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# The changes in growth and metabolic adaptation responses in Java plum seedlings exposed to *Cassia javanica* extract under salinity

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**Abstract:** Developing and employing new, sustainable, and eco-friendly biostimulants that enhance plant growth and alleviate the harmful effects of environmental challenges is a major focus for many researchers. Salt stress is a critical constraint on plant growth and a limiting factor in crop productivity, particularly during the early developmental stages in the nurseries. *Syzygium cumini* (L.) Skeels (Java plum) is an important fruit tree and widely cultivated in gardens as an ornamental plant. This study was designed to develop *Cassia javanica* subsp. *nodosa* leaf extract (CLE) as a new sustainable and eco-friendly biostimulant capable of triggering the metabolic adaptation to salt stress in Java plum seedlings grown in nurseries. CLE successfully mitigated reductions in growth, biomass yield, and secondary metabolite production caused by salinity. Although salt stress depressed morphological characters and biomass yield, CLE foliar spray enhanced these parameters. Moreover, CLE enhanced the ferric reducing antioxidant potential, catalase, and superoxide dismutase enzyme activities, increased phenolic content, and reduced hydrogen peroxide ($H_2O_2$) accumulation and lipid peroxidation. Additionally, CLE application increased seedling biomass and stimulated antioxidant activity, osmoprotectant accumulation, and overall tolerance to salinity stress. These observations provide new insights into CLE's potential as an eco-friendly biostimulant for enhancing salt tolerance in Java plum seedlings.

**Keywords:** abiotic stress; toxicity; osmotic stress; nursery application; osmoregulation; sustainable biostimulants

In nurseries, procedures are intended to standardise all practices for each plant species in order to produce high-quality seedlings (El-Serafy 2020). During the early stages of a tree's life, careful management techniques are therefore essential to ensure the production of high-quality, marketable trees and to enhance proper growth and development. *Syzygium cumini* (L.) Skeels (Java plum), an important member of the Myrtaceae family, is widely cultivated as a fruit tree and valued for its medicinal properties, particularly in humid regions. Diabetic patients frequently consume its fruits (Ayyanar and Subash-Babu 2012). Because of its many benefits, it can be utilised effectively in several agroforestry systems (Sarvade et al. 2016) and is also favoured as an attractive ornamental tree in gardens. Additionally, Java plum thrives in marginal soils and is well adapted to tolerate adverse climatic conditions (Tewari et al. 2017).

Water shortages are a global problem that is worsening, as water is essential for all metabolic processes that take place in plant cells (Attia et al. 2022, Ghanem et al. 2022). To compensate for freshwater scarcity, saline water has increasingly been used for crop irrigation. Salinity is an abiotic stress that

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alters plant physiological and morphological traits, thereby reducing agricultural output (El-Serafy et al. 2021, Sheta et al. 2024). Salts affect plant development by disrupting hormonal balance, causing ionic toxicity, inducing osmotic stress, reducing nutrient availability, and producing reactive oxygen species (Kumar et al. 2020). Consequently, plants exposed to salt stress may wilt, dry up, and eventually die. In response to osmotic stress, plants develop osmotically active compounds known as osmoprotectants, which lower cellular osmotic potential under saline conditions. These osmoprotectants preserve cell turgor by absorbing water and functioning as free-radical scavengers (Ahmad and Sharma 2008, El-Serafy et al. 2024). Carbohydrates, proline, proteins, and amino acids are among osmoprotective metabolites affected by salt stress (Rasool et al. 2013, Youssef et al. 2022, Atteya et al. 2022b, Bahgat et al. 2023). Recently, considerable efforts have been devoted to identifying new and sustainable plant growth promoters to enhance plant growth under saline conditions and ameliorate the detrimental effects of salt stress (El-Serafy et al. 2024, Alayafi et al. 2025).

*Cassia javanica nodosa* trees belong to the family Fabaceae and are widely cultivated in gardens and landscapes. The wood and seed gum of this tree have commercial value, and its leaves have shown antidiabetic activity (Kumavat and Gopalkrishnan 2024). The leaves of *C. javanica* contain glycosides, saponins, alkaloids, flavonoids, tannins, and phytosterols (Khurm et al. 2021) and exhibit antimicrobial, antioxidant, antidiabetic, and anticancer activities (Lavanya et al. 2018). A preliminary experiment was done to evaluate the superoxide radical-scavenging activity of *C. javanica* leaf extract (CLE), which revealed strong antioxidant potential, suggesting its applicability as an antioxidant source across various sectors (Kaur and Arora 2011). Highly antioxidant plants can also be employed to mitigate ROS-induced damage in plant cells under stress (El-Serafy et al. 2023, 2024, Alayafi et al. 2025). In addition to their therapeutic uses, *Cassia* species are also commonly used for many purposes, such as food, gums, timber, dyes, and fuelwood (Khurm et al. 2021). *C. nigleaf's* leaf powder, for example, has been utilised as a potential pesticide against grain storage pests (Belmain et al. 2001).

However, limited information is available on the use of *C. javanica* extracts in agricultural practices, particularly regarding their application as a growth promoter. Therefore, this study is the first to evaluate the adaptive responses of Java plum seedlings to saline stress as affected by CLE foliar application as a natural antioxidant. Our hypothesis is that CLE supplementation to plant foliage may be beneficial for Java plum seedlings irrigated with saline water. Accordingly, our object was to assess the adaptive strategies of *Syzygium cumini* (L.) Skeels seedlings subjected to CLE foliar spray under saline conditions.

## MATERIAL AND METHODS

**Experiment location.** A pot investigation was done in the greenhouse of the Horticulture Department, Agriculture Faculty, Tanta University (30°47'18"N, 31°00'06"E), at 8 m a.s.l., from June to October 2024. Java plum seedlings were provided from the Nursery of the Horticulture Department, Agriculture Faculty, Tanta University, and cultivated in plastic pots. Before pot preparation, samples of experimental soil were collected for analysis. The results were as follows: sand 21.5%, silt 38.9%, clay 39.6%, electrical conductivity 1.8 dS/m, pH 8.3, $Mg^{2+}$ 139.73 mg/L, $Ca^{2+}$ 290 mg/L, N 0.26%, P 0.014%, and K 0.06%.

**Planting methodology.** Uniform seedlings of *Syzygium cumini* were planted on 20 June 2024 in plastic pots inside the greenhouse. After root establishment, the pots were divided into groups for respective treatments.

***Cassia* extract preparation.** The young leaves of *Cassia javanica* subsp. *nodosa* were used in this experiment. To prepare the aqueous CLE, fresh leaves were blended with water at a 1:2 *w*/*v* ratio and filtered. A concentration of 40% of the stock solution was prepared. Java plum seedlings received CLE foliar sprays three times: on 1 July, 1 August, and 1 September. Seedlings not treated with CLE spray or saline water were identified as control seedlings. The chemical analysis of CLE was as follows: FRAP 170 μmol/g; total chlorophyll 1.82 mg/g FW (fresh weight); polyphenols 13.6%; total carbohydrates 2.1 mg/g DW (dry weight); ascorbic acid 2.31 mg/g FW; total protein 1.66 mg/g DW; total alkaloid 5.11%; flavonoids 1.6%, and tannins 0.25%.

**Treatments.** The pots were subjected to salinity and CLE treatments as follows: (1) irrigation with tap water (control, T1); (2) irrigation with 20 dS/m NaCl saline water (T2); (3) foliar spray with CLE (T3), and (4) combined saline water irrigation and CLE foliar spray (T4). Each treatment (group) was replicated three times, and each replicate consisted

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of 12 pots, each with one seedling. Seedlings were gradually acclimated to saline irrigation, beginning with 5 dS/m on 1st July and increasing every 3 days to reach the required level of 20 dS/m. After that, all pots were irrigated every 2 days to maintain field capacity. NPK fertiliser (15% N, 5% P, 5% K) was applied at 5 g/kg soil (Zafar et al. 2021). On 20th October, growth traits of both above- and below-ground parts were measured across all treatments.

**Physio-biochemical analysis.** Total chlorophyll (mg/g FW) was measured using methanol (Dere et al. 1998). Total carbohydrates in leaves were assessed *via* the Anthrone protocol (Leyva et al. 2008). Total protein content was determined using a nitrogen-to-protein conversion factor (Mariotti et al. 2008). Phenolic content in leaves was assessed as described by Dewanto et al.'s (2002) method and expressed as mg gallic acid (GAE)/g DW. Ascorbic acid content (mg/100 g DW) in seedling leaves was measured according to the methods of AOAC (1995). Ferric reducing antioxidant potential (FRAP; Umol/g FW) was determined using the Benzie and Strain (1996) method. Catalase (CAT) activity was assessed following the Aebi (1984) and Hadwan (2018) protocols and reported as U/mg protein. The activity of superoxide dismutase enzyme (SOD, U/mg protein) was assessed with the nitro blue tetrazolium method of Giannopolitis and Ries (1977). Proline content was assessed using the procedure of Ábrahám et al. (2010) and presented in mg/100 g FW. Lipid peroxidation in the cell membrane was assessed as malondialdehyde (MDA) according to Landi (2017) and presented in mg/g FW. Hydrogen peroxide ($H_2O_2$; μmol/g FW) generated in plant leaves was measured using the method of Patterson et al. (1984). Nitrogen (N% %) was measured using the micro-Kjeldahl method, while phosphorus (P; mg/100 g DW) and potassium (K; mg/100 g DW) concentrations were determined using Estefan et al. (2013) description.

**Statistical analysis.** This pot investigation was arranged in a randomised complete design (RCD) in four treated groups; each group was replicated three times, and each replicate consisted of 12 pots with one seedling per pot. The collected results were analysed statistically using COSTAT version 6.4 software (CoHort Software, Monterey, USA). The Duncan's test was used to estimate differences in mean values among treatments at $P \leq 0.05$.

## RESULTS

**Growth parameters.** Results in Figure 1 indicate that seedlings' height was significantly depressed in the T2 treatment, which showed lower fresh and dry weights than the other treatments. The CLE treatment (T3) increased seedlings' height and biomass, presenting the tallest seedlings with the heaviest weights, and mitigated the negative impacts of saline water in T4. Conversely, root length showed the opposite trend (Table 1): T2 seedlings had the long-

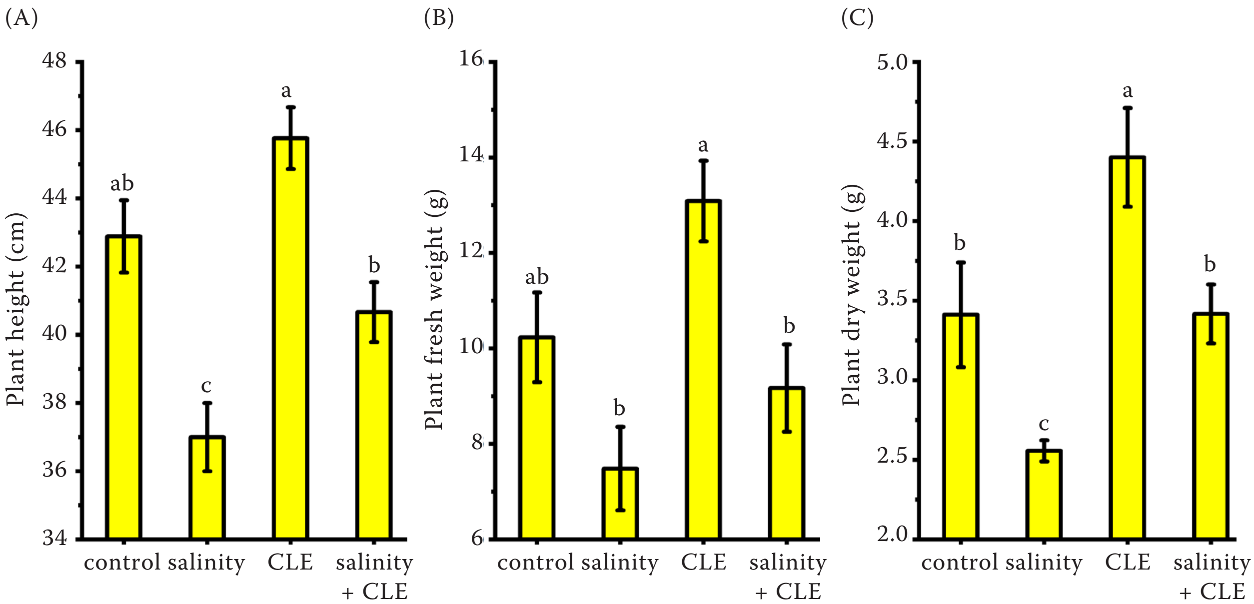


Figure 1. Effect of *Cassia javanica* leaf extract (CLE) foliar application on (A) plant height; (B) plant fresh weight, and (C) plant dry weight of *Syzygium cumini* L. seedlings irrigated with saline water. The same letters are significantly different at the $P \leq 0.05$ level

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Table 1. Effect of *Cassia javanica* leaf extract (CLE) foliar application on the root and shoot traits, and leaf area of *Syzygium cumini* L. seedlings irrigated with saline water

| Treatment | Root length (cm) | Root fresh weight (g) | Root dry weight (g) | Shoot fresh weight (g) | Shoot dry weight (g) | Leaves area ($cm^2$) |
|---|---|---|---|---|---|---|
| Control (T1) | 22.21 ± 1.25$^{b}$ | 2.68 ± 0.17$^{b}$ | 0.94 ± 0.03$^{b}$ | 7.57 ± 0.31$^{b}$ | 2.47 ± 0.30$^{ab}$ | 71.0 ± 1.32$^{b}$ |
| Salinity (T2) | 25.89 ± 0.59$^{a}$ | 1.81 ± 0.16$^{c}$ | 0.60 ± 0.02$^{c}$ | 5.30 ± 0.26$^{c}$ | 1.96 ± 0.05$^{b}$ | 49.86 ± 1.20$^{d}$ |
| CLE (T3) | 20.78 ± 0.40$^{b}$ | 3.72 ± 0.25$^{a}$ | 1.34 ± 0.13$^{a}$ | 9.47 ± 0.39$^{a}$ | 3.06 ± 0.19$^{a}$ | 88.56 ± 1.90$^{a}$ |
| Salinity + CLE (T4) | 22.23 ± 0.66$^{b}$ | 3.10 ± 0.22$^{b}$ | 1.04 ± 0.10$^{b}$ | 6.47 ± 0.68$^{bc}$ | 2.37 ± 0.13$^{b}$ | 65.72 ± 1.11$^{c}$ |

The same letters within a column are significantly different at the $P \leq 0.05$ level

est roots, with significant differences as compared with the other treatments. But T3 seedlings had the shortest roots.

The fresh and dry weights of both above- and belowground parts were significantly lower in T2 seedlings relative to T1 (Table 1). In contrast, T3 seedlings exhibited the highest weights among all treatments. Furthermore, T4 seedlings significantly recorded higher fresh and dry weights than those of T2 seedlings. Regarding leaf area, T2 seedlings presented the smallest leaves (49.86 $cm^2$). Meanwhile, CLE treatment significantly increased leaf area when applied alone (T3) and with saline water (T4).

## Physicochemical analysis

**Chlorophyll, carbohydrates, and protein.** The adverse influence of salinity on chlorophyll content was noticed in Java plum leaves (Figure 2), as T2 seedlings showed lower chlorophyll content. In contrast, T3 seedlings presented the highest chlorophyll content. Java plum seedlings in the T2 treatment showed significantly lower carbohydrate and protein levels than the control (Figure 2). The CLE foliar application alone (T3) resulted in the maximum carbohydrates and protein values, while T4-seedlings presented significantly higher levels than T2-seedlings.

**Total phenols and ascorbic acid.** In T2-seedlings, total phenols reached 52.96 mg gallic acid/g DW and ascorbic acid reached 13.84 mg/100 g DW (Table 2). These values were further enhanced following T4 treatment, which recorded significantly higher levels of total phenols and ascorbic acid than all other treatments. The lowest levels of both metabolites were recorded in T1 seedlings.

**Ferric ion reducing power assay.** Concerning the FRAP assay, T3-treated seedlings significantly exhib-

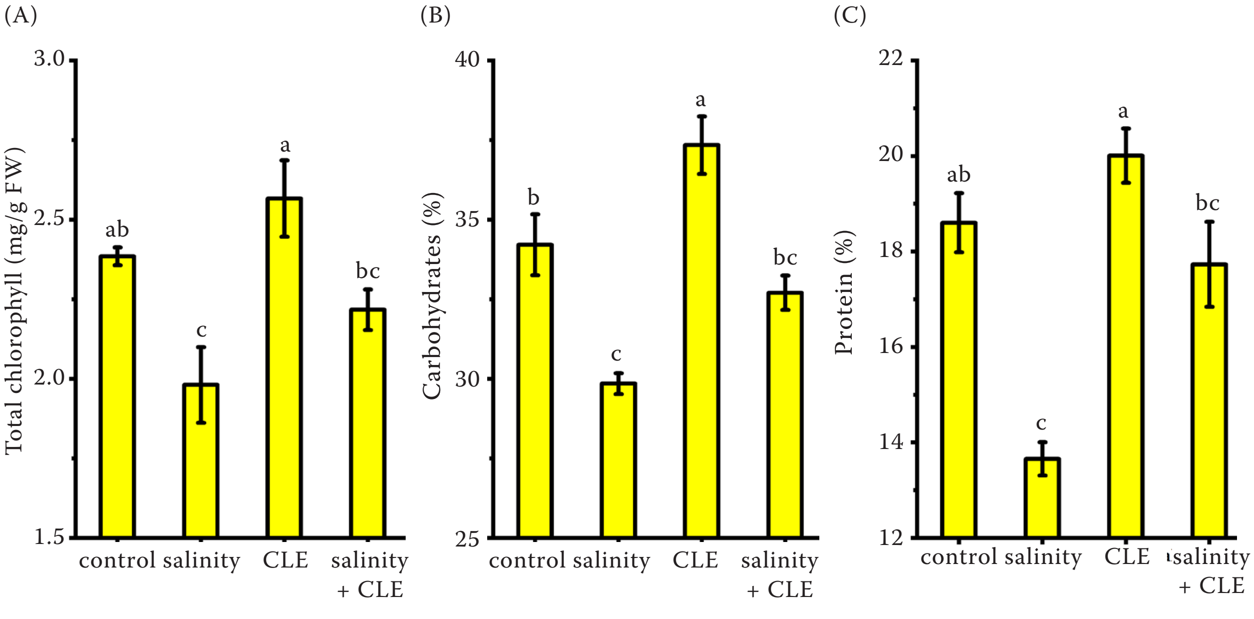


Figure 2. Effect of *Cassia javanica* leaf extract (CLE) foliar application on (A) total chlorophyll; (B) carbohydrates, and (C) protein content in the leaves of *Syzygium cumini* L. seedlings irrigated with saline water. The same letters are significantly different at the $P \leq 0.05$ level. FW – fresh weight

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Table 2. Effect of *Cassia javanica* leaf extract (CLE) foliar application on total phenols, ascorbic acid, ferric ion reducing power assay (FRAP), catalase (CAT), and superoxide dismutase (SOD) content in the leaves of *Syzygium cumini* L. seedlings irrigated with saline water

| Treatment | Total phenols (mg gallic acid/g DW) | Ascorbic acid (mg/100 g DW) | FRAP (U mol/g FW) | CAT (U/mg protein) | SOD (U/mg protein) |
|---|---|---|---|---|---|
| Control (T1) | 45.59 ± 0.92$^{c}$ | 10.38 ± 0.49$^{c}$ | 102.94 ± 1.29$^{b}$ | 6.02 ± 0.20$^{c}$ | 9.26 ± 0.37$^{d}$ |
| Salinity (T2) | 48.48 ± 0.98$^{c}$ | 13.84 ± 0.50$^{ab}$ | 83.10 ± 1.74$^{c}$ | 8.32 ± 0.72$^{b}$ | 13.32 ± 0.28$^{c}$ |
| CLE (T3) | 52.96 ± 0.72$^{b}$ | 12.59 ± 0.13$^{b}$ | 120.53 ± 1.84$^{a}$ | 9.63 ± 0.25$^{b}$ | 17.23 ± 0.51$^{b}$ |
| Salinity + CLE (T4) | 56.29 ± 1.11$^{a}$ | 14.86 ± 0.29$^{a}$ | 104.07 ± 1.77$^{b}$ | 12.27 ± 0.30$^{a}$ | 20.76 ± 0.36$^{a}$ |

The same letters within a column are significantly different at the $P \leq 0.05$ level. DW – dry weight; FW – fresh weight

ited the highest FRAP values (Table 2), but this value decreased in T4 seedlings. T2 seedlings showed the lowest FRAP value in their leaves among all treatments.

**Antioxidant enzyme activity.** Seedlings that received T2 treatment presented significantly lower activities of CAT and SOD than T3 and T4-seedlings, although T1-seedlings remained the lowest overall (Table 2). CLE treatment enhanced enzyme activities in T3, and more so in T4. The highest CAT (12.3 U/mg protein) and SOD (20.8 U/mg protein) activities were recorded by T4-seedlings.

**Proline and malondialdehyde.** Salinity (T2) resulted in significantly greater proline accumulation and lipid peroxidation (MDA) than in T1 (Figure 3). The lowest proline and MDA levels were observed in the T3 seedlings. Moreover, T4-seedlings showed significantly less proline and MDA than T2-seedlings.

**$H_2O_2$ content.** The results in Figure 3 showed that $H_2O_2$ levels in Java plum leaves were affected by saline water irrigation and CLE foliar spray. The $H_2O_2$ generated in Java plum leaves was significantly higher in the T2 treatment than in all other treatments. However, in T4 seedlings, $H_2O_2$ generation was significantly lower. Seedlings treated with CLE alone (T3) presented $H_2O_2$ levels similar to those detected in T1.

**Elements concentration.** Element concentrations in Java plum leaves treated with CLE spray showed a significant increase compared with the other treatments (Table 3). In salt-stressed seedlings, the nutrient contents were the lowest for N, P, and K (2.19%, 4.61 mg/100 g, and 44.31 mg/100 g, respectively). However, these values were further increased following CLE foliar application for salt-stressed seedlings

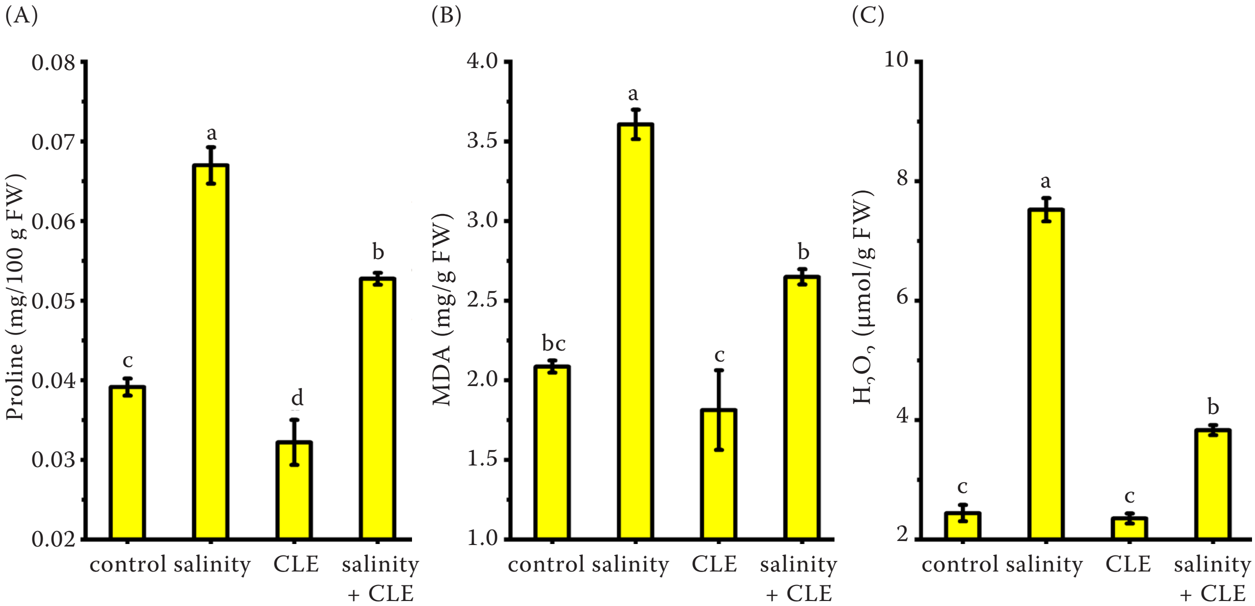


Figure 3. Effect of *Cassia javanica* leaf extract (CLE) foliar application on (A) proline; (B) malondialdehyde (MDA), and (C) hydrogen peroxide ($H_2O_2$) content in leaves of *Syzygium cumini* L. seedlings irrigated with saline water. The same letters are significantly different at the $P \leq 0.05$ level. FW – fresh weight

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Table 3. Effect of *Cassia javanica* leaf extract (CLE) foliar application on nitrogen (N), phosphorus (P), and potassium (K) of *Syzygium cumini* L. seedlings irrigated with saline water

| Treatment | N (% DW) | P (mg/100 g DW) | K (mg/100 g DW) |
|---|---|---|---|
| Control (T1) | 2.98 ± 0.10[ab] | 5.38 ± 0.14[b] | 48.1 ± 1.0[ab] |
| Salinity (T2) | 2.19 ± 0.06[c] | 4.61 ± 0.16[c] | 44.3 ± 0.60[c] |
| CLE (T3) | 3.20 ± 0.09[a] | 6.53 ± 0.23[a] | 50.2 ± 0.80[a] |
| Salinity + CLE (T4) | 2.84 ± 0.14[bc] | 5.72 ± 0.11[b] | 45.7 ± 0.45[bc] |

The same letters within the column are not significant at the $P \leq 0.05$ level. DW – dry weight

(T4), reaching 2.84%, 5.72 mg/100 g, and 45.74 mg/100 g, respectively. Significantly higher nutrient concentrations were detected in T3 seedlings.

## DISCUSSION

Limited water resources have increased the demand for high-quality crop cultivation while maximising productivity per unit of water. This study reported the impacts of CLE on Java plum seedlings irrigated with saline water (Figure 4). Saline water depressed the seedling growth, as indicated by decreased plant height and biomass.

An improvement in the root length was observed in salted seedlings; however, CLE-treated seedlings exhibited the shortest but thickest roots. Under salt stress, morphological changes take place in root length, thickness, and proliferation (Acosta-Motos et al. 2017). Foliar spraying increases element absorption, promoting root initiation and increasing root weight (Sheha et al. 2023, Al-Saif et al. 2024, Mahdy et al. 2024). These findings explain why the root length and weight of Java plum seedlings showed differences as affected by the treatments applied in this study.

The decline in growth traits of seedlings irrigated with saline water is attributed to osmotic stress, which causes a reduction in the expansion and division of plant cells and a depression in photosynthesis (Pardo 2010). In contrast, the CLE application enhanced plant growth. Kaur and Arora (2011) reported that a high antioxidant potency in *C. javanica* leaf extract, noting that water extract exhibited the strongest antioxidant activity. *Cassia* contains several important compounds, e.g., anthracene derivatives (anthraquinones), sterols, flavonoids, piperidine alkaloids, phenylpropanoids, pentacyclic triterpenoids, and γ-naphthopyrones, that contribute to plant growth and enhance stress resistance.

Bi et al. (2017) previously stated that sinapic acid, a phenylpropanoid compound, is extracted from

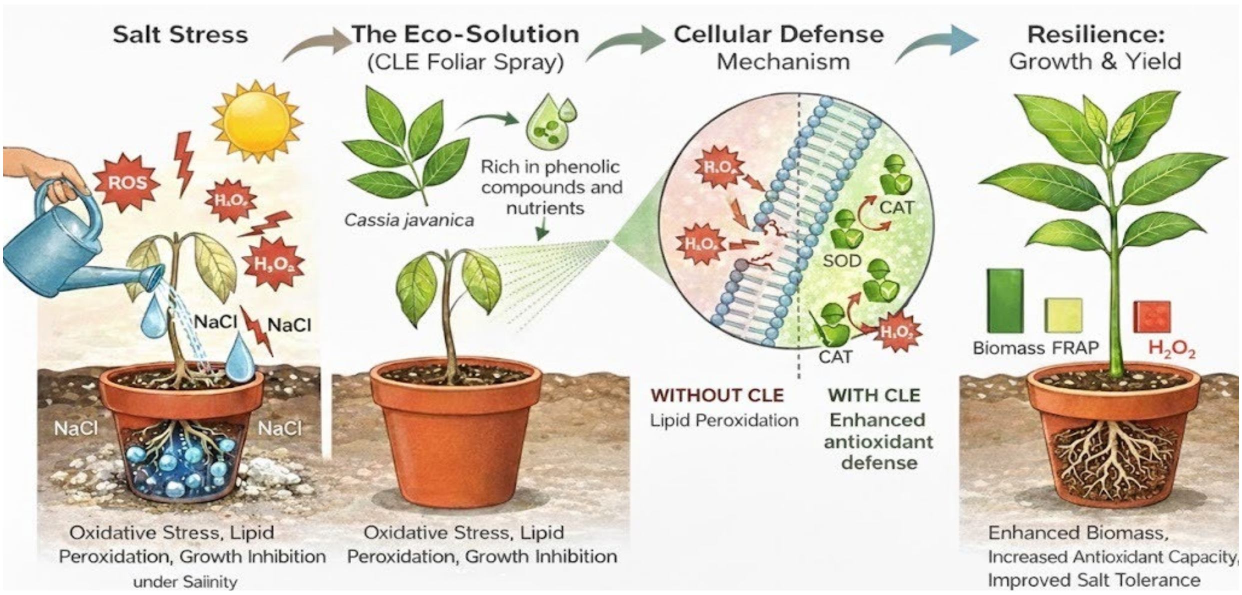


Figure 4. The response of *Syzygium cumini* L. seedlings to *Cassia javanica* leaf extract (CLE) foliar application under saline conditions

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*C. javanica* leaves. The carbon skeleton of phenylalanine serves as the source of a wide range of chemicals produced through phenylpropanoid metabolism, which contribute to plant survival, defence, and structural support (Vogt 2010). Sinapic acid, a typical precursor for soluble secondary metabolites, is a natural member of the phenylpropanoid family (Shahidi and Naczk 2004). In *Arabidopsis*, the buildup of these soluble phenylpropanoids and sinapic acid esters also provides defence against several stresses (König et al. 2014). Sinapic acid stimulated the root length and weight of *Arabidopsis* seedlings by 44% and 20%, respectively (Bi et al. 2017). The same authors emphasised the importance of sinapic acid during the early stages of plant development. Additionally, *C. javanica* leaves contain chrysophanol (chrysophanic acid) (Arya and Yadav 2011), a naturally occurring anthraquinone compound found in various plants and known for its potential anti-inflammatory, anticancer, and antimicrobial activities (Prateeksha et al. 2019). Chrysophanol supplementation caused an enhancement in the growth, proteomic analysis, photosynthesis, sucrose content, and defence-related gene expression in cabbage seedlings exposed to *Botrytis cinerea* (Liu et al. 2016). A reduction in chlorophyll content in seedling leaves was detected following salt stress. Similar reductions in chlorophyll content under saline conditions were observed (Atteya et al. 2021, 2022a, Bahgat et al. 2023). Salinity stimulates chlorophyllase activity, which contributes to chlorophyll degradation and decreases nitrogen absorption (Paul and Lade 2014). The fact that CLE improved chlorophyll levels may help clarify how this extract mitigates the detrimental effects of salinity on growth. Increased growth is associated with higher chlorophyll concentrations (Alshallash et al. 2022, Atteya et al. 2022b). CLE is rich in N and Mg (Al-Menaie et al. 2010), which are essential for photosynthesis. The observed increases in leaf pigments and carbohydrate concentrations in Java plum seedlings highlight the important effects of CLE treatments.

An increase in total carbohydrates, proteins, and antioxidant activity in seedling leaves following CLE treatment was observed. Osmoprotectant generation is a strategy for plant resistance against stress conditions (Rasool et al. 2013). Osmoprotectants preserve water absorption, membrane stability, and protein integrity, consequently preserving cell turgor. Carbohydrates accumulated in plant cells function as osmoprotectants by stimulating water and nutrient absorption, photosynthesis, and essential metabolic processes (Zali and Ehsanzadeh 2018, El-Nagar et al. 2025).

Under saline conditions, proteins and cell membranes are oxidatively damaged by $H_2O_2$ (Ahmad et al. 2011). An elevation in $H_2O_2$ concentrations in saline treatments was detected; this increase was accompanied by increased MDA concentration, indicating greater protein and membrane deterioration and lipid peroxidation (Eraslan et al. 2007). High levels of lipid peroxidation caused by ROS under stressful conditions have also been reported previously (El-Serafy et al. 2021, 2023, Gururani et al. 2023).

There was a slight increase in the antioxidant system of the CLE-treated salted seedlings compared to the salted seedlings without CLE. Antioxidant status increments may be due to the natural antioxidant content present in *C. javanica* leaves (Kaur and Arora 2011, Lavanya et al. 2018), which contributed to improving the antioxidant activity of the T4 seedlings. These improvements may collaborate in depressing the oxidative burst under stress conditions (Kaur et al. 2014, Gémes et al. 2016).

Proline is associated with enhanced resilience to various environmental stressors (Liang et al. 2013, Shafi et al. 2019). CLE application reduced free proline content in stressed seedlings, suggesting a role for CLE in alleviating the detrimental effects of salt stress. The observations obtained provide evidence that the aqueous extract of *Cassia javanica* leaves, when applied as a foliar spray, has the potential to serve as a sustainable, green source of antioxidants that stimulate plant growth by mitigating the adverse effects of salinity in Java plum seedlings. *Cassia javanica* leaf extract not only improved the antioxidant system in salt-stressed Java plum but also reduced ROS generation and membrane damage, while increasing the levels of important osmoprotectants.

In conclusion, the present study investigated the use of *Cassia javanica* leaf extract as a foliar-applied biostimulant and evaluated its effectiveness in stimulating Java plum seedling growth under saline conditions. Foliar application of CLE significantly maintained plant fresh and dry weights in salted seedlings by 34.6% and 33%, respectively, compared with salt-treated seedlings. The results indicated a potential ameliorative effect of CLE under salt stress, as evidenced by increased enzymatic and non-enzymatic antioxidant activity, reduced ROS generation, increased accumulation of osmoprotectants, and improved nutrient homeostasis in seedling

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leaves. Aqueous leaf extract of *Cassia javanica* can be further developed as an effective biostimulant, pending additional studies across different crops and under varying stress conditions.

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## REFERENCES


Ábrahám E., Hourton-Cabassa C., Erdei L., Szabados L. (2010): Methods for determination of proline in plants. In: Sunkar R. (ed.): Plant Stress Tolerance: Methods and Protocols. Totowa, Humana Press, 317–331.

Acosta-Motos J.R., Ortuño M.F., Bernal-Vicente A., Diaz-Vivancos P., Sanchez Blanco M.J., Hernandez J.A. (2017): Plant responses to salt stress: adaptive mechanisms. Agronomy, 7: 18.

Aebi H. (1984): Catalase *in vitro*. Methods in Enzymology, 105: 121–126.

Ahmad P., Nabi G., Jeleel C.A., Umar S. (2011): Free radical production, oxidative damage and antioxidant defense mechanisms in plants under abiotic stress. In: Ahmad P., Umar S. (eds.): Oxidative Stress: Role of Antioxidants in Plants. New Delhi, Studium Press, 19–53.

Ahmad P., Sharma S. (2008): Salt stress and phyto-biochemical responses of plants. Plant, Soil and Environment, 54: 89–99.

Alayafi A.H., Dahab A.A., El-Sheshtawy A.A., Sharma A., Elhakem A., Youssef S.M., El-Serafy R.S. (2025): Stimulatory effect of *Delonix regia* flower extract in protecting *Syzygium cumini* seedlings from salinity. Plants, 14: 875.

Al-Menaie H.S., Al-Ragam O., Al-Shatti A., Mathew M., Suresh N. (2010): The effects of different treatments on seed germination of the *Cassia fistula* L. and *Cassia nodosa* Buch.-Ham. ex Roxb. in Kuwait. African Journal of Agricultural Research, 5: 230–235.

Al-Saif A.M., Ahmed M.E.M., Taha M.A., Sharma A., El-Sheshtawy A.A., Abouelsaad I.A., El-Serafy R.S., Mahdy R.M. (2024): Preharvest applications improve the postharvest storage and quality of tomato fruits by enhancing the nutritional value and antioxidant system. Horticulturae, 10: 1248.

Alshallash K.S., Mohamed M.F., Dahab A.A., Abd El-Salam H.S., El-Serafy R.S. (2022): Biostimulation of *Plectranthus amboinicus* (Lour.) spreng. with different yeast strains: morphological performance, productivity, phenotypic plasticity, and antioxidant activity. Horticulturae, 8: 887.

AOAC (1995): Official Method of Analysis. 16$^{th}$ Edition. Arlington, Association of Official Analytical Chemists International.

Arya V., Yadav J.P. (2011): Comparative assessment of relative antioxidant activity of sequential leaf extracts of *Cassia occidentalis* L. and *Cassia tora* L. Pharmacologyonline, 1: 529–543.

Atteya A.K.G., Albalawi A.N., El-Serafy R.S., Albalawi K.N., Bayomy H.M., Genaidy E.A.E. (2021): Response of *Moringa oleifera* seeds and fixed oil production to vermicompost and NPK fertilizers under calcareous soil conditions. Plants, 10: 1998.

Atteya A.K.G., El-Serafy R.S., El-Zabalawy K.M., Elhakem A., Genaidy E.A.E. (2022a): Brassinolide maximized the fruit and oil yield, induced the secondary metabolites, and stimulated linoleic acid synthesis of *Opuntia ficus-indica* oil. Horticulturae, 8: 452.

Atteya A.K.G., El-Serafy R.S., El-Zabalawy K.M., Elhakem A., Genaidy E.A.E. (2022b): Exogenously supplemented proline and phenylalanine improve growth, productivity, and oil composition of salted moringa by upregulating osmoprotectants and stimulating antioxidant machinery. Plants, 11: 1553.

Attia M.A.E.H., El-Enin M.M.A., Tahoun A.M.A., Abdelghany F.I.M., El-Serafy R.S. (2022): Productivity of some barley cultivars as affected by supplemental irrigation under rainfed conditions. Australian Journal of Crop Science, 16: 665–667.

Ayyanar M., Subash-Babu P. (2012): *Syzygium cumini* (L.) Skeels: a review of its phytochemical constituents and traditional uses. Asian Pacific Journal of Tropical Biomedicine, 2: 240–246.

Bahgat A.R., Dahab A.A., Elhakem A., Gururani M.A., El-Serafy R.S. (2023): Integrated action of rhizobacteria with *Aloe vera* and moringa leaf extracts improves defense mechanisms in *Hibiscus sabdariffa* L. cultivated in saline soil. Plants, 12: 3684.

Belmain S., Neal G., Ray D., Golob P. (2001): Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. Food and Chemical Toxicology, 39: 287–291.

Benzie I.F., Strain J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Annals of Biochemistry, 239: 70–76.

Bi B., Tang J., Han S., Guo J., Miao Y. (2017): Sinapic acid or its derivatives interfere with abscisic acid homeostasis during *Arabidopsis thaliana* seed germination. BMC Plant Biology, 17: 1–12.

Dere S., Günes T., Sivaci R. (1998): Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. Turkish Journal of Botany, 22: 13–17.

Dewanto V., Wu X., Adom K.K., Liu R.H. (2002): Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agriculture and Food Chemistry, 50: 3010–3014.

El-Nagar A.H., Ghanem K.Z., Hassan F.A.S., Fetouh M.I., El-Serafy R.S., Moussa M.M. (2025): The changes in growth, yield, and biologically active compounds of essential oil in *Trachyspermum ammi* L. upon rhizobacteria and seaweed applications. Plant, Soil and Environment, 71: 565–580.

El-Serafy R.S. (2020): Phenotypic plasticity, biomass allocation, and biochemical analysis of cordyline seedlings in response to oligochitosan foliar spray. Journal of Soil Science and Plant Nutrition, 20: 1503–1514.

El-Serafy R.S., Dahab A.A., Ghanem K.Z., Elhakem A., Bahgat A.R., Venkatesh J., El-Sheshtawy A.A., Badawy A. (2024): As a natural

https://doi.org/10.17221/374/2025-PSE

antioxidant: *Sesbania grandiflora* leaf extract enhanced growth and yield performance, active ingredients and tolerance of *Hibiscus sabdariffa* L. under salt-affected soil. Journal of Soil Science and Plant Nutrition, 24: 3406–3420.

El-Serafy R.S., El-Sheshtawy A.A., Atteya A.K., Al-Hashimi A., Abbasi A.M., Al-Ashkar I. (2021): Seed priming with silicon as a potential to increase salt stress tolerance in *Lathyrus odoratus*. Plants, 10: 2140.

El-Serafy R.S., El-Sheshtawy A.A., Dahab A.A. (2023): Fruit peel soil supplementation induces physiological and biochemical tolerance in *Schefflera arboricola* L. grown under heat conditions. Journal of Soil Science and Plant Nutrition, 23: 1046–1059.

Eraslan F., Inal A., Savasturk O., Gunes A. (2007): Changes in antioxidative system and membrane damage of lettuce in response to salinity and boron toxicity. Scientia Horticulturae, 114: 5–10.

Estefan G., Sommer R., Ryan J. (2013): Methods of Soil, Plant, and Water Analysis: A Manual for the West Asia and North Africa Region. 3rd Edition. Beirut, International Center for Agricultural Research in the Dry Areas, 84–105.

Gémes K., Kim Y.J., Park K.Y., Moschou P.N., Andronis E., Valassaki C., Roussis A., Roubelakis-Angelakis K.A. (2016): An NADPH-oxidase/polyamine oxidase feedback loop controls oxidative burst under salinity. Plant Physiology, 172: 1418–1431.

Ghanem K.Z., Hasham M.M., El-Sheshtawy A.A., El-Serafy R.S., Sheta M.H. (2022): Biochar stimulated actual evapotranspiration and wheat productivity under water deficit conditions in sandy soil based on non-weighing lysimeter. Plants, 11: 3346.

Giannopolitis C.N., Ries S.K. (1977): Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology, 59: 309–314.

Gururani M.A., Atteya A.K., Elhakem A., El-Sheshtawy A.A., El-Serafy R.S. (2023): Essential oils prolonged the cut carnation longevity by limiting the xylem blockage and enhancing the physiological and biochemical levels. PLoS One, 3: e0281717.

Hadwan M.H. (2018): Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochemistry, 19: 7.

Kaur G., Sharma A., Guruprasad K., Pati P.K. (2014): Versatile roles of plant NADPH oxidases and emerging concepts. Biotechnology Advances, 32: 551–563.

Kaur P., Arora S. (2011): Superoxide anion radical scavenging activity of *Cassia siamea* and *Cassia javanica*. Medicinal Chemistry Research, 20: 9–15.

Khurm M., Wang X., Zhang H., Hussain S.N., Qaisar M.N., Hayat K., Saqib F., Zhang X., Zhan G., Guo Z. (2021): The genus *Cassia* L.: ethnopharmacological and phytochemical overview. Phytotherapy Research: PTR, 35: 2336–2385.

König S., Feussner K., Kaever A., Landesfeind M., Thurow C., Karlovsky P., Gatz C., Polle A., Feussner I. (2014): Soluble phenylpropanoids are involved in the defense response of *Arabidopsis* against *Verticillium longisporum*. New Phytologist, 202: 823–837.

Kumar A., Singh S., Gaurav A.K., Srivastava S., Verma J.P. (2020): Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Frontiers in Microbiology, 11: 1216.

Kumavat U.C., Gopalkrishnan B. (2024): Differentiation of two allied pink *Cassia* species based on physicochemical and chemotaxonomic studies. World Journal of Pharmacological Science Research, 3: 113–121.

Landi M. (2017): Commentary to: "Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds" by Hodges et al., Planta (1999) 207: 604–611. Planta, 245: 1067.

Lavanya B., Maheswaran A., Vimal N., Vignesh K., Uvarani K.Y., Varsha R. (2018): An overall view of *Cassia* species phytochemical constituents and its pharmacological uses. International Journal of Pharmacology Science Research, 3: 2455–4685.

Leyva A., Quintana A., Sánchez M., Rodríguez E.N., Cremata J., Sánchez J.C. (2008): Rapid and sensitive anthrone-sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: method development and validation. Biologicals, 36: 134–141.

Liang X., Zhang L., Natarajan S.K., Becker D.F. (2013): Proline mechanisms of stress survival. Antioxidants and Redox Signalling, 19: 998–1011.

Liu S.Y., Liao C.K., Lo C.T., Yang H.H., Lin K.C., Peng K.C. (2016): Chrysophanol is involved in the biofertilization and biocontrol activities of *Trichoderma*. Physiological and Molecular Plant Pathology, 96: 1–7.

Mahdy R.M., Al-Saif A.M., Ahmed M.E.M., Abd El-Bary T.S., Sharma A., El-Sheshtawy A.A., El-Serafy R.S., Abd El-Ghany T.S. (2024): Evaluation of two different methods of fulvic acid application (seed priming and foliar spray) on growth, yield, and nutritional quality of pea (*Pisum sativum* L.). Plants, 13: 3380.

Mariotti F., Tomé D., Mirand P.P. (2008): Converting nitrogen into protein – beyond 6.25 and Jones' factors. Critical Reviews in Food Science and Nutrition, 48: 177–184.

Pardo J.M. (2010): Biotechnology of water and salinity stress tolerance. Current Opinion Biotechnology, 21: 185–196.

Patterson B.D., Macrae E.A., Ferguson I.B. (1984): Estimation of hydrogen peroxide in plant extracts using titanium (IV). Analytical Biochemistry, 139: 487–492.

Paul D., Lade H. (2014): Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. Agronomy for Sustainable Development, 34: 730–752.

Prateeksha Yusuf M.A., Singh B.N., Sudheer S., Kharwar R.N., Siddiqui S., Abdel-Azeem A.M., Fraceto L.F., Dashora K., Gupta V.K. (2019): Chrysophanol: a natural anthraquinone with multifaceted biotherapeutic potential. Biomolecules, 9: 68.

Rasool S., Hameed A., Azooz M.M., Siddiqi T.O., Ahmad P. (2013): Salt stress: causes, types and responses of plants. In: Ahmad P., Azooz M.M., Prasad M.N.V. (eds.): Ecophysiology and Responses of Plants under Salt Stress. New York, Springer, 1–24.

Sarvade S., Gautam D.S., Bhalawe S., Bisen P.K. (2016): An overview of potential multipurpose agroforestry tree species, *Syzygium cuminii* (L.) Skeels in India. Journal of Applied Natural Science, 8: 1714–1719.

Shafi A., Zahoor I., Mushtaq U. (2019): Proline accumulation and oxidative stress: diverse roles and mechanism of tolerance and adaptation under salinity stress. In: Akhtar M.S. (ed.): Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches 2. Singapore, Springer Nature Singapore Pte Ltd., 269–300.

Shahidi F., Naczk M. (2004): Cereals, legumes, and nuts. In: Kakade A. (ed.): Phenolics in Food and Nutraceuticals. Boca Raton, CRC Press, 117–166.

Sheha A.M., Abou El-Enin M.M., El-Hashash E.F., Rady M.M., El-Serafy R.S., Shaaban A. (2023): The productivity and overall benefits of faba bean-sugar beet intercropping systems interacted with foliar applied nutrients. Journal of Plant Nutrition, 46: 1683–1700.

Sheta M.H., Abd El-Wahed A.H.M., Elshaer M.A., Bayomy H.M., Ozaybi N.A., Abd-Elraheem M.A.M., El-Sheshtawy A.A., El-Serafy R.S., Moustafa M.M.I. (2024): Green synthesis of zinc and iron nanoparticles using *Psidium guajava* leaf extract stimulates cowpea growth, yield, and tolerance to saline water irrigation. Horticulturae, 10: 915.

Tewari S.K., Singh D., Nainwal R.C. (2017): Horticultural management of *Syzygium cumini*: *Syzygium cumini* and other underutilized species. In: Nair K.N. (ed.): The Genus *Syzygium*. Boca Raton, CRC Press, 215–236.

Vogt T. (2010): Phenylpropanoid biosynthesis. Molecule Plant, 3: 2–20.

Youssef S.M., El-Serafy R.S., Ghanem K.Z., Elhakem A., Abdel Aal A.A. (2022): Foliar spray or soil drench: microalgae application impacts on soil microbiology, morpho-physiological and biochemical responses, oil and fatty acid profiles of Chia plants under alkaline stress. Biology, 11: 1844.

Zafar Z., Rasheed F., Atif R.M., Maqsood M., Gailing O. (2021): Salicylic acid-induced morpho-physiological and biochemical changes triggered water deficit tolerance in *Syzygium cumini* L. saplings. Forests, 12: 491.

Zali A.G., Ehsanzadeh P. (2018): Exogenously applied proline as a tool to enhance water use efficiency: case of fennel. Agriculture Water Management, 197: 138–146.

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