

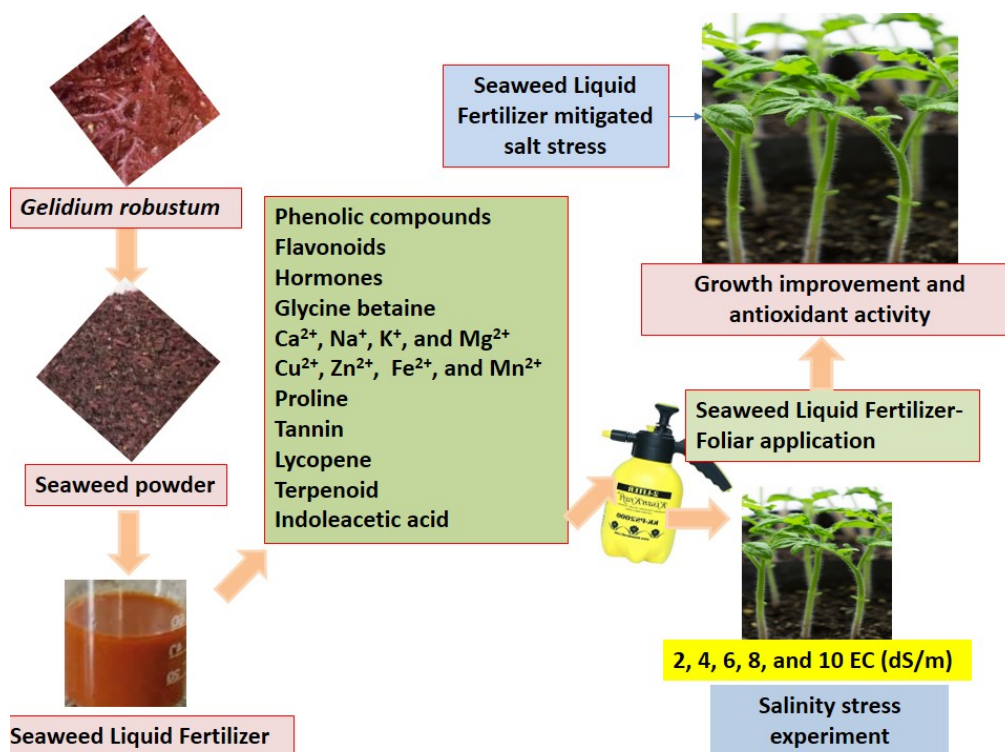
Mitigating Salt Stress in Tomato Plants via *Gelidium robustum* Seaweed Extract

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GRAPHICAL ABSTRACT



Mitigating Salt Stress in Tomato Plants via *Gelidium robustum* Seaweed Extract

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Salinity stress, a major abiotic factor, poses a threat to crops. Thus, strategies to mitigate adverse effects on crops are warranted. This study analyzed the effects of the red seaweed *Gelidium robustum* extract on tomato plants (*Solanum lycopersicum*) subjected to salt stress. The composition of seaweed was analyzed by analytical methods, showing that it consists of protein, phenolic compounds, flavonoids, glycine betaine, elements, tannins, proline, and growth hormones. The tomato plants were cultivated under salinity stress (2 to 10 dS/m) and treated with seaweed liquid fertilizer (SLF) at various concentrations (2.5% to 10%). The tomato plants treated with SLF presented significant growth-promoting activity. Morphological analysis revealed improved shoot, root, and leaf growth. Compared with no treatment, SLF increased the total protein and fat contents in tomato leaves. Similarly, treatment of tomato plants with SLF improved their flavonoid and phenolic compound contents and antioxidant activity. SLF altered the biochemical profile, alleviated stress, and enhanced defenses. Overall, SLFs have the potential to mitigate salinity stress in tomato plants due to the antioxidant defense, and the application of red seaweed is useful for improving agriculture in salt-contaminated agricultural soils.

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Keywords: Seaweed; Seaweed liquid fertilizer; Tomato plant; Salinity stress; Stress mitigation

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INTRODUCTION

Plant biostimulants have broad applications in agriculture. These microorganisms or substances improve plant growth performance, improve disease resistance, and mitigate abiotic stress (Calvo *et al.* 2014). Biostimulants such as glycine betaine, protein hydrolysate, mycorrhizal fungi, bacterial supernatant, microalgae, or seaweed extracts (Plaut *et al.* 2013; du Jardin 2015; Van Oosten *et al.* 2017) have been used to improve plant growth. Among these biostimulants, seaweed extracts (SWEs) have been shown to improve plant growth and mitigate abiotic stress through a wide range of bioactive secondary metabolites that act as the major component in commercial SWE preparations (Di Stasio *et al.* 2018; El Boukhari *et al.* 2020). SWEs are used to improve the quality of crops (Mattner *et al.* 2013), mitigate abiotic stress, including temperature, and salt stress (Xu and Leskovar 2015; Elansary *et al.* 2016), and improve the bioavailability of nutrients (Papenfus *et al.* 2013; Halpern *et al.* 2015). In addition, SWEs improve the vegetative growth of plants and stimulate rhizospheric microbial populations (Mansori *et al.* 2015). Supplemented SWEs have been shown to improve the proliferation of soil microbes,

enhanced nutrient cycling, mitigated salt stress, and increased enzymatic activity (Ali *et al.* 2024; Kumar *et al.* 2024).

Salinity is an important abiotic stress that alters several physiological mechanisms involved in plant growth and development, including increasing the production of reactive oxygen species (ROS), decreasing shoot and root biomass, decreasing photosynthetic activity, and increasing the biosynthesis of antioxidant compounds (He *et al.* 2021; Shariatinia *et al.* 2021). Several approaches have been proposed to mitigate the negative impact of salinity stress on crops. Crop rotation, nutrient and irrigation management, reduced tillage, cropping-tolerant crops, mulching, and phytoremediation are used to reduce salt stress (Cuevas *et al.* 2019). Biostimulants can play a significant role in reducing the deleterious effects of salt stress. These mitigating effects are associated with reducing oxidative stress by scavenging ROS, reducing ionic imbalances, enhancing the accumulation of osmo-protectants, reducing lipid peroxidation, and improving antioxidant mechanisms through increased production of phenolic compounds and nonenzymatic antioxidants (Trivedi *et al.* 2018; Liu *et al.* 2019; Zou *et al.* 2019). However, such positive impacts are significantly associated with increased sugar accumulation, photosynthetic activity and nutritional status, leading to improvements in crop yield and plant biomass (Chrysargyris *et al.* 2018; De Carvalho *et al.* 2019; Rosa *et al.* 2021).

Tomatoes constitute one of the major crop species and are cultivated widely in several countries. They are used for the preparation of sauces, canned fruit, paste, and several culinary products and are considered common vegetables. In tomato, the taste of the fruit has been found to vary based on the growing conditions, the types of fertilizers used for cultivation, and the agricultural practices that significantly influence flavor. These factors affect the sugar content and composition of tomato fruits, acidity, and level of proline, which significantly influence fruit quality and taste (Cai *et al.* 2024). The application of chemical fertilizers significantly improves plant growth and meets the global food demand, and their unrestricted use poses risks to animals and the environment, including water pollution, soil degradation, and nutrient imbalances (Pahalvi *et al.* 2021). To promote eco-friendly sustainable agriculture, the adoption of eco-friendly methods, such as biobased amendments, including the use of organic fertilizers, is very useful (Priya *et al.* 2023). Seaweeds are attractive alternatives and have attracted increasing interest in recent years to improve crop productivity and mitigate salt, drought, and water stress by improving antioxidant mechanisms, either by stimulating enzymatic or nonenzymatic antioxidant molecular mechanisms.

Previous studies have shown that the combined application of *Mycorrhiza* and seaweed extracts significantly enhances seedling growth parameters in tomato and pepper (Bayram *et al.* 2025; Calvo *et al.* 2014; Poveda *et al.* 2019)". These findings support the potential of seaweed-based biostimulants to improve plant growth and mitigate abiotic stresses, including salinity, in Solanaceae crops. Among algal groups, brown algae have been widely studied for their growth-promoting and biostimulant properties. Seaweed extracts or seaweed fertilizers are reservoirs of bioactive compounds, including polysaccharides, phytohormones, vitamins, minerals, and micronutrients, which improve plant growth, increase nutrient uptake potential and improve resistance to mitigate abiotic stresses (Battacharyya *et al.* 2015; Prisa and Spagnuolo 2022; Spagnuolo *et al.* 2023). Red seaweeds are rich of phenolic compounds which exhibited antioxidant activity and mitigated abiotic stress. Recently reported seaweeds, in terms of their extracts, were *Gelidium corneum* (Matias *et al.* 2023), and *Gelidium sesquipedale* (Nogot *et al.* 2024). The application of these SWE is increasing in agriculture as part of an eco-friendly and

sustainable friendly approach. The hypothesis of this work is that antioxidant-rich red seaweed can be used to mitigate salt stress in tomato plant. There has been a need for studies on the use of red seaweed for plant growth and salt stress mitigation. Considering these research gaps, this study was designed to use seaweed liquid fertilizer to improve the growth, yield and quality of tomato plants under salt stress. The aim of the work was to analyze the proximate, phytochemical, hormone, and element composition of *G. robustum* extract and its growth profile, and salt stress mitigation effect on tomato plants.

EXPERIMENTAL

Seaweed

The red seaweed *Gelidium robustum* was collected from a marine environment for testing as a biostimulant agent. This mixture was used for the preparation of seaweed liquid fertilizer.

Preparation of Seaweed Liquid Fertilizer

The collected seaweed was cleaned by repeatedly washing with freshwater, and impurities were removed. It was dried for 48 h in an oven at 60°C and stored in silica gel until the extraction procedure was started. The dried seaweed powder was mixed with sterilized distilled water at a 1:20 ratio (distilled water/volume ratio). The mixture was heated for 2 h at 80 °C for the extraction of bioactive secondary metabolites. The mixture was subsequently filtered using Whatman no. 1 filter paper, and the resulting mixture was considered seaweed liquid fertilizer (SLF). The final concentration was fixed at 10% for the foliar treatment.

Proximate, Phytochemical, Hormone and Element Contents of the *G. robustum* Liquid Fertilizer

The SLFs were dried at 40 °C, and the total yield was calculated. The mixture was reconstituted (1.0 mg/mL) in distilled water and stored at –20 °C, after which composition analysis was performed. The total phenolic content of the SLF was estimated. Briefly, the SLF (0.2 mL) was mixed with 4.8 mL of double distilled water. The amount of total phenolic compounds in the SLF was determined as described previously with slight modifications. Then, 0.5 mL of 1 N of formaldehyde reagent was added, and the mixture was mixed for 5 seconds. Then, 1 mL of 10% sodium carbonate was added to the reaction mixture. The mixture was incubated for 10 min, and the total phenolic content was assayed at 765 nm. The results are expressed as gallic acid equivalents per gram. Gallic acid was prepared at 100 to 500 g/mL, and the experiment was performed in triplicate (Malar *et al.* 2020). The total flavonoid content of the SLFs was evaluated as described previously with slight modifications. Briefly, 0.2 mL of SLF was mixed with 3.8 mL of double distilled water and 0.5 mL of 5% NaNO₂ and stirred for three min. Then, 0.25 mL of AlCl₃ solution (10%) was added, and the mixture was incubated for 5 min. Then, 1.0 mL of 2 M NaOH was added, and the final volume was adjusted to 15 mL. The absorbance of each sample was read at 510 nm, and quercetin was used as a standard at 20 to 100 mg/L. The amount of flavonoid content in the SLF was expressed as mg quercetin/g (Al-Dhabi *et al.* 2020). Hormonal analysis of SLFs was performed as described previously by Benítez García *et al.* (2020). The amount of glycine betaine in SLFs was estimated as suggested by Grieve and Grattan (1983). Elemental analysis of the SLF was carried out according to the methods

developed by Nazarudin *et al.* (2021). The determinations of macronutrients (Ca^{2+} , Na^{+} , K^{+} , and Mg^{2+}) and micronutrients (Cu^{2+} , Zn^{2+} , Fe^{2+} , and Mn^{2+}) were performed *via* an atomic absorption spectrophotometer (ICE 3000, Thermo Scientific, USA). The samples were digested using nitric acid and perchloric acid mixture at 3:1 ratio and analysis was performed following the guidelines provided by AOAC (1990). The total protein content of the SLFs was determined *via* the Folin-phenol method (Lowry *et al.* 1951). The proline (Bates *et al.* 1973), lipid (Van Handel 1985), tannin (Makkar *et al.* 1993), lycopene (Nagata and Yamashita 1992), terpenoid (Fan and He 2006), and indoleacetic acid (Rauf *et al.* 2021) contents were analyzed.

Tomato Plants and Seaweed Treatment

In this study, a completely randomized block design with six blocks was used. Each block consisted of five treatment groups, with eight tomato plants per group, consisting of 30 plants. A hand sprayer was used for foliar application, ensuring total leaf coverage. Foliar application was performed every two days, with a total of five treatment groups for 30 days. After 30 days of treatment, the growth and response of the tomato plants were analyzed.

Tomato Seed Treatment with a Seaweed Liquid Fertilizer and Growth Conditions

Tomato seeds were collected from the market, and they were sterilized with 2% (v/v) sodium hypochlorite solution for 3 min at ambient temperature (28 ± 1 °C). They were further rinsed with filtered water three times. The seeds were subsequently treated with SLF at various concentrations (2%, 4%, 6%, 8%, and 10%). Water alone was added to the control seeds. Specimens were incubated at ambient temperature (28 ± 1 °C) for 60 min in an orbital shaker. The maximum concentration of SLF (10%) used in this study did not inhibit seed germination, confirming the results of preliminary studies. The seeds were soaked for 24 h and placed on wet filter paper 30 min. The seeds were subsequently sown in sterilized trays filled with sand and peat at a 1:1 ratio. All trays were placed in a growth chamber with 14 h of daylight and 10 h of night at 28 °C/24 °C and 75% relative humidity in a randomized design. Experimental and control seeds were treated with filtered tap water until complete emergence and further irrigated with sufficient nutrients. The plant growth was monitored, and the 4-leaf level tomato plants were harvested and used for salt stress tolerance experiments.

Salinity Stress Experiment

The experimental pots were irrigated with Hoagland nutrient solution (Hoagland and Arnon 1950) with five different salt concentrations. To the Hoagland nutrient solution, sodium chloride was added to reach electrical conductivity values of 2, 4, 6, 8, and 10 dS/m. For the control experiments, only Hoagland nutrient solution was applied. SLF was added at concentrations of 2.5%, 5%, 7.5%, and 10%.

Analysis of Plant Growth

The harvested seedlings were subjected to plant growth performance analysis. The shoot mass, root length, and fresh biomass were analyzed.

Analysis of Antioxidant Activity

The antioxidant activity of the leaves was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay with slight modifications. Briefly, 0.5 mL of plant extract was mixed with 0.5 mL of 2 mM DPPH prepared in methanol. The mixture was stirred for 30 min at ambient temperature. The absorbance of the sample and standard was assayed at 517 nm. The antioxidant activity was expressed as mg ascorbic acid/g fresh weight (mg AA/g FW).

Determination of Hydrogen Peroxide

The amount of hydrogen peroxide in the leaves was estimated as described previously (Velikova *et al.* 2000). Briefly, 0.2 g of leaves were homogenized with a glass homogenizer using 0.1 mL of 0.1% of trichloroacetic acid for 10 min. Then, the mixture was centrifuged at 5000×g for 10 min at 4 °C. Then, 0.5 mL extract was then mixed with 0.5 mL of phosphate buffer (0.01 M, pH 7.2) and 1.0 mL of 1 M KI. The absorbance of each sample was subsequently read at 390 nm, and hydrogen peroxide was used for the preparation of a standard curve (10 to 100 nm). The results are expressed as nmol/g fresh weight.

Analysis of Total Protein and Soluble Sugars

The total protein content of the plant leaf extract was assayed *via* the Bradford method (Bradford 1976). Briefly, 0.1 mL of leaf extract was mixed with 1.9 mL of Bradford reagent. The mixture was mixed and incubated for 2 min, and the absorbance was read at 595 nm against a reagent blank. Bovine serum albumin (BSA) was prepared at various concentrations (100 to 1000 µg/mL), and the results are expressed in mg of protein. The amount of soluble sugar in the leaves was tested by adding 0.1 g of leaves to 2 mL of double distilled water, and then the mixture was homogenized.

The amount of soluble sugars in the SLF was estimated as described previously by DuBois *et al.* (1956), with slight modifications. Briefly, 0.2 mL of SLF was mixed with 0.2 mL of phenol (80%). Then, 2 mL of concentrated sulfuric acid was added, and the mixture was mixed. The mixture was allowed to stand for 15 min and then placed in a water bath at 25 °C. The absorbance was read at 490 nm against a reagent blank. Glucose was used for the preparation of the standard curve (10 mg to 100 mg), and the result was expressed as mg glucose/g.

Determination of Chlorophyll and Carotenoid Contents

The amounts of chlorophylls *a* and *b*, and carotenoids were determined as described previously by Lichtenthaler (1987). A total of 0.05 g of leaf biomass was ground with 5 mL of 80% acetone and centrifuged at 3000×g for 10 min at ambient temperature. The supernatant was subsequently collected, and the absorbance was read at 470 nm, 642 nm, and 662 nm *via* a UV–visible spectrophotometer. The analysis was performed in triplicate, and the mean value was taken into consideration. The results were obtained by interpreting the values following Eqs. 1-3:

$$\text{Chlorophyll } a \text{ (}\mu\text{g mL}^{-1}\text{)} = 12.5 A_{663} - 2.79 A_{646} \quad (1)$$

$$\text{Chlorophyll } b \text{ (}\mu\text{g mL}^{-1}\text{)} = 21.5 A_{646} - 2.79 A_{663} \quad (2)$$

$$\text{Total carotenoids} = \frac{1000A_{470} - 1.82ch(a) - 85.02ch(b)}{198} \quad (3)$$

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to determine the effects of SLF on different salinity levels. All the data were analyzed *via* two-way ANOVA followed by Tukey's multiple range tests.

RESULTS AND DISCUSSION

Composition of Seaweed Liquid Fertilizer

Compositional analysis of *Gelidium robustum* aqueous extract revealed significant amounts of total protein (0.21 ± 0.02 mg/g FW), lipids (0.12 ± 0.01 mg/g FW), phenols (4.9 ± 0.2 mg/g FW), flavonoids (7.1 ± 0.14 mg/g FW), and glycine betaine (0.13 ± 0.02 μ g/g DW). The *G. robustum* aqueous extract consisted of significant amounts of Ca^{2+} (135 ± 1.5 mg/g DW), Na^+ (720 ± 10.2 mg/g DW), K^+ (126 ± 12.5 mg/g DW), Mg^{2+} (35 ± 0.8 mg/g DW), Cu^{2+} (0.7 ± 0.03 mg/g DW), Zn^{2+} (0.24 ± 0.01 mg/g DW), Fe^{2+} (23 ± 1.1 mg/g DW), and Mn^{2+} (1.2 ± 0.2 mg/g DW). The amount of tannins (153 ± 11.5 mg/g FW) was high in seaweed liquid fertilizer (SLF), and it had a low amount of proline (0.007 ± 0 mg/g FW). The lycopene level was 0.13 ± 0.02 mg/g FW, and the growth hormone level was detected from SLF. The extract contained gibberellic acid (13.4 ± 1.1 mg/100 mL), CK (0.102 ± 0.0 mg/100 mL), and auxin (0.09 ± 0.0 mg/100 mL). SLF is rich in protein, phenol, tannins, flavonoids, terpenes, micro- and macronutrients, and growth hormones were also determined. These essential elements have been shown to improve plant growth and to suppress salt stress. Terpenes are considered antioxidant secondary metabolites (Aziz *et al.* 2018), and they were detected in the *G. robustum* aqueous extract. Seeds are rich in tannins, and the tannins detected in the SLF showed growth-promoting activity and antioxidant effects. The increased tannin content in the SLF improved nutrient uptake and potassium ions, which positively influenced the plant growth and salt stress mitigation effects (Baatour *et al.* 2018). Seaweed liquid fertilizer consists of flavonoids and phenolic compounds that improved the antioxidant activity and helped to mitigate salt stress, which is consistent with previous reports (Naikoo *et al.* 2019). In this study, hormones and elements were detected in the SLF and have been identified as signaling molecules that improved the phytohormonal activity of plants and improve plant growth (Polat *et al.* 2023). SLF are characterized by growth hormones and growth-promoting molecules. Cytokinin-rich SLFs reportedly improve the photosynthetic potential of plants and increase the absorption of nutrients, ionic absorption, and growth-promoting activity (Jupri *et al.* 2019).

Effect of SLF on Tomato Growth

Compared with those of the control plants, the root length, shoot biomass, and leaf diameter of the tomato plants decreased significantly when the salt concentration increased ($P < 0.001$) to 10 dS/m (Tables 1 to 3). The salinity-stress in tomato plants treated with SLF *via* foliar feeding presented promising tomato growth traits. Salinity stress causes severe oxidative damage to plants, which can lead to major physiological damage, reduced growth, and mineral nutrient imbalances and can affect nutrient uptake. Salinity is a harmful stress to plants and affects their growth and yield. The results obtained in this study are consistent with those of previous reports (Bello *et al.* 2021), where salt stress can negatively influence absorptive processes and metabolic functions such as the absorption of mineral elements, transport, and assimilation, which affect the synthesis of hormones.

Under salinity stress conditions, elevated ROS generation and oxidative bursts can occur in cells, causing disorders in the regulation of growth hormones (Hasanuzzaman *et al.* 2021). On the other hand, the potential of SLF was utilized to minimize harmful effects either through the induction of physiological immunity or the inhibition of water salinity. A mixture of microalgae and seaweed extract reduced the impact of salt stress and increased the growth of milkweed (*Calotropis procera* Ait) when it was cultured under various stress conditions (7.5 dS/m to 30 ds/m) (Jafarlou *et al.* 2021). Seaweed liquid fertilizer (0.5% to 4% SLF) increased the growth of *Lavandula officinalis* under salt stress at various concentrations of sodium chloride (40 mM to 80 mM).

Table 1. Effects of the SLF on the Root Lengths of Tomato Plants Grown at Various Salinity Levels

SLF	Root Length (cm)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	19.4±1.1 ^a	17.1±2.3 ^a	16.5±2.4 ^a	13.5±2.2 ^a	11.2±2.3 ^a
2.5% SLF	24.3±1.4 ^b	15.6±3.5 ^b	17.3±1.1 ^b	13.3±1.4 ^a	12.7±1.1 ^b
5% SLF	25.8±1.1 ^b	19.4±2.2 ^c	18.4±2.8 ^c	16.7±1.1 ^c	15.6±2.2 ^c
7.5% SLF	33.9±4.2 ^c	20.3±1.6 ^c	18.9±1.5 ^c	17.4±2.5 ^d	16.8±1.7 ^d
10% SLF	34.2±2.3 ^c	25.9±3.2 ^d	24.8±2.4 ^d	18.9±1.2 ^e	17.5±1.5 ^e
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Table 2. Effect of the SLF on the Shoot Mass of Tomato Plants Growing at Various Salinity Levels

SLF	Shoot Mass (g)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	2.05±0.5 ^a	1.7±0.2 ^a	1.3±0.2 ^a	1.1±0.1 ^a	1.02±0.2 ^a
2.5% SLF	2.4±0.3 ^a	1.9±0.1 ^a	1.6±0.1 ^b	1.4±0.2 ^b	1.3±0.1 ^a
5% SLF	2.7±0.2 ^b	2.5±0.2 ^b	1.7±0.2 ^b	1.5±0.1 ^b	1.6±0.2 ^b
7.5% SLF	2.8±0.4 ^b	2.9±0.4 ^c	1.8±0.2 ^b	1.7±0.2 ^c	1.9±0.1 ^c
10% SLF	3.2±0.2 ^c	3.0±0.3 ^c	2.2±0.1 ^c	2.0±0.1 ^d	2.0±0.2 ^c
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Table 3. Effects of the Addition of the SLF on the Leaf Area of Tomato Plants Growing at Various Salinity Levels

SLF	Leaf Area (cm)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	89.2±4.2 ^a	76.2±1.8 ^a	73.4±3.2 ^a	64.5±2.3 ^a	53.8±1.3 ^a
2.5% SLF	92.5±1.4 ^b	77.5±2.1 ^b	78.4±1.2 ^b	65.1±1.1 ^a	58.4±2.2 ^b
5% SLF	95.3±1.2 ^c	78.4±1.9 ^b	80.4±2.2 ^c	74.1±2.8 ^b	60.2±1.1 ^c
7.5% SLF	96.2±1.3 ^c	82.4±2.2 ^c	82.1±1.9 ^c	78.4±3.2 ^c	65.3±2.2 ^d
10% SLF	104±2.5 ^d	84.2±1.9 ^c	89.2±2.2 ^d	82.5±1.3 ^d	67.9±1.3 ^e
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Owing to the mitigating effects of seaweed extracts, the contents of chlorophylls *a* and *b* and other traits are stable in *L. officinalis* (Korkmaz and Çiçek 2024). In black grams (*Vigna mungo* L.), the SLF prepared from brown algae, *Sargassum* sp., had a stimulatory effect on black gram growth traits, and 10% SLF had a significant salt stress (50 to 150 mM NaCl, treated for five days) mitigation effect (Afeeza and Dilipan 2024), which was similar to the present results.

Antioxidant Activity of Tomato Leaves

Salinity treatments (2 to 10 dS/m) of tomato plants through foliar application resulted in a significant increase in antioxidant activity ($P < 0.001$). Priming tomato seeds with SLF significantly improved leaf development. In the control plants, the antioxidant activity was lower, and the SLF-treated plants (5% SLF, 7.5% SLF, and 10% SLF) presented significant antioxidant activity (Table 4). Moreover, a lower concentration of SLF had an insignificant effect on antioxidant activity compared with that of the control. Antioxidants and bioactive secondary metabolites constitute the first-line defense mechanism that alleviates abiotic stress, including stress, in plants (Isah 2019). Seaweed extract has been applied to minimize the oxidative damage caused by salinity stress in plants. The SLF derived from *Ascophyllum umnodosum* mitigated abiotic stress by increasing the antioxidant capacity, flavonoid and phenolic contents, and proline accumulation. In addition, seaweed extracts improve growth-promoting traits. These extracts are enriched with antioxidant flavonoid compounds, phenols, proteins, and carbohydrates (Elansary *et al.* 2016). The presence of bioactive phytochemicals from seaweed extracts derived from *Padina gymnospora* improved overall plant growth by alleviating stress and optimizing secondary metabolite production and membrane permeability, which improved abiotic stress tolerance by altering turgor pressure and water capacity in plants (Khan *et al.* 2022). Seaweed extract has been reported to induce the production of secondary metabolites and may improve the accumulation of antioxidant compounds and trigger the antioxidant system. The improved antioxidant activity of the seaweed extract in lettuce can be attributed to the stimulatory effect of the extract, which improves the antioxidant power in lettuce through the effective stimulation of the synthesis of antioxidant enzymes (Colla *et al.* 2017). Seaweed extracts increase antioxidant activity in cowpea (Vasantharaja *et al.* 2019) and pepper plants (Ashour *et al.* 2021).

Table 4. Effect of SLF on Antioxidant Activity of Tomato Plants Growing at Various Salinity Levels

SLF	Antioxidant Activity (mg AA/mg FW)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	8.9±0.2 ^a	7.7±1.1 ^a	14.2±1.1 ^a	18.3±1.1 ^a	19.3±1.2 ^a
2.5% SLF	9.3±0.1 ^a	10.5±1.2 ^b	19.3±2.3 ^b	20.3±1.1 ^b	20.4±1.3 ^a
5% SLF	24.5±0.2 ^b	29.4±2.2 ^c	25.3±1.1 ^c	37.2±1.3 ^c	36.3±2.2 ^b
7.5% SLF	35.4±0.3 ^c	34.3±1.0 ^d	38.5±1.2 ^d	39.3±2.2 ^d	42.5±1.3 ^c
10% SLF	36.3±0.2 ^d	37.2±2.2 ^e	40.2±2.2 ^e	42.5±1.3 ^e	46.5±1.6 ^d
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Effect of SLF on Hydrogen Peroxide Content in Tomato Plant under Salt Stress

The variation in H₂O₂ content in tomato leaves significantly varied among the salinity treatments ($P < 0.01$). At 2 dS/m salt stress, the control tomato leaves presented 316 ± 12.2 nmol/g FW H₂O₂, and SLF mitigated the generation of H₂O₂ at 2.5% SLF (318 ± 4.7 nmol/g FW), whereas at 10% SLF, it declined significantly (245 ± 2.5 nmol/g FW) (Table 5). In the present study, the application of SLF significantly decreased the H₂O₂ levels in tomato leaves; in particular, the higher concentrations of SLF resulted in lower H₂O₂ levels than the lower SLF concentrations (2.5% to 5% SLF). Salinity stress can cause hyperproduction of ROS because of the resulting oxidative stress (Debnath *et al.* 2011). The oxidation or reduction process results in the generation of superoxide, singlet oxygen, hydrogen peroxide or hydroxyl radicals (Mittler 2002). Reactive oxygen species are considered triggers that cause dysfunction in DNA, lipids, and proteins (Das and Roychoudhury 2014). In this study, foliar application of SLF reduced H₂O₂ generation in a dose-dependent manner under salt stress. The reduction in H₂O₂ in the tomato leaves sprayed with SLF tested under salt stress conditions could be attributed to the availability of antioxidant molecules in the SLF, which was greater at higher concentrations of SLF spray. These findings are consistent with the results of Patel *et al.* (2018), who reported that the extract of *Kappaphycus alvarezii* reduced H₂O₂ production under salt stress in *Triticum durum* plants, increased total phenols, and increased the expression of catalase and superoxide dismutase genes.

Table 5. Effect of SLF on Hydrogen Peroxide Content in Tomato Leaves under Salt Stress

SLF	H ₂ O ₂ Conc. (nmol/gFW)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	316 ± 12.2^a	327 ± 14.8^a	416 ± 4.7^a	493 ± 6^a	527 ± 6^a
2.5% SLF	318 ± 4.7^a	358 ± 10.2^b	407 ± 2.4^b	482 ± 5^b	519 ± 4^b
5% SLF	303 ± 2.5^b	275 ± 11.3^c	348 ± 1.3^c	443 ± 7^c	489 ± 5^c
7.5% SLF	266 ± 1.7^c	269 ± 11.1^d	322 ± 11^d	429 ± 5^d	474 ± 4^d
10% SLF	245 ± 2.5^d	272 ± 10.2^d	384 ± 7^e	408 ± 8^e	453 ± 8^e
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Effects of SLF on Tomato Leaf Protein and Sugar Contents

The total protein and sugar contents of tomato leaves grown under salinity stress were lower than those of the control leaves. However, the total protein (Table 6) and sugar (Table 7) contents of the tomato plants treated with SLF were significantly improved because of the stress mitigation effect. In the control plants (10 dS/m salt stress), the total protein content was 0.9 ± 0.2 mg protein/g FW, and further SLF application at the 10% level significantly improved the total protein content in the tomato leaves (2.9 ± 0.2 mg protein/g FW), whereas 2.5% SLF had an insignificant effect on the total protein content (1.2 ± 0.1 mg protein/g FW). Proteins are important macromolecules and are very important in plant metabolism. The sugar content was 4.3 ± 0.4 mg glucose/g FW in the control (2 dS/m salt stress), and it improved in tomato leaves treated with 2.5% SLF (5.1 ± 0.2 mg glucose/g) or 10% SLF (9.2 ± 0.2 mg glucose/g). There are several transports, catalytic, defense, and structural proteins in plants (Shukla *et al.* 2019). In the present study, at higher

concentrations of SLF, the total protein content significantly increased compared with that of control. The application of *S. latifolium* extract in barley cultivation increased the total protein content after stress with 75 and 150 mM NaCl (Sofy *et al.* 2017). Under salt stress, the generated ROS decreases the oxidation of proteins, nucleic acids, and lipids; thus, the amount of protein generated decreases under salt stress (Sachdev *et al.* 2021). Seaweed extract has a protective effect against salt stress by increasing the production and accumulation of several enzymes, including superoxide dismutase, antioxidant enzymes, and lipoxygenase enzymes; thus, the overall protein content of seaweed extract-treated plants has increased (Chanthini *et al.* 2022). In plants, improvements in sugar and protein contents are associated mainly with stress resistance (Du *et al.* 2020). The improvement in protein and sugar levels in the tomato leaves revealed that SLF significantly improved the salt stress response of the tomato leaves.

Table 6. Effect of the SLF on the Total Protein Content in Tomato Leaves under Salt Stress

SLF	Total Proteins (mg protein/g FW)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	2.8±0.4 ^a	2.5±0.4 ^a	1.8±0.2 ^a	1.2±0.2 ^a	0.9±0.2 ^a
2.5% SLF	3.5±0.3 ^b	2.7±0.2 ^a	2.1±0.1 ^b	1.91±0.1 ^b	1.2±0.1 ^a
5% SLF	4.1±0.2 ^c	3.3±0.1 ^b	2.6±0.2 ^c	2.8±0.2 ^c	1.6±0.2 ^b
7.5% SLF	4.8±0.2 ^d	3.6±0.2 ^b	2.7±0.4 ^c	3.0±0.2 ^d	2.4±0.3 ^c
10% SLF	5.3±0.3 ^e	4.2±0.3 ^c	3.1±0.2 ^d	3.4±0.3 ^e	2.9±0.2 ^d
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Table 7. Effect of SLF on Soluble Sugars in Tomato Leaves under Salt Stress

SLF	Soluble Sugars (mg glucose/g FW)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	4.3±0.4 ^a	3.7±0.2 ^a	3.3±0.1 ^a	2.9±0.2 ^a	2.5±0.3 ^a
2.5% SLF	5.1±0.2 ^b	3.9±0.1 ^b	4.2±0.2 ^b	3.2±0.2 ^a	2.9±0.2 ^b
5% SLF	7.3±0.2 ^c	4.8±0.2 ^c	4.7±0.2 ^b	4.9±0.5 ^b	3.5±0.1 ^c
7.5% SLF	8.9±0.1 ^d	6.7±0.1 ^d	6.5±0.1 ^c	6.1±0.3 ^c	4.9±0.2 ^d
10% SLF	9.2±0.2 ^e	8.9±0.2 ^e	6.5±0.3 ^c	6.3±0.2 ^c	5.4±0.6 ^e
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Effect of SLF on Tomato Leaf Pigment under Salinity Stress

Salinity stress reduces the amount of chlorophyll and carotenoids in tomato plants. Moreover, the application of the seaweed SLF significantly increased the total chlorophyll content in tomato leaves subjected to 5%, 7.5%, and 10% SLF under salinity stress (Table 8). In this study, variations in the total carotenoid level of leaves were observed between tomato leaves treated with 2 dS/m and those treated with 10 dS/m. However, no significant difference was observed between the control and 2 dS/m salt treatment at 2.5% SLF (Table 9). In plants, reduced pigment synthesis leads to the disruption of several important cellular functions and an imbalance in energy biosynthesis (Sharma *et al.* 2020). Moreover, compared with those of the control tomato plants (salt-stressed plants), the chlorophylls *a*

and *b* levels and carotenoid contents of the salt-stressed tomato plants were increased in response to foliar SLF. The increased biosynthesis of these pigments in tomato plants is due to the presence of amino acids, minerals, and bioactive compounds. Seaweed extract (*A. nodosum*) significantly reduces oxidative blast in cells *via* several molecular mechanisms (Jain and Sirisha 2020; Elnahal *et al.* 2022). The generation of ROS affects the production of photosynthetic pigments in plants (Verma *et al.* 2013). Biostimulants effectively increase the amount of photosynthetic pigments in plants and play a significant role in the biosynthesis of chlorophyll (Carillo *et al.* 2019). The present findings are consistent with previous experimental results. Foliar application of SLF can improve tomato leaf pigments, thus increasing photosynthesis, by increasing the net photosynthetic rate, water use, and stomatal conductance (de Carvalho *et al.* 2019). In addition, seaweed extracts are rich in plant growth-promoting molecules such as gibberellins, auxins, and polyamines, which promote plant leaf growth and pigment production (Gupta *et al.* 2021). The presence of cytokinin in seaweed extracts has a protective effect on chloroplasts and improves the production of endogenous cytokinin in plants (Alhasan *et al.* 2021). Seaweed extracts are rich in elements, including Mg, which is essential for the synthesis of chlorophyll, and K plays a protective role in photosynthesis, improving shoot growth and water transport (Chrysargyris *et al.* 2018).

Table 8. Effect of the SLF on the Photosynthetic Pigments of Tomato Leaves Growing under Salt Stress

SLF	Chlorophylls a+b (µg/mL)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	0.83±0.02 ^a	0.72±0.05 ^a	0.65±0.03 ^a	0.53±0.03 ^a	0.39±0.02 ^a
2.5% SLF	1.1±0.05 ^a	0.8±0.04 ^a	0.72±0.01 ^a	0.67±0.02 ^b	0.42±0.01 ^a
5% SLF	1.5±0.02 ^b	1.1±0.02 ^b	0.81±0.02 ^b	0.72±0.03 ^c	0.48±0.02 ^b
7.5% SLF	1.7±0.4 ^c	1.2±0.03 ^b	0.83±0.03 ^b	0.75±0.02 ^c	0.59±0.03 ^c
10% SLF	2.2±0.2 ^d	1.5±0.02 ^c	1.1±0.02 ^c	0.89±0.03 ^d	0.68±0.02 ^d
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

Table 9. Effect of the SLF on the Photosynthetic Pigments of Tomato Leaves Growing under Salt Stress

SLF	Total Carotenoids (µg/mL)				
	2 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m
Control	2.1±0.01 ^a	2.61±0.03 ^a	2.64±0.02 ^a	2.54±0.02 ^a	2.34±0.09 ^a
2.5% SLF	2.2±0.05 ^a	2.7±0.04 ^b	2.72±0.04 ^b	2.6±0.01 ^a	2.41±0.02 ^b
5% SLF	3.4±0.01 ^b	3.1±0.02 ^c	3.84±0.03 ^c	2.71±0.03 ^b	2.59±0.04 ^c
7.5% SLF	3.8±0.4 ^c	4.5±0.03 ^d	3.85±0.04 ^c	2.8±0.03 ^c	2.53±0.04 ^c
10% SLF	3.2±0.3 ^b	3.2±0.03 ^c	3.4±0.03 ^d	2.7±0.02 ^b	2.44±0.03 ^b
Different lower-case letters next to numerical data in the column of the tables indicate significant variation between control and treatment groups.					

The present findings indicate that foliar application of SLF tended to increase the pigment content, which was reflected in the potential photosynthesis rate, readily improving plant vegetative growth. These results are consistent with those of the effects of seaweed extracts derived from *Ulva* on mung bean (Castellanos-Barriga *et al.* 2017) and the effects of *Sargassum vulgare* extracts on red radish plant leaf pigments (Mahmoud *et al.* 2019).

CONCLUSIONS

1. The aqueous extract of *Gelidium robustum* contains a rich blend of bioactive compounds, including proteins, phenols, flavonoids, tannins, glycine betaine, essential micro- and macronutrients, and growth hormones, which collectively contribute to its plant growth-promoting properties.
2. Foliar application of *G. robustum* liquid fertilizer (7.5to10%) effectively mitigated salinity stress in tomato plants by enhancing root length, shoot biomass, and leaf area, even under high salinity conditions.
3. These beneficial effects were associated with increased antioxidant activity, reduced hydrogen peroxide accumulation, and elevated levels of total proteins, soluble sugars, phenols, and flavonoids, demonstrating that SLF enhances both plant growth and stress tolerance through physiological and biochemical modulation.

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Conflict of Interest

The authors do not have any conflict of interest in publication of this research article.

Use of Generative AI

Authors did not use any AI tools in the preparation of text, and data analysis.

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