PLANT SCIENCE TODAY
Vol 13(sp8): 01–09
https://doi.org/10.14719/pst.16357

eISSN 2348-1900

RESEARCH ARTICLE

Sargassum-derived biostimulant improves drought tolerance and yield performance in *Zea mays* L.

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Received: 26 June 2026; Accepted: 30 July 2026; Available online: Version 1.0: 28 September 2026

Cite this article: Sivaranjani K, Senthil K, Sherene JRT, Kiruthika N, Yuvaraja A, Santwana P, Baskar M, Senthilkumar S. *Sargassum*-derived biostimulant improves drought tolerance and yield performance in *Zea mays* L. Plant Science Today. 2026;13(sp8):01–09. https://doi.org/10.14719/pst.16357

Abstract

Drought stress, a major constraint in maize (*Zea mays* L.) productivity around the world. This study assessed the effectiveness of a phosphoric acid (H_3PO_4)-hydrolysed *Sargassum*-based biostimulant in improving drought tolerance and sustaining maize productivity under water-deficit conditions. A field experiment was conducted during the 2025–26 rabi season at Sakkampatty, Theni District, Tamil Nadu, India, using a randomised block design (RBD) with nine treatments and three replications. Biochemical characterisation of the biostimulant identified phenolics, flavonoids, tannins, terpenoids, phytosterols, carbohydrates, proteins and biologically active functional groups. The biostimulant significantly improved physiological traits, growth, yield attributes and grain yield compared with the drought-stressed control. Among the drought-stressed treatments, application at 3.0 kg ha^{-1} (T_7) recorded the highest chlorophyll stability index (CSI), membrane stability index (MSI), relative water content (RWC), proline accumulation, antioxidant enzyme activities and grain yield (39.58 g $plant^{-1}$), representing an approximately 115 % increase over the drought-stressed control (T_2). These findings suggest that the H_3PO_4-hydrolysed *Sargassum* biostimulant has potential as an eco-friendly approach for enhancing drought tolerance and sustaining maize productivity under water-limited conditions. Multi-location and multi-season studies are required to validate its effectiveness across diverse agro-climatic conditions.

Keywords: biostimulant; drought stress; grain yield; maize; physiological responses; *Sargassum*

Introduction

By 2050, global food demand is expected to increase substantially, while climate change, declining soil fertility, environmental degradation and water scarcity continue to threaten sustainable agricultural production. The global biostimulants market, valued at approximately USD 3.3 billion in 2021, is projected to reach about USD 9.3 billion by 2030, reflecting the increasing adoption of sustainable and eco-friendly agricultural technologies (1–3). In India, where agriculture supports more than 50 % of the population, drought remains a major constraint, affecting nearly 68 % of the cultivated area and causing significant yield losses. Tamil Nadu, characterised by semi-arid climatic conditions and variable rainfall, faces increasing challenges from water scarcity, particularly in maize cultivation (4–6). Seaweed-based biostimulants, particularly those derived from *Sargassum* spp., have attracted considerable attention because of their rich composition of bioactive compounds that promote plant growth, enhance nutrient uptake and improve tolerance to abiotic stresses.

Maize (*Zea mays* L.) is one of the world's most important cereal crops, cultivated on more than 200 million hectares with an annual production exceeding 1.2 billion tonnes (7). In India, maize occupies approximately 10 million hectares, producing over 35 million tonnes and plays a vital role in the food, feed, starch, bioethanol and industrial sectors. Despite its high yield potential, maize is highly sensitive to drought, particularly during vegetative growth, flowering and grain-filling stages, with yield losses commonly ranging 20–50 % depending on the severity and duration of water stress. Improving drought tolerance in maize is therefore essential for sustainable crop production. Marine macroalgae represent promising sources of biostimulants because they contain a wide range of bioactive compounds, including polysaccharides, phenolics, flavonoids, terpenoids, phytosterols, amino acids, vitamins and minerals (8–10). Among them, brown seaweeds of the genus *Sargassum* are particularly valuable because of their abundance and high concentrations of plant growth-promoting metabolites (2, 11). These bioactive compounds have been reported to enhance nutrient uptake, antioxidant capacity, osmotic

adjustment, root development and tolerance to environmental stresses (6). However, many of these compounds remain bound within complex polysaccharide matrices, limiting their extraction efficiency and bioavailability. Hydrolysis and depolymerisation of seaweed polysaccharides can enhance the release of low-molecular-weight bioactive compounds, potentially improving their bioavailability and biological activity (12). Although several studies have reported the beneficial effects of seaweed-based biostimulants, limited information is available on phosphoric acid (H_3PO_4)-hydrolysed *Sargassum* formulations evaluated under field-induced drought conditions in maize. Based on the bioactive composition of *Sargassum* and the enhanced release of low-molecular-weight metabolites through H_3PO_4 hydrolysis, we hypothesised that the developed biostimulant would improve drought tolerance in maize by enhancing plant water status, membrane stability, antioxidant defence and photosynthetic performance, thereby improving growth and grain yield under water-deficit conditions. Accordingly, this study aimed to evaluate the effectiveness of a H_3PO_4-hydrolysed *Sargassum* biostimulant under field conditions and compare its performance with two commercially available biostimulants for mitigating drought stress and improving maize productivity.

Materials and Methods

Collection and preparation of seaweed biomass

Fresh thalli of *Sargassum* were collected from the coastal waters of Mandapam (9.28° N, 79.12° E), Ramanathapuram District, Tamil Nadu, India, during January 2025 (winter season). The collection site experiences a tropical maritime climate with average seawater temperatures of 27–29 °C, salinity of approximately 33–35 practical salinity units (PSU) and seawater pH ranging 8.0–8.2. The collected seaweed materials were immediately transported to the laboratory in clean polyethylene bags for further processing. The samples were washed carefully, first with seawater and then with distilled water, to remove adhering salts, sand, epiphytes, shells and other foreign materials. The cleaned biomass was shade-dried at room temperature (28 ± 2 °C) for seven days until a constant weight was reached (8, 13). The dried material was then pulverised using a Wiley mill and sieved through a 0.2 mm mesh to obtain a fine, homogeneous powder. The powdered *Sargassum* biomass was stored in airtight polyethylene containers under dry conditions until used for biostimulant formulation development. Proper drying and storage helped preserve the phytochemical constituents and prevent microbial spoilage of the biomass (6, 14).

Preparation of *Sargassum*-based biostimulant

Preparation of reaction

Ten grams of finely ground *Sargassum* biomass were mixed with 100 mL of 5 % (v/v) H_3PO_4 solution in a 250 mL conical flask, maintaining a solid-to-liquid ratio of 1 : 10 (w/v). The mixture was homogenised using a magnetic stirrer and incubated at 30 °C for 24 hr under continuous agitation to facilitate acid-assisted hydrolysis of the seaweed biomass. Following incubation, the reaction mixture was processed for extraction of soluble bioactive constituents (9, 12).

Extraction of phytochemicals

Following acid-assisted hydrolysis, the reaction mixture was subjected to extraction to obtain soluble bioactive constituents from the *Sargassum* biomass. The hydrolysate was processed to recover phenolic compounds, flavonoids, tannins, terpenoids, phytosterols, carbohydrates, proteins, amino acids and mineral constituents released during hydrolysis (8). The resulting extract was used for subsequent formulation, biochemical characterisation and field evaluation.

Biostimulant formulation

Following extraction, the reaction mixture was centrifuged at 10000 × g for 10 min at 4 °C to separate insoluble residues from the liquid extract. The supernatant containing the soluble bioactive constituents was collected and neutralised to pH 6.8–7.0 using 1 N potassium hydroxide (KOH) under continuous stirring. The neutralised extract was later filtered through Whatman No. 1 filter paper and stored in sterile, amber-coloured bottles at 4 °C until further biochemical characterisation, Fourier transform infrared (FTIR) analysis and field evaluation under drought stress conditions in maize (1, 6).

Biochemical characterisation of biostimulant formulation

The formulation was characterised biochemically to determine the levels of major bioactive compounds involved in plant growth promotion and stress tolerance. Total phenolic content was measured using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE) mL^{-1} (15). Tannin content was estimated using the Folin–Denis method using tannic acid as the standard and expressed as mg tannic acid equivalents (TAE) mL^{-1} (16).

Total flavonoid content was quantified using the aluminium chloride colorimetric assay with quercetin as the reference standard and values were expressed as mg quercetin equivalents (QE) mL^{-1} (17). Terpenoid content was determined spectrophotometrically using linalool as the standard, with results expressed as mg linalool equivalents (LE) mL^{-1} (18). Phytosterol content was estimated and expressed as mg cholesterol equivalents (CE) mL^{-1} (19).

Total carbohydrate content was measured using the phenol-sulfuric acid method with glucose as the standard (20). Protein content was determined using the Bradford dye-binding assay with bovine serum albumin as the reference protein (21). All biochemical analyses were performed in triplicate and the results are presented as mean ± standard deviation.

Fourier transform infrared spectral analysis

Fourier transform infrared spectroscopy was used to identify the main functional groups present in the formulation and to assess the chemical changes brought about by hydrolysis. The extract was dried and finely ground before analysis. About 1–2 mg of the dried sample was mixed with spectroscopic-grade potassium bromide (KBr) and pressed into a translucent pellet using a hydraulic press.

The FTIR spectrum was recorded with an FTIR spectrophotometer over a wavelength range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. The resulting spectra were examined for characteristic absorption bands corresponding to hydroxyl (-OH), carbonyl (C=O), carboxyl (-COOH), ether (C-O-C), phosphate (P-O) and other functional groups typically associated with bioactive compounds from seaweed (6, 9, 13).

Study area and experimental design

The field experiment was performed at Sakkampatty Village in Theni District, Tamil Nadu, India (10.012° N, 77.476° E; 289 m above mean

sea level (AMSL) during the rabi season of 2025–26. This area falls within the Southern agroclimatic zone of Tamil Nadu and has a tropical semi-arid climate. The region receives an average annual rainfall of 835 mm and has a mean annual temperature of 28.4 °C. The experimental soil was sandy clay loam in texture, with a pH of 7.81, electrical conductivity (EC) of 0.17 dS m^{-1}, organic carbon content of 0.18 %, exchangeable calcium of 400 mg kg^{-1} and exchangeable magnesium of 384 mg kg^{-1}.

The experiment was set up in a randomised block design (RBD) with nine treatments and three replications. A total of 27 plots were established to evaluate the effectiveness of the *Sargassum*-based biostimulant formulation on drought tolerance and productivity in maize (*Z. mays*). The treatments included T_1: irrigated control without stress (untreated control), T_2: untreated control subjected to drought stress at 50 % irrigation, T_3: biostimulant formulation applied at 1.0 kg ha^{-1} under non-stress conditions, T_4: biostimulant formulation applied at 2.5 kg ha^{-1} under non-stress conditions, T_5: biostimulant formulation applied at 2.0 kg ha^{-1} under drought stress (50 % irrigation), T_6: biostimulant formulation applied at 2.5 kg ha^{-1} under drought stress (50 % irrigation), T_7: biostimulant formulation applied at 3.0 kg ha^{-1} under drought stress (50 % irrigation), T_8: acadian stimplex® applied at 2.5 kg ha^{-1} as a commercial check under drought stress (50 % irrigation) and T_9: radifarm® (valagro) applied at 2.5 kg ha^{-1} as a commercial check under drought stress (50 % irrigation). These treatments were imposed to compare the performance of different biostimulant application rates with untreated controls and commercially available biostimulants under both optimal and water-deficit conditions.

Crop establishment and biostimulant application

Maize was cultivated following the recommended agronomic practices of Tamil Nadu Agricultural University. The recommended fertiliser dose and standard crop management practices were uniformly adopted across all treatments. The *Sargassum*-based biostimulant was applied as a foliar spray at the respective treatment doses at the knee-high stage (30 days after sowing (DAS)) and pre-tasselling stage (50 DAS). The commercial biostimulants acadian stimplex® and radifarm® were each applied at 2.5 kg ha^{-1} as commercial check treatments. In the drought-stressed control (T_2), only water was sprayed, whereas the irrigated control (T_1) was maintained under optimum soil moisture conditions throughout the crop growth period. The effectiveness of the treatments was evaluated using physiological, biochemical, growth and yield parameters under drought stress conditions.

Drought stress imposition and soil moisture monitoring

Drought stress was imposed by maintaining soil moisture at 50 % of field capacity (FC) during the critical growth stages of maize. Before initiating the stress treatment, the FC of the experimental soil was determined using the gravimetric method. Soil moisture was monitored periodically by collecting soil samples from the root zone (0–30 cm depth) and determining moisture content on a dry-weight basis. The irrigated control (T_1) was maintained at optimum soil moisture throughout the crop growth period, whereas the drought-stressed treatments (T_2–T_9) received irrigation sufficient to maintain approximately 50 % FC. Irrigation schedules were adjusted periodically based on soil moisture measurements to ensure uniform drought stress across all stressed treatments. The effects of drought stress and biostimulant application were evaluated through physiological, biochemical, growth, nutrient uptake and yield responses of maize.

Physiological and biochemical parameters

Physiological and biochemical parameters associated with drought tolerance were assessed at critical growth stages to evaluate the effectiveness of the *Sargassum*-based biostimulant under moisture-deficit conditions. Fully expanded, healthy leaves were collected from representative plants in each treatment and immediately processed for analysis. The evaluated parameters included chlorophyll stability index (CSI), relative water content (RWC), proline content, membrane stability index (MSI), catalase (CAT) activity, peroxidase (POD) activity and total carotenoid content. Chlorophyll stability index was determined (22). Relative water content was estimated (23). Proline content was determined using the acid ninhydrin method (24). Membrane stability index was measured (25). Catalase and POD activities were determined (26). Total carotenoid content was estimated (27). All analyses were performed according to the respective standard protocols.

Growth and yield parameters

Growth and yield observations were recorded at different stages of crop development to evaluate the effects of the *Sargassum*-based biostimulant on maize growth and productivity under drought stress. Five representative plants were randomly selected and tagged in each treatment plot for periodic observations. Plant height was measured from the base of the plant to the tip of the uppermost fully expanded leaf. Leaf area index (LAI) was determined (28). Crop vigour was assessed visually based on plant establishment, greenness, uniformity and overall growth performance.

At harvest, yield attributes including cob length, cob diameter, number of grain rows per cob, number of grains per cob and 100-grain weight were recorded (28). Grain yield and stover yield were measured at physiological maturity and expressed as kg ha^{-1} after appropriate moisture correction. Harvest index (HI) was calculated as the ratio of grain yield to total biological yield and expressed as a percentage.

Statistical analysis

The experiment was conducted using a RBD comprising nine treatments and three replications. Data obtained for biochemical characteristics, physiological parameters, growth attributes, nutrient uptake, yield components and grain yield were subjected to analysis of variance (ANOVA) appropriate for an RBD (29). Treatment means were compared using the critical difference (CD) test at the 5 % level of significance ($p \leq 0.05$) and the standard error of difference [SE(d)] was calculated. All statistical analyses were performed using IBM SPSS Statistics Version 24.0.

Results

Biochemical characterisation of *Sargassum* extract

The biochemical composition of the H_3PO_4-hydrolysed *Sargassum* extract is presented in Table 1. The extract contained flavonoids (69.4 mg QE mL^{-1}), phenolics (38.9 mg GAE mL^{-1}), terpenoids (23.5 mg LE mL^{-1}), tannins (21.4 mg TAE mL^{-1}), phytosterols (19.5 mg CE mL^{-1}), carbohydrates (12.8 mg mL^{-1}) and proteins (7.4 mg mL^{-1}). Among the quantified constituents, flavonoids were presented at the highest concentration, followed by phenolics,

Table 1. Biochemical characterisation of *Sargassum* extract

Biochemical constituent	Content
Total phenolics (mg GAE mL^{-1})	38.9 ± 0.8
Total flavonoids (mg QE mL^{-1})	69.4 ± 1.2
Total tannins (mg TAE mL^{-1})	21.4 ± 0.5
Total terpenoids (mg LE mL^{-1})	23.5 ± 0.7
Total phytosterols (mg CE mL^{-1})	19.5 ± 0.4
Total carbohydrates (mg mL^{-1})	12.8 ± 0.3
Total proteins (mg mL^{-1})	7.4 ± 0.2

Values are expressed as mean ± standard deviation (n = 3).

GAE: gallic acid equivalents; QE: quercetin equivalents; TAE: tannic acid equivalents; LE: linalool equivalents; CE: cholesterol equivalents.

whereas proteins were detected at the lowest concentration.

Fourier transform infrared characterisation of *Sargassum* extract

The FTIR spectrum of the H_3PO_4-hydrolysed *Sargassum* biostimulant is presented in Fig. 1 and Table 2. A broad absorption band at 3350.29 cm^{-1} was assigned to O–H stretching vibrations of (-OH) groups. A weak absorption band at 2327.94 cm^{-1} corresponded to atmospheric CO_2, which is commonly observed as an instrumental artifact in FTIR spectroscopy. The prominent band at 1629.62 cm^{-1} was assigned to C=O and COO- stretching vibrations, while the strong bands at 1084.15 cm^{-1} and 999.00 cm^{-1} were attributed to P–O and C–O–C stretching vibrations respectively. Overall, the FTIR spectrum indicated the presence of (–OH), (C=O), carboxylate (COO-), (P–O) and ether/glycosidic (C–O–C) functional groups in the biostimulant formulation.

Effect of *Sargassum* biostimulant on physiological parameters of maize

The *Sargassum*-based biostimulant significantly affected the CSI, MSI and RWC of maize at different intervals following the second application (Table 3). Among all treatments, T_4 (2.5 kg ha^{-1} under non-stress conditions) recorded the highest CSI throughout the observation period, reaching 86.27 % at 36 days after the second application (DASA). Under drought-stress conditions, T_7 (3.0 kg ha^{-1})

Table 2. Fourier transform infrared peak assignments of *Sargassum* extract

Wavenumber (cm^{-1})	Functional group
3350.29	O–H stretching
2327.94	Atmospheric CO_2 band (instrumental artifact)
1629.62	C=O and COO- stretching
1084.15	P–O stretching
999.00	C–O–C stretching

recorded a significantly higher CSI (82.30 %) than the drought-stressed control (T_2), which recorded 54.82 %.

A similar trend was observed for MSI. Treatment, T_4 recorded the highest MSI (86.30 %), followed by T_7 (82.30 %), whereas the drought-stressed control (T_2) recorded the lowest value (54.80 %). Relative water content was also highest in T_4 (73.5 %) at 36 DASA, followed by T_7 (70.5 %), T_8 (70.0 %) and T_6 (69.0 %), while T_2 recorded the lowest RWC (58.5 %).

The CSI, MSI and RWC values recorded in T_7 were significantly higher than those of the drought-stressed control (T_2), exceeding the respective CD values of 2.74, 3.17 and 1.01.

Effect on proline content and antioxidant enzyme activities

Application of the *Sargassum*-based biostimulant significantly influenced proline accumulation, CAT activity and POD activity in maize under drought stress (Table 4). At 36 DASA, T_7 (3.0 kg ha^{-1} under drought stress) recorded the highest proline content (24.56 µmol g^{-1}), followed by T_8 (23.47 µmol g^{-1}) and T_6 (22.43 µmol g^{-1}), whereas the lowest value was observed in T_1 (16.98 µmol g^{-1}).

Catalase activity was highest in T_7 (6.81 units mg^{-1} protein) at 28 DASA and remained significantly higher than that of the drought-stressed control (T_2). Similarly, POD activity increased in response to biostimulant application, with T_8 recording the highest activity (0.99 units mg^{-1} protein) at 36 DASA, followed closely by T_7 (0.95 units mg^{-1} protein). The drought-stressed control (T_2) recorded lower CAT and POD activities than the biostimulant-treated plants.

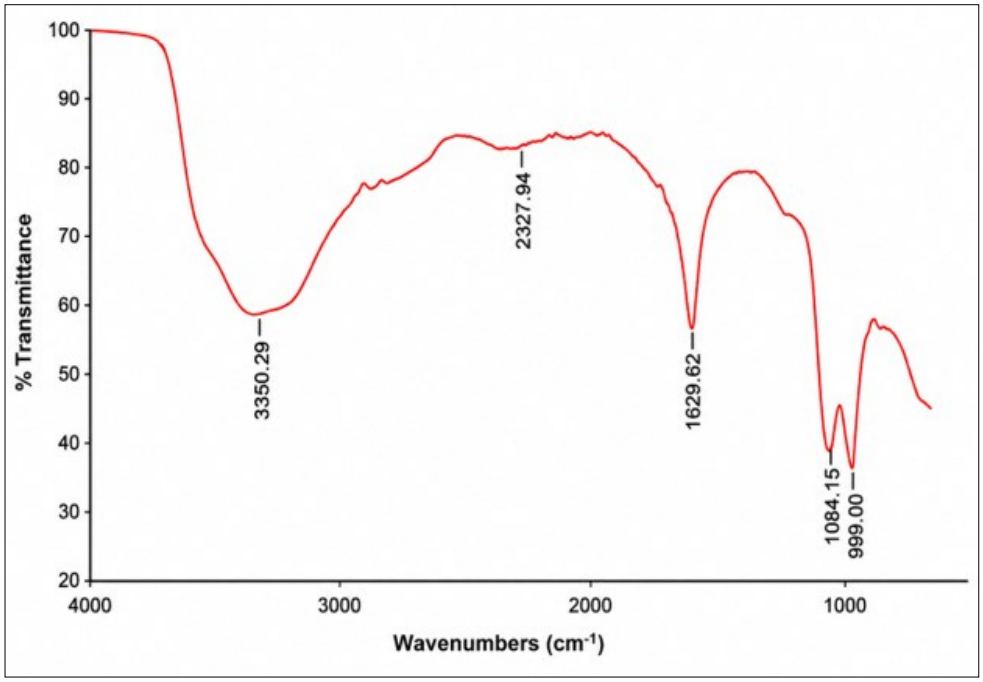


Fig. 1. Fourier transform infrared spectrum of *Sargassum* extract showing characteristic absorption bands.

Table 3. Effect of the *Sargassum*-based biostimulant on chlorophyll stability index (CSI), membrane stability index (MSI) and relative water content (RWC) of maize at different days after the second application (DASA)

	Chlorophyll stability index				Membrane stability index				Relative water content			
Treatments	14th DASA	21st DASA	28th DASA	36th DASA	14th DASA	21st DASA	28th DASA	36th DASA	14th DASA	21st DASA	28th DASA	36th DASA
T_1	64.19^{c}	61.20^{c}	63.58^{d}	67.81^{d}	64.30^{c}	61.20^{c}	63.80^{d}	67.80^{d}	68.0^{b}	69.5^{b}	68.5^{c}	68.0^{c}
T_2	46.83^{e}	49.63^{d}	52.33^{e}	54.82^{e}	46.80^{e}	49.70^{d}	52.30^{e}	54.80^{e}	61.5^{f}	60.0^{f}	59.5^{f}	58.5^{f}
T_3	60.30^{d}	63.49^{c}	71.90^{c}	75.35^{c}	60.30^{d}	63.80^{c}	71.80^{c}	75.40^{c}	67.0^{c}	65.5^{d}	64.5^{e}	63.8^{e}
T_4	79.42^{a}	81.67^{ab}	85.90^{a}	86.27^{a}	79.60^{a}	81.30^{b}	85.70^{a}	86.30^{a}	70.5^{a}	73.0^{a}	73.0^{a}	73.5^{a}
T_5	66.57^{c}	79.52^{b}	71.20^{c}	67.29^{d}	66.70^{c}	79.60^{b}	71.20^{c}	67.30^{d}	63.0^{e}	62.0^{e}	65.0^{de}	67.0^{cd}
T_6	73.46^{b}	83.54^{a}	81.60^{b}	81.75^{b}	78.50^{ab}	80.90^{b}	82.50^{b}	81.80^{b}	65.0^{d}	70.5^{b}	69.5^{b}	69.0^{b}
T_7	78.19^{a}	81.07^{ab}	83.47^{ab}	82.30^{b}	78.60^{ab}	80.90^{b}	82.50^{b}	82.30^{b}	69.0^{ab}	71.0^{b}	69.5^{b}	70.5^{b}
T_8	74.47^{b}	82.75^{ab}	84.20^{ab}	79.65^{b}	73.40^{b}	82.10^{ab}	83.00^{ab}	79.70^{b}	66.0^{cd}	70.5^{b}	70.0^{b}	70.0^{b}
T_9	73.46^{b}	83.54^{a}	81.60^{b}	79.81^{b}	74.10^{b}	82.70^{a}	81.30^{b}	79.80^{b}	64.0^{de}	67.0^{c}	67.0^{cd}	67.0^{cd}
Mean	68.08	73.37	74.82	74.7	69.14	73.58	74.9	75.02	66	67.7	67.4	67.5
SE (d)	1.41	1.72	1.55	1.36	1.42	1.58	1.64	1.48	0.45	0.52	0.48	0.5
CD (p = 0.05)	2.86	3.47	3.13	2.74	3.04	3.38	3.52	3.17	0.91	1.05	0.97	1.01

Values are treatment means of three replications (n = 3). Means followed by different superscript letters within a column differ significantly at $p \leq 0.05$ according to the CD test.

Table 4. Effect of *Sargassum*-based biostimulant on proline content and antioxidant enzyme activities of maize under drought stress at different days after the second application (DASA)

	Proline content (µmol g^{-1} FW)				Catalase activity (Units mg^{-1} protein)				Peroxidase activity (Units mg^{-1} protein)			
Treatments	14th DASA	21st DASA	28th DASA	36th DASA	14th DASA	21st DASA	28th DASA	36th DASA	14th DASA	21st DASA	28th DASA	36th DASA
T_1	4.42^{f}	7.28^{f}	17.18^{f}	16.98^{f}	5.30^{f}	5.27^{f}	5.53^{f}	6.86^{a}	0.36^{f}	0.49^{f}	0.66^{f}	0.86^{c}
T_2	5.70^{a}	9.17^{c}	19.18^{c}	20.01^{e}	5.23^{f}	4.48^{g}	5.27^{g}	5.90^{f}	0.51^{e}	0.53^{e}	0.71^{e}	0.74^{f}
T_3	5.39^{de}	8.38^{e}	18.49^{e}	19.18^{f}	5.52^{e}	5.55^{e}	5.72^{e}	6.90^{a}	0.52^{e}	0.60^{d}	0.63^{g}	0.69^{g}
T_4	4.74^{e}	8.76^{d}	18.03^{e}	20.98^{d}	6.28^{c}	6.62^{a}	6.74^{b}	6.71^{c}	0.71^{a}	0.83^{a}	0.94^{a}	0.85^{c}
T_5	5.35^{e}	9.14^{c}	19.21^{c}	20.15^{e}	6.29^{c}	6.20^{b}	6.46^{d}	6.64^{d}	0.68^{b}	0.64^{c}	0.72^{d}	0.81^{d}
T_6	5.54^{c}	9.57^{b}	19.62^{b}	22.43^{c}	6.43^{b}	6.10^{c}	6.64^{c}	6.52^{e}	0.65^{c}	0.69^{b}	0.87^{c}	0.89^{b}
T_7	5.57^{bc}	9.78^{a}	19.69^{a}	24.56^{a}	6.46^{a}	6.30^{b}	6.81^{a}	6.69^{c}	0.69^{ab}	0.77^{a}	0.91^{b}	0.95^{a}
T_8	5.61^{ab}	9.60^{b}	19.64^{ab}	23.47^{b}	6.26^{c}	6.19^{b}	6.45^{d}	6.67^{cd}	0.68^{b}	0.64^{c}	0.94^{a}	0.99^{a}
T_9	5.39^{d}	9.26^{c}	19.14^{c}	21.45^{d}	6.13^{d}	6.09^{c}	6.26^{d}	6.61^{d}	0.63^{d}	0.66^{c}	0.70^{e}	0.87^{bc}
Mean	5.30	8.99	18.91	21.02	5.89	5.82	6.19	6.64	0.60	0.65	0.79	0.85
SE(d)	0.06	0.11	0.12	0.31	0.07	0.09	0.08	0.04	0.04	0.04	0.04	0.03
CD (p = 0.05)	0.12	0.21	0.24	0.64	0.14	0.18	0.16	0.08	0.08	0.08	0.09	0.07

Values are treatment means of three replications (n = 3). Means followed by different superscript letters within a column differ significantly at $p \leq 0.05$ according to the CD test.

The increases in proline content, CAT and POD activity in T_7 compared with T_2 exceeded the respective CD values, indicating statistically significant treatment effects.

Effect on growth parameters of maize

The *Sargassum*-based biostimulant significantly influenced plant height, number of leaves, LAI and crop vigour at 36 DASA (Table 5). Among all treatments, T_4 (2.5 kg ha^{-1} under non-stress conditions) recorded the highest plant height (69.0 cm), number of leaves (45.4), LAI (0.60) and crop vigour (90 %). Under drought-stress conditions, T_7 (3.0 kg ha^{-1}) recorded significantly higher plant height (65.0 cm), LAI (0.55) and crop vigour (80 %) than the drought-stressed control (T_2), which recorded the lowest values for all growth parameters. The increases in plant height, LAI and crop vigour observed in T_7 compared with T_2 exceeded the respective CD values of 2.52, 0.027 and 2.89, indicating significant treatment effects.

Effect on cob characteristics and yield components

Application of the *Sargassum*-based biostimulant significantly influenced cob characteristics and yield components of maize (Table 6). Among all treatments, T_4 (2.5 kg ha^{-1} under non-stress conditions) recorded the highest cob length (5.49 cm), cob diameter (4.99 cm), cob weight (45.55 g), grain yield per plant (42.86 g), grain yield per plot (6.25 kg), 100-grain weight (35.72 g) and HI (48.65 %). Under drought-stress conditions, T_7 (3.0 kg ha^{-1}) recorded the highest cob length (5.21 cm), cob diameter (4.25 cm), cob weight (42.09 g), grain yield per plant (39.58 g), grain yield per plot (5.84 kg), 100-grain weight (33.84 g) and HI (46.82 %).

The drought-stressed control (T_2) recorded the lowest values for all yield parameters. Compared with T_2, T_7 increased grain yield per plant by approximately 115 % and the difference exceeded the CD, indicating a statistically significant treatment effect.

Economics of maize cultivation

The economic investigation showed significant differences among the treatments (Table 7). Among all treatments, T_4 (2.5 kg ha^{-1} under non-stress conditions) recorded the highest gross return (₹ 142800 ha^{-1}), net return (₹ 79300 ha^{-1}) and benefit : cost (B : C) ratio (2.25). Under drought-stress conditions, T_7 (3.0 kg ha^{-1}) recorded the highest gross return (₹ 131600 ha^{-1}), net return (₹ 67800 ha^{-1}) and B : C

Table 5. Effect of *Sargassum*-based biostimulant on growth parameters of maize at 36 days after second application under drought stress

Treatments	Plant height (cm)	Number of leaves	Leaf area index	Crop vigour (%)
T_1	54.2^{f}	35.2^{f}	0.49^{c}	75.0^{c}
T_2	44.0^{g}	20.4^{g}	0.29^{e}	50.0^{f}
T_3	58.0^{e}	31.5^{f}	0.48^{c}	70.0^{d}
T_4	69.0^{a}	45.4^{a}	0.60^{a}	90.0^{a}
T_5	49.5^{f}	29.8^{f}	0.37^{d}	60.0^{e}
T_6	63.5^{d}	35.8^{e}	0.50^{c}	70.0^{d}
T_7	65.0^{c}	39.2^{c}	0.55^{b}	80.0^{b}
T_8	64.0^{cd}	38.5^{cd}	0.52^{b}	85.0^{ab}
T_9	67.0^{b}	42.8^{b}	0.48^{c}	90.0^{a}
Mean	59.3	35.4	0.47	74.4
SE(d)	1.25	0.79	0.013	1.36
CD (p = 0.05)	2.52	1.59	0.027	2.89

Values are treatment means of three replications (n = 3). Means followed by different superscript letters within a column differ significantly at $p \leq 0.05$ according to the CD test.

Table 6. Effect of the *Sargassum*-based biostimulant on cob characteristics and yield components of maize under drought stress

Treatment	Cob length	Cob diameter	Cob weight (g)	Grain yield plant^{-1} (g)	Grain yield plot^{-1} (kg)	100-grain weight (g)	Harvest index (%)
T_1	3.60^{f}	3.43^{f}	36.25^{e}	28.65^{f}	4.12^{f}	27.54^{f}	38.62^{f}
T_2	3.48^{g}	2.38^{h}	24.95^{h}	18.42^{h}	2.85^{h}	22.16^{h}	31.28^{h}
T_3	4.96^{c}	3.56^{e}	36.89^{e}	30.17^{e}	4.38^{e}	28.40^{e}	39.18^{e}
T_4	5.49^{a}	4.99^{a}	45.55^{a}	42.86^{a}	6.25^{a}	35.72^{a}	48.65^{a}
T_5	4.62^{d}	3.54^{e}	32.78^{g}	24.65^{g}	3.72^{g}	25.84^{g}	35.41^{g}
T_6	5.01^{c}	3.91^{d}	40.31^{d}	35.24^{d}	5.28^{d}	31.63^{d}	43.76^{d}
T_7	5.21^{b}	4.25^{b}	42.09^{b}	39.58^{b}	5.84^{b}	33.84^{b}	46.82^{b}
T_8	5.10^{bc}	4.04^{c}	41.42^{c}	38.74^{c}	5.72^{bc}	33.25^{c}	45.95^{c}
T_9	4.74^{d}	3.74^{d}	38.96^{de}	36.92^{d}	5.36^{d}	32.18^{d}	44.37^{d}
Mean	**4.69**	**3.76**	**37.69**	**32.80**	**4.84**	**30.06**	**41.56**
SE(d)	**0.03**	**0.04**	**0.07**	**0.08**	**0.04**	**0.06**	**0.09**
CD (p = 0.05)	**0.07**	**0.09**	**0.15**	**0.17**	**0.08**	**0.13**	**0.19**

Values are treatment means of three replications (n = 3). Means followed by different superscript letters within a column differ significantly at $p \leq 0.05$ according to the CD test.

Table 7. Effect of the *Sargassum*-based biostimulant on the economics of maize cultivation

Treatments	Cost of cultivation (₹ ha^{-1})	Gross return (₹ ha^{-1})	Net return (₹ ha^{-1})	B : C ratio
T_1	58500^{f}	92800^{f}	34300^{f}	1.59^{f}
T_2	56800^{g}	64100^{g}	7300^{g}	1.13^{g}
T_3	60200^{e}	98600^{e}	38400^{e}	1.64^{e}
T_4	63500^{d}	142800^{a}	79300^{a}	2.25^{a}
T_5	61500^{c}	84300^{f}	22800^{f}	1.37^{f}
T_6	62300^{b}	118400^{d}	56100^{d}	1.90^{d}
T_7	63800^{b}	131600^{b}	67800^{b}	2.06^{b}
T_8	64500^{a}	127800^{c}	63300^{c}	1.98^{c}
T_9	64200^{ab}	121500^{d}	57300^{d}	1.89^{d}
Mean	**61700**	**109544**	**47400**	**1.76**
SE(d)	**157.92**	**144.02**	**52.12**	**0.006**
CD (p = 0.05)	**334.79**	**305.31**	**110.49**	**0.013**

Values are treatment means of three replications (n = 3). Means followed by different superscript letters within a column differ significantly at $p \leq 0.05$ according to the CD test.

B : C ratio: benefit cost ratio.

ratio (2.06) among the stressed treatments. In contrast, the drought-stressed control (T_2) recorded the lowest economic returns, with a net return of ₹ 7300 ha^{-1} and a B : C ratio of 1.13.

Discussion

The higher concentrations of bioactive compounds observed in the H_3PO_4-hydrolysed *Sargassum* extract suggest that acid hydrolysis facilitated the release of metabolites from the seaweed biomass. Brown seaweeds contain structurally complex polysaccharides, including alginate, fucoidan and laminarin, which serve as reservoirs of diverse bioactive compounds. Acid hydrolysis can promote partial depolymerisation of these polysaccharides, releasing low-molecular-weight oligosaccharides, phenolics, flavonoids, terpenoids, phytosterols, carbohydrates and proteins. Previous studies have reported that depolymerised seaweed polysaccharides exhibit greater solubility and biological activity than native polymers and that extraction methods influence the biochemical composition and efficacy of seaweed-based biostimulants (6, 9, 12, 13). Therefore, the biochemical profile observed in the present study is consistent with the proposed effects of H_3PO_4 hydrolysis.

The FTIR spectrum further supported the chemical characteristics of the H_3PO_4-hydrolysed *Sargassum* biostimulant. The broad absorption band at 3350.29 cm^{-1} indicated (-OH) groups associated with polysaccharides and phenolic compounds, whereas the band at 1629.62 cm^{-1} corresponded to (C=O) and carboxylate (COO-) stretching vibrations characteristic of alginate and other (-COOH)-containing constituents of brown seaweeds. The strong absorption band at 1084.15 cm^{-1} was attributed to (P–O)-related stretching vibrations (P–O), while the weak band at 2327.94 cm^{-1} represented atmospheric CO_2, a common instrumental artifact in FTIR analysis. These spectral characteristics are consistent with

previous reports on brown seaweed-derived biostimulants and indicate the retention of major bioactive functional groups following H_3PO_4 hydrolysis (8).

The higher CSI, RWC and MSI observed in biostimulant-treated maize plants suggest improved physiological adaptation to drought stress. Drought stress is known to reduce chlorophyll content and photosynthetic efficiency through oxidative damage to the photosynthetic apparatus. Phenolics and flavonoids present in seaweed extracts have been reported to possess antioxidant properties that may help protect photosynthetic pigments and cellular components under stress conditions. In agreement with previous studies, seaweed-derived biostimulants have been shown to improve chlorophyll retention, photosynthetic performance and plant water status under drought stress (2, 4, 30, 31). The higher CSI, RWC and MSI values observed in the present study are consistent with these reports and suggest that the H_3PO_4-hydrolysed *Sargassum* biostimulant contributed to improved physiological performance under moisture-deficit conditions.

The increased RWC and proline accumulation observed in biostimulant-treated plants suggest improved physiological responses to drought stress. Seaweed-derived oligosaccharides have been reported to act as signalling molecules that activate stress-responsive pathways, while proline plays an important role in osmotic adjustment, stabilisation of proteins and membranes and protection against oxidative stress under water-deficit conditions (24). Similar increases in proline accumulation and improved plant water status following seaweed biostimulant application have been reported in previous studies (32, 33). The concurrent increases in RWC, proline accumulation and CSI observed in the present study are consistent with these findings and suggest improved drought adaptation in maize treated with the H_3PO_4-hydrolysed *Sargassum* biostimulant.

The higher MSI observed in treatments T_4 and T_7 is consistent with the improved physiological performance of maize under drought stress. Drought-induced oxidative stress can disrupt membrane integrity through lipid peroxidation, resulting in increased membrane permeability and electrolyte leakage. Phenolics, flavonoids and terpenoids present in seaweed extracts have been reported to possess antioxidant properties that may help protect cellular membranes under abiotic stress. Previous studies have also shown that seaweed-derived biostimulants enhance antioxidant defence systems and contribute to improved membrane stability in plants exposed to environmental stress (6, 34). The higher MSI recorded in the present study is therefore consistent with the reported protective effects of seaweed biostimulants under drought conditions.

The increased CAT and POD activities observed in biostimulant-treated plants indicate an enhanced antioxidant response under drought stress. Antioxidant enzymes play an important role in detoxifying reactive oxygen species (ROS) generated during water-deficit conditions, thereby helping to minimise oxidative damage. Previous studies have reported increased CAT and POD activities following the application of seaweed-derived biostimulants, which has been associated with improved stress tolerance in crops (6, 34). The higher CAT and POD activities recorded in treatments T_7 and T_8 are consistent with these reports and may have contributed to the improved physiological performance observed under drought stress.

The improved plant height and grain yield observed in treatments T_4 and T_7 are consistent with the enhanced physiological and biochemical responses recorded in the present study. Improved CSI, plant water status, MSI, proline accumulation and antioxidant enzyme activities are likely to have contributed to better crop growth and productivity under both optimum and drought conditions. Previous studies have similarly reported that seaweed-derived biostimulants improve crop growth and yield through coordinated physiological and biochemical responses rather than a single mechanism (11, 33). Among the drought-stressed treatments, T_7 was the most effective in mitigating drought-induced yield losses, whereas T_4 recorded the highest productivity under non-stress conditions. These findings indicate that the H_3PO_4-hydrolysed *Sargassum* biostimulant was effective under both irrigation regimes, with T_7 showing superior performance under drought stress.

Compared with the commercial biostimulants such as acadian stimplex® (T_8) and radifarm® (T_9), the H_3PO_4-hydrolysed *Sargassum* biostimulant (T_7) showed comparable or slightly superior performance for several physiological traits and grain yield under drought stress. These findings suggest that the developed formulation has potential as an alternative seaweed-based biostimulant. The use of locally available *Sargassum* biomass and a relatively simple extraction process may provide practical advantages. However, further studies are required to optimise the formulation, evaluate its shelf life and validate its performance across different environments before large-scale commercial application.

The economic analysis demonstrated that the H_3PO_4-hydrolysed *Sargassum* biostimulant improved the profitability of maize cultivation under both optimum and drought conditions. Treatments T_4 and T_7 recorded higher gross returns, net returns and B : C ratio than the untreated drought-stressed control, reflecting the yield advantages obtained with biostimulant application. These findings indicate that, in addition to improving physiological performance and grain yield, the developed biostimulant has potential economic benefits for maize production under water-limited conditions.

Although significant improvements were observed in physiological, growth, yield and economic parameters, this study has certain limitations. Reactive oxygen species, hydrogen peroxide (H_2O_2), lipid peroxidation (malondialdehyde (MDA)) and molecular responses were not directly assessed. Therefore, the proposed mechanisms underlying drought tolerance are based on physiological and biochemical responses and should be regarded as plausible interpretations rather than direct mechanistic evidence. In addition, some treatment comparisons were not significant at certain observation stages, indicating that treatment responses may vary with crop developmental stage and environmental conditions. Further studies incorporating ROS and MDA measurements, molecular analyses and multi-location, multi-season field evaluations are required to validate the mechanisms and broader applicability of the developed biostimulant.

Overall, the findings of the present study suggest a close association between the biochemical characteristics of the H_3PO_4-hydrolysed *Sargassum* extract and the improved physiological, biochemical and agronomic performance of maize under drought stress. The enhanced availability of bioactive compounds following acid hydrolysis was associated with improved CSI, RWC, MSI, proline accumulation, antioxidant enzyme activities and grain yield. Among the treatments, T_7 was the most effective under drought stress, whereas T_4 achieved the highest productivity under optimum irrigation. These findings indicate that the developed *Sargassum*-based biostimulant has potential for

improving drought tolerance and maize productivity under the field conditions evaluated in this study. However, further multi-location and multi-season studies are required to confirm its broader applicability and long-term effectiveness.

Conclusion

The findings of the present study suggest that the phosphoric acid (H_3PO_4)-hydrolysed *Sargassum*-based biostimulant improved the physiological, biochemical and agronomic performance of maize under drought stress. Biostimulant application was associated with higher chlorophyll stability index (CSI), membrane stability index (MSI), relative water content (RWC), proline accumulation and antioxidant enzyme activities, which corresponded with improved plant growth, grain yield and economic returns compared with the untreated drought-stressed control. Among the drought-stressed treatments, T_7 (3.0 kg ha^{-1}) was the most effective in mitigating drought-induced yield losses, whereas T_4 (2.5 kg ha^{-1} under non-stress conditions) recorded the highest overall productivity and economic returns. These findings indicate that the developed *Sargassum*-based biostimulant has potential as an eco-friendly approach for improving maize productivity under water-limited conditions. Further multi-location and multi-season studies, together with detailed physiological and molecular investigations, are required to validate its broader applicability and better understand the mechanisms underlying drought tolerance.

Acknowledgements

The authors sincerely acknowledge the technical and laboratory support provided by the staff of the Department of Soil Science and Agricultural Chemistry, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, during the conduct of this study. The authors also acknowledge the institutional facilities and support provided for the successful completion of this research work.

Authors' contributions

SK[1] conducted the experiment, collected and analysed the data and prepared the original manuscript draft. SK[2] conceptualised and supervised the study, provided overall guidance and reviewed and edited the manuscript. SJRT, BM, KN, YA, SP and SS critically reviewed the manuscript and contributed to data interpretation and scientific discussion. All authors read and approved the final manuscript. SK[1] stands for Sivaranjani K and SK[2] stands for Senthil K.

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT for limited assistance in language refinement and manuscript editing and Grammarly for grammar and language checking. All scientific content, interpretation and conclusions were developed, reviewed and verified by the authors and take full responsibility for the content of this publication.

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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