



RESEARCH ARTICLE

Biochemical and microbial interactions of *Sargassum wightii* extract and plant growth-promoting rhizobacteria consortia enhance growth and yield of tomato (*Solanum lycopersicum* L.)

K Oviya¹, J Jaipriyanka¹, S Mahalakshmi¹, M Jayanthi¹ & T Porchelvan²

¹Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India

²Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India

*Correspondence email - sasioviya2503@gmail.com

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Abstract

The increasing need for sustainable agricultural practices has intensified interest in seaweed-based biostimulants and plant growth-promoting rhizobacteria (PGPR) as eco-friendly alternatives to chemical fertilisers. This study examined the individual and combined effects of *Sargassum wightii* extract and PGPR strains (*Azospirillum lipoferum* TSA-6 and *Bacillus megaterium* TSB-3) on the growth and yield of tomato (PKM-1) under pot culture conditions. Seaweed extracts obtained through hot-water and solvent extraction were screened for phytochemicals and endogenous growth hormones (IAA, GA₃ and cytokinin). Among the six species analysed, *S. wightii* exhibited the highest levels of auxin (3.7 mg L⁻¹) and cytokinin (4.9 mg L⁻¹). Preliminary screening using crude extract showed that a 2.5 % concentration significantly enhanced germination, seedling vigour, root and shoot length and leaf area index. In the pot experiment, the integrated treatment comprising 75 % recommended dose of fertilisers (RDF) + 2.5 % *S. wightii* extract + *A. lipoferum* + *B. megaterium* (T8) recorded superior growth and yield attributes, including increased dry matter production, fruit number, fruit weight and total fruit yield, comparable to 100 % RDF. The results demonstrate that the combined application of *S. wightii* extract and PGPR can effectively enhance tomato productivity while reducing fertiliser usage by 25 %. This integrated biostimulant strategy offers a sustainable approach to improving crop performance and reducing chemical fertiliser dependence.

Keywords: *Azospirillum lipoferum*; *Bacillus megaterium*; biostimulant; PGPR; *Sargassum wightii*; seaweed extract; sustainable agriculture; tomato yield

Introduction

Bio-based agricultural amendments, including microbial inoculants and natural plant biostimulants, have emerged as promising tools to improve crop performance without the drawbacks associated with synthetic fertilisers. Seaweed-based biofertilisers and plant growth-promoting rhizobacteria (PGPR) have emerged as promising tools for improving soil fertility and enhancing nutrient uptake in crops. Seaweeds are a crucial element of the marine ecology and can function as an organic biostimulant for terrestrial plants.

Recent developments in seaweed-derived liquid organic biostimulants have significantly improved their biochemical efficacy, positioning them as sustainable alternatives to synthetic fertilisers and contributing to their growing adoption in modern agriculture (1, 2). Brown seaweeds, especially *Sargassum wightii*, are rich sources of macro- and micronutrients, amino acids, phytohormones and polysaccharides that stimulate plant growth. Their chemical composition varies by species; thus, elemental characterisation plays a crucial role in selecting nutrient-rich seaweeds. Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) is widely used for structural and

elemental analysis due to its precision in nutrient mapping. The seaweed extract formulation with the addition of PGPR microorganisms will increase the nutrient uptake of crop plants and enrich the soil in an eco-friendly way (3). The seaweed extract-based bio stimulants, having plant growth - promoting capacity and aiding in improving the overall plant resilience, are highly desirable (4).

Over the last 40 years, studies on *Azospirillum*-plant interactions have identified a wide array of mechanisms demonstrating the beneficial impacts of this bacterium on plant growth. Multiple phytohormones, plant regulators, nitrogen fixation, phosphate solubilisation, a variety of small-sized molecules and enzymes, enhanced membrane activity, proliferation of the root system, enhanced water and mineral uptake, mitigation of environmental stressors and competition against pathogens have been studied, leading to the concept of the multiple mechanisms hypothesis (5).

Plant growth-promoting *Bacillus* strains regulate physiologic and metabolic balance, induce resistance against drought stress and solubilise minerals for efficient plant uptake. Even though its advantages for growing crops have been widely

acknowledged and researched in lab settings, its success when applied in fields differs greatly. Water stress can be alleviated by using osmo-tolerant gram-positive rods, *Bacillus* strains, as inoculants, which can improve the plant's ability to use water effectively (6). Different *Bacillus* species can enhance plant growth, yield and biomass by improving nutrient availability and solubilisation, thereby promoting nutrient uptake. They also contribute to nitrogen fixation, phosphorus solubilisation and the synthesis of growth-promoting substances such as indole-3-acetic acid (IAA), gibberellins, siderophores, carotenoids and nitric oxide. *Bacillus* spp. also improve fruit and crop quality by stimulating the production of phytohormones, flavonoids, phenols, antioxidants and other beneficial metabolites (7).

Tomato (*Solanum lycopersicum* L.) is a significant vegetable crop grown within protected environments (8). Tomatoes are a staple in the human diet and among the world's most valuable crops, both nutritionally and economically (9). Rich in vitamin C and K, folate and antioxidants such as lycopene, they are essential to a healthy diet and have been associated with a reduced risk of heart disease and cancer. The integration of PGPR with seaweed extracts may enhance nutrient availability, stimulate hormonal balance and improve the physiological efficiency of crops. In particular, the combination may address phosphorus limitation, a major constraint in tomato production through solubilisation by PGPR and improved nutrient absorption supported by seaweed-derived compounds.

Tomato, an economically important crop, often faces constraints such as phosphorus deficiency, environmental stress and reduced nutrient availability. Although previous studies have explored the individual effects of seaweed extracts or PGPR, research combining *S. wightii* extract with a dual PGPR consortium (*Azospirillum* sp. and *Bacillus* sp.) remains limited, with most studies focusing on single treatments, lacking integrated biochemical and microbial analysis and rarely evaluating synergistic effects on growth, yield, nutrient uptake and stress tolerance. Therefore, the novelty of the present study lies in evaluating the combined biochemical and microbial synergy of *S. wightii* extract and a PGPR consortia, integrating seaweed-derived metabolites with dual microbial mechanisms to enhance nutrient mobilisation and examining their complementary effects on plant growth, yield and soil nutrient status. Accordingly, this study aims to investigate the combined effect of *Azospirillum* sp. and *Bacillus* sp. with *S. wightii* seaweed extract on the growth and yield of tomato, to develop a sustainable, eco-friendly biostimulant strategy to improve crop productivity and reduce dependence on chemical fertilisers.

Materials and Methods

Collection of *Sargassum wightii*

Fresh and healthy fronds of *S. wightii* were collected from the intertidal region of the Mandapam coast, Ramanathapuram district, Tamil Nadu, India (9° 14'N; 79° 14'E), along the Gulf of Mannar. The collected samples were washed thoroughly with seawater and then with tap water to remove adhering sand, salts and epiphytes. The cleaned seaweeds were transported to the laboratory in sterile polythene bags, shade-dried for one week and powdered using an electric grinder. The dry form of *S. wightii* used for extract preparation is shown in Fig. 1, while its surface

morphology as observed under scanning electron microscopy (SEM) is presented in Fig. 2. The powdered material was stored at room temperature until further analysis. Species identification was confirmed at the Centre for Advanced Studies in Marine Biology, Annamalai University.

Extraction of seaweeds by the hot water method (Crude extract)

To prepare the extract, 10 g of powdered *S. wightii* sample was mixed with 100 mL of distilled water and heated for 30 min. After heating, the mixture was allowed to cool for a few minutes and filtered through a muslin cloth. The filtrate obtained was considered the 100 % crude or hot-water extract. The extract was stored under refrigerated conditions for further use. Different concentrations of the crude extract, viz., 1 %, 2.5 %, 5 % and 7.5 %, were prepared and used for the study, along with a control treatment.

Extraction of seaweeds using different solvents

The collected seaweed samples were thoroughly washed with distilled water to remove adhering salts, sand particles and epiphytes and subsequently shade-dried at room temperature until a constant weight was achieved. The dried samples were then pulverised into a fine powder using a mechanical grinder. Approximately 50 g of powdered seaweed material was subjected to Soxhlet extraction using methanol, ethyl acetate and chloroform as solvents. Extraction was carried out for 6–8 h at the respective boiling temperatures of each solvent until the solvent in



Fig. 1. Dry form of *Sargassum wightii*.

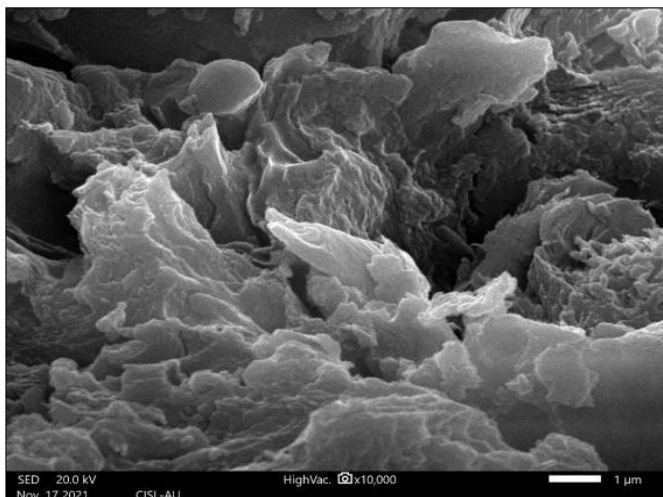


Fig. 2. SEM image of *Sargassum wightii*.

the siphon tube became colourless, indicating complete extraction. The obtained extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C to remove the solvents. The resulting semi-solid residues were further dried to constant weight and the extraction yield was calculated as a percentage of the initial dry weight of the sample. The dried extracts were reconstituted in their respective solvents to obtain a stock concentration of 100 mg/mL and stored at 4 °C in airtight containers until further use. All extractions were performed in triplicate to ensure reproducibility of the results.

Estimation of plant growth hormones by standard protocol

Estimation of auxin in seaweeds

The auxin content in the seaweed extract was estimated using the Salkowski colourimetric method. Briefly, 1 mL of the extract was mixed with 2 mL of Salkowski reagent (a mixture of 2 % FeCl₃ and 35 % perchloric acid). The reaction mixture was incubated in the dark for 30 min at room temperature, during which a pink-reddish colour developed due to the formation of an IAA-Fe complex. The absorbance was recorded at 530 nm using a UV-Visible spectrophotometer. A standard curve was prepared using known concentrations of IAA (10–100 µg mL⁻¹) and the IAA content in the samples was calculated accordingly. Results were expressed as µg IAA g⁻¹ fresh weight (10).

Estimation of cytokinin in seaweeds

Cytokinin content was quantified using the phenol sulfuric acid colourimetric method. One mL of the seaweed extract was mixed with 1 mL of 5 % phenol solution, followed by the rapid addition of 5 mL of concentrated sulfuric acid. The tubes were allowed to stand for 10 min, followed by an additional incubation of 20–30 min at room temperature for colour development. The yellow–orange chromophore formed was measured at 490 nm. A calibration curve was generated using zeatin standards (10–100 µg mL⁻¹) and cytokinin concentration was expressed as µg zeatin equivalents g⁻¹ fresh weight (11).

Estimation of gibberellin in seaweeds

Gibberellin content was determined using the zinc acetate-potassium ferrocyanide colourimetric method. One mL of the extract was mixed with 2 mL of 2 % zinc acetate solution, followed by the addition of 2 mL of 10 % potassium ferrocyanide. The mixture was centrifuged at 3000 rpm for 10 min to obtain a clear supernatant. The absorbance of the supernatant was measured at 254 nm. A standard curve was prepared using GA₃ (10–100 µg mL⁻¹) and the gibberellin concentration in the samples was calculated in terms of µg GA₃ equivalents g⁻¹ fresh weight (12).

Phytochemical analysis of the seaweed *Sargassum wightii*

The seaweed *S. wightii* was tested for the presence of phyto-chemicals by using standard methods (13).

Carbohydrate test

The crude extract of *S. wightii* was mixed with 2 mL of iodine solution. The appearance of dark blue or purple colour indicates the presence of carbohydrates.

Protein test

Ninhydrin 0.2 % solution was boiled with 2 mL of crude extract. Proteins and amino acids are present, as shown by the production of the violet colour.

Glycoside test

The crude *S. wightii* extract was combined with 2 mL of chloroform, followed by 2 mL of concentrated H₂SO₄ and gently shaken. The presence of glycosides, namely the glycone part of the glycoside, is indicated by the steroidal ring's reddish-brown colour.

Quinone test

A total of 2 mL concentration of concentrated hydrochloric acid (HCl) was added to the crude seaweed extract and the mixture was gently heated. The development of a yellow colour indicates the existence of quinones.

Terpenoid test

The crude extract of *S. wightii* was dissolved in 2 mL of chloroform. The dissolved crude extract was evaporated to dryness and to this extract, add 2 mL of conc. H₂SO₄ and heated for about 2 min. The presence of terpenoids was indicated by the formation of a greyish colour.

Saponin test

In a test tube, the crude extract was mixed with 5 mL of distilled water and it was shaken vigorously. The stable foam formation was considered an indication of the presence of saponins.

Flavonoid test

A few fragments of magnesium ribbon were mixed with crude extract of *S. wightii* and drops of conc. HCl were added to it. After a few minutes, the pink scarlet colour appears, which indicates the presence of flavonoids.

Phenol and tannin test

Crude extract of *S. wightii* was mixed with 2 mL of 2 % solution of FeCl₃. The presence of phenols and tannins was indicated by the appearance of blue-green or black colouration.

Alkaloid test

The crude extract was mixed with 2 mL of 1 % HCl and heated gently. To this mixture, Mayer's and Wagner's reagents were added. The presence of alkaloids was indicated by the formation of turbidity resulting in precipitate.

Preparation of pots and seed inoculation

Experimental design and randomisation

The pot experiment was conducted using a completely randomised design (CRD) with nine treatments and three replications, resulting in a total of 27 experimental units. Treatments were assigned to pots based on a computer-generated randomisation sequence to eliminate positional bias. Pots were periodically rotated to avoid micro-environmental variation. No blocking was used, as the experiment was carried out under uniform environmental conditions.

Preparation of pots

Cement pots of size 1 × 2 × 2 ft were filled with a sterilised field soil and sand mixture (1:1, w/w). Each pot received 25 seeds initially and seedlings were later thinned to maintain five uniform plants per pot.

Seed surface sterilisation

Tomato seeds (Var. PKM-1) were surface sterilised with 80 % ethanol for 30 s, followed by 0.1 % mercuric chloride for 1–2 min and subsequently rinsed 3–4 times with sterile distilled water to remove all traces of sterilants.

Plant growth-promoting rhizobacteria strain authentication and viability confirmation

The plant growth-promoting rhizobacteria (PGPR) strains, *Azospirillum lipoferum* and *Bacillus megaterium*, were obtained from an authenticated microbial collection. Strain identity was confirmed by morphological, biochemical and culture-based characterisation prior to use. The pure culture of *A. lipoferum* maintained on agar slants is shown in Fig. 3, while the isolated culture of *B. megaterium* obtained from the rhizosphere soil of tomato is presented in Fig. 4. The viability of each inoculant was verified using serial dilution and plate count techniques, ensuring a final population of 1×10^9 CFU mL⁻¹ at the time of application.

Compatibility testing for consortium treatments

For treatments involving microbial consortia, compatibility between *A. lipoferum* and *B. megaterium* was assessed using the cross-streak method on nutrient agar. The absence of any inhibitory zones confirmed that both strains were compatible and could be applied together without antagonism.

Seed inoculation procedure

Surface-sterilised seeds were coated with carrier-based inoculants containing either individual PGPR or their combinations. The inoculated seeds were shade-dried for 30 min before sowing. For treatments involving *S. wightii* extract, the required concentration (2.5 %) was applied as per the treatment structure. The treatments are as follows:

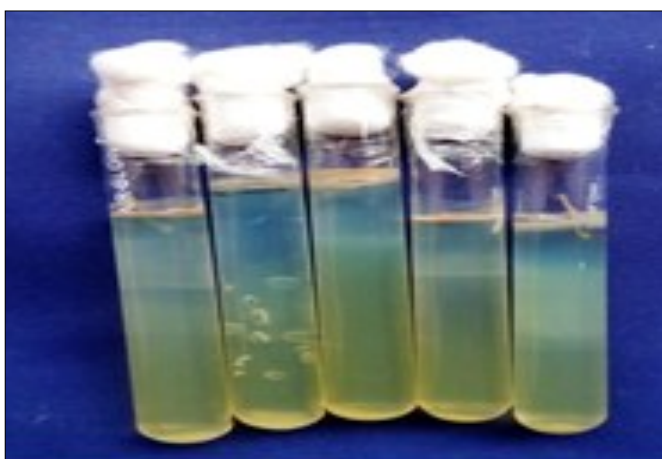


Fig. 3. Pure culture of *Azospirillum lipoferum* maintained in agar slant.



Fig. 4. Isolated culture of *Bacillus megaterium* from the rhizosphere soil of tomato.

T₁ – 100 % RDF

T₂ – 75 % RDF + 2.5 % *Sargassum wightii*

T₃ – 75 % RDF + *Azospirillum lipoferum*

T₄ – 75 % RDF + *Bacillus megaterium*

T₅ – 75 % RDF + 2.5 % *Sargassum wightii* (SWb6) + *Azospirillum lipoferum*

T₆ – 75 % RDF + 2.5 % *Sargassum wightii* (SWb6) + *Bacillus megaterium*

T₇ – 75 % RDF + *Azospirillum lipoferum* + *Bacillus megaterium*

T₈ – 75 % RDF + 2.5 % *Sargassum wightii* (SWb6) + *Azospirillum lipoferum* + *Bacillus megaterium*

T₉ – Control

Seaweed extract application

The seaweed liquid fertiliser (SLF) prepared from *S. wightii* was applied as a foliar spray at 15-day intervals throughout the experiment. Each pot received 25 mL of the respective SLF concentration until the foliage was uniformly wetted. Applications were made in the early morning to prevent leaf scorch.

Determination of growth parameters of tomato variety PKM- 1 under pot conditions

Soil characteristics

Soil used for the experiment was collected from the topsoil layer (0–15 cm), air-dried and sieved through a 2-mm mesh. Physicochemical properties were analysed following standard procedures. The soil had a pH of 6.8, electrical conductivity (EC) of 0.42 dS m⁻¹, organic carbon content of 0.64 %, available nitrogen of 248 kg ha⁻¹, available phosphorus of 21.4 kg ha⁻¹ and available potassium of 312 kg ha⁻¹. Based on particle-size distribution, the soil was classified as sandy loam.

Environmental conditions

The experiment was conducted under a CRD with nine treatments and three replications, making a total of 27 pots. Pots were arranged randomly in an open (ambient) environment and re-randomised weekly to avoid positional bias. The study was carried out under natural environmental conditions with an average temperature of 28 ± 2 °C and relative humidity of 65–70 %. All pots were irrigated uniformly every day to maintain adequate soil moisture throughout the experimental period.

Experimental design and plant material

Tomato variety PKM-1 seeds were sown in pots filled with prepared soil. The experiment was laid out in a Completely randomised design (CRD) with three replications per treatment.

Biometric observations

Five plants per treatment were randomly selected for recording biometric observations at 25, 50, 75 and 100 days after sowing (DAS).

Plant height

Plant height was measured from the soil surface to the tip of the terminal bud and expressed in centimetres.

Germination percentage

Germination percentage was calculated on the 7th day after sowing using the formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total seeds sown}} \times 100$$

Vigour index

The vigour index was estimated on the 15th DAS using:

$$\text{Vigour Index} = \text{Germination (\%)} \times \text{Shoot length (cm)}$$

Number of fruits

The number of fruits per plant was recorded at harvest and averaged across the five sample plants.

Fruit weight

Average fruit weight was obtained by weighing representative fruits and expressed as g fruit⁻¹.

Fruit yield

Fruit yield was measured as g plant⁻¹ and further extrapolated to tonnes ha⁻¹ using the standard spacing of 45 × 30 cm.

Dry matter production (DMP)

At 25, 50, 75 and 100 DAS, five plants per treatment were uprooted carefully, washed and dried. Samples were sun-dried for 24 h and subsequently oven-dried at 80 °C until a constant weight was obtained. Dry matter production was expressed as g plant⁻¹.

Harvest index (HI)

Harvest Index was calculated at the final harvest to assess the proportion of assimilates allocated to economic yield. Total above-ground biomass (shoot + leaves + fruits) was recorded after drying. HI was calculated using the formula:

$$\text{Harvest index} = \frac{\text{Fruit yield (g plant}^{-1}\text{)}}{\text{Total above ground biomass (g plant}^{-1}\text{)}}$$

Microbial population and biochemical analysis

Rhizosphere soil samples were collected at each sampling stage for the determination of microbial population using standard serial dilution and plating techniques. Available N, P and K content and their uptake by plants were estimated following established analytical procedures.

Statistical analysis

The experiment was conducted using CRD. All data were analysed using one-way analysis of variance (ANOVA) to determine the significance of treatment effects. When the ANOVA results were significant, treatment means were separated using Tukey's honest significant difference (HSD) post-hoc test at a 5 % significance level ($p \leq 0.05$). Statistical analyses were carried out using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Before performing ANOVA, the assumptions of normality and homogeneity of variance were verified using the Shapiro-Wilk test and Levene's test, respectively (14).

Result

Estimation of growth hormones in seaweeds

The six different seaweeds were screened for their efficiency in the production of growth hormones, viz., auxin, cytokinin and gibberellin (GA₃) and the results are given in Table 1. The highest quantity of the growth hormone auxin (3.7 mg/L) and cytokinin (4.9 mg/L) was present in *S. wightii* (SWb6), while gibberellin-GA₃ (3.8 mg/L) was observed more in *Kappaphycus alvarezii* (SWr3). The least amount of the growth hormones auxin (2.7 mg/L), gibberellin (2.5 mg/L) and cytokinin (2.3 mg/L) were seen in *Padina gymnospora* (SWb4).

Analysis of phytochemical components in *Sargassum wightii* by using different solvents

The phytochemical analysis of brown algae *S. wightii* was estimated by using different solvents. There were four different solvents, viz., hot water, ethyl acetate, petroleum ether and methanol, used to identify the compounds present in it. Totally ten phytochemicals were identified from these various solvent extractions. Most of the phytochemicals, viz., carbohydrate, protein, quinines, saponins, flavonoids and anthraquinones were shown to be positive in the hot water extract. The flavonoids were shown to be positive in ethyl acetate, whereas protein and phenols were observed to be positive in chloroform and methanol shows positive for the phytochemicals carbohydrate, protein, quinines and saponins. The remaining phytochemicals were shown to be negative for all the solvents. The results were interpreted in Table 2.

Table 1. Estimation of growth hormones in seaweeds

Sl. No.	Name of the seaweeds	Auxin (mg/ L)	GA ₃ (mg/L)	Cytokinin (mg/L)
1.	<i>Acanthophora spicifera</i>	-	2.7	4.1
2.	<i>Halymenia dilate</i>	3.4	3.1	2.9
3.	<i>Kappaphycus alvarezii</i>	3.2	3.8	3.1
4.	<i>Padina gymnospora</i>	2.7	2.5	2.3
5.	<i>Stechospermum marginatum</i>	2.9	3.0	-
6.	<i>Sargassum wightii</i>	3.7	3.3	4.9

Table 2. Phytochemical analysis of compounds in *Sargassum wightii* by using different solvents

Sl. No.	Phytochemicals	Hot water	Ethyl acetate	Chloroform	Methanol
1.	Carbohydrate	+	-	-	+
2.	Proteins	+	-	+	+
3.	Glycosides	-	-	-	-
4.	Quinines	+	-	-	+
5.	Terpenoids	-	-	-	-
6.	Saponins	+	-	-	+
7.	Flavonoids	+	+	-	-
8.	Anthraquinones	+	-	-	-
9.	Phenols	-	-	+	-
10.	Alkaloids	-	-	-	-

Absent (-); Positive (+).

Relationship between solvent extract phytochemicals and growth-promoting activity

In addition to the qualitative identification of phytochemicals, the results were examined in relation to the plant growth responses, which were interpreted in Table 3. Extracts that contained carbohydrates, proteins and saponins, particularly the hot-water and methanol extracts, were associated with higher germination percentage and improved seedling vigour. These compounds are known to support metabolic activity during early growth and their presence in these extracts may have contributed to the observed enhancement in plant performance. The ethyl acetate extract, which showed the presence of flavonoids, corresponded with a moderate increase in shoot elongation, suggesting a possible role of flavonoids in facilitating auxin-related growth processes. Although proteins and phenols were detected in the chloroform extract, this extract showed comparatively weaker growth responses, indicating that the presence of these compounds alone may not be sufficient to promote growth. Overall, the observed plant growth responses corresponded with the phytochemical composition of each solvent extract, indicating that specific groups of compounds may contribute to the differential growth-promoting effects of *S. wightii* extracts.

Effect of crude extract of *Sargassum wightii* on the growth parameters of tomato

The seaweed (*Sargassum wightii*-SWb6) was extracted with hot water. The crude extract obtained from the algae was used to analyse the growth parameters of the tomato crop. The results obtained were represented in Table 4. The growth parameters viz., germination percentage (98 %), root length (29 cm), shoot length (62 cm) and leaf area index (3.2268) were observed to be

maximum in the concentration 2.5 %, followed by the concentration 1 %. The lowest values for growth parameters, including germination percentage (86 %), root length (19 cm), shoot length (46 cm) and leaf area index (3.0212), were recorded at the 7.5 % concentration of seaweed crude extract. However, the control treatment showed comparatively higher values than the 5 % and 7.5 % extract concentrations.

Influence of integrated seaweed-PGPR biostimulation in seed germination and seedling vigour in tomato (PKM-1)

The influence of PGPR inoculation and *S. wightii* extract on plant height, germination percentage and vigour index of tomato variety PKM-1 is presented in Table 5 and Fig. 5. Significant variations were observed among the nine treatments evaluated.

The highest plant height (17.60 cm), germination percentage (96.54 %) and vigour index (1738.80) were recorded in Treatment T8 (75 % RDF + 2.5 % *S. wightii* extract (SWb6) + *A. lipoferum* TSA-6 + *B. megaterium* TSB-3). This treatment was statistically on par with T1 (100 % RDF), which recorded a plant height of 16.50 cm, germination percentage of 95.53 % and vigour index of 1730.32. The results clearly indicate that the combined application of seaweed extract with PGPR enhances early seedling performance, even under reduced fertiliser inputs.

Combined effects of seaweed extract and PGPR inoculation on the number of fruits, fruit weight and fruit yield of tomato (PKM-1)

These findings demonstrate that the combined application of seaweed extract and PGPR significantly enhances yield potential even under reduced fertiliser inputs. The results of PGPR inoculation on fruiting parameters of tomato are presented in Table 6 and Fig. 6,

Table 3. Correlation between phytochemical composition of *Sargassum wightii* extracts and plant growth responses

Solvent extract	Major phytochemicals	Observed plant response	Possible relationship
Hot water	Carbohydrates, proteins, saponins, flavonoids	Highest germination and vigor index	Nutrient-rich extract enhancing metabolic activity
Methanol	Carbohydrates, proteins, quinones	Moderate growth	Supports early seedling metabolism
Ethyl acetate	Flavonoids	Slight increase in shoot length	Flavonoids may facilitate auxin transport
Chloroform	Proteins, phenols	Weak growth	Low presence of growth-promoting phytochemical groups

Table 4. Effect of crude extract of *Sargassum wightii* on the growth parameters of tomato

Growth parameters				
Extract Concentration (%)	Germination percentage (%)	Root length (cm)	Shoot length (cm)	Leaf area index (%)
1 %	96	26	57	3.0774
2.5 %	98	29	62	3.2268
5 %	94	23	52	3.1662
7.5 %	86	19	46	3.0212
Control (0)	90	20	48	3.0224
SED	0.32	0.20	0.54	0.29
CD ($p=0.05$)	0.61	0.43	1.09	0.06

Table 5. Effect of seaweed *Sargassum wightii* and plant growth promoting rhizobacterial (PGPR) inoculation on the plant height, germination percentage and vigour index of tomato variety PKM-1 under pot culture condition

Treatments	Plant height (cm)	Germination (%)	Vigour index
T1- 100 % RDF	16.50	95.53	1730.32
T2- 75 % RDF + 2.5 % <i>Sargassum wightii</i>	13.64	72.00	1250.30
T3- 75 % RDF + <i>Azospirillum lipoferum</i> (TSA-3)	11.10	65.58	1242.53
T4- 75 % RDF + <i>Bacillus megaterium</i> (TSB-3)	10.38	54.10	1049.12
T5- 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Azospirillum lipoferum</i> (TSA-6)	15.15	85.35	1574.07
T6- 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Bacillus megaterium</i> (TSB-3)	15.30	88.10	1626.02
T7- 75 % RDF + <i>Azospirillum lipoferum</i> (TSA-6) + <i>Bacillus</i>	14.20	76.20	1478.92
T8- 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Azospirillum lipoferum</i> (TSA-6) + <i>Bacillus megaterium</i> (TSB-3)	17.60	96.54	1738.80
T9- Control	9.89	52.50	1029.33
S.Ed	0.10	0.58	3.80
CD ($p=0.05$)	0.34	1.25	8.40

Table 6. Effect of seaweed *Sargassum wightii* and plant growth-promoting rhizobacterial (PGPR) inoculation on the number of fruits, fruit weight and fruit yield of tomato variety PKM-1 under pot culture conditions

Sl. No.	Treatments	No. of fruits (plant ⁻¹)	Fruit weight (g) (plant ⁻¹)	Fruit yield (g) (plant ⁻¹)	Fruit Yield (ton) (plant ⁻¹)
1.	T1- 100 % RDF	22.20	34.80	1480.76	18.52
2.	T2- 75 % RDF + 2.5 % <i>Sargassum wightii</i>	16.55	27.50	968.45	11.90
3.	T3- 75 % RDF + <i>Azospirillum lipoferum</i> (TSA-6)	13.48	25.65	874.50	9.10
4.	T4- 75 % RDF + <i>Bacillus megaterium</i> (TSB-3)	10.60	22.30	740.58	6.05
5.	T5- 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Azospirillum lipoferum</i> (TSA-6)	19.78	30.95	1239.60	17.45
6.	T6- 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Bacillus megaterium</i> (TSB-3)	20.65	32.75	1356.80	17.90
7.	T7- 75 % RDF + <i>Azospirillum lipoferum</i> (TSA-6) + <i>Bacillus megaterium</i>	17.95	29.70	1097.43	15.18
8.	T8- 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Azospirillum lipoferum</i> (TSA-6) + <i>Bacillus megaterium</i> (TSB-3)	22.50	35.15	1489.63	18.60
9.	T9- Control	9.5	13.50	650.40	4.06
	S.Ed	0.17	0.36	0.36	0.34
	CD (p=0.05)	0.36	0.65	0.65	0.76

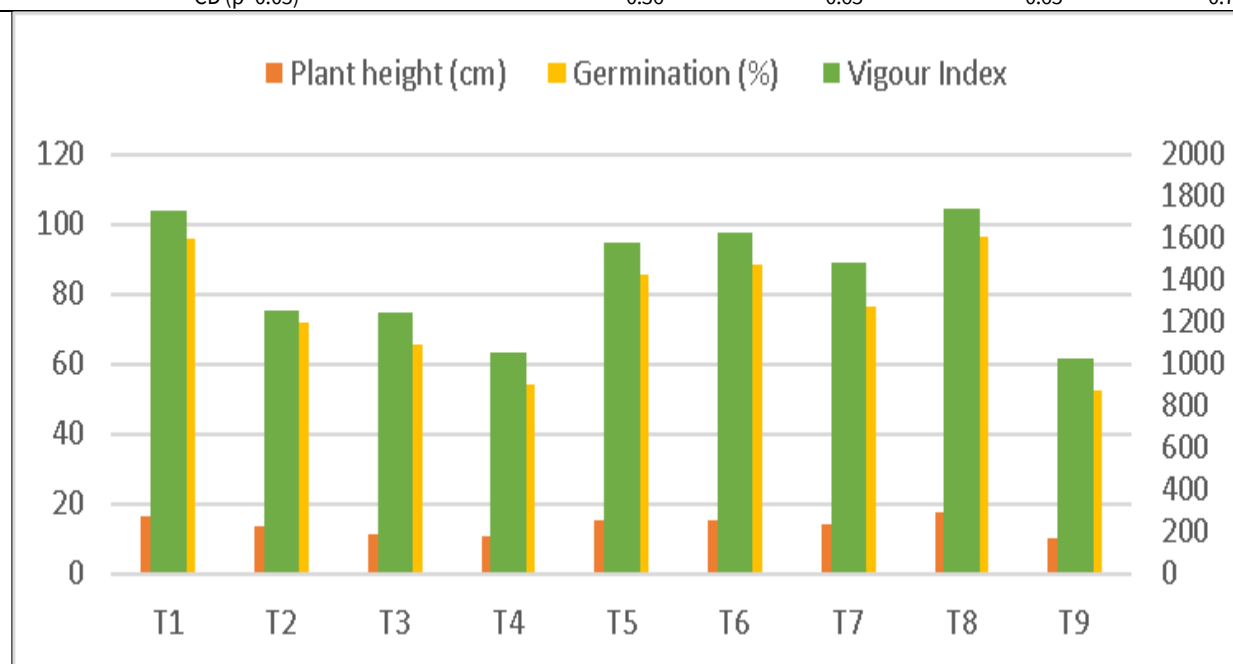


Fig. 5. Effect of seaweed *Sargassum wightii* and plant growth promoting rhizobacterial (PGPR) inoculation on the plant height, germination percentage and vigour index of tomato variety PKM-1 under pot culture condition.

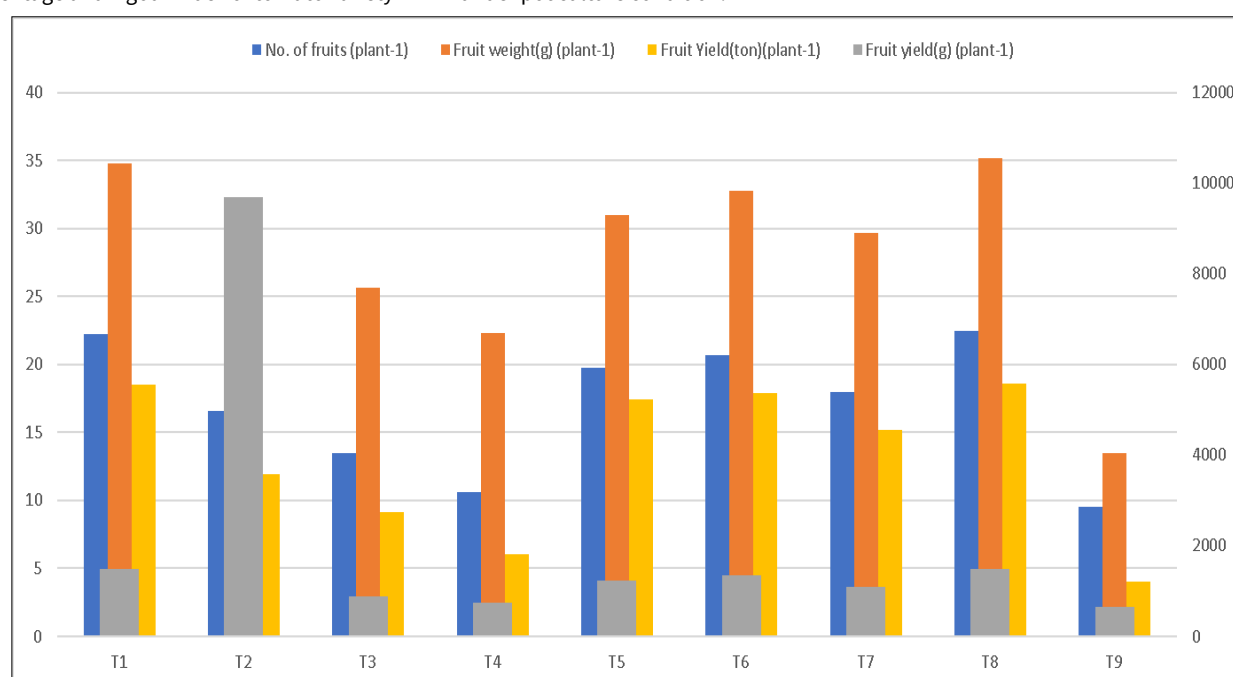


Fig. 6. Effect of seaweed *Sargassum wightii* and plant growth promoting rhizobacterial (PGPR) inoculation on the number of fruits, fruit weight and fruit yield of tomato variety PKM-1 under pot culture condition.

showing significant improvement in all yield attributes. The highest number of fruits (22.50 per plant), fruit weight (35.15 g per fruit) and fruit yield (1489.63 g per plant; equivalent to 18.60 t ha⁻¹) were recorded in T8 (75 % RDF + 2.5 % *S. wightii* + TSA-6 + TSB-3). Harvest index could not be calculated in the present study because total above-ground biomass was not recorded; however, the fruit yield data clearly reflect the treatment-related differences in productivity. This treatment performed on par with T1 (100 % RDF), while the lowest values were observed in T9 (control) across all yield parameters. Overall, these results show that integrating seaweed extract with PGPR enhances yield potential and improves productivity even under reduced fertiliser inputs.

Bioinoculant seaweed interaction enhances dry matter productivity in tomato (PKM-1)

The dry matter production (DMP) of tomato as influenced by PGPR inoculation and *S. wightii* extract is presented in Table 7 and Fig. 7. Among the nine treatments, T8 (75 % RDF + 2.5 % *S. wightii* + TSA-6 + TSB-3) recorded the highest dry matter accumulation (7.35 g plant⁻¹) at harvest, while the lowest DMP (2.90 g plant⁻¹) was observed in T9 (control). Similar to earlier parameters, T8 was statistically comparable to T1 (100 % RDF), indicating that the combined application of seaweed extract and PGPR enhanced biomass production even under reduced fertiliser levels. Although

soil microbial population, enzyme activity and nutrient availability were not measured in the present study, the improved biomass accumulation under T8 suggests enhanced nutrient uptake and physiological activity, which may be influenced by the PGPR and seaweed application. An overall view of the tomato crops grown under pot culture conditions is provided in Fig. 8.

Discussion

The overdependence on chemical fertilisers has raised serious environmental concerns, including soil degradation, water pollution and the decline of soil organic matter (15). Such ecological challenges underscore the need for sustainable alternatives capable of maintaining crop productivity without compromising environmental integrity. In this context, seaweed-based fertilisers have gained global attention as eco-friendly biostimulants. Seaweed extracts are non-toxic, biodegradable and free from harmful residues, making them suitable substitutes for conventional chemical fertilisers (16). Similarly, several studies have highlighted that the excessive application of synthetic fertilisers and pesticides reduces soil fertility and adversely affects soil biological activity. Organic inputs such as seaweed manure help restore nutrient balance and

Table 7. Effect of seaweed *Sargassum wightii* and plant growth-promoting rhizobacterial (PGPR) inoculation on the plant dry matter production of tomato variety PKM- 1 under pot culture conditions

Sl. No.	Treatments	Plant dry matter production (g plant ⁻¹)			
		25 DAP	50 DAP	75 DAP	Harvest
1.	T1– 100 % RDF	2.02	2.68	4.80	7.29
2.	T2– 75 % RDF + 2.5 % <i>Sargassum wightii</i>	1.56	2.63	3.75	5.86
3.	T3– 75 % RDF + <i>Azospirillum lipoferum</i> (TSA-6)	1.40	2.30	3.45	4.33
4.	T4– 75 % RDF + <i>Bacillus megaterium</i> (TSB-3)	1.20	2.22	3.10	3.90
5.	T5– 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Azospirillum lipoferum</i> (TSA-6)	1.86	2.56	4.25	6.34
6.	T6– 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Bacillus megaterium</i> (TSB-3)	1.89	2.62	4.76	7.03
7.	T7– 75 % RDF + <i>Azospirillum lipoferum</i> (TSA-6) + <i>Bacillus megaterium</i> (TSB-3)	1.83	2.42	4.65	6.32
8.	T8– 75 % RDF + 2.5 % <i>Sargassum wightii</i> (SWb6) + <i>Azospirillum lipoferum</i> (TSA-6) + <i>Bacillus megaterium</i> (TSB-3)	2.04	2.70	4.84	7.35
9.	T9– Control	1.09	1.19	2.10	2.90
S.Ed		0.01	0.02	0.02	0.02
CD (p=0.05)		0.03	0.04	0.05	0.05

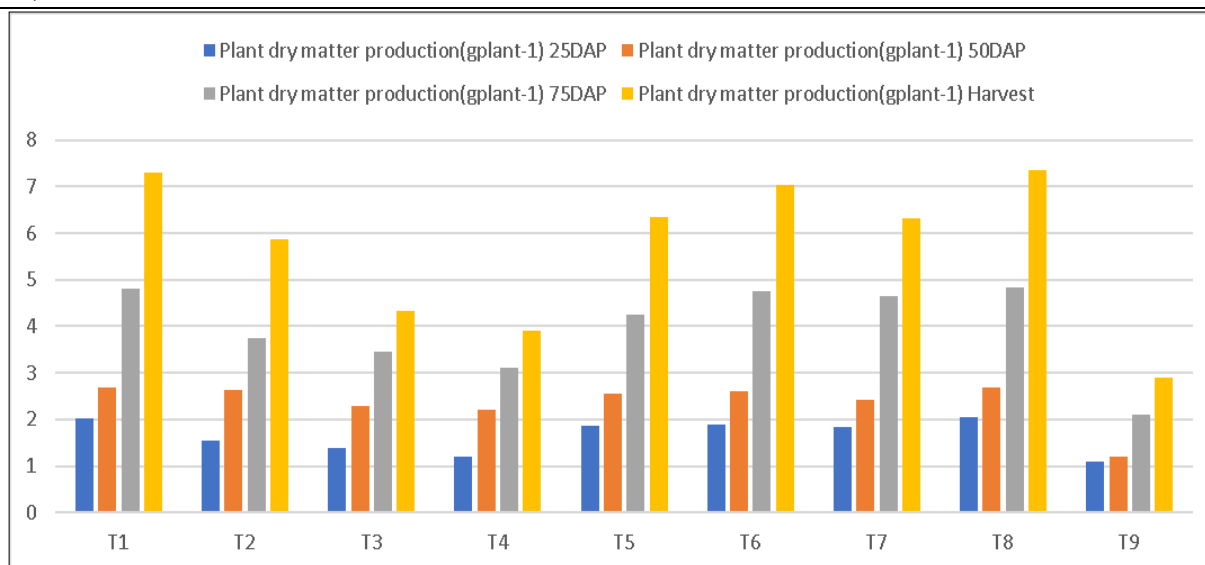


Fig. 7. Effect of seaweed *Sargassum wightii* and plant growth promoting rhizobacterial (PGPR) inoculation on the plant dry matter production of tomato variety PKM- 1 under pot culture condition.



Fig. 8. Overall view of tomato crops under pot culture condition.

improve soil health (17).

Macroalgal extracts are particularly rich in bioactive compounds, phytohormones, micronutrients and polysaccharides that stimulate crop growth, stress tolerance and pest resistance (18). Their application has been shown to reduce the accumulation of heavy metals and toxic residues, common outcomes of prolonged chemical fertiliser use, which otherwise impair plant growth and productivity (19). The results of the present study reaffirm the potential of seaweed extracts as sustainable biostimulants for vegetable crops such as tomato.

Seaweeds are found to contain an adequate amount of plant growth substances such as cytokinins, IAA, gibberellins and gibberellin-like substances (20). The application of seaweed extracts to the horticulture crops promoted the growth and yield to a considerable level (21).

The various plant growth hormones were identified in the extracts of seaweeds, such as auxin, gibberellin, cytokinins, abscisic acid, etc. (22).

Seaweeds were considered as bio-stimulants in *Calibrachoa*, *Capsicum annum* and *Lycopersicum esculentum* (23, 24).

In our investigations, different seaweeds, i.e., three red algae and three brown algae, were collected and screened for the efficiency in the production of growth hormones, namely, auxin, cytokinin and GA_3 in the crude extract. Among the six seaweeds, the brown algae *S. wightii* produced the maximum amount of all growth hormones. A similar result was obtained regarding the growth hormones in seaweeds by previous researchers (24). Marine macro algae (seaweeds) are an important factor in the marine living resources of the world. In many crops like vegetables, trees, flowering plants and grain crops, seaweed extracts are effective as a fertiliser. Extracts of seaweed are known to stimulate cell division, cell elongation, differentiation and protein synthesis, which describes the stimulatory effect of seaweed on seed germination (25).

The use of seaweed extract on many crops shows a physiological change due to the presence of cytokinins (26).

The application of seaweed liquid fertiliser (SLF) shows an increase in plant growth, grain yield and also quality of the

product. The use of SLF spray on plants at 2.5 % concentration showed a significant increase per plot in fruit yield (20.47 %), fruit length (31.77 %), fruit diameter (18.26 %) and number of fruits (37.47 %) (27).

The application of 1.5 % *S. wightii* and 1.0 % of *Ulva lactuca* on cluster beans shows that growth was increased, but the higher concentrations retarded the growth due to stress and wilting of leaves (28).

The foliar application of SLF (*Rosenvingeia intricata*) at 20 % concentration shows a maximum number of fruits (7.33 ± 0.47), fruit length (14.2 ± 0.4 cm), fruit weight (12.43 ± 0.56) (29).

The minimum yield parameters were shown in 100 % concentration of SLF. Hence, in this experimental study, foliar application of *S. wightii* as a liquid fertiliser to tomato plants shows a maximum growth and yield in 2.5 % concentration. The highest growth parameters of root length, shoot length and leaf area index (29 cm and 62 cm) were shown in the control. At 2.5 % concentration of SLF, the yield parameters viz., number of fruits (14.20), fruit length (13.84 cm), fruit dry weight (13.48 g) and yield (12.40 t/ha) followed by 1 % concentration. The least yield parameters were observed in the control.

Brown algae are found to be rich in bioactive compounds such as polysaccharides, peptides, omega fatty acids, carotenoids, phenolics, vitamins, minerals and consist of mainly 90 % water (30).

The current research studied the phytochemical analysis of *S. wightii* by a standard protocol (31). It shows various phytoconstituents viz., carbohydrates, proteins, tannins, saponins, flavonoids, phenols, glycosides, terpenoids, quinones, coumarins and anthraquinones.

In this study, the crude extract of *S. wightii* was prepared at concentrations of 1 %, 2.5 %, 5 % and 7.5 %, along with a control. Among the treatments, the 2.5 % concentration exhibited substantially higher growth parameters, including germination percentage (98 %), root length (29 cm), shoot length (62 cm) and leaf area index (3.2268). Higher concentrations (≥ 5 %) negatively affected growth, with the lowest values recorded at 7.5 %, suggesting that excessive extract concentrations may exert inhibitory effects.

Similarly, yield attributes of tomato, including number of fruits (14.20), fruit length (13.84 cm), fruit dry weight (13.48 g) and total yield (12.40 t/ha) are maximised at 2.5 % SLF, followed closely by the 1 % concentration. Interestingly, the control treatment showed the least yield performance, demonstrating the positive influence of seaweed biostimulants on reproductive development.

In addition to seaweed extracts, PGPR play a central role in sustainable crop production. PGPR enhance nutrient cycling, increases plant nutrient uptake, suppresses phytopathogens, improves soil microbial activity and stimulates phytohormone production (32). They offer an eco-friendly alternative to chemical inputs and are increasingly recognised as key components of integrated nutrient management strategies (33). Previous studies have shown that inoculation with *Azotobacter*, *Pseudomonas*, *Azospirillum* and *Bacillus* enhances growth and yield in tomato (34).

The combined application of *S. wightii* extract and PGPR in the present experiment exhibited strong synergistic effects on tomato growth, yield and soil microbial populations. Among all treatments, T₈ (75 % RDF + 2.5 % *S. wightii* extract + *A. lipoferum* + *B. megaterium*) recorded the highest values for plant growth and yield parameters. These findings indicate that integrating seaweed-based biostimulants with PGPR, even under reduced fertiliser application (75 % RDF), can effectively enhance crop performance. The combined use of seaweed extract and PGPR is not only highly efficient but also contributes to environmentally sustainable tomato cultivation.

A direct comparison of the key treatments clearly highlights the superior performance of T₈ (75 % RDF + *S. wightii* extract + PGPR) over both T₁ (100 % RDF) and T₉ (control). T₈ recorded the highest growth and yield attributes despite receiving 25 % less chemical fertiliser, indicating that the synergistic action of seaweed-derived phytohormones and PGPR mediated nutrient mobilisation compensated for reduced fertiliser inputs. T₁, which received the full recommended dose of fertilisers, performed moderately but still lower than T₈, demonstrating that chemical fertilisation alone could not match the combined biostimulant microbial effects. In contrast, T₉ exhibited the lowest plant growth and yield due to the absence of nutrient inputs and microbial stimulation. The clear yield hierarchy T₈ > T₁ > T₉ confirms that integrating seaweed extract with PGPR not only reduces fertiliser dependency but also enhances crop productivity beyond conventional fertilisation alone.

Although the study demonstrated significant improvements in tomato growth under pot culture conditions, these controlled environments do not fully capture the variability of field soils, fluctuating weather, root-zone interactions and natural microbial activity. Hence, the outcomes cannot be directly generalised to field agriculture. Future field trials will be essential to validate the consistency, scalability and agronomic practicality of the combined application of *S. wightii* extract and PGPR.

Even though biomass data were not recorded in the present experiment, fruit yield results provide a reliable estimate of treatment performance. Future studies should include biomass partitioning to enable accurate Harvest Index estimation.

Conclusion

Overall, the study highlights that the combination of seaweed-based bio-stimulants and PGPR can significantly enhance tomato growth and yield while reducing the dependency on synthetic fertilisers. These findings support the adoption of integrated organic approaches for sustainable vegetable production and provide scientific evidence for the use of *Sargassum wightii* extracts along with PGPR strains in commercial agriculture.

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Authors' contributions

KO conducted the experiment, recorded data, performed data analysis and wrote the manuscript. SM supervised the experiment and formulated the experimental design. JJ, MJ and TP made corrections to the manuscript. All authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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