Evaluation of Nutrient Composition and Biostimulant Properties of Seaweeds for Improving Soil Microbial Population and Tomato Plant Growth

Venci Candida Xavier James,[a] Arokya Glory Pushpa Thiraviam,[a] Munirah Abdullah Al-Dosary,[b] Ashraf Atef Hatamleh,[b] Najat A. Bukhari,[b] Selvaraj Arokiyaraj,[c] and Moni Kalaiyarasi [d],*

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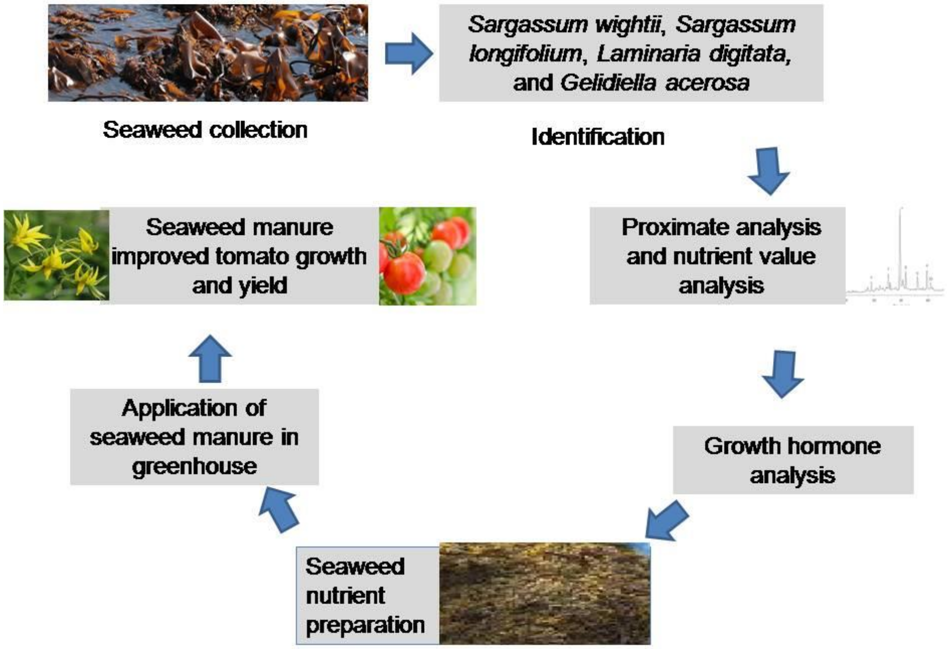
GRAPHICAL ABSTRACT



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Venci Candida Xavier James,[a] Arokya Glory Pushpa Thiraviam,[a] Munirah Abdullah Al-Dosary,[b] Ashraf Atef Hatamleh,[b] Najat A. Bukhari,[b] Selvaraj Arokiyaraj,[c] and Moni Kalaiyarasi [d,]*

The nutrient composition and biostimulant properties of seaweed were determined as solid biofertilizer for cultivating tomato seedlings in a greenhouse. Seaweeds (*Sargassum wightii*, *Sargassum longifolium*, *Laminaria digitata*, and *Gelidiella acerosa*) were collected from rocky areas and turned into a powder, and their nutrient compositions were analyzed. The brown seaweed showed indoleacetic acid (IAA), gibberellic acid (GA_3), indole butyric acid (IBA), and abscisic acid (ABA). The amount of IAA ranged from 0.52 to 21.5 µg/mL. Compared with the other brown algae, the *G. acerosa* extract presented the maximum amount of GA_3 (149 µg/mL). The amount of IBA ranged from 1.5 to 15.3 µg/mL, and the ABA level was high in *S. wightii* (2.5 µg/mL). All algae powders were subjected to biofertilizer preparation and their biostimulant properties were studied. The algal biostimulant improved flower cluster number, fruit number, shoot dry weight, and root dry weight in tomato plants in a greenhouse. Macroalgae fertilizer improved urease, phosphatase, invertase, and catalase activities ($p<0.05$) and the microbial population in the soil. The results showed positive effects of biostimulants on soil physicochemical and biological properties.

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Keywords: Seaweed; Nutrients; Minerals; Growth promoters; Tomato plant; Soil enzymes; Microbial population

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INTRODUCTION

Seaweeds are used as fertilizers for legume plants and in horticulture because they have various mineral components, and these minerals are absent in other fertilizers. Seaweed also contains various growth-stimulating phytohormones that induce plant growth. In addition, various macro-and micronutrients present in seaweed can improve plant hormone synthesis and promote plant growth (Ateweberhan *et al*. 2008). Iodine, iron, aluminium, calcium, manganese, sulphur, phosphorus, dissolved nitrogen, chlorine, titanium, barium, boron, cobalt, potassium, and copper are the major minerals found in seaweed. *Ulva lactuca* contains increased amounts of K, N, Fe, and Mg and micronutrients such as Mn, Cu, B, Zn, Al, Cr, Ni, Pb, and Cd. *Caulerpa racemosa* contains minerals such as Ca, Mg, and ammonia (Villares *et al*. 2007). Species such as *Laminaria shinzii*, *Ascophylum nodosum*, *Gracilaria chilensis*, *Ecklonia maxima*, and *Durvillaea potatorum*, are the major sources of minerals and are used for the preparation of fertilizers (Crouch *et al*. 1992; Craigie 2011). Because of their increased availability, seaweeds offer an inexpensive solution for improving crop yield and subsequently decreasing the applications of inorganic fertilizers in the field. Biostimulants prepared from seaweed improve crop yield and plant growth, increase biotic and abiotic stress tolerances, and increase the bioavailability of nutrients (Shukla *et al*. 2019; Wadduwage *et al*. 2023). The application of seaweed extract was found to improve abiotic stresses, such as salt stress and freezing, and drought stress in tomato, soybean, and *Arabidopsis* (Nair *et al*. 2012; Martynenko *et al*. 2016; Santaniello *et al*. 2017; Goni *et al*. 2018; Jithesh *et al*. 2019). Foliar application of seaweed extracts increased soil fertilization and improved yield in several crops, including sugarcane, wine grapes, vegetables, and strawberries (Khan *et al*. 2009; Arioli *et al*. 2015; Shukla *et al*. 2019; Muniswami *et al.* 2023).

The application of seaweed fertilizer, which is environmentally friendly, can improve soil productivity and fertility (Ali *et al*. 2021). Seaweed extracts increase the total chlorophyll content in plants, including tomato plants, while improving their photosynthetic potential, stomatal conductance, transpiration rate, and antioxidant properties, which are positively correlated with increases in fruit yield and fresh weight (Carillo *et al*. 2020; Franzoni *et al*. 2022). The application of seaweed extracts may improve crop quality and yield by increasing the accumulation of beneficial secondary metabolites, including antioxidant compounds, alcohols, simple sugars, proline, and abscisic acid. In addition, the seaweed extract was found to mitigate the negative impacts of abiotic stress (El Khattabi *et al*. 2023). These extracts improved the quality characteristics of tomato fruits and reduced the accumulation of several toxic ions under abiotic stress (Di Stasio *et al*. 2018). The biostimulant properties of *Padina gymnospora* extract have been reported, and it improves nutrient uptake and absorption, and promotes tomato plant growth (Hernández-Herrera *et al*. 2014, 2016). However, the selection of seaweed extract is based on the type of crop and environmental conditions (Bose *et al*. 2014; Rai *et al*. 2021; Khan *et al*. 2022).

The soil treated with seaweed extract increases nutrients for the soil microbiome. Seaweed fertilizer improved the soil bacterial population (Sunarpi *et al*. 2020; Prasedya *et al*. 2022). In agriculture, the soil rhizosphere microbiome plays an important role in plant nutrition and breeding (Yang *et al*. 2017; Mendes *et al*. 2018). The available nutrients in the soil environment regulate the population of the soil microbiome (Krid *et al*. 2023). The application of *Ascophyllum nodosum* extract increased the shoot and root length, berry yield, physiological activity, and microbial population in the rhizosphere of strawberry plants (Alam *et al.* 2013). In carrots, the application of biostimulants is positively related to microbial activity and plant growth (Alam *et al*. 2014). The use of seaweed-based natural products has been gaining momentum in improved crop production systems owing to their growth hormones and bioactive components. The phytostimulatory characteristics result in improved crop yield in several commercial important crops. The phytoelicitor activity of seaweed extract, which was not completely elucidated, included improved plant defence systems in plants that significantly contribute to disease resistance, pest resistance, and abiotic stresses including salinity, drought, and cold. Treatment by seaweed extracts causes changes in the microbiome components of soil and plant in support of sustainable plant growth. Seaweed extract contain various phytohormones which helps in overall plant growth and crop production. The nutrient composition, and phytochemical components of seaweed varied widely. Since seaweed extracts are organic, they are highly suited for crop production and environmental management. The authors hypothesized that the application of seaweed fertilizer would improve plant growth, microbial activity, and the microbial population. This study used tomato plants and applied seaweed fertilizer in a greenhouse. The plants growing in the pots were supplemented with seaweed fertilizer at specific time intervals. The plant biology, root growth, shoot growth, chlorophyll content of leaves, fruit yield, and fruit quality were analysed. The aim of this study was to analyse the effects of seaweed extract on plant growth, yield, and rhizosphere-associated microbes in tomato plants in a greenhouse.

EXPERIMENTAL

Seaweed Collection and Processing

Four seaweed samples were collected in the early morning on the rocky shore, approximately 10 m from the shore along the Kanniyakumari coast, India (8° 5' 17.9016" N and 77° 32' 18.4272" E), in October 2022. The seaweeds were cleaned of extraneous substances, such as sand particles, epiphytes, and barnacles by repeatedly washing with seawater. The collected seaweeds were identified with monographs, taxonomic books, and reference from herbaria. To prepare samples for analytical experiments, fresh seaweed was washed with tap water and frozen at -80 °C for 12 h. The samples were lyophilized, and the macroalgae were finely powdered with a blender. The sample was labelled and stored in a deep freezer at -80 °C until further use.

Proximate Composition and Mineral Content of Macroalgae

The collected seaweeds (*Sargassum wightii*, *Sargassum longifolium*, *Laminaria digitata*, and *Gelidiella acerosa*) were subjected to proximate and mineral analyses. The crude protein, ash, dry matter, crude fat, and dietary fibre contents were determined as described by the AOAC (AOAC 2006).The crude protein content of each sample was determined *via* a Kjeltec system (N×6.25). The ash content of each sample was determined through gravimetric method. Briefly, seaweeds were placed in a muffle furnace (Carbolite, UK) for 5 h, and the ash content was calculated. The moisture content of the algae was determined by drying 5 g of seaweed at 110 °C until a constant weight was obtained in a hot air oven.

To determine the fat content, 50 g of seaweed was extracted with petroleum ether. The seaweed was hydrolyzed with 0.26 N sulfuric acid, incubated at 100 °C, and treated with 0.31 N sodium hydroxide for 30 min. The amount of dietary fibre was determined *via* the enzymatic-gravimetric method. The results are expressed as the percentage dry weight. To determine the mineral composition of the seaweed, 5 g of dried sample was dissolved in nitric acid (1 mL) and hydrogen peroxide and digested in a microwave oven. The mixture was subsequently shaken vigorously and filtered through Whatman number 1 filter paper. The amounts of minerals, such as calcium (Ca), magnesium (Mg), potassium (K), manganese (Mn), and iron (Fe), were analyzed using an atomic absorption spectrophotometer (Hitachi Z-5000, Tokyo, Japan) equipped with an air-acetylene burner. The amounts of phosphorus (P), sodium (Na), and selenium (Se) from the seaweed were tested *via* inductively coupled plasma–mass spectrometry (ICP-MS) (Perkin Elmer ELAN 9000, Wellesley, MA, USA). The mineral composition of the seaweed was expressed as mg/100 g dry weight.

Analysis of Plant Growth Hormones in the Seaweed Extracts

Approximately 1.0 g of each of the four algal powder samples was infused in 10 mL of methanol (99.9% purity) and 1.0 mmol/L citric acid. The mixture was sonicated and incubated at 4 °C for 2 days. After 2 days, the mixture was centrifuged for 10 min at 5000 g at 4 °C. Then, the clear supernatant was collected, and methanol (2 mL) was added and mixed. The mixture was incubated for 1 h and then centrifuged for 10 min at 5000 × g at 4 °C. It was filtered through a 0.2-μm filter and diluted appropriately for analysis. The amounts of indoleacetic acid (IAA), gibberellic acid (GA_3), indole butyric acid (IBA), and abscisic acid (ABA) in the extract were determined, and the results were compared with those of standards (>99% purity). The standard solutions were prepared with methanol, and the stock solutions were prepared at 1 mg/mL and stored at 4 °C until use. All the solvents were sonicated for 30 min prior to the experiments. High-performance liquid chromatography (Brea, CA, USA) was used to detect phytohormones. It was equipped with a Beckman Coulter 166 ultraviolet-visible (UV/VIS)detector system. Methanol and water (70:30, v/v) were used as the mobile phase, and the flow rate used was 0.5 mL/min. Approximately 20 µL of each of the four algal extracts were injected and detected at 280 nm.

Seaweed Fertilizer Preparation

A solid fertilizer from seaweed was prepared as described previously by Prasedya *et al*. (2022). The biomass from all four seaweeds (500 g) (*S. wightii*, *S. longifolium*, *L. digitata*, and *G. acerosa*) was first fermented with a starter bacterium, and fermentation was carried out in Erlenmeyer flasks for 40 days as previously described (Bzdyk *et al*. 2018). After 40 days of fermentation, the fermented biomass was used as solid fertilizer. It was applied as an enriched nutrient to the soil before the transplantation of tomato plants in a greenhouse environment.

Tomato Seeds

Tomato (*Lycopersicon esculentum*) seeds were purchased from a vegetable market and used for this study. The tomato seeds were dried prior to the experiment. The seeds were carefully planted into seedling trays containing fertile agricultural soil. The trays were maintained for twenty days at 23 ± 1 °C in a greenhouse.

Greenhouse Experiment

Twenty-day-old tomato seedlings were used in this study. The seedlings were subsequently transplanted to round plastic pots containing agricultural soil and seaweed fertilizer (10:1, w/w). The concentration of seaweed biostimulant was fixed as described previously (Arioli *et al*. 2020). Seaweed fertilizer was applied directly to the potted soil in each set of experiments, which was irrigated daily. The tomato seedlings (control and experimental) were maintained in a greenhouse. The experiment was performed in a randomized complete block design with two duplicates for each set of experiments. For each set of experiments, 12 plants were used. The flowering and harvest time points were analyzed after 60 and 90 days (60 DAP and 90 DAP, respectively). The flowering development time (days of DAP) and fruit development were monitored daily.

Plant Growth Analysis

Plant growth was analyzed by observing flower cluster and fruit numbers, root length, and shoot length. The dry weights of the root and shoot samples were analyzed by drying the samples in an oven at 70 °C until a constant dry weight was reached, and the results were recorded with an electronic balance (Shimadzu, Japan). The growth profile was analyzed after 60 DAP and 90 DAP.

Soil Macronutrient Analysis

The soil samples were collected from the experimental and control pots 60 DAP and 90 DAP. Approximately 300 g of soil sample was collected from the top layer in each pot. The sample was dried for five days at room temperature. The unwanted debris were subsequently removed. The amount of organic matter in each soil sample was quantified using the dichromate wet oxidation technique by Walkley and Black (1934) method. The total nitrogen in each soil sample was determined *via* the micro-Kjeldahl method. The amounts of nitrogen and potassium were analyzed using the inductively coupled plasma-optical emission spectroscopy(ICP–OES) technique.

Soil Enzyme Activity

The urease activity of the soil was determined as described previously by Zantua and Bremner (2002). Approximately 5 g of each soil sample was mixed with 50 mL of 0.1 M phosphate buffer (pH 7.0). The mixture was incubated for 24 h, after which the amount of NH_4^+ ions released into the soil solutions was determined. Urease activity was expressed as U/g soil. Invertase activity was determined *via* the procedure reported by Balasubramanian *et al*. (1970). Glucose was used as the positive control. The amount of phosphatase activity in the soil was determined as described previously by Tabatabai and Bremner (1969). The amount of phosphatase activity was determined using colorimetric method in which *p*-nitrophenol phosphate and *p*-nitrophenol were used as standards.

Determination of the Microbial Population in the Soil

The microbial population was determined from pots treated with seaweed fertilizer and the control. Briefly, 5 g of each soil sample was mixed with 95 mL of 0.9% saline and shaken for 30 min at room temperature. The mixture was subsequently centrifuged at 4 °C for 30 min at 5000×g. The supernatant was diluted with sterile demineralized water and used as a sample. To determine the soil bacteria on nutrient agar (Himedia, India), the sample was spread and incubated for 24 h at 37 °C. The fungi were isolated *via* potato dextrose agar media and incubated for 4 to 5 days at 32 °C. The sample was spread on starch casein agar medium and incubated for 5to10 days at 28 °C (Balasubramanian *et al*. 2021).

Statistical Analysis

Analysis of variance was performed with SPSS (version 22, IBM, Armonk, NY, USA). The effects of fertilizer on plant growth, enzyme activity, and the microbial population were analyzed using statistical methods. The results were compared through the Tukey test, and the differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Proximate Analysis and Mineral Composition of Macroalgal Biomass

The proximate compositional analysis (ash, carbohydrate, crude fibre, total dietary fibre, moisture, lipid, and protein) based on the dry weight (DW) of seaweed was determined, and the results are presented in Table 1. The composition of the samples varied widely according to the seaweed species. Overall, the ash content ranged from 4.1 ± 0.2 to 21.4 ± 1.02% DW. In brown algae, the ash content has been reported to vary from 15% to 45% (Øverland *et al*. 2019). Moreover, the range of ash content varies from sampling location. Bikker *et al*. (2020) analyzed the ash content of brown seaweeds in Ireland and France. The ash content ranged from 18.9% to 37.4% in *A. nodosum* and *L. digitata*. The total carbohydrate content of the seaweed ranged from 14.2 ± 0.1% DW (*L. digitata*) to 48.2 ± 2.4% DW (*G. acerosa*). The seaweed extract resulted in increased carbohydrate contents (up to 48.2 ± 2.4% DW) and low-fat contents (1.05 ± 0.09% to 1.92 ± 0.2% DW). The carbohydrate and fat contents of the *L. digitata* biomass observed in this study were similar to those reported previously (Costa *et al*. 2022). The biochemical composition of wild macroalgal species varies with season, time of harvest, nutrient availability, water current, and pollution (Coaten *et al*. 2023). The mineral components of the seaweed were analyzed, and the results are expressed as mg/100 g DW in Table 1. Compared with the other seaweeds, *L. digitata* contained greater amounts of microminerals. Seaweeds are rich sources of several nutrients, vitamins, and plant growth hormones that directly influence cellular processes and improve crop yield and plant growth (Khan *et al*. 2009). A brown alga, *A. nodosum*, collected from the North Atlantic Ocean was used as a biostimulant. It is rich in several polysaccharides, vitamins and minerals, lipids, polyphenols, and proteins (Holdt and Kraan 2011). In the present study, the collected brown algae presented increased levels of carbohydrates, proteins, and micronutrients, however it varied among seaweed (*S. latissima*, *A. esculenta*, *L. digitata*, and *L. hyperborean*), which highlight the importance of brown algae in biostimulant preparation. The biochemical composition of *S. latissima*, *A. esculenta*, *L. digitata*, and *L. hyperborean* was reported previously. The amount of laminarin, alginate, mannitol, ash, proteins, moisture, metals, nitrogen, total carbon and polyphenolics varied based on season and harvest time (Schiener *et al*. 2015).

Table 1. Proximate Composition and Mineral Content of *Sargassum wightii*, *Sargassum longifolium*, *Laminaria digitata*, and *Gelidiella acerosa*

Composition	*S. wightii*	*S. longifolium*	*L. digitata*	*G. acerosa*
Ash (DW%)	18.31 ± 1.1	20.5 ± 0.4	4.1 ± 0.2	21.4 ± 1.02
Carbohydrate (DW%)	42.1 ± 1.3	43.2 ± 0.8	14.2 ± 0.1	48.2 ± 2.4
Crude fibre (DW%)	9.03 ± 0.9	9.5 ± 0.11	10.3 ± 0.2	11.04 ± 2.2
Total dietary fibre (DW%)	3.03 ± 0.17	4.08 ± 0.52	5.1 ± 0.21	5.2 ± 0.2
Moisture (DW%)	14.04 ± 0.25	9.5 ± 0.13	10.3 ± 0.3	10.2 ± 0.4
Lipid (DW%)	1.05 ± 0.09	1.3 ± 0.14	1.92 ± 0.2	1.07 ± 0.25
Protein (DW%)	7.06 ± 0.28	10.52 ± 1.4	6.2 ± 0.1	12.4 ± 1.5
Sodium (mg/100 g DW)	6549.3 ± 129	5092 ± 0.9	4021 ± 10.4	4098 ± 4.5
Potassium (mg/100 g DW)	1908.3 ± 87.3	2052 ± 53	1409 ± 10.5	802.4 ± 2.2
Calcium (mg/100 g DW)	908.3 ± 20.7	958 ± 92	792 ± 19	802.3 ± 2.1
Magnesium (mg/100 g DW)	139 ± 10.2	209 ± 2.7	94.5 ± 2.5	303.7 ± 12.3
Selenium (mg/100 g DW)	1.21 ± 0.02	0.92 ± 0.04	0.39 ± 0.09	2.87 ± 0.4
Iron (mg/100 g DW)	302 ± 2.2	397 ± 3.1	206.4 ± 2.8	408.4 ± 10.5

Plant Growth Promoters in Seaweed Extracts

The brown seaweed contained IAA, GA_3, IBA, and ABA. The amount of IAA ranged from 0.52 ± 0.03 to 21.5 ± 1.5µg/mL. Compared with the other brown algae, the *G. acerosa* extract presented the maximum amount of GA_3 (149.1 ± 2.4 µg/mL). The amount of IBA ranged from 1.5 ± 0.12 to 15.3 ± 1.2 µg/mL, and the ABA level was high in *S. wightii* (2.5 ± 0.3 µg/mL) (Table 2). Similar to terrestrial plants, seaweeds are rich sources of various plant growth promoters. These plant growth promoters respond to physiological and developmental processes and provide adequate support to overcome biotic and abiotic stresses. Recently, plant growth promoters have been characterized from seaweeds with the aim of agronomic uses (Verma *et al*. 2016). In *Spyridia filamentosa*, the extract increased the IBA, KN, ABA, and IAA contents (Spagnuolo *et al*. 2022), which was similar to the plant growth promoter values obtained in this study. The plant growth-promoting properties of the seaweed used in this study were comparable with those reported previously (Sanderson *et al*. 1987). The seaweed *Ascophyllum nodosum* has been widely used as a source of phytohormones (Fan *et al*. 2013; Mattner *et al*. 2018; EL Boukhari *et al*. 2020; Shukla *et al*. 2021), and the brown algae characterized in this study presented comparable plant growth-promoting activities. It has been reported that plant growth promoters in seaweeds are not exclusively based on metabolic processes but may have specific physiological effects on growth in response to abiotic stress, especially environmental stimuli. In *Ulva fasciata* Delile, the amount of ABA was greater than that in *Dictyotahumifusa* Hörnig, and the production was greater in response to environmental stimuli. The available ABA in the extract improved the quality of the tomato plants. The ABA reportedly plays a typical role in various developmental processes, including shoot and root development, seed germination, and photosynthesis (Luo *et al*. 2014; Wang *et al*. 2021). In this study, the phytohormone levels of all four brown algae were studied considering the use of all the extracts to understand their synergistic activity. However, the biostimulant activity of macroalgal combinations can differ from that of individual biostimulants (Colla *et al*. 2017). The combined effects of these three biostimulants on plants have been reported previously, and they improved the total yield, dry biomass, mineral composition, and chlorophyll content of *Diplotaxis tenuifolia* (Giordano *et al*. 2020). The amount of fatty acids, polysaccharide, phytohormones, salicylic acid, (+)-abscisic acid, indole-3-acetic acid, vitamins, and mineral nutrients varied widely among the seaweeds. The increased amount of these compounds improved biostimulant properties (Benítez García *et al*. 2020; Yang *et al*. 2023; Mughunth *et al*. 2024).

Table 2. Plant Growth-Promoting Properties of Brown Seaweed

Seaweed	IAA (µg/mL)	GA3 (µg/mL)	IBA (µg/mL)	ABA (µg/mL)
S. wightii	14.3 ± 0.5^a	25.3 ± 0.3^a	10.2 ± 0.5^a	2.5 ± 0.3^a
S. longifolium	8.2 ± 0.3^b	18.5 ± 0.49^b	1.5 ± 0.12^b	ND
L. digitata	0.52 ± 0.03^c	3.2 ± 0.18^c	4.9 ± 0.2^c	ND
G. acerosa	21.5 ± 1.5^d	149.1 ± 2.4^d	15.3 ± 1.2^d	1.02 ± 0.02^b

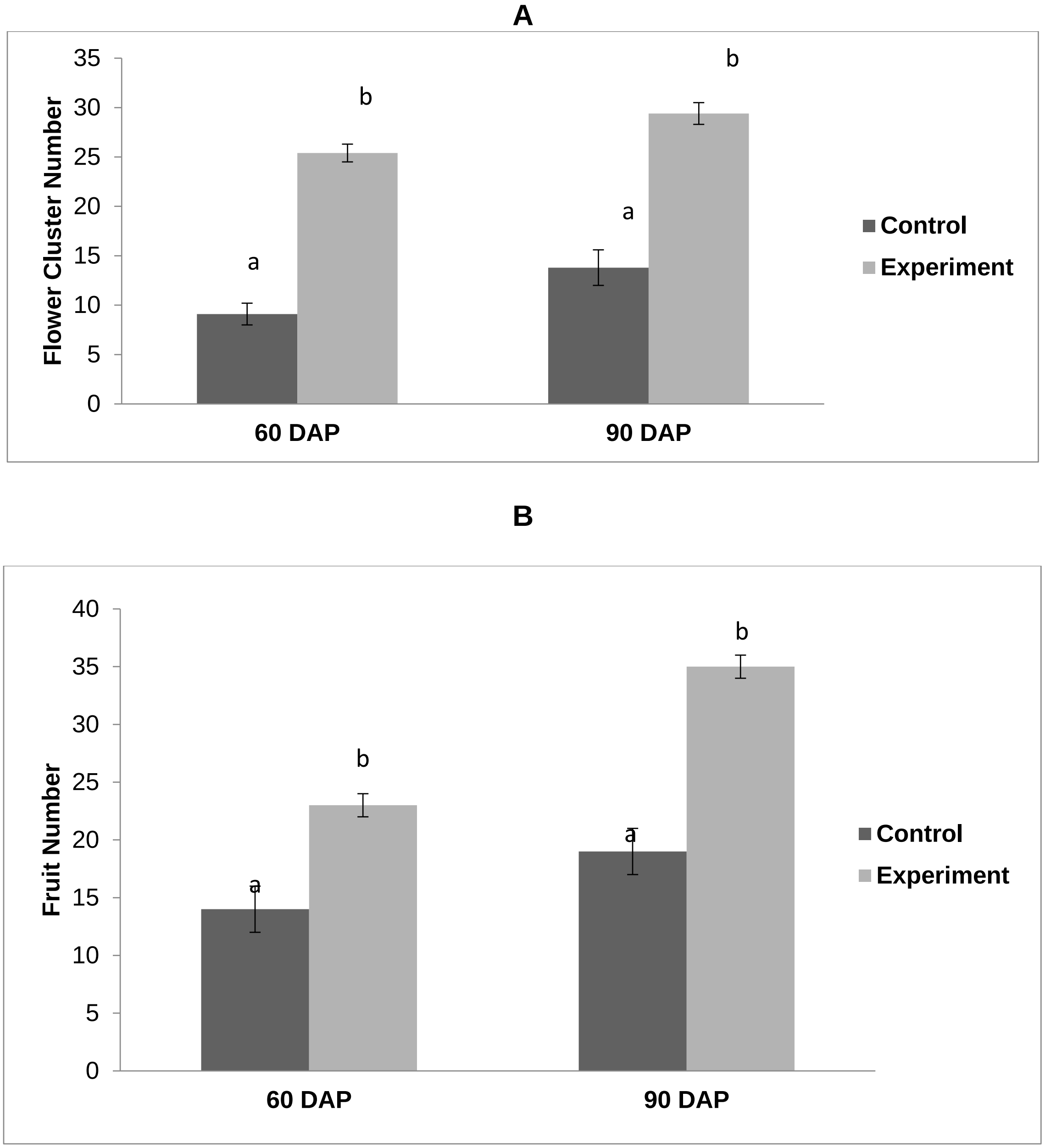
Indoleacetic acid (IAA), gibberellic acid (GA_3), indole butyric acid (IBA), and abscisic acid (ABA)
Mean value within the same column followed by the different lower case letter(s) are statistically significant.

Synergistic Effects of Seaweed Biostimulants on Tomato Plants in a Greenhouse

The synergistic effect of the seaweed biostimulant on tomato plants was evaluated, and the plant growth-stimulating activity after 60 DAP and 90 DAP in a greenhouse is shown in Fig. 1. In the greenhouse experiments, the biostimulant significantly improved the growth of the tomato plants. The soil application of the seaweed-biostimulant increased the values of the observed morphological factors analyzed in this study. Compared with those of the control groups, the number of flower clusters increased 51% after 60 DAP. The fruit number increased significantly at 60 DAP (35%) and 90 DAP (52%) compared with that of the control ($p<0.05$). The shoot dry weight increased significantly at 60 DAP (15%) and 90 DAP (95%) ($p<0.05$). Similarly, the dry weight of the roots was greater in the tomato plants treated with the biostimulant than in the control plants at 60 DAP and 90 DAP. In the present study, the early growth phase (60 DAP) resulted in improved growth compared with the later stages (90 DAP).

Macroalgae manure has a wide range of biostimulant properties during the vegetative phase of plant growth, including shoot and root elongation, stimulation of root cell division, and hair development, an increased root-to-shoot ratio, and increased leaf surface area. Several studies have revealed that horticultural plants grow in terrestrial environments treated with seaweed extract or seaweed manure and exhibit a wide range of biostimulant properties during the early vegetative phase of plant growth compared with the latter stages (El-Naggar *et al*. 2005; Mulbry *et al*. 2007; Hernández-Herrera *et al*. 2016; Akila *et al*. 2019; Baroud *et al*. 2021). The beneficial role of macroalgal biostimulants observed in plants was attributed mainly to the presence of various macro- and micro-nutrients in the macroalgal biostimulant, and most of the minerals were available in soluble form. These macro- and micro-nutrients, along with plant growth hormones, improve the vegetative growth of plants (Ahmed *et al*. 2021). In addition, seaweed fertilizer isa rich source of various bioactive secondary metabolites that can directly influence plant physiology and metabolic processes (Pirian *et al*. 2018; Benítez-García *et al*. 2020). The increased level of root architecture observed in this study could be due to the supply of adequate nutrients by seaweed nutrients.

A trace level of phytohormones is present in seaweed biomass, which improves plant growth in a greenhouse (Kumari *et al*. 2013). Seaweed extracts improved seed germination rates and increases in seedling vigor by positively affecting root size and density. The seaweed extracts improved rooting architecture, and this could be a result of auxins in the extracts (Loconsole *et al*. 2024). Seaweed extracts improved water and nutrient uptake, which ultimately improved the overall growth of plants. Seaweeds improved the absorption of minerals and potassium uptake in the leaves. Seaweed extracts improved phytohormonal activity and chlorophyll content of leaves and inhibited chlorophyll degradation by the activity of betaines (Chrysargyris *et al*. 2018). The availability of various levels of phytohormones, including cytokinins, and the induction of host hormonal synthesis led to a steep increase in early flowering, fruit size, and yield. In addition, the seaweed extracts can modulate the expression of growth hormone genes, including cytokinin, auxin, and gibberellins (Ghaderiardakani *et al*. 2019).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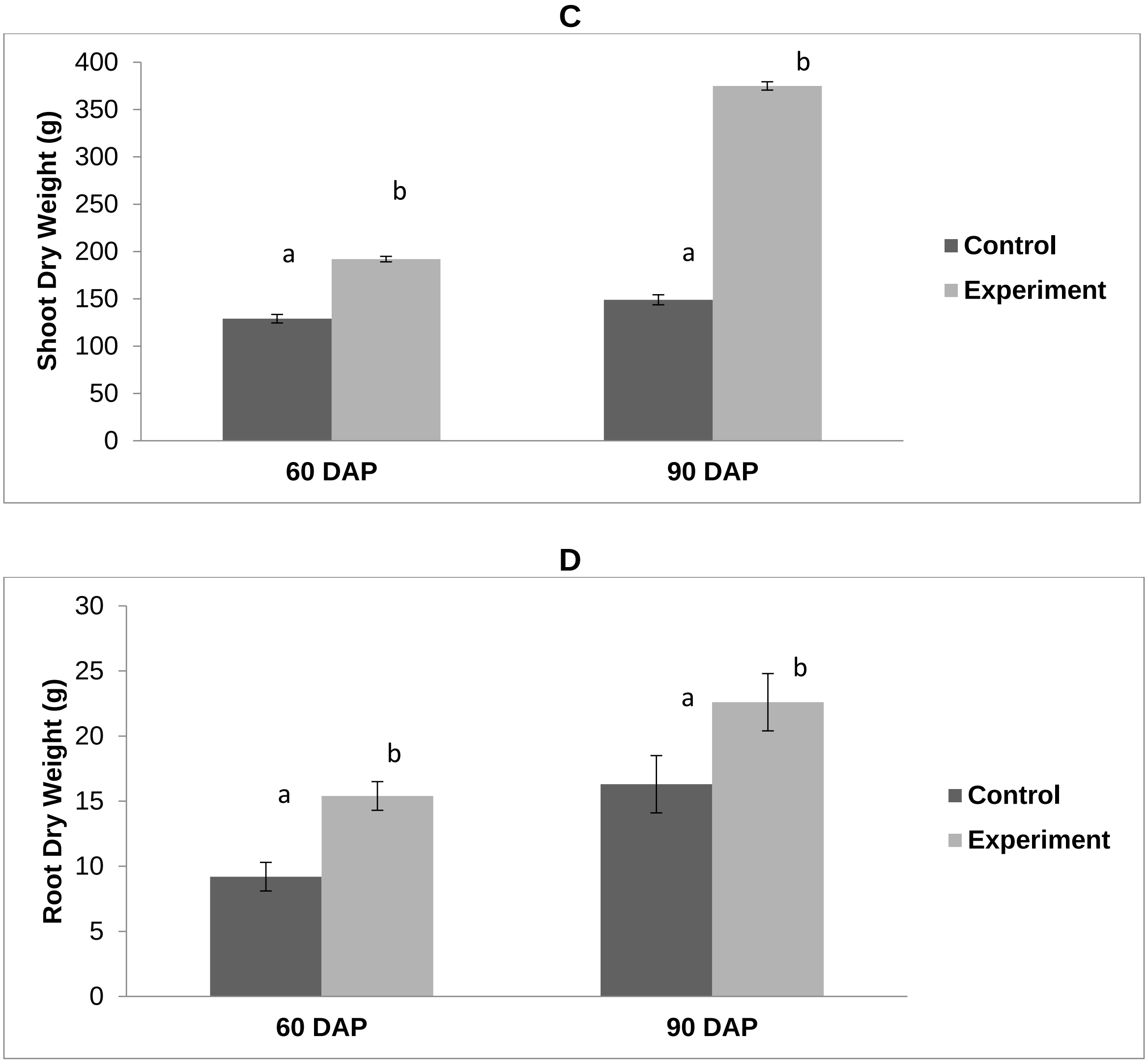

Fig. 1. The effects of seaweed extract on flower cluster number (A), fruit number (B), shoot dry weight (C), and root dry weight (D) in tomato plants in a greenhouse. Mean ± SD values followed by different lowercase letters indicate statistically significant differences($p<0.05$). Mean value after prefixed DAP followed by the different lower case letter(s) are statistically significant.

Effect of Seaweed Manure on the Improvement of Soil Nutrients

The physicochemical properties of the soil growth media used in the greenhouse experiments are depicted in Table 3.The pH of the control soil sample was 7.21 ± 0.02, and the experimental soil used in this study was sandy. The three major nutrients, N, P, and K were significantly higher in the soil treated with macroalgal nutrients. It was previously reported that supplemented organic fertilizers generally improved essential macronutrients (N, P, and K) in soils (Moe *et al*. 2019; Adekiya *et al*. 2020). Similarly, Tursun (2022) used organic seaweed fertilizer to improve the growth and essential oil composition of coriander. In addition, Ashour *et al*. (2021) used commercial seaweed liquid fertilizer and reported improved antioxidant activities in hot pepper (*Capsicum annuum*). The improvement in NPK in the soil indicated improved fertility. The amount of NPK increased continuously at 60 DPI and 90 DPI. The increased level of NPK after 90 DPI showed that the availability of macro- and micro-nutrients would benefit the next plantation. In agriculture, P is considered a major nutrient factor, and the increase in P in the soil was consistent with the findings of a previous study indicating that it improved crop yields (Cassidy *et al*. 2013). The application of seaweed fertilizers improved soil enzyme activities and the growth of *Malus hupehensis* Rehd. seedlings (Wang *et al*. 2016) and soil fertility (de Sosa *et al*. 2023).The seaweed residues in the supplemented soil improved soil fertility and vine productivity (de Sosa *et al*. 2023). In addition, the seaweed (*Ulva ohnoi*) was used for the preparation of compost and the compost manure improved crop yield (Cole *et al*. 2016). The applied seaweed acted as a nitrogen source, increased sweet corn yield and quality, and improved soil quality (Possinger and Amador 2016).

Table 3. Macronutrient Composition of Soil Treated with Seaweed Biostimulants in a Greenhouse

Properties	Control	60 DAP	90 DAP
Total nitrogen	1.19 ± 0.02^{a}	1.87 ± 0.02^{b}	2.48 ± 0.03^{c}
Total phosphorus	0.41 ± 0.01^{a}	0.59 ± 0.04^{b}	0.71 ± 0.09^{c}
Total potassium	0.74 ± 0.02^{a}	0.82 ± 0.11^{b}	1.53 ± 0.22^{c}

Mean values within the same row followed by the different lower case letter(s) are statistically significant.

Soil Enzyme Activity

Seaweed soil fertilizer improved the soil enzyme activities in the greenhouse. The application of seaweed fertilizer increased the activity of the tested soil enzymes (Table 4). The soil urease activity and soil phosphatase activity increased significantly ($p<0.05$). Compared with the control treatment, the control treatment had no significant effect on urease activity. Catalase activity increased marginally at 60 DPI and was statistically insignificant ($p>0.05$). Moreover, soil invertase activity was significantly greater in the seaweed-treated soil than in the control ($p<0.05$). Soil enzymes are produced by microorganisms and are closely related to the microbial community and activity. They are very important in catalyzing the enzymatic reactions required for nutrient cycling and organic matter decomposition (Demisie *et al*. 2014). The cultivation method, type of organic amendment, crop type, and climate conditions are all significant factors that influence enzymatic reactions. The available organic matter in the soil indicates microbial activity and soil fertility. In this study, phosphatase activity was assayed, and it was improved by macroalgal supplementation. It is a useful enzyme in agriculture because it hydrolyses organic phosphorus from the environment into inorganic phosphate. Plants utilize this inorganic phosphate directly (Amador *et al*. 1997). Several workers have shown an increase in soil enzyme activities after organic fertilizers, such as composts and manures, are applied (Akça and Namlı 2015; Chathurika *et al*. 2019). In agreement with these previous findings, soil enzyme activities significantly increased with the application of seaweed manure. In this study, the soil enzyme activity of urease and phosphatase was correlated with the application of organic fertilizer and the number of days of application. The application of inorganic fertilizers to the soil affects enzyme activities and soil fertility dynamics. The activities of soil enzymes, such as phosphatase, urease, catalase, and invertase, increase when plants are supplied with manure (Yang *et al*. 2008), and these results are consistent with those of the present study. Moreover, enzymatic activity in soil depends on several factors, such as the soil pH, organic matter level, content of biogenic elements, and diversity of microorganisms. The brown algal seaweed fertilizer has been used in the okra planting field for a long period (2 years). The continuous application of algal residues improved soil fertility, soil enzyme activity and soil bacterial diversity. The soil microbiome is involved in nitrogen fixation, polysaccharide degradation, and enzyme production (Liu *et al*. 2023).

Table 4. Effects of Weed Fertilizer on Soil Enzyme Activity in Greenhouses

Enzymes	60 DAP	90 DAP	Control
Urease (U/g)	259.4 ± 10.1^{a}	268.5 ± 5.9^{b}	241 ± 11.3^{c}
Phosphatase (U/g)	8759 ± 14.7^{a}	9057 ± 20.3^{b}	8067 ± 102.4^{c}
Invertase (U/g)	18.3 ± 1.1^{a}	25.4 ± 1.2^{b}	15.3 ± 1.1^{c}
Catalase (U/g)	9.2 ± 0.86^{a}	14.8 ± 1.7^{b}	8.58 ± 0.54^{a}

Mean values within the same row followed by the different lower case letter(s) are statistically significant.

Effect of Seaweed Manure on the Soil Microbial Population

In this study, the prepared seaweed manure improved the soil microbial population. In agricultural fields, plant roots are in contact with soil microbial structures. These interactions improve nutrient acquisition, enhance growth, facilitate disease suppression, and mitigate stress (El Boukhari *et al*. 2020). The application of seaweed nutrients to the soil in the current study improved the microbial communities after 60 days and 90 days of the experiment. Seaweed nutrients significantly ($p<0.05$) improved the bacterial, fungal, and actinomycete populations, and the results are expressed in CFU/g soil (Table 5, Fig. 2). Moreover, the bacterial population was greater in the soil treated with seaweed nutrients than in the control. Overall, these findings show that seaweed nutrient application stimulated bacterial growth to a greater extent than fungal or actinomycetes did. The predominance of bacteria depends on the type of organic compost used as a soil amendment. Macroalgae supply nutrients in the form of carbohydrates, carbon, and energy for microbial populations (Illera-Vives *et al*. 2020). Seaweed manure improved the soil microbial population, thus improving the soil biogeochemical cycles. The present result was corroborative with previous findings. Wang *et al*. (2017) used seaweed fertilizer prepared from *L. nigrescens* and *L. flavicans*, which increased the bacterial and fungal populations compared with those of the control. In addition, seaweed extracts prepared from *D. potatorum* and *A. nodosum* improved the microbiological processes of the soil by increasing the total microbial load and increasing the availability of nitrogen. The available N-content in soil treated with seaweed extract improved the bacterial population and improved soil health (Hussain *et al*. 2021). The seaweed extracts improved nutrient recycling and microbial population. The seaweed biostimulants influenced the exudate composition, which directly affected rhizosphere associated microorganisms. The pattern and composition of root exudates affect the population size, structure, and activity (Ali *et al*. 2021).

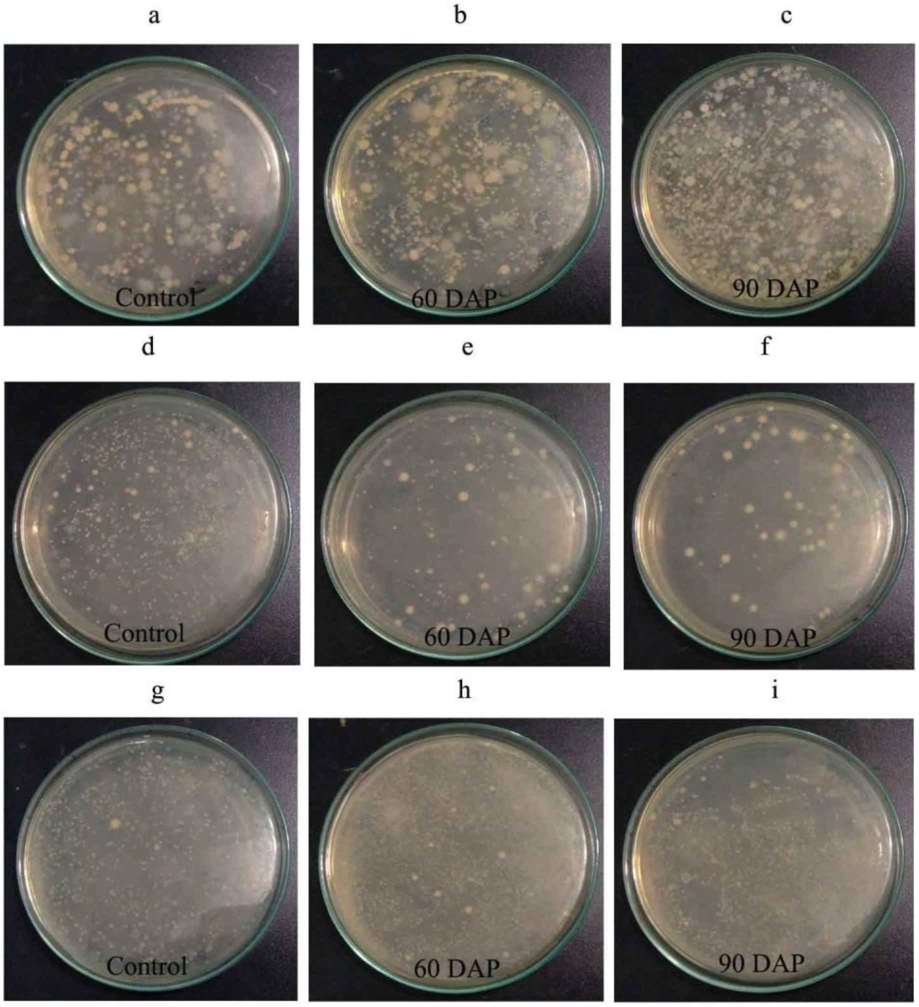

Fig. 2. Growth of bacteria on nutrient agar plates (a through c), Fungi on potato dextrose agar media (d through f), and Actinomycetes on starch casein agar media (g through i)

Table 5. Effect of Seaweed on the Microbial Population in the Soil Grown in the Greenhouse Environment

Experiment	Untreated Control	60 DAP	90 DAP
Bacteria (×10^7)	1.82 ± 0.21^a	2.76 ± 0.15^b	3.42 ± 0.22^c
Fungi (×10^5)	1.01 ± 0.2^a	1.42 ± 0.14^b	1.44 ± 0.25^b
Actinomycetes (×10^3)	0.53 ± 0.08^a	0.54 ± 0.1^a	0.82 ± 0.11^b

Mean values within the same row followed by the different lower case letter(s) are statistically significant.

CONCLUSIONS

1. Proximate analyses of seaweed revealed that brown algae are rich of macronutrients, micronutrients, and minerals. The presence of phytohormones, including indoleacetic acid, gibberellic acid, indole butyric acid, and abscisic acid was detected.

2. Seaweed fertilizer was prepared using *Sargassum wightii*, *Sargassum longifolium*, *Laminaria digitata*, and *Gelidiella acerosa.*
3. The supplemented algal biostimulant improved flower cluster and fruit number, shoot dry and root dry weight in tomato plants in a greenhouse. Macroalgae fertilizer improved soil enzyme activities and the microbial population.

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