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Evaluation of the effects of a blended seaweed extract of *Gracilaria salicornia* (Rhodophyta) and *Sargassum swartzii* (Heterokontophyta) on the growth and grain yield of rice (*Oryza sativa*) farmed in the lower Gangetic and Eastern Coastal Plains and Hills of India

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Abstract

Concentrated seaweed extract (CSE), prepared from *Gracilaria salicornia* (red) and *Sargassum swartzii* (brown), was characterized for its macro- and micronutrients and amino acids, evaluated for plant growth-regulating properties and tested as a biostimulant. Its effects on vegetative growth, grain and straw yield were assessed in rice varieties Satabdi (IET4786) and CO-51 (IET21605) in farmers' fields in West Bengal (Lower Gangetic Plains) and Tamil Nadu (Eastern Coastal Plains and Hills), India. The control received the recommended NPK at 100% (150:60:60 kg ha⁻¹) applied as basal applications in three splits. In T1, basal nitrogen was reduced to 50% and a nitrogen-enriched nano formulation (NEN) was applied as a foliar spray at 2 mL L⁻¹ three times. In T2, the T1 regimen was supplemented with CSE at 2 mL L⁻¹ applied as a foliar spray three times. For Satabdi, grain yields were 4,391 kg ha⁻¹ (T2) and 4,208 kg ha⁻¹ (T1), representing 15.98% and 11.15% increases ($p=0.012$ by ANOVA with Tukey HSD) over the control (3,786 kg ha⁻¹); CO-51 showed comparable improvements in grain yield (T1: 10.58%, T2: 18.27%). The combined application of NEN and CSE increased grain yield relative to the control under the tested conditions, although the incremental benefit of adding CSE over NEN alone was modest, and its independent effect could not be isolated. Noted reductions in nitrogen input costs are specific to these study conditions and may vary regionally.

Keywords Biostimulant, Seaweed extracts, Nitrogen-enriched nano formulation, Rice variety, Satabdi (IET4786), CO-51 (IET21605)



1 Introduction

Fertilizers play a crucial role in agriculture to increase yield and ensure the constant productivity of any agricultural crop throughout the season. During the last three decades, several innovations like agronomic management practices supporting sustainable crop production systems and intensification and testifying to developments in the selection of crops and cultivars and improvements in fertilizer and nutrient management were introduced to increase production [1–3]. Reducing synthetic fertilizer inputs via the use of plant biostimulants is a promising and environmentally friendly approach for enhancing flowering, plant growth, fruit set, crop productivity, and nutrient use efficiency in addition to improving tolerance to a wide range of abiotic stressors [4–7]. Biostimulants are a broad term that includes extracts, lysates, purified natural compounds or microorganisms that are applied to crops in lesser amounts to improve plant disease resistance and/or vigor [8–10]. When biostimulants are applied to plants, seeds or soils in specific formulations, they have the capacity to modify the physiological processes of plants and associate microflora in a way that provides potential benefits to their growth and development through the modulation of biochemical, physiological, and molecular responses to increase crop productivity, nutrient-use efficiency (NUE), water use efficiency and increase tolerance to biotic stress [11–16]. Biostimulants derived from brown and red seaweeds may be sources of nutrients and function as physiological elicitors, promoting the growth and development of many agricultural crops, including rice [17, 18].

Rice is a staple food, and more than half of the world's population consumes it every day. Rice is farmed extensively across Asian and African countries [19, 20], but Asia has the largest share of its production globally [21]. However, increasing prices of inorganic fertilizer is one of the major concerns in rice farming in many developing countries [22]. Nano urea is enriched with nitrogen through a process that involves formulating urea into nanoscale particles (18–30 nm) and stabilizing them in polymer matrices. These nanoscale particles have a significantly larger surface area (10,000 times more than 1 mm urea prill) and a higher number of particles per unit volume, allowing for more efficient absorption by plants while reducing the total amount of fertilizer needed. Seaweeds such as *Gracilaria salicornia* and *Sargassum swartzii* are among the few that are available at low cost in India and are normally used for the production of phycocolloids. The estimated wild stock (drift) of *G. salicornia* is more than 1000 t dry per year [23]. However, based on the data collected from industries, it is estimated to be about 5000 t dry per year (drift) and its current market price is approximately USD 200 t⁻¹ dry (Eswaran - personal communication). Regarding *S. swartzii*, it is also sourced from wild-stocks through hand cutting leaving the hold-fast for regrowth and its estimated quantity is about 3000 t dry per year and procured at USD 300 (dry) t⁻¹. Therefore, both these seaweeds are sourced through wild collection; however, research institutes are engaged in developing cultivation techniques for the sustainable supply of biomass for industrial application - with the support of the Indian Government (Ganesh - personal communication). The combined use of red seaweed (*Kappaphycus alvarezii*) and brown seaweed (*Sargassum wightii*) extracts was demonstrated to have beneficial effects on crops [24] and to increase the nutrient-use-efficiency (NUE) in mustard plant [25]. Ramamoorthy and Shanmugam [26] reported that a blended extract of *Gracilaria salicornia* and *Sargassum swartzii* were found to mitigate salt-induced stress in green gram due to its stress elicitor elicitors, such as proline aspartic acid, glutamic acid, and other amino acids, viz.,

arginine, alanine, methionine, isoleucine, and leucine amino acid. A nitrogen-enriched nano formulation (NEN) is the latest version of a nano urea and it is currently being evaluated on a wide range of crops across different agroclimatic regions of India to assess its efficacy. In India, the rice varieties Satabdi (IET4786) and CO-51 (IET21605) are considered important due to their high-yielding possibilities, early maturity, and resistance to diseases like leaf blast. Satabdi (IET4786) is a popular high-yielding variety known for its adaptability and desirable grain quality and it is economically important due to its finer grain quality and higher market price compared to some other varieties. CO-51 (IET21605) is a significant variety in Southern India, particularly Tamil Nadu, praised for its high yield, short duration, and good grain quality. Hence, these two species were selected to study their response to 50% of conventional nitrogen fertilizer input applied through soil, along with NEN in combination with concentrated seaweed extract (CSE) through foliar spray in terms of plant growth and grain yield.

2 Materials and methods

2.1 Preparation of concentrated seaweed extract (CSE)

Gracilaria salicornia and *Sargassum swartzii* were sourced from Mandapam (9.2770° N, 79.1252° E, Tamil Nadu, India) and herbaria of these seaweeds' specimen (*Gracilaria salicornia*: MKU-SBS-HER-1168, dated 09.04.2023 and *Sargassum swartzii*: MKU-SBS-HER-1169, dated 09.04.2023) have been deposited with Department of Plant Science, Madurai Kamaraj University, Madurai, India. Seaweed extracts were manufactured by following the method done by Ramamoorthy and Shanmugam (2025). Aqueous extracts of both seaweeds prepared by soaking 1.0 t (dry) of each seaweed separately in water (1:6 ratio) for 60 min at room temperature ($\approx 30^\circ\text{C}$). The extract obtained was first filtered in 40 mesh (= 400 microns) followed by a finer filtration (= 100 microns) and concentrated through a multiple effect evaporator (Alfa Laval India Limited, Pune, India) at 75–80 °C at 350–400 mm Hg vacuum pressure to obtain 400 L of concentrated extracts containing total soluble solids (TSS) of $\approx 30\%$ from each seaweed. Then, both extracts were blended at a 1:1 ratio, homogenized and stabilized with food-grade preservatives to obtain concentrated seaweed extract (CSE) with a TSSs of $\approx 30\%$ (Fig. 1).

2.2 Characterization of CSE

2.2.1 Physicochemical properties of the CSE

Physical parameters such as pH, total soluble solids content, specific gravity, ash content and organic matter content were determined by following the methods described in the Fertilizer Control Order of India [27] and reported the average value of 10 replicates with SD. Ash content was tested by the gravimetric dry ashing method, which involves incinerating the sample in a muffle furnace at high temperatures (typically 550 °C to 600 °C) to remove organic matter. The remaining inorganic residue (ash) is then weighed.

2.3 Estimation of nitrogen and phosphorus in CSE

A total of 1.0 g of oven-dried CSE ($60 \pm 2^\circ\text{C}$ for 15 h) was added to 40 mL of concentrated H_2SO_4 with 15 g of K_2SO_4 and digested in a sand bath ($400 \pm 10^\circ\text{C}$) until it turned colorless [28]. The final volume was made to 200 mL, of which 25 mL aliquot was transferred to a Kel Plus semi automatic nitrogen analyzer (Pelican Instruments, India) and distilled for 15 min. The distillate was supplemented with a few drops of methyl red

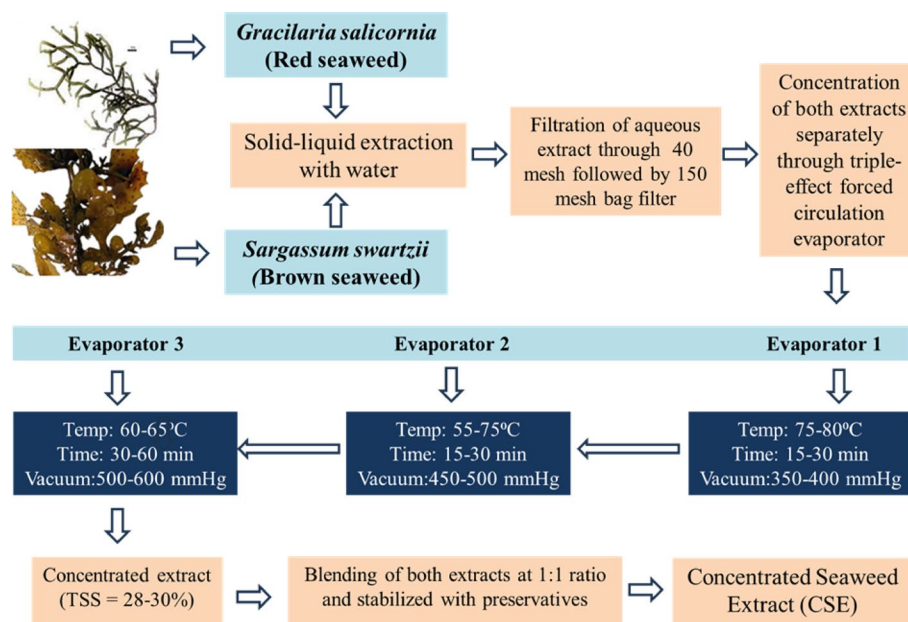


Fig. 1 Schematic representation of the step-by-step process used for the production of concentrated seaweed extract (CSE) derived from *Gracilaria salicornia* (red seaweed) and *Sargassum swartzii* (brown seaweed)

indicator and titrated against 0.1 N HCl, and the nitrogen content was estimated by the Kjeldahl method [29]. Phosphorus was estimated gravimetrically, 1.0 g of CSE was diluted to 200 mL distilled water, and a 25 mL aliquot was further diluted with 100 mL of distilled water. Approximately 30 mL of citric molybdic acid reagent was added to it and boiled for 3 min. Approximately 10 mL of quinoline solution was added to CSE mixture at room temperature and incubated overnight to obtain the precipitation of phosphorus. The solution was filtered through a sintered 30 mL glass gooch crucible (grade 4), then the residue was oven dried, and the total phosphorus content was calculated [30] and reported the average value of samples ($n = 10$, $\pm$ SD).

2.4 Quantification of micronutrients in the CSE

The elemental nutrients Ca, Mg, S, Fe, Zn, Cu and Mn were analyzed [31]. A total of 1.0 g of oven-dried CSE (60 ± 2 °C for 15 h) was incinerated in a muffle furnace at 450 ± 5 °C for 120 min, and the sample was wet with 5 mL of HNO_3 and evaporated at 90 ± 2 °C. The sample was loaded once again in the muffle furnace for 60 min to obtain ash. The ash was dissolved in 10 mL HCl, and the volume was adjusted to 50 mL with distilled water, followed by filtration with Whatman 541 filter paper. The filtered sample was subsequently analyzed for micronutrients by an atomic absorption spectrophotometer (Model Agilent 240 AA, USA) reported the average value of 10 samples with SD.

2.5 Quantification of amino acids and plant growth regulators in CSE

Amino acids were content of CSE were analyzed by a standard method i.e. liquid chromatography system (Model-Agilent 1260 Infinity, USA) equipped with a Quaternary pump (G7111B), a standard autosampler (G1729A), a thermostat column compartment, a diode array, a multiple wavelength detector (G7115A) and a Poroshell 120 EC-C18 column (100 mm $\times$ 4.6 mm, particle size 2.7 μm). Standard solutions with a mixture of 19 amino acids (25, 100, 250 and 1000 nmol mL^{-1}) were procured from Agilent

Technologies (Waldbronn, Germany) and subjected to HPLC with LC-grade acetonitrile, methanol, and borate buffer with Millipore water. The retention values of the CSE were compared against those of standard amino acids obtained from Sigma Aldrich, Mumbai (India). Similarly, the levels of plant growth regulators, such as auxin, cytokinin and gibberellic acid, were determined via comparison with their respective standards, such as indole-3-acetic acid, kinetin, and gibberellic acid (Sigma Aldrich, India), via HPLC (Make HPLC 1260 Agilent system, USA) following previously described methods [32] reported the average value of 10 samples with SD.

2.6 Quantification of alginic acid in CSE

The amount of alginic acid present in CSE was determined by a colloidal titration method with 1% sodium carbonate at 80 °C, and the pH was neutralized with 0.1 N hydrochloric acid. 2 mL of liquid ammonia and 10 mL of N-trimethylated glycol chitosan with a few drops of 0.1% toluidine blue as an indicator were added and titrated against 0.0025 N polyvinyl sulfonate until the blue color of the solution turned reddish-purple. The precipitate was dried, and alginic acid was quantified [33] and reported the average value of 10 samples with SD.

2.7 Evaluation of the effects of CSE on the growth and yield of rice

2.7.1 Material source and design of the trial plot

The trial was carried out at farmers' field on 1.0 ha area for each control, T1 (50% reduced recommended fertilizer dose (RFD) of nitrogen+NEN) and T2 (T1+CSE) plants two rice varieties tested in two different agroclimatic zones in India, The rice variety Satabdi (IET4786), sourced from Bengal Seeds and Agro Industries, was tested at the lower Gangetic Plain at Jaguli of West Bengal state (22.9276°N, 88.5505°E), and this zone was characterized by sandy clay loam with a mixture of sand (61.24%), clay (24.56%) and silt (14.20%), pH 6.65, electrical conductivity (EC 0.29 dSm⁻¹), and a filtration rate of 1.25 cm h⁻¹. A trial on the rice variety CO-51 (IET21605), obtained from Tamil Nadu Agricultural University, was conducted in the Northeast Zone at TV Malai of Tamil Nadu state (12.745.98°N, 79.5505°E), characterized by red series loam with a mixture of sand (50.40%), clay (10.50%) and silt (40.25), pH 6.8, EC 0.66 dSm⁻¹.

2.7.2 Nitrogen-enriched nano (NEN) formulation

Sample of NEN was provided by Indian Farmers Fertiliser Cooperative Limited (IFFCO), it is a newer version of commercial 'nano urea liquid' with nano particle size of 30–40 nm and 4.0% nitrogen in it. The particle size distribution proportion of NEN in solid state is 30–40 nm (measured by TEM – transmission electron microscopy) and when it is dispersed in water, it becomes hydrated and particle sizes increase to 30–50 nm (measured by DLS – dynamic light scattering), and these distribution metrics ensure the nano urea has the intended nanoscale properties which are critical for foliar uptake and increase the nutrient use efficiency in crops. The NEN was diluted to a concentration of 0.2% (2 mL L⁻¹) and applied over one hectare via foliage and a total of three sprays (150 L, 300 L and 300 L at 21, 45 and 60 DAT respectively) were given [34].

2.7.3 Treatments

In both locations the trials (two trials in two consecutive years) were conducted in farmers' fields. The recommended fertilizer dose (RFD) of NPK for rice, as prescribed by the Government of India, is 150–60–60 kg ha⁻¹ [35] and this dose was split into three applications and applied to the soil by the broadcasting method at 21, 45, and 60 days after transplanting (DAT) and this was served as the control treatment. In Treatment 1 (T1), 50% of the RFD of nitrogen i.e. 75 kg ha⁻¹ and 100% of phosphorus and 100% of potassium were applied in the same manner as the control, i.e., through soil application at 21, 45, and 60 DAT, along with foliar application of 0.2% NEN at 21 DAT (150 L ha⁻¹), 45 DAT (300 L ha⁻¹), and 60 DAT (300 L ha⁻¹). In Treatment 2 (T2), in addition to the inputs applied in T1, a 0.2% CSE solution was applied as a foliar spray at 21 DAT (150 L ha⁻¹), 45 DAT (300 L ha⁻¹), and 60 DAT (300 L ha⁻¹), following the same schedule as the NEN application in T1.

2.7.4 Leaf area index (LAI) and Tillers

For each treatment, ten rice plants were randomly marked for measuring plant height (cm) and leaf area (cm) via a digital leaf area meter (Clikzz Technology, India). The sample number of 10 plants is too small for a one-hectare rice field, but the aim was also to investigate how rice plants respond to NEN alone and in combination with CSE in terms of vegetative growth like LAI, tiller, and plant height. The leaf area index (LAI) was computed based on the formula by Watson and French [36].

$$\text{Leaf area (cm}^2\text{)} = \text{Maximum leaf length} \times \text{maximum leaf width} \times k$$

where $k = 0.75$ was the coefficient).

$$\text{Leaf area index} = \text{Leaf area} / \text{ground area}$$

The number of tillers per hill was scored from 10 randomly selected and marked plants from 1.0 m² quadrats from each trial plot at 30 and 60 DAT.

2.7.5 Straw and grain yield of rice

The grain and straw yields obtained from rice by each treatment were measured and recorded. The weight of the grains were cleaned and sundried and expressed as yield = kg per hectare and the straw yield was obtained by deducting the grain yield from the total biomass obtained and recorded as yield per hectare.

2.8 Scanning electron microscopy - energy dispersive spectroscopy (SEM–EDS)

Elemental analysis (SEM–EDS) indicated the presence of multiple nutrients across plant tissues in T2-treated plants. However, this analysis was qualitative to semi-quantitative and does not establish causality between treatment and nutrient uptake efficiency. Leaves, stems, and roots of the rice variety CO-51 (IET21605) were fixed in 3% glutaraldehyde, washed three times with 0.1 µm phosphate buffer (pH 6.8) and dehydrated through a graded series of alcohol (10, 25, 50 and 70%) at 15 min intervals and final dehydration was performed with 100% alcohol for 2 h. The dehydrated samples were mounted on a stub, treated with a critical point drier (CPD) and examined through SEM–EDS (Model- Joel JSM-56010 with INSA–EDS). Electron micrographs were taken

selectively from the targeted region of the section, and the data were recorded by INSA-EDS systems. (Indian National Science Academy – Early-Career Development Scheme). By following the operational procedure, an energy dispersive X-ray spectrum was developed, and the detected elements in the samples were restored in the form of primary data with a spectrum and analyzed.

2.9 Economics and crop production efficiency

The gross and net return of control and treated plants of two rice varieties, cultivated at one hectare were analyzed. For the computation of input costs and current market prices were considered and analyzed to establish the economic returns. Gross monetary return (GMR) was estimated using the minimum support prices (MSPs) set by the Directorate of Economics and Statistics, Government of India (<https://desagri.gov.in>).

$$\text{Gross monetary return (GMR) (USD ha}^{-1}\text{)} = \text{grain yield (kg ha}^{-1}\text{)} \\ \times \text{selling price (USD ha}^{-1}\text{)}$$

$$\text{Net monetary return (NMR) (USD ha}^{-1}\text{)} = \text{GMR (USD ha}^{-1}\text{)} \\ - \text{cost of cultivation (USD ha}^{-1}\text{)}$$

$$\text{Benefit} = \text{NMR (USD ha}^{-1}\text{)} / \text{cost of cultivation (USD ha}^{-1}\text{)}$$

2.10 Statistical analyses

Statistical analyses were performed by SPSS Version 22, and the mean comparisons between the treatments were drawn up through the Duncan multiple range test (DMRT). Ten random samples were taken to measure the plant height, leaf area index, and number of tillers. Value were expressed as means $\pm$ SD, *p*-value. Mean comparisons between treatments were drawn via Duncan's multiple range test (DMRT), and analysis of variance (ANOVA) was performed via Fisher's least significant difference (LSD) test at $p \leq 0.05$ to determine the significance of the difference between the means of the control and different treatments.

3 Results

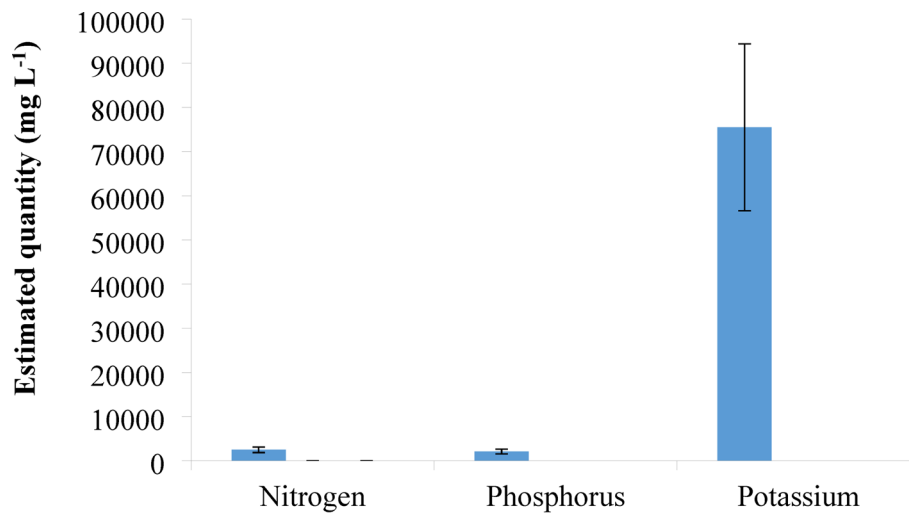
3.1 Characterization of the biostimulant

3.1.1 Macro- and micronutrients

The concentrated seaweed extract (CSE) that was used for trials in both locations contained a total soluble solids (TSS) of $29.15 \pm 1.24\%$. The CSE was evaluated to contain nitrogen (N), phosphorus (P), and potassium (K) at 2500, 2100 and 75,000 mg L⁻¹ level respectively (Fig. 2) and 3500 ± 170 mg L⁻¹, 2500 ± 140 mg L⁻¹ and $12,000 \pm 100$ mg L⁻¹ corresponded to plant secondary nutrients calcium, magnesium, and sulfur respectively (Fig. 3a). Micronutrients, i.e., Fe, Zn, Cu, Mn, Mo, Ni and B were also present in CSE, i.e. 1.1 mg L⁻¹ (Mo) to 75 mg L⁻¹ (Fe) (Fig. 3b).

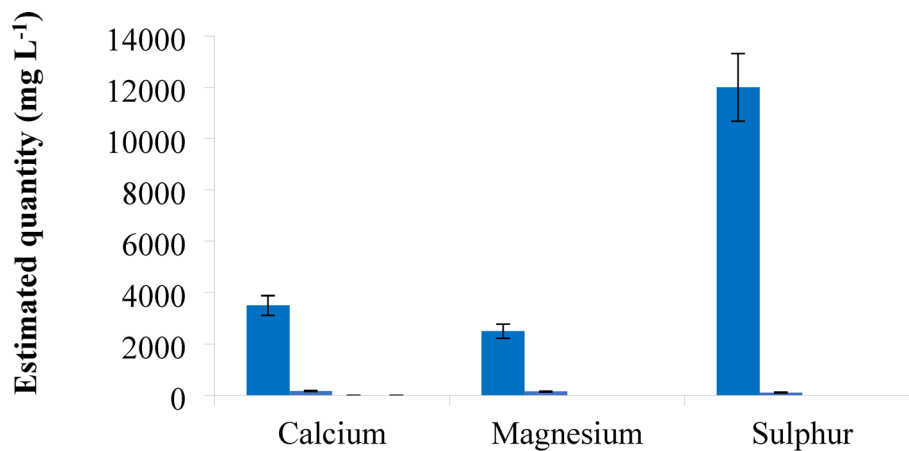
3.1.2 Endogenous growth hormones and vitamins

The CSE was found to contain significant amounts of plant growth regulators such as gibberellic acids (335.0 ± 75.5 mg L⁻¹), auxins (18.5 ± 10.5 mg L⁻¹) and cytokinins (560.0 ± 150.5 mg L⁻¹). The prominent level of cytokinins was one of the unique



Plant primary nutrients present in CSE

Fig. 2 Estimated quantities of primary macronutrients (N, P, and K) in the concentrated seaweed extract (CSE) manufactured from *Gracilaria salicornia* (red seaweed) and *Sargassum swartzii* (brown seaweed) expressed in mg L⁻¹. Values represent mean $\pm$ standard deviation (SD) ($n = 10$)



Secondary macronutrients present in CSE

Fig. 3 a Estimated quantities of secondary macronutrients (Ca, Mg, and S) in the concentrated seaweed extract (CSE) manufactured from *Gracilaria salicornia* (red seaweed) and *Sargassum swartzii* (brown seaweed) expressed in mg L⁻¹. Values represent mean $\pm$ standard deviation (SD) ($n = 10$). **b** Estimated quantities of micronutrients (B, Ni, Mo, Mn, Cu, Zn, and Fe) in the concentrated seaweed extract (CSE) produced from *Gracilaria salicornia* (red seaweed) and *Sargassum swartzii* (brown seaweed) expressed in mg L⁻¹. Values represent mean $\pm$ standard deviation (SD) ($n = 10$)

characteristics of CSE; it is 30 times greater than that of auxins and 1.67 time higher than gibberellic acids content (Fig. 4a). Three essential vitamins, i.e., C, B2, B6 and trace of B12, were found to be present in CSE at 18.5 ± 4.4 mg L⁻¹, 0.21 ± 0.0 mg L⁻¹ and 0.11 ± 0.0 mg L⁻¹ respectively (Fig. 4b).

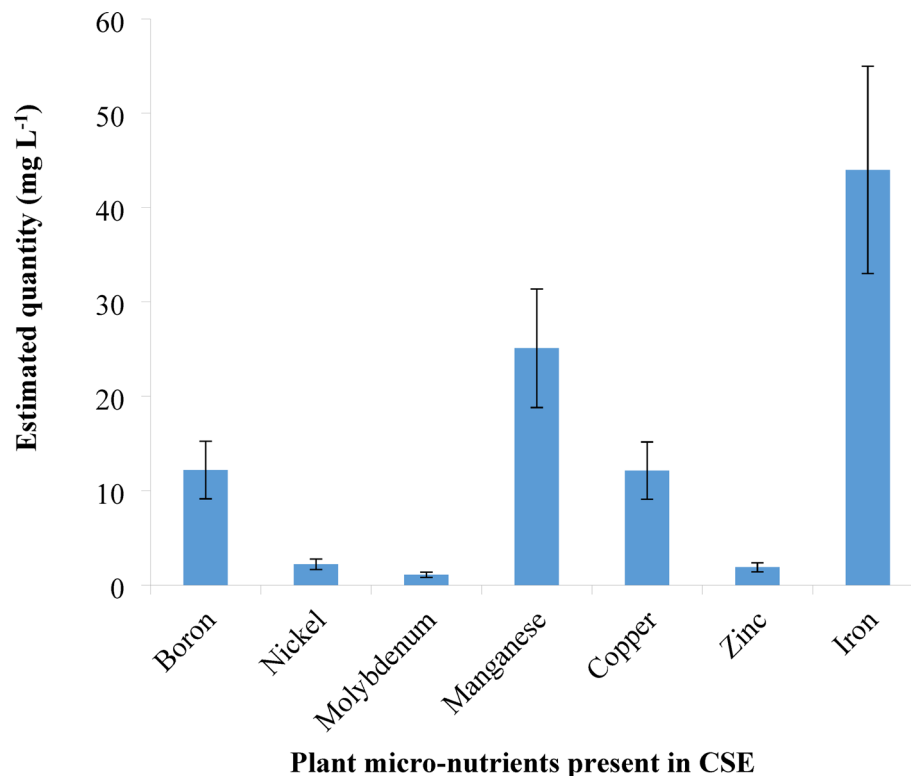


Fig. 3 (continued)

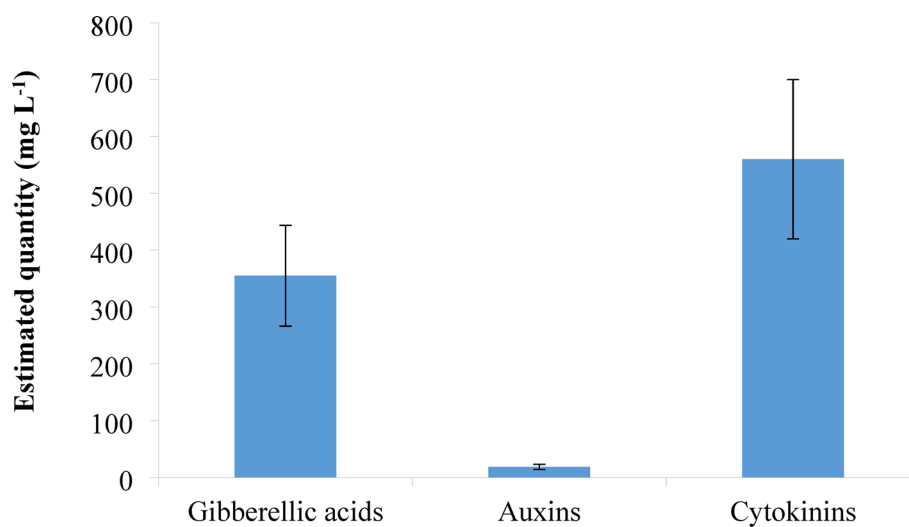
3.1.3 Amino acids and alginic acid in CSE

The CSE contained $2.12 \pm 0.15\%$ alginic acid (from the *Sargassum* component) and 19 amino acids in it. Among the amino acids, hydroxyproline was found to be at higher level (102 mg L^{-1}), but other amino acids are in the range of $12.55\text{--}18.99 \text{ mg L}^{-1}$, viz. arginine ($12.55 \pm 1.10 \text{ mg L}^{-1}$), glutamic acid ($13.22 \pm 2.0 \text{ mg L}^{-1}$), proline ($13.39 \pm 0.50 \text{ mg L}^{-1}$), leucine ($15.95 \pm 1.50 \text{ mg L}^{-1}$), alanine ($16.38 \pm 2.50 \text{ mg L}^{-1}$), aspartic acid ($16.86 \pm 0.10 \text{ mg L}^{-1}$) and methionine ($18.99 \pm 1.20 \text{ mg L}^{-1}$). The extent of remaining amino acids acid concentrations were less than 10 mg L^{-1} , ranging from $0.92 \pm 0.10 \text{ mg L}^{-1}$ (asparagine) to $9.74 \pm 0.15 \text{ mg L}^{-1}$ (histidine), and these amino acids could make CSE a more potent biostimulant product (Fig. 5).

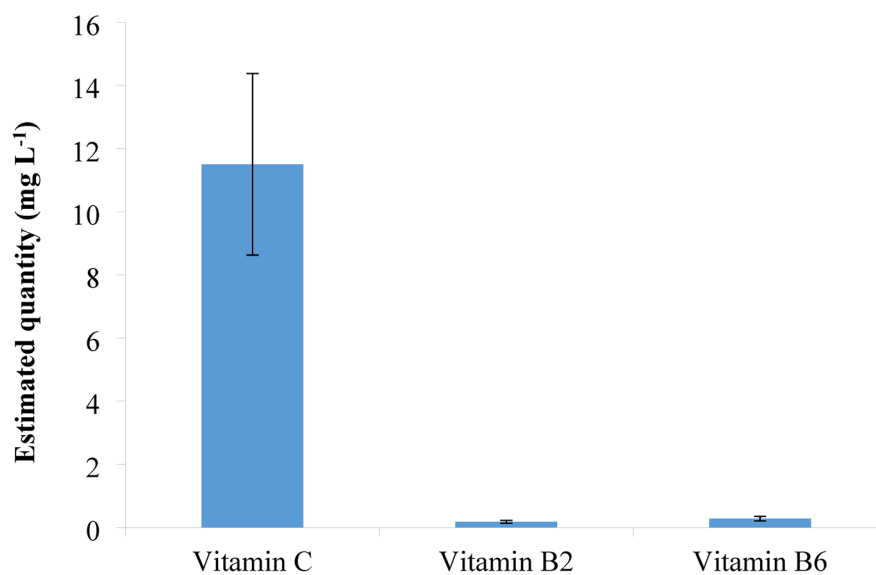
3.2 Influence of CSE on the growth and yield of the two rice varieties

3.2.1 Plant height

Plant height, tiller number, and dry matter accumulation were generally higher in T1 and T2 treatments compared to the control. Differences became more pronounced at later growth stages (60 and 90 DAT). However, early-stage differences (30 DAT) were limited, and some parameters such as leaf area index at harvest did not show significant differences, likely due to senescence effects. Plants receiving NEN + CSE showed greater plant height. Plant height during initial growth stage i.e. at 30 DAT was recorded lower without much variations among control and treated plants of two test varieties tested, however, significant difference in plant height treated plants was observed at 60 and 90 DAT ($p < 0.05$) compared to control ones. The plant height at 60 DAT of Satabdi (IET4786) was $81.86 \pm 8.24 \text{ cm}$, $74.04 \pm 2.42 \text{ cm}$ and $68.75 \pm 1.82 \text{ cm}$ in T2, T1 and control respectively and at 90 DAT were $100.23 \pm 9.23 \text{ cm}$ (T2), $95.90 \pm 2.11 \text{ cm}$ (T1) $84.20 \pm 3.23 \text{ cm}$



Plant growth regulators present in CSE



Vitamins present in CSE

Fig. 4 **a** Estimated quantities of plant growth hormones - gibberellic acid, auxins and cytokinins - in the concentrated seaweed extract (CSE) produced from red seaweed *Gracilaria salicornia* and brown seaweed *Sargassum swartzii*. Values are expressed as mean $\pm$ standard deviation (SD) in mg L⁻¹ ($n = 10$). **b** Estimated quantities of vitamins (vitamin C, vitamin B2 and vitamin B6) in the concentrated seaweed extract (CSE) produced from red seaweed *Gracilaria salicornia* and brown seaweed *Sargassum swartzii*. Values are expressed as mean $\pm$ standard deviation (SD) in mg L⁻¹ ($n = 10$)

(control). A similar trend in plant height was recorded in CO-51 (IET21605), i.e., at 60 DAT: 93.65 ± 6.00 (T2) and 88.010 ± 3.50 (T1) and 77.12 ± 6.20 (control) and at 90 DAT, it was 112.10 ± 3.22 cm, 101.40 ± 2.12 cm and 90.50 ± 3.12 cm in T2, T1 and the control respectively) (Supplementary Fig. 1).

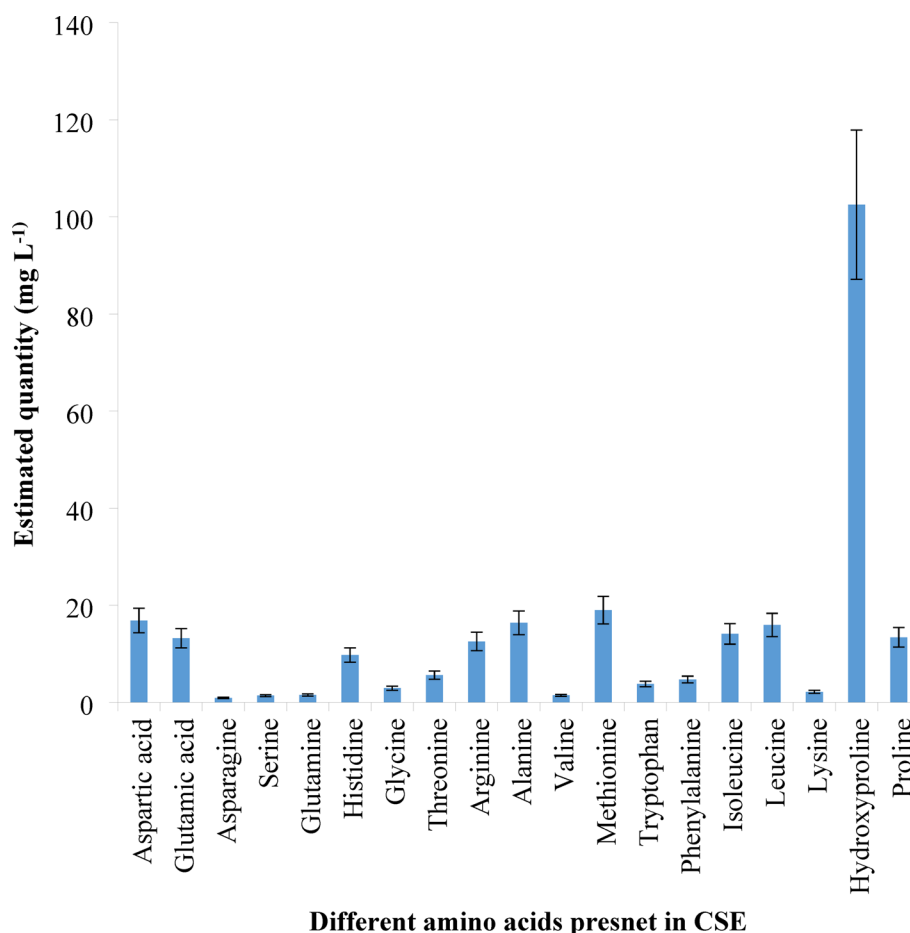


Fig. 5 Amino acid profile of the concentrated plant extract (CSE) produced from *Gracilaria salicornia* (red seaweed) and *Sargassum swartzii* (brown seaweed). Values are expressed as mean $\pm$ standard deviation (SD) in mg L⁻¹ ($n=10$)

3.2.2 Leaf area index (LAI) and dry matter accumulation (DMA)

The leaf area indices of CO-51 (IET21605) at 60DAT were 4.93 ± 0.42 , 4.31 ± 0.21 and 3.37 ± 0.11 in the T2, T1 and control groups, respectively and they were 4.31 ± 0.21 (T2), 4.00 ± 0.23 (T1) and 3.21 ± 0.21 (control) in the case of the Satabdi (IET4786). However, there was no significant improvement in the LAI in either rice variety at the time of harvest and it could be due to the initiation of the senescence process (Supplementary Fig. 2). It was also recorded that dry matter accumulation was found increased at significant level in both varieties of rice at the harvest stage, i.e. in Satabdi (IET4786), it was, 795 ± 8.2 (T1), 854 ± 10.2 (T2) and 688.2 ± 28.3 g m² (control) and in the rice variety CO-51 (IET21605), it was tested to be 1097.43 ± 15.14 , 1166 ± 17.24 and 920.87 ± 12.24 g m² in T1, T2 and control plants respectively.

3.2.3 Influence of NEN and CSE on tillers

There was a significant difference in the number of tillers formed by the T1 and T2 plants both rice varieties evaluated ($p < 0.05$) compared to their control plants. Satabdi (IET4786), which was grown in the lower Gangetic plains, developed 19.32 ± 2.24 tillers per hill in response to T2 treatment, but in CO-51 (IET21605), in the Eastern Coastal Plains and Hills, developed a slightly greater number of tillers per hill (26.36 ± 3.21).

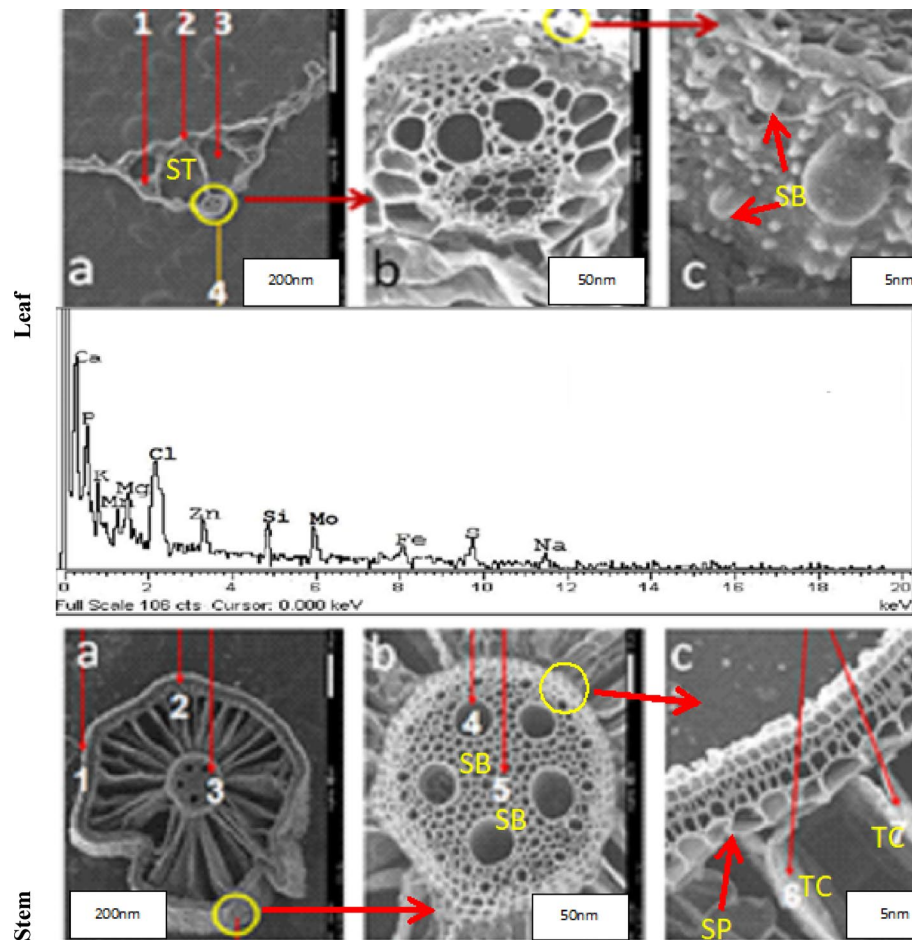


Fig. 6 Scanning electron microscopic (SEM) images of the leaf, stem, and root of rice variety CO-51 (IET21605) treated with a concentrated seaweed extract (CSE) prepared from a blend of seaweed *Gracilaria salicornia* and *Sargassum swartzii*. Encircled regions in both low and high magnification images indicate the targeted areas selected for energy-dispersive microanalysis (EDM). The corresponding EDM spectra reveal the presence of elemental constituents in the respective tissues of the rice variety CO-51 (IET21605)

These data clearly showed that CO-51 (IET21605) was more responsive than Satabdi (IET4786). However, the combination of NEN and CSE was effective for both varieties regardless of the extreme geographic conditions (Fig. Supplementary Fig. 3).

3.2.4 Elemental distribution in T2 plant of CO-51 (IET21605)

Rice variety CO-51 (IET21605) treated with NEN and CSE (T2, 90 DAT) was evaluated to contain different nutrients at varying levels. SEM images of the leaves, stems, and roots along with the spectra generated by energy dispersive spectroscopy (EDS) are shown in Fig. 6. The EDS analysis revealed a variable percentage of elements in the leaves, stems, and roots. A total of 13 different elements, viz. N, P, K, Ca, Mg, S, Si, Na, Cl, Mn, Zn, Mo, and Fe were detected, of which Ca, N, K, Si and Na were found at higher levels (7.20 to 28.56%) within the total elemental content in CSE (Fig. 7).

3.2.5 Grain and straw yield

The yields of grain and straw were significantly higher in NEN alone (T1) and NEN + CSE-treated rice plants (T2) than in the control (Table 1). The grain yield of rice

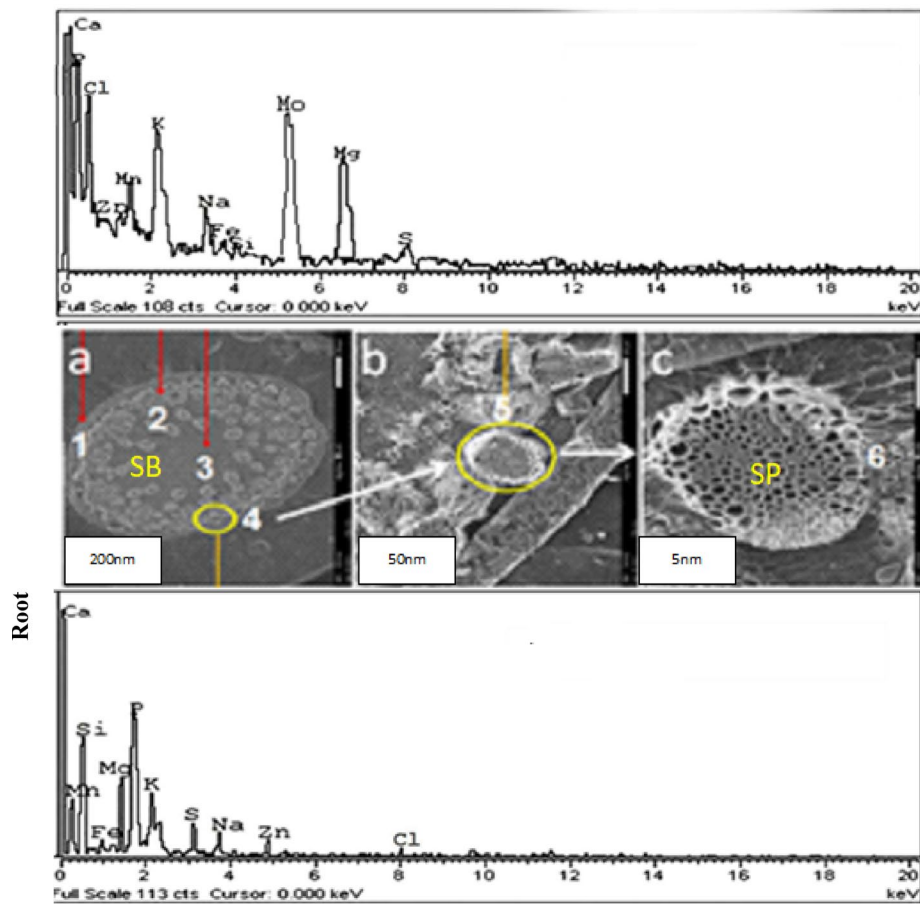


Fig. 6 (continued)

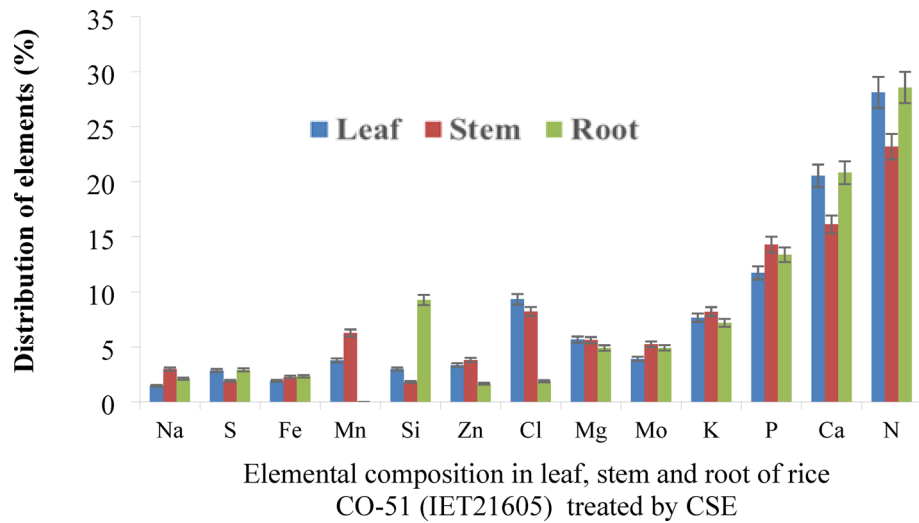


Fig. 7 SEM-EDS analysis showing the distribution of various nutrients in the leaf, stem, and root of variety CO-51 (IET21605) at 90 DAT, treated with concentrated seaweed extract (CSE) produced from red seaweed *Gracilaria salicornia* and brown seaweed *Sargassum swartzii*. Data are presented as mean $\pm$ SD ($n = 10$)

Table 1 Influence of NEN and CSE on grain and straw yield of rice varieties Satabdi (IET4786) and CO-51 (IET21605) cultivated in Lower Gangetic Plains and Eastern Coastal Plains and Hills respectively

Treatment	Satabdi (IET 4786)		CO-51 (IET21605)			
	Grain yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)	Grain yield increase (%)	Grain yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)	Grain yield increase (%)
Control: 100% recommended dose of fertilizer NPK (150/60/60 kg ha ⁻¹)	3786	5536	--	5066	7903	--
T1: 50% N and 100% PK + Foliar application of NEN at 2 mL L ⁻¹	4208*	6120*	11.15*	5602*	8907*	10.58*
T2: 50% N and 100% PK + Foliar application of NEN at 2 mL L ⁻¹ and CSE at 2 mL L ⁻¹	4391*	6347*	15.98*	5992*	9288*	18.27*
SEm (±)	52.33	71.10		70.02	101.5	
CD @ 5%	157.0	213.0		210.0	304.0	

Treatments were applied on 27–30 and 40–45 day after transplantation (DAT)

NEN – Nitrogen-enriched nanoformulation; CSE – Concentrated seaweed extract

* Statistically significant at $p < 0.05$ level

was higher in both T1 and T2 treatments compared to the control in both cultivars. In Satabdi (IET4786), grain yield increased from 3786 kg ha⁻¹ (control) to 4208 kg ha⁻¹ in T1 and 4391 kg ha⁻¹ in T2, corresponding to increases of 11.15% and 15.98% ($p < 0.05$), respectively under the tested conditions. In CO-51 (IET21605), yields increased from 5066 kg ha⁻¹ (control) to 5602 kg ha⁻¹ (T1) and 5992 kg ha⁻¹ (T2), representing increases of 10.58% and 18.27%, respectively. While both treatments improved yield relative to the control, the incremental benefit of CSE over NEN alone (T2 vs. T1) was modest and varied between cultivars. Both Satabdi (IET4786) and CO-51 (IET21605) showed significant increases in straw yield with foliar NEN and the combined NEN + CSE treatments compared with the control. In Satabdi, straw yield rose from 5536 kg ha⁻¹ (control) to 6120 kg ha⁻¹ with T1 (NEN) and to 6347 kg ha⁻¹ with T2 (NEN + CSE); both increases were statistically significant (SEm = 71.10; CD5% = 213.0). In CO-51, straw yield increased from 7903 kg ha⁻¹ (control) to 8907 kg ha⁻¹ with T1 and to 9288 kg ha⁻¹ with T2, also significant (SEm = 101.5; CD5% = 304.0). The patterns of straw response mirrored grain-yield improvements, with T2 producing the largest gains in both varieties. The timing of foliar applications (27–30 and 40–45 DAT) corresponded with active vegetative and reproductive transitions, suggesting that enhanced nitrogen availability from the NEN and bio-stimulant effects of CSE promoted above-ground biomass accumulation. No evidence in the data indicates that increased straw resulted from a trade-off with grain; rather, both biomass components increased concurrently, indicating improved overall biomass production and nutrient use efficiency.

3.3 Cost–benefit analysis

A cost–benefit analysis of NEN and CSE application is summarized in Table 2. Nitrogen input cost for the control (100% NPK) was USD 75 ha⁻¹, while N input costs for T1 and T2 were substantially lower by 35.5% and 22.3%, respectively. Both treatments produced higher net returns than the control after deducting N-related input costs. In Satabdi, net returns were USD 2,434 (control), USD 2,740 (T1, +USD 306, +12.6%) and USD 2,851 (T2, +USD 418, +17.2%). In CO-51, net returns were USD 3,282 (control), USD 3,664 (T1, +USD 382, +11.6%) and USD 3,912 (T2, +USD 630, +19.2%). Although

Table 2 Cost-benefit analysis of combined use of NEN and CSE in farming of two rice varieties

Treatment	Farm input cost Vs Economic return (USD)	Satab-di (IET 4786)	CO-51 (IET21605)
Control	Total input cost of recommended dose of nitrogen per hectare (150 kg urea x USD 0.5 kg ⁻¹) (USD)	75	75
	Total grain yield per hectare (kg)	3786	5066
	Total gross return per hectare (grain yield x USD 0.66 kg ⁻¹) (USD)	2509	3357
	Net return per hectare (Gross return - Input cost) (USD)	2434	3282
T1	Cost of 50% use of nitrogen per hectare (USD)	37.5	37.5
	Cost of nitrogen-enriched nano formulation per hectare (0.2% of 750 L in total) @ USD 5.42 L ⁻¹) (USD)	11	11
	Total input cost of nitrogen per hectare (USD)	48	48
	Total grain yield per hectare (kg)	4208	5602
T2	Total gross return per hectare (grain yield x USD 0.66 kg ⁻¹) (USD)	2788	3712
	Net return per hectare (Gross return - Input cost) (USD)	2740	3664
	Increase in net return over control per hectare (USD)	306	382
	Cost of 50% use of nitrogen per hectare (USD)	37.5	37.5
	Cost of nitrogen-enriched nano formulation per hectare (0.2% 750 L in total) @ USD 5.42 L ⁻¹) (USD)	11	11
	Cost of concentrated seaweed extract (CSE) per hectare ((0.2% 750 L in total) @ USD 6.02 L ⁻¹) (USD)	9,9	9,9
	Total input cost of nitrogen and CSE per hectare (USD)	58	58
	Total grain yield per hectare (kg)	4391	5992
	Total gross return per hectare (grain yield x USD 0.66 kg ⁻¹) (USD)	2910	3971
	Net return per hectare (Gross return - Input cost) (USD)	2851	3912
	Increase in net return over control per hectare (USD)	418	630

T2 required slightly higher inputs than T1, the additional CSE cost was more than offset by yield gains, making T2 the most profitable option; a full economic appraisal should also account for labor, application, and straw value.

4 Discussion

The study presents the nutritional profile of CSE and it was found to contain macro- and micronutrients, endogenous plant growth hormones (auxins, cytokinins and gibberellic acids), 19 amino acids and trace of amounts of vitamins and vitamin analogs in it. The distribution of nutritional elements across the rice plants from roots to leaves in response to the application of CSE in combination with NEN contributed to improved growth. CSE made from the red seaweed *G. salicornia* and brown seaweed *S. swartzii* may serve as a supplementary source of nutrients under the tested conditions. Vitamin B12 were detected at lower quantities in CSE and it could be a bacterial product extracted during the process [37] or a biologically inactive analog (pseudo-vitamin B12) reported in red algae [38].

Fertilizers have low uptake efficiency, and they are applied in copious quantities in agriculture [39] and inorganic NPK-based fertilizers undergo rapid chemical changes into unavailable forms [40], therefore, it often leads to a negative impact on environmental health [40, 41]. Recently, the use of nitrogen-enriched products, popularly referred to as 'nano urea' for foliar applications, has gained importance because of its ability to improve the efficiency of nutrient uptake through the gradual release of various elements without causing any adverse effects on the health and growth of crops and on the soil [42]. Nano fertilizers have gained importance in agriculture because, compared with conventional fertilizers, they effectively deliver nutrients to plants at lower prices

[42, 43]. Reducing the recommended dosage of NPK to 75% with two rounds of nano urea improved the grain and straw yields of rice [44]. Efficient absorption of nano urea through foliar application improved the grain yield to 15–21% [45]. Nano fertilizers have been reported to have greater transport and delivery efficiency of nutrients through the nanopores of plasmodesmata (50–60 nm) to all parts of the plant [46]. The merits and demerits of the use of nano urea in agriculture have been reported [47–50]. There are some reports on the beneficial effect of use of nano urea in Indian agriculture [51–53]. The present study supports the use of nano urea only through foliar application in conjunction with a reduction (50%) in RFD of nitrogen through the conventional method of basal application. However, more detailed research on the long-term usage of nano urea over many cycles of single cropping and its influences on plant health and yield is needed [42, 50, 54].

With regard to straw, the observed increases in straw yield with NEN and NEN + CSE likely reflect improved nitrogen supply dynamics and enhanced metabolic stimulation from the seaweed extract. NEN can improve N uptake and reduce losses, while CSE contains phytohormones and signaling compounds that promote cell expansion and biomass accumulation; together they produced additive or synergistic effects on vegetative growth, as shown by the larger straw yields in T2 than T1. Both T1 and T2 treatments differed significantly from the control; however, differences between T1 and T2 were not consistently tested for statistical significance (or specify if they were). From an agronomic perspective, higher straw yield has dual implications: it represents increased total productivity and additional fodder or residue value for farmers, but it may also raise lodging risk if not managed. CSE and MEN may have associated with improvement in grain and straw yield, however, future work should report lodging observations, straw quality (C: N), and economic valuation to fully assess practical benefits.

When the soil application of N was reduced to 50% of its RFD with a combination of NEN and CSE, significant improvements in plant height, leaf area index, number of tillers, biomass and grain yield were shown for two popular varieties of rice cultivated in India under two extreme agroclimatic conditions, thus, the Lower Gangetic Plains are inland, flat alluvial regions with higher soil fertility, wider temperature variation, and predominantly rice-based intensive cropping, whereas the Eastern Coastal Plains and Hills are coastal and hilly areas with diverse soils, narrower temperature ranges, and agriculture often constrained by cyclones, salinity, and coastal flooding. Nano urea plays a significant role in plant growth by optimizing nutrient requirements during the vegetative and reproductive phases of rice crops [40, 42]. In this study, the use of CSE along with NEN, the latest version of conventional nano urea found to increase the vegetative growth and the grain yield significantly level in both rice varieties assessed over control plants. Dry matter accumulation is one of the indices used to determine efficient metabolic activities to correlate the growth of any crop. In the present study, the combination of NEN and CSE significantly improved dry matter accumulation, possibly because of the efficient intake of nitrogen from NEN through foliar application, as reported earlier [42, 55–57]. The observed increases in growth and yield under T1 and T2 treatments may be associated with improved nutrient availability and uptake resulting from foliar application of the nitrogen-enriched nano formulation (NEN), however, it requires further studies. The additional improvements observed in T2 could be linked to the presence of bioactive compounds in the concentrated seaweed extract (CSE), including plant

growth regulators, amino acids, and micronutrients. However, the specific contribution of CSE relative to NEN cannot be conclusively separated in this study, as no treatment with CSE alone was included. Therefore, the observed effects should be interpreted as combined treatment effects rather than independent contributions. CSE may have contributed to improved growth responses due to its composition, although the mechanisms were not directly investigated.

Reducing the use of fertilizers by replacing organic fertilizers is gaining importance in modern agriculture [58]. In this context, reducing the application of fertilizers to 75% [44] and then to 50% by compensating with foliar application of nano urea has been demonstrated in a wide range of crops [59]. Precautionary farming to reduce the application of synthetic fertilizers gradually with simultaneous practices involving the use of organic fertilizers is one of the possible environmentally friendly approaches for restoring soil quality [60]. Therefore, seaweed-based biostimulants for application as foliar and soil amendment products were developed for use in agriculture [61, 62]. While T2 showed higher yields than T1, the magnitude of improvement suggests that NEN contributed the majority of the observed effect under the present conditions. Therefore, future studies should include CSE-only treatment and multi-season trials to better understand the independent and long-term effects of these inputs. Significant differences in the growth and grain yield of rice were observed between T2 (soil application of 50% NPK + NEN + CSE) and T1 (soil application of 50% NPK + NEN), which could be due primarily to the combined influence of CSE, but further research is required and ascertain its mechanism of action.

A number of seaweeds [10, 63], brown algae [64, 65], green algae [66–69], and cyanobacteria [70] have been investigated for their ability to stimulate the growth and metabolic activities of many crops for sustainable agriculture. The biostimulant developed from the present study was derived from a combination of two different seaweeds; therefore, they are expected to have different nutrient contents that are responsible for positive impacts on the rice studied. Micronutrients like zinc, boron, manganese, and iron are crucial to enhancing photosynthesis, better tillering, protein synthesis, grain filling and yields in rice [71]. Zinc deficiency is the most common issue, impacting nitrogen use and growth of rice, while other micronutrients ensure overall plant health and resistance to stresses [72]. CSE was evaluated to contain all important micronutrients including zinc, which could have contributed to the growth and grain yield of both rice varieties studied. Biostimulant CSE was also found to contain 19 amino acids including stress elicitors, such as proline, aspartic acid, glutamic acid, and other amino acids, viz., arginine, alanine, methionine, isoleucine, and leucine, therefore it has potential to mitigate abiotic stress in plants [5, 10, 62], however, further studies are needed to better understand this aspect. The results of this study revealed that CSE contains plant nutrients and PGR at significant levels and could influence the ability of plants to absorb nutrients well from the soil, as they were found distributed in different plant parts. The SEM study on rice variety CO-51 (IET21605) provided a basic understanding of the bioaccumulation and distribution of elemental nutrients. It was also observed that the cost of nitrogen input was reduced when combined use of NEN and CSE with 19.21% of higher economic return in CO-51 (IET21605). The economic analysis considered only selected input costs (nitrogen, NEN, and CSE) and did not include labor, application costs, or potential revenue from straw, which may influence overall profitability.

4.1 Limitation the study

The limitations of this study are: The experimental design did not include treatment with CSE alone, making it difficult to distinguish the individual effects of CSE from those of NEN. Trials were conducted in two locations and over a single cropping cycle. Results may vary across seasons, soil types, and broader agroecological conditions. The study did not evaluate long-term impacts on soil health, nutrient dynamics, or sustainability of repeated nano fertilizer use. Although CSE contains plant growth regulators and nutrients, the study did not directly investigate physiological or molecular mechanisms underlying the observed responses. SEM–EDS analysis provides indicative elemental distribution but does not quantify nutrient uptake efficiency or bioavailability. Cost–benefit calculations were based on specific input prices and may not be generalizable across regions or time. The composition of CSE may vary depending on harvesting conditions, season, and processing methods, which were not extensively evaluated.

5 Conclusion

The combined application of nitrogen-enriched nano formulation (NEN) and concentrated seaweed extract (CSE) was associated with increased growth and grain yield in two rice cultivars under the conditions tested. The incremental gain from T2 over T1 was smaller than the gain from control to T1. Yield improvements ranged from approximately 10–18% relative to the control, with slightly higher values observed when CSE was included. These results suggest that partial substitution of conventional nitrogen fertilizer with foliar-applied NEN, with or without CSE, may improve nitrogen use efficiency and economic returns under certain conditions. However, further studies are required to (i) isolate the individual effects of CSE, (ii) evaluate long-term impacts on soil and crop systems, and (iii) validate these findings across diverse agroecological environments before broader recommendations can be made.

Abbreviations

CSE	Concentrated seaweed extract
DAT	Days after transplantation
INSA-EDS	Indian National Science Academy – Early-Career Development Scheme
LAI	Leaf area index
NEN	Nitrogen-enriched nano formulation
RFD	Recommended fertilizer dose
SEM-EDS	Scanning Electron Microscopy–Energy Dispersive X-ray Spectroscopy

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44279-026-00620-5>.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Author contributions

SM: Project implementation, seaweed nutrient analysis. Data curation and manuscript drafting. TS: Investigation, Funding acquisition, Formal analysis, Data curation. YK: Methodology, investigation, formal analysis, conceptualization. AS: Project administration, Funding acquisition, Project monitoring. TS: Project administration, funding acquisition, project monitoring, conceptualization. SK: Scanning electron microscopy image, SEM–EDS analysis and data curation. TS: statistical analysis, literature, draft preparation; GD: Manuscript review, data analysis and presentation, critical editing.

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Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethics approval and consent to participate

The seaweeds *Gracilaria salicornia* and *Sargassum swartzii* used in this study were procured from local fishermen in Tamil Nadu, India, who harvest seaweeds from natural habitats for in accordance with the guidelines and regulations of the Govt. of India and Local Administration. The collected seaweed samples were taxonomically identified by Prof. Rengasamy (retired Professor, University of Madras, India). Voucher specimens of *Gracilaria salicornia* (MKU-SBS-HER-1168, dated 09.04.2023) and *Sargassum swartzii* (MKU-SBS-HER-1169, dated 09.04.2023) have been deposited in the Department of Plant Science, Madurai Kamaraj University, Madurai, India.

Consent for publication

Yes.

Competing interests

The authors declare no competing interests.

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