunle, C. O. & Paul, M.S. 2021. Role of secondary metabolites in salt and heavy metal stress mitigation by halophytic plants: An overview. *Handbook of bioremediation* 307–327. <https://doi.org/10.1016/B978-0-12-819382-2.00020>
- Jia, T., Ito, H. & Tanaka, A. 2016. Simultaneous regulation of antenna size and photosystem I/II stoichiometry in *Arabidopsis thaliana*. *Planta* **244**, 1041–1053. doi: 10.1007/s00425-016-2568-5
- Jmii, G., Sayari, M., Mars, M., Gharsallaoui, S. & Haouala, R. 2022. *Nicotiana glauca*, a Key Plant for Tomato Growth Enhancement and for the Weed *Cynodon dactylon* Control. *Tunisian Journal of Plant Protection* **17**(2), 77–96.
- Kapadia, C., Sayyed, R. Z., El Enshasy, H.A., Vaidya, H., Sharma, D., Patel, N., ... & Zuan, A.T.K. 2021. Halotolerant microbial consortia for sustainable mitigation of salinity stress, growth promotion, and mineral uptake in tomato plants and soil nutrient enrichment. *Sustainability* **13**(15), 8369. <https://doi.org/10.3390/su13158369>
- Khalili, S., Hamidi, Y. & Delfanian, R. 2022. Evaluation of antioxidant capacity of ethanol, acetone, ether and aqueous extract of mistletoe leaves. *Journal of Biological Studies* **5**(1), 103–112. <https://doi.org/10.62400/jbs.v5i1.6385>
- Khedr, N.M., Ibrahim, A.A., El-Metwally, M., Eldakroory, S. & Soliman, M.I. 2024. Phytochemical analysis, antioxidant activity, antimicrobial evaluation, and cytotoxicity effects of wild medicinal plants. *SABRAO Journal of Breeding & Genetics* **56**(4), 1152–1162. <http://doi.org/10.54910/sabrao2024.56.4.21>
- Kiani, R., Arzani, A. & Mirmohammady Maibody, S.A.M. 2021. Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, *Aegilops cylindrica* and their amphidiploids. *Frontiers in plant science* **12**, 646221. doi: 10.3389/fpls.2021.646221
- Kiranmai, M., Kumar, C.M. & Mohammed, I. 2011. Comparison of total flavonoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2**(3), 254–261. [http://rjpbcs.com/pdf/2011_2\(3\)/31.pdf](http://rjpbcs.com/pdf/2011_2(3)/31.pdf)
- Kumar, K., Debnath, P., Singh, S. & Kumar, N. 2023. An overview of plant phenolics and their involvement in abiotic stress tolerance. *Stresses* **3**(3), 570–585. doi: 10.3390/stresses3030040
- Kumari, R., Bhatnagar, S., Mehla, N. & Vashistha, A. 2022. Potential of organic amendments (AM fungi, PGPR, Vermicompost and Seaweeds) in combating salt stress: A review. *Plant Stress* **6**, 100111. <https://doi.org/10.1016/j.stress.2022.100111>
- Maeda, K., Johkan, M., Tsukagoshi, S. & Maruo, T. 2020. Effect of salinity on photosynthesis and distribution of photosynthates in the Japanese tomato ‘CF momotaro york’ and the Dutch tomato ‘Endeavour’ with low node-order pinching and a high-density planting system. *The Horticulture Journal* **89**(4), 454–459. <https://doi.org/10.2503/hortj.UTD-167>
- Moazz Ali, M., Javed, T., Mauro, R.P., Shabbir, R., Afzal, I. & Yousef, A.F. 2020. Effect of seed priming with potassium nitrate on the performance of tomato. *Agriculture* **10**(11), 498. <https://doi.org/10.3390/agriculture10110498>
- Muslu, A.S. 2024. Improving salt stress tolerance in *Zea mays* L. by modulating osmolytes accumulation and antioxidant capacity with Rutin. *Anatolian Journal of Botany* **8**(1), 21–29. <https://doi.org/10.30616/ajb.1387695>

- Mzibra, A., Aasfar, A., Benhima, R., Khouloud, M., Boulif, R., Douira, A., ... & Meftah Kadmiri, I. 2021. Biostimulants derived from Moroccan seaweeds: seed germination metabolomics and growth promotion of tomato plant. *Journal of Plant Growth Regulation* **40**, 353–370. <https://doi.org/10.1007/s00344-020-10104-5>
- Ojha, S., Raj, A., Roy, A. & Roy, S. 2018. Extraction of total phenolics, flavonoids and tannins from *Paederia foetida* L. Leaves and their relation with antioxidant activity. *Pharmacognosy Journal* **10**(3). doi: 10.5530/pj.2018.3.88
- Ors, S., Ekinci, M., Yildirim, E., Sahin, U., Turan, M. & Dursun, A. 2021. Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. *South African Journal of Botany* **137**, 335–339. <https://doi.org/10.1016/j.sajb.2020.10.031>
- ÖzdenefeÖzdenefe, M.S., Takcı, A.M. & Kayış, F.B. 2023. Antibacterial, Antioxidant, Antidiabetic Potentials and Chemical Composition of *Nicotiana glauca* Graham Leaf Extract. *Journal of Anatolian Environmental and Animal Sciences* **8**(4), 700–706. <https://doi.org/10.35229/jaes.1325678>
- Panuccio, M.R., Chaabani, S., Roula, R. & Muscolo, A. 2018. Bio-priming mitigates detrimental effects of salinity on maize improving antioxidant defense and preserving photosynthetic efficiency. *Plant Physiology and Biochemistry* **132**, 465–474. doi: 10.1016/j.plaphy.2018.09.033
- Pascual, P.R.L., Carabio, D.E., Abello, N.F.H., Remedios, E.A. & Pascual, V.U. 2021. Enhanced assimilation rate due to seaweed biostimulant improves growth and yield of rice bean (*Vigna umbellata*). *Agronomy Research* **19**(4), 1863–1872 doi: 10.15159/ar.21.106
- Qian, X., Liu, L., Croft, H. & Chen, J. 2021. Relationship between leaf maximum carboxylation rate and chlorophyll content preserved across 13 species. *Journal of Geophysical Research: Biogeosciences* **126**(2), e2020JG006076.
- Rady, M.O., Semida, W.M., Abd El-Mageed, T.A., Hemida, K.A. & Rady, M.M. 2018. Up-regulation of antioxidative defense systems by glycine betaine foliar application in onion plants confer tolerance to salinity stress. *Scientia Horticulturae* **240**, 614–622. <https://doi.org/10.1016/j.scienta.2018.06.069>
- Rady, M.M., Talaat, N.B., Abdelhamid, M.T., Shawky, B.T. & Desoky, E.S.M. 2019. Maize (*Zea mays* L.) grains extract mitigates the deleterious effects of salt stress on common bean (*Phaseolus vulgaris* L.) growth and physiology. *The Journal of Horticultural Science and Biotechnology* **94**(6), 777–789. <https://doi.org/10.1080/14620316.2019.1626773>
- Rafi, M., Meitary, N., Septaningsih, D.A. & Bintang, M. 2020. Phytochemical profile and antioxidant activity of *Guazuma ulmifolia* leaves extracts using different solvent extraction. *Indonesian Journal of Pharmacy* **31**(3), 171–180.
- Rahman, A., Alam, M.U., Hossain, M.S., Mahmud, J.A., Nahar, K., Fujita, M. & Hasanuzzaman, M. 2022. Exogenous gallic acid confers salt tolerance in rice seedlings: Modulation of ion homeostasis, osmoregulation, antioxidant defense, and methylglyoxal detoxification systems. *Agronomy* **13**(1), 16. <https://doi.org/10.3390/agronomy13010016>
- Ramzan, M., Haider, S.T.A., Hussain, M.B., Ehsan, A., Datta, R., Alahmadi, T.A., ... & Alharbi, S.A. 2024. Potential of kaempferol and caffeic acid to mitigate salinity stress and improving potato growth. *Scientific Reports* **14**(1), 21657. doi: 10.1038/s41598-024-72420-0
- Roşca, M., Mihalache, G. & Stoleru, V. 2023. Tomato responses to salinity stress: From morphological traits to genetic changes. *Frontiers in plant science* **14**, 1118383. <https://doi.org/10.3389/fpls.2023.1118383>
- Rossi, L., Zhang, W., Lombardini, L. & Ma, X. 2016. The impact of cerium oxide nanoparticles on the salt stress responses of *Brassica napus* L. *Environmental Pollution* **219**, 28–36. <https://doi.org/10.1016/j.envpol.2016.09.060>

- Roupshael, Y. & Colla, G. 2018. Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. *Frontiers in plant science* **9**, 1655. <https://doi.org/10.3389/fpls.2018.01655>
- Roupshael, Y. & Colla, G. 2020. Biostimulants in agriculture. *Frontiers in plant science* **11**, 40. doi: 10.3389/fpls.2020.00040
- Sarov, A., Kostenarov, K. & Tzvetanova, E. 2024. An optimization model for evaluating the economic effect of foliar treatment with biostimulants on spring rape. *Agronomy Research* **22**(2), 939–955. <https://doi.org/10.15159/ar.24.094>
- Sassine, Y.N., Sajyan, T.K., El Zarzour, A., Abdelmawgoud, A.M.R., Germanos, M. & Alturki, S.M. 2022. Integrative effects of biostimulants and salinity on vegetables: Contribution of bioumik and Lithovit®-urea50 to improve salt-tolerance of tomato. *Agronomy Research* **20**(4), 793–804. <https://doi.org/10.15159/ar.22.074>
- Shahzadi, A., Noreen, Z., Alamery, S., Zafar, F., Haroon, A., Rashid, M., ... & Fiaz, S. 2024. Effects of biochar on growth and yield of Wheat (*Triticum aestivum* L.) under salt stress. *Scientific Reports* **14**(1), 20024. <https://doi.org/10.1038/s41598-024-70917-2>
- Sharma, A. & Chetani, R. 2017. A review on the effect of organic and chemical fertilizers on plants. *International Journal for Research in Applied Science & Engineering Technology (IJRASET)* **5**, 677–680. <http://www.ijraset.com/files/serve.php?FID=6329>
- Sheteiwy, M.S., Shao, H., Qi, W., Daly, P., Sharma, A., Shaghaleh, H., ... & Lu, H. 2021. Seed priming and foliar application with jasmonic acid enhance salinity stress tolerance of soybean (*Glycine max* L.) seedlings. *Journal of the Science of Food and Agriculture* **101**(5), 2027–2041. <https://doi.org/10.1002/jsfa.10822>
- Shin, Y.K., Bhandari, S.R., Cho, M.C. & Lee, J.G. 2020. Evaluation of chlorophyll fluorescence parameters and proline content in tomato seedlings grown under different salt stress conditions. *Horticulture, Environment, and Biotechnology* **61**, 433–443. <https://doi.org/10.1007/s13580-020-00231-z>
- Shukla, P.S., Mantin, E.G., Adil, M. & Critchley, A.T. 2019. Ascophyllum nodosum-based biostimulants: Sustainable applications in agriculture. *Frontiers in Plant Science* **10**, 655. <https://doi.org/10.3389/fpls.2019.00655>
- Supraja, K.V., Behera, B. & Balasubramanian, P. 2020. Efficacy of microalgal extracts as biostimulants through seed treatment and foliar spray for tomato cultivation. *Industrial crops and products* **151**, 112453. <https://doi.org/10.1016/j.indcrop.2020.112453>
- Suryaman, M., Sunarya, Y., Istarimila, I. & Fudholi, A. 2021. Effect of salinity stress on the growth and yield of mungbean (*Vigna radiata* (L.) R. Wilczek) treated with mangosteen pericarp extract. *Biocatalysis and Agricultural Biotechnology* **36**, 102132. doi: 10.1016/j.bcab.2021.102132
- Taha, R. 2016. Improving salt tolerance of *Helianthus annuus* (L.) plants by *Moringa oleifera* leaf extract. *Egypt. J. Agron* **38**, 117–140. doi: 10.21608/agro.2016.301
- Tanaka, R. & Tanaka, A. 2011. Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1807**(8), 968–976. <https://doi.org/10.1016/j.bbabi.2011.01.002>
- Tang, C.J., Luo, M.Z., Zhang, S., Jia, G.Q., Sha, T.A.N.G., Jia, Y.C., ... & Diao, X.M. 2023. Variations in chlorophyll content, stomatal conductance, and photosynthesis in *Setaria* EMS mutants. *Journal of Integrative Agriculture* **22**(6), 1618–1630. doi: 10.1016/j.jia.2022.10.014
- Tarchoun, N., Saadaoui, W., Mezghani, N., Pavli, O.I., Falleh, H. & Petropoulos, S.A. 2022. The effects of salt stress on germination, seedling growth and biochemical responses of tunisian squash (*Cucurbita maxima* Duchesne) germplasm. *Plants* **11**(6), 800. <https://doi.org/10.3390/plants11060800>

- Uçarlı, C. 2020. Effects of salinity on seed germination and early seedling stage. *Abiotic stress in plants* **211**, 10.5772.
- Vali, A., Zahedi, H., Alipour, A., Sharghi, Y. & Naeini, M.R. 2024. Differential effects of foliar and seed priming glycine betaine application on quinoa physiology under varying salinity level. *South African Journal of Botany* **175**, 253–267. doi: 10.1016/j.sajb.2024.10.009
- Wu, L., Huo, W., Yao, D. & Li, M. 2019. Effects of solid matrix priming (SMP) and salt stress on broccoli and cauliflower seed germination and early seedling growth. *Scientia Horticulturae* **255**, 161–168. doi: 10.1016/j.scienta.2019.05.007
- Zhang, L., Miras-Moreno, B., Yildiztugay, E., Ozfidan-Konakci, C., Arikan, B., Elbasan, F., ... & Lucini, L. 2021. Metabolomics and physiological insights into the ability of exogenously applied chlorogenic acid and hesperidin to modulate salt stress in lettuce distinctively. *Molecules* **26**(20), 6291. <https://doi.org/10.3390/molecules26206291>
- Zulfiqar, F., Akram, N.A. & Ashraf, M. 2020. Osmoprotection in plants under abiotic stresses: New insights into a classical phenomenon. *Planta* **25**(1), 3. doi: 10.1007/s00425-019-03293-1
- Zuluaga, M.Y.A., Monterisi, S., Rouphael, Y., Colla, G., Lucini, L., Cesco, S. & Pii, Y. 2023. Different vegetal protein hydrolysates distinctively alleviate salinity stress in vegetable crops: A case study on tomato and lettuce. *Front. Plant Sci.* **14**. doi: 10.3389/fpls.2023.1077140
- Zuzunaga-Rosas, J., Calone, R., Mircea, D.M., Shakya, R., Ibáñez-Asensio, S., Boscaiu, M., ... & Vicente, O. 2024. Mitigation of salt stress in lettuce by a biostimulant that protects the root absorption zone and improves biochemical responses. *Frontiers in Plant Science* **15**, 1341714. <https://doi.org/10.3389/fpls.2024.1341714>
- Zuzunaga-Rosas, J., Silva-Valdiviezo, D., Calone, R., Luput, I., Ibáñez-Asensio, S., Boscaiu, M., Moreno-Ramón, H. & Vicente, O. 2023. Biochemical responses to salt stress and biostimulant action in tomato plants grown in two different soil types. *Horticulturae* **9**, 1209. doi: 10.3390/horticulturae9111209