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RESEARCH ARTICLE

Exploring the potential of seaweed extract in paddy seed presoaking: A pathway to improve crop performance

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Abstract

Paddy (*Oryza sativa* L.) is a globally important staple crop, and achieving high yield is closely linked to effective seed treatments. In this study, seeds of Improved Kavuni CO 57 were treated with seaweed extracts (SE) from *Sargassum myricocystum* (brown algae) and *Kappaphycus alvarezii* (red algae) at various concentrations to assess their impact on seed performance. The treated seeds were evaluated for physiological and biochemical improvements. Notably, seeds soaked in a 0.5% methanol extract of *Sargassum myricocystum* (T6) showed significant improvements compared to the control group, including a higher germination rate (94%), increased root length (19.51 cm), enhanced shoot length (9.29 cm), higher dry matter production (0.155 g/seedling), and a marked increase in seedling vigor index (2707). Biochemical analysis revealed significant enhancements in enzyme activities, with α-amylase (2.41 mg maltose min^{-1}), catalase (3.15 µmol H_2O_2 reduced min^{-1} g^{-1}), and peroxidase (0.332 moles tetra guaiacol min^{-1} g^{-1}) all exhibiting higher levels in treated seeds. Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified key secondary metabolites in the treated seeds, with hexadecanoic acid (21.14%) and octadecanoic acid (10.86%) as dominant compounds. These compounds, known for their antimicrobial, antiviral, antibacterial, and antifungal properties, suggest enhanced resilience in the treated plants. Overall, the findings highlight the potential of SE as a sustainable alternative to conventional seed treatments, offering a promising approach for enhancing crop growth and yield in organic and sustainable agricultural systems.

Keywords

improved kavuni CO 57; *Kappaphycus alvarezii*; paddy; presoaking; seaweed; *Sargassum myricocystum*

Introduction

Paddy (*Oryza sativa* L.) is one of the world's most important staple crops and remains a key source of food for more than half of the global population (1). Organic produce, particularly organic rice, has experienced unprecedented demand in recent years due to increasing consumer awareness (2). Organic rice production emphasizes the use of natural inputs and ecological farming approaches, which contribute to improved soil health and biodiversity (3). In this context, organic seed production for paddy holds significant potential, enabling farmers to operate sustainably while improving the quality of their output.

Quality seeds are essential for maximizing agricultural production and yield, particularly in organic farming systems, which often limit or prohibit the use of conventional inputs. Organic seed production requires careful selection of high-quality seeds and practices that enhance genetic diversity, pest resistance, and local adaptation. These factors are critical in developing resilient crops capable of withstanding both biotic and abiotic stresses, which is particularly important for yields in organic systems that depend on biodiversity and natural regulatory mechanisms (4, 5).

Presoaking technology is an advanced agronomic technique used to improve seed germination and crop establishment. It involves submerging seeds in water or nutrient solutions prior to sowing, which induces physiological changes that promote earlier and more predictable germination (6). Presoaking offers numerous benefits for many crops, including rice, especially in terms of enhancing seed viability and increasing resilience to environmental stresses (7).

The advantage of presoaking seeds is that it improves the seed's water absorption capacity (8). Water absorption activates the necessary metabolic processes that must occur before germination, such as the mobilization of stored carbohydrates and proteins (9). Experiments have demonstrated that this process reduces the germination time, leading to faster seedling establishment. This is particularly crucial in paddy cultivation, where timely germination allows plants to take advantage of favorable weather conditions, enabling them to grow to their full potential and thereby increasing yields (10).

SE derived from marine algae (brown, red, and green) contain bioactive substances, including hormones (auxins, cytokinins, abscisic acid, and gibberellins), vitamins, and minerals, all of which can positively influence plant growth and physiological responses (11). Treating seeds with SE can promote seed germination vigour and enhance resistance to environmental stress (12, 13). It has been reported that presowing treatments with SE improve germination percentages, root development, and overall plant robustness, which is especially beneficial for organic paddy cultivation.

Several studies have shown that SE regulate germinating seeds in various physiological ways, such as enhancing water uptake, hormonal signaling, and nutrient absorption (14). These extracts promote early root development, which aids in anchorage and nutrient uptake once the plant is established. The auxins and cytokinins in SE are critical for stimulating cell division and elongation, which ultimately strengthens root and shoot growth (15). Pre-soaking seeds in SE also accelerates water uptake, reducing germination times. This approach enables agro-scientists to promote more balanced and uniform plant distribution, which is essential for maximizing yields. By shortening the germination lag phase, it minimizes competition among seedlings, contributing to healthier crop establishment.

The Improved Kavuni CO 57 variety of paddy represents an innovative advancement in rice cultivation, specifically developed to address the challenges posed by modern agricultural demands. This variety is distinguished by its high yield potential, nutritional benefits, and adaptability to a range of climatic conditions. The Kavuni CO 57 variety is also well-suited for organic farming practices, aligning with the growing global trend toward sustainable and environmentally friendly agriculture (16). Its resilience to various biotic and abiotic stresses enables farmers to reduce their reliance on synthetic agrochemicals, fostering a more sustainable food production system. This adaptability not only benefits the ecosystem but also aids organic farmers in maintaining soil health and biodiversity.

This study examines the effects of presoaking paddy seeds of the improved Kavuni variety with SE derived from two species, using water and methanol as solvents at different concentrations. By applying seaweed extract treatments prior to sowing, the study seeks to explore their potential in enhancing the physiological and biochemical performance of rice seedlings. Physiological parameters such as germination percentage and seedling vigor were assessed, alongside key biochemical markers.

The selection of *Sargassum myricocystum* (brown algae) and *Kappaphycus alvarezii* (red algae) was based on their known bioactive properties and previous research indicating their potential in promoting plant growth. *Sargassum myricocystum* is recognized for its rich composition of growth-promoting compounds such as alginates, fucoidans, and phytohormones, which are beneficial for enhancing seed vigor and resilience. *Kappaphycus alvarezii*, on the other hand, is valued for its high carrageenan content and various micronutrients, which contribute to improved germination rates, root development, and stress tolerance in plants.

Furthermore, metabolite profiling was performed using GC-MS to gain deeper insight into the metabolic changes induced by seaweed extract treatments. GC-MS provides a comprehensive analysis of small molecules, enabling the identification of potential metabolites that may contribute to enhanced seed performance (17).

In this context, the present study aims to standardize presoaking techniques to improve seed germination and seedling vigor in the Improved Kavuni CO 57 variety. This study addresses a specific research gap by investigating the effectiveness of presoaking paddy seeds, conducting both physiological and biochemical analyses, and identifying metabolic compounds in presoaked seeds compared to non-soaked seeds. Paddy serves as an ideal model for this research due to its agricultural significance and the need to improve its germination rates and overall crop performance. The findings from this research will contribute to sustainable farming practices and provide valuable insights into enhancing paddy cultivation through presoaking.

Materials and Methods

Seed materials and seaweed

A total of 2 kg of Improved Kavuni CO 57 paddy seeds were collected from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore. The laboratory experiment was conducted at the Department of Seed Science and Technology, Agricultural College and Research Institute, TNAU, Madurai, during the 2023-24 period. The seaweeds *Sargassum myricocystum* (brown algae) and *Kappaphycus alvarezii* (red algae) were collected from the Mandapam coast in Ramanathapuram, Tamil Nadu.

Preparation of seaweed extract

The seaweed was initially washed with seawater to remove macroscopic epiphytes and sand particles, followed by a rinse with fresh water to eliminate any adhering salt. It was then shade-dried for one day, sun-dried for one week, and finally oven-dried (Model: Binder FD 115, Company: Binder GmbH, Germany) at 50°C for 24 hours. The dried seaweed was ground using a Willey mill, and the pulverized powder was sieved through a 0.25 mm mesh to obtain a fine powder.

For the preparation of SE, 10 g of the seaweed powder was homogenized with 100 ml of a solvent mixture (water and methanol, 1:1) in a conical flask. The flask was kept at ambient temperature, and the homogenized material was placed in a water bath at 60°C for 1 hour. Afterward, the mixture was centrifuged (Eppendorf 5804, Company: Eppendorf AG, Germany) at 3000 rpm for 20 minutes. The supernatant was then collected as the seaweed extract (18).

Experimental Design

In this experiment, the seeds were subjected to presoaking in seaweed extract at different concentrations for 28 hours. The treatment details are as follows : T_0-Control (Without soaking) it's an absolute control, T_1- Seeds soaked with water, T_2- Seeds soaked with 5 % *Sargassum myricocystum* water extract, T_3- Seeds soaked with 7.5 % *Sargassum myricocystum* water extract, T_4- Seeds soaked with 10 % *Sargassum myricocystum* water extract, T_5 - Seeds soaked with 0.25 % *Sargassum myricocystum* methanol extract, T_6- Seeds soaked with 0.5 % *Sargassum myricocystum* methanol extract, T_7- Seeds soaked with 0.75 % *Sargassum myricocystum* methanol extract, T_8 - Seeds soaked with 5 % *Kappaphycus alvarezii* water extract, T_9- Seeds soaked with 7.5 % *Kappaphycus alvarezii* water extract, T_{10}- Seeds soaked with 10 % *Kappaphycus alvarezii* water extract, T_{11}- Seeds soaked with 0.25 % *Kappaphycus alvarezii* methanol extract, T_{12}- Seeds soaked with 0.5 % *Kappaphycus alvarezii* methanol extract, T_{13}- Seeds soaked with 0.75 % *Kappaphycus alvarezii* water extract. SE at higher concentrations were found to affect seed quality parameters.

Assessment of seed physiological quality parameters

A standard germination test was conducted using the roll towel method, following ISTA guidelines (19). Four sets of 100 seeds were used in this experiment. The seeds were placed on moistened germination paper and incubated in a germinator set at 25 ± 2°C with a relative humidity of 95 ± 2%. After 14 days, the germination percentage was assessed and expressed as a percentage (20).

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100 \qquad \text{……..(Eqn. 1)}$$

The average root and shoot lengths were measured in cm to evaluate seedling growth.

To determine the dry weight, ten healthy seedlings, selected for measuring root and shoot lengths, were placed in paper covers and air-dried in the shade for 24 hours. They were then dried in a hot air oven at 85 ± 1°C for an additional 24 hours. The average weight was recorded in grams per ten seedlings.Additionally, the seedling vigour index was calculated, and the mean values were presented as whole numbers (21).

$$\text{Vigour index I} = \text{Germination (\%)} \times \text{Total seedling length (cm)} \qquad \text{……..(Eqn. 2)}$$

$$\text{Vigour index II} = \text{germination (\%)} \times \text{Dry matter production (g per 10 seedlings)} \qquad \text{……..(Eqn. 3)}$$

Biochemical analysis during seed germination

Biochemical assays such as α -amylase, catalase and peroxidase were carried out by (22-24)

α -amylase (mg maltose min^{-1})

The fresh and aged seeds were pre-germinated using the top-of-paper method and allowed to undergo radicle emergence. In this method, 500 mg of pre-germinated seeds were homogenized in 1.8 mL of ice-cold 0.02 M sodium phosphate buffer (pH 6.0). The homogenate was then centrifuged at 20,000 rpm for 20 minutes to collect the extract. Subsequently, 1 mL of a 0.0067% starch solution was added to the extract and incubated for 10 minutes at 25°C. The reaction was terminated by adding 1 mL of iodine-HCl solution containing 60 mg KI and 6 mg I_2 dissolved in 100 mL of 0.05 N HCl. The color change was observed at 620 nm, and the enzyme activity was calculated and expressed as milligrams of maltose per minute (22).

Catalase (µmol of reduced H_2O_2 g^{-1} min^{-1})

The enzyme extract was prepared by finely grinding 0.5 g of pre-germinated seed sample with 5 mL of ice-cold 50 mM phosphate buffer (pH 7.0). The homogenized sample was then centrifuged at 15,000 rpm for 20 minutes at 4°C. The collected supernatant was used as the enzyme extract. To 0.3 mL of enzyme extract, 1.5 mL of 50 mM potassium phosphate buffer and 1.2 mL of 12.5 mM hydrogen peroxide were added. The mixture was then incubated for 10-15 minutes. Subsequently, the optical density (OD) values at 240 nm were measured every 15 seconds for 1 minute using a UV-Vis spectrophotometer. Based on the reduction of H_2O_2, catalase activity was calculated and compared with known concentrations of hydrogen peroxide using a standard curve. The enzyme activity was calculated as the amount of H_2O_2 reduced (initial reading – final reading = quantity of H_2O_2 reduced) and expressed as µmol of reduced H_2O_2 per gram per minute (23).

Peroxidase (m mol tetra guaiacol min^{-1} g^{-1})

A 0.5 g sample of pre-germinated seeds was homogenized with 1.5 mL of 60 mM phosphate buffer using a pestle and mortar. The sample was then centrifuged at 10,000 rpm for 10 minutes at 4°C. To 0.1 mL of enzyme extract, 0.5 mL of 1% hydrogen peroxide and 0.5 mL of 96 mM guaiacol were added. The mixture was then diluted with 0.4 mL of water to make a final volume of 3 mL and incubated at 25°C for 10 minutes. The optical density (OD) value was recorded at 470 nm using a UV-Vis spectrophotometer, based on the coefficient of oxidized tetra-guaiacol. Peroxidase activity was calculated from the absorbance and expressed as mmol of tetra-guaiacol per minute per gram (24).

$$\text{Peroxidase activity} = \text{Difference in OD value} \;/\; 10 \text{ min} \times 1000 \;/500 \times 60 \quad \text{……..(Eqn. 4)}$$

Identification of metabolic compounds through GC-MS analysis

Paddy seeds were presoaked with 0.5% *Sargassum myricocystum* methanol extract (T6) (the best treatment) and water-soaked seeds (T2) were used for GC-MS analysis. The extraction protocol for polar compounds was modified and carried out as follows: 50 mg of seed material was ground using a grinder. For primary metabolite profiling, solute extraction was performed with 400 µL of methanol (–20°C), containing 200 nmol of cis-inositol as an internal standard. The mixture was processed in a thermomixer (Grant Instruments) at 70°C for 10 minutes at 950 rpm. Subsequently, 200 µL of chloroform was added, and the solution was shaken for another 5 minutes at 70°C and 950 rpm. Finally, 400 µL of ultra-pure water was added, followed by vortexing for 20 seconds and centrifugation for 10 minutes at 7400×g. A 50 µL aliquot of the methanol supernatant was dried in a speed vacuum for subsequent GC-MS analysis (25).

The samples were subjected to GC-MS analysis using an Agilent 7890A Gas Chromatograph (GC) and an Agilent 5975C Mass Spectrometer (MS) (Agilent Technologies, USA), widely used for analyzing complex chemical mixtures and detecting volatile and semi-volatile compounds with high sensitivity. It is commonly applied in environmental testing, food safety, and forensic analysis.

A 1 µL aliquot of the reaction mixture was injected directly into the GC-MS system. The operating conditions were as follows: the initial temperature was set to 80°C for 1 minute, then raised to 250°C at a rate of 8°C per minute, followed by a further increase to 300°C at 12°C per minute, where it was held for 5 minutes. The total run time for the GC was 30 minutes, and the injector temperature was maintained at 240°C.

Statistical design

The observed data were recorded and subjected to ANOVA at a 5% level of significance. The percentage values were transformed to arc-sine values before analysis. The Critical Difference (CD) was calculated at both 1% and 5% probability levels and tested for statistical significance. Graphs were generated using Microsoft Excel (2019). The data were further analyzed using Principal Component Analysis (PCA) in R software.

Results

Efficacy of seaweed extract on seedling growth parameters

The results demonstrated that seeds presoaked with SE exhibited significant improvements ($P < 0.05$) in seedling attributes, such as germination percentage, root and shoot lengths (cm), and vigour indices I and II, compared to untreated seeds. The most pronounced increase in germination (94%) was observed in seeds presoaked in 0.5% *Sargassum myricocystum* methanol extract (T6) for 28 hours, followed by T5 (92%) compared to the control (Table 1) (Fig. 1). Furthermore, seeds presoaked with 0.5% *Sargassum myricocystum* methanol extract (T6) showed substantial improvements in root length (19.51 cm), shoot length (9.29 cm), dry matter production (0.155 g per 10 seedlings), and seedling vigour index (2707). Statistically significant differences in germination and seedling vigour were observed between presoaked and untreated seeds. Overall, the presoaking treatments increased germination rates and seedling vigour relative to the control. However, higher concentrations of soaking agents tended to negatively affect germination and seed quality parameters.

Effects of presoaking on biochemical changes during germination

The activities of α-amylase (mg maltose min^{-1}), catalase (µmol H_2O_2 reduced $min^{-1}g^{-1}$), and peroxidase (mmoles of tetra-guaiacol $min^{-1}g^{-1}$) in paddy seeds were significantly influenced by the presoaking treatments with SE, in comparison to untreated seeds. Seeds soaked in 0.5% *Sargassum myricocystum* methanol extract (T6) for 28 hours exhibited the highest α-amylase activity at 2.41 mg maltose min^{-1}, followed by T5 (0.25% *Sargassum myricocystum* methanol extract for 28 hours) at 2.38 mg maltose min^{-1}, while non-soaked seeds showed only 2.15 mg maltose min^{-1} (Fig. 2A). This increase in α-amylase activity was associated with higher germination rates. Catalase activity also increased due to the nutrient content in the extracts, with T6 recording 3.15 µmol H_2O_2 reduced $min^{-1}g^{-1}$, followed by T5 at 3.12 µmol H_2O_2 reduced $min^{-1}g^{-1}$, and the lowest value in non-soaked seeds at 2.88 µmol H_2O_2 reduced $min^{-1}g^{-1}$ (Fig. 2B). Peroxidase levels were similarly higher in T6 (0.332 mmoles of tetra-guaiacol $min^{-1}g^{-1}$), with T5 close behind at 0.328 mmoles of tetra-guaiacol $min^{-1}g^{-1}$, com-

Table 1. Effect of seed presoaking on physiological parameters of Improved kavuni CO 57.

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings^{-1})	Vigour index I	Vigour index II
T_0	74	16.74	7.54	0.130	1797	9.6
T_1	74	16.83	7.67	0.131	1813	9.7
T_2	84	17.66	8.21	0.140	2173	11.8
T_3	88	18.44	8.54	0.144	2374	12.7
T_4	76	17.03	7.84	0.134	1890	10.2
T_5	92	19.12	9.16	0.151	2602	13.9
T_6	94	19.51	9.29	0.155	2707	14.6
T_7	82	17.49	8.05	0.138	2094	11.3
T_8	84	17.54	8.16	0.138	2159	11.6
T_9	86	18.35	8.34	0.141	2295	12.1
T_{10}	80	17.19	7.95	0.136	2011	10.9
T_{11}	90	18.48	8.70	0.144	2446	13.0
T_{12}	90	18.87	8.92	0.148	2501	13.3
T_{13}	80	17.38	8.02	0.136	2032	10.9
SEd	1.792	0.424	0.157	0.003	36.637	0.185
CD (0.05)	3.276	0.868	0.322	0.007	75.032	0.378

T_0-Control (Without soaking) **T_1**- Seeds soaked with water **T_2**- Seeds soaked with 5 % *Sargassum myricocystum* water extract **T_3**- Seeds soaked with 7.5 % *Sargassum myricocystum* water extract **T_4**- Seeds soaked with 10 % *Sargassum myricocystum* water extract **T_5**- Seeds soaked with 0.25 % *Sargassum myricocystum* methanol extract **T_6**- Seeds soaked with 0.5 % *Sargassum myricocystum* methanol extract **T_7**- Seeds soaked with 0.75 % *Sargassum myricocystum* methanol extract **T_8**- Seeds soaked with 5 % *Kappaphycus alvarezii* water extract **T_9**- Seeds soaked with 7.5 % *Kappaphycus alvarezii* water extract **T_{10}**- Seeds soaked with 10 % *Kappaphycus alvarezii* water extract **T_{11}**- Seeds soaked with 0.25 % *Kappaphycus alvarezii* methanol extract **T_{12}**- Seeds soaked with 0.5 % *Kappaphycus alvarezii* methanol extract **T_{13}**- Seeds soaked with 0.75 % *Kappaphycus alvarezii* water extract.

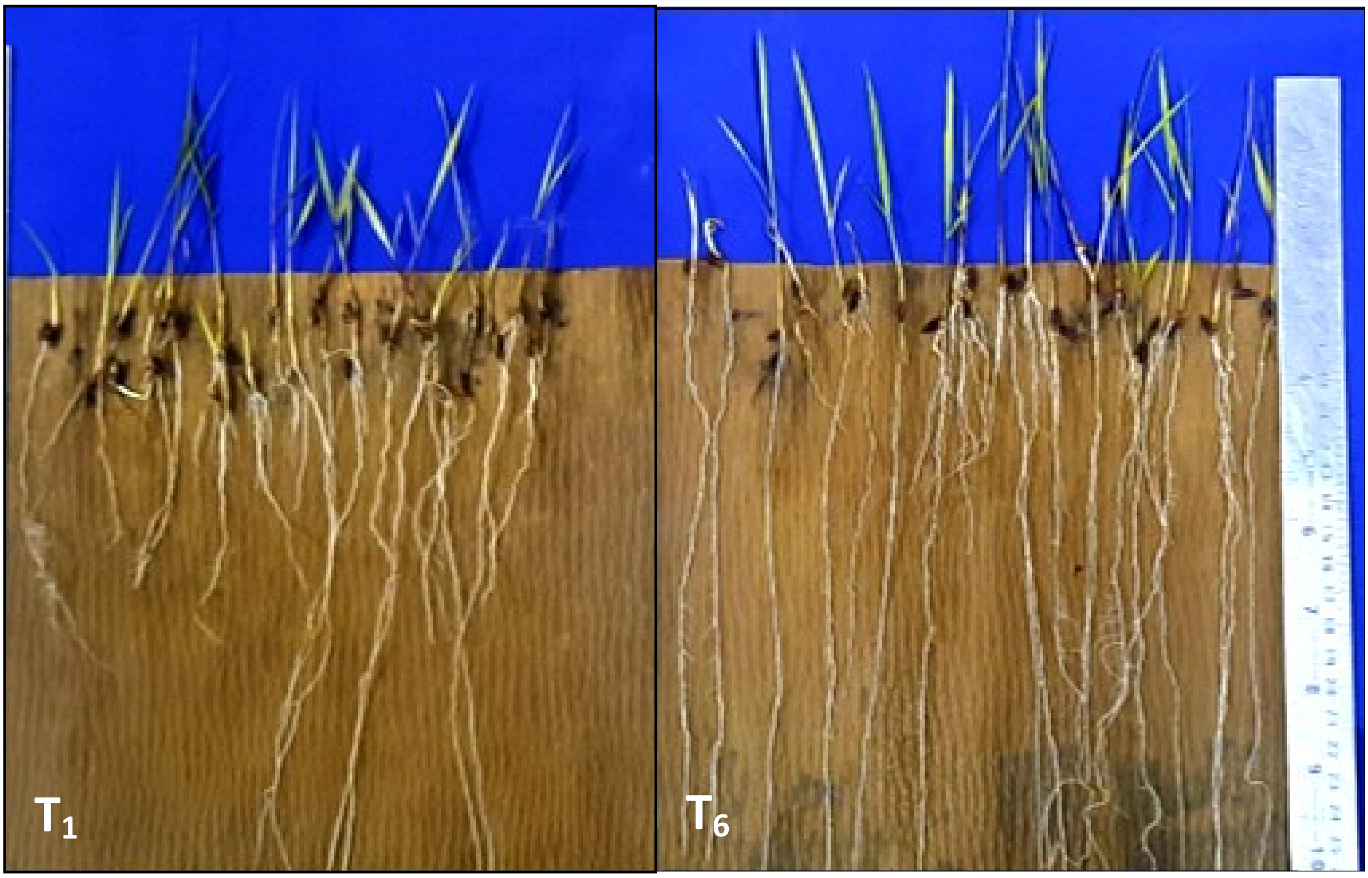

Fig. 1. Effect of seed presoaking on germination and seedling vigour of improved Kavuni CO 57.

pared to 0.308 mmoles of tetra-guaiacol min^{-1}g^{-1} in untreated seeds (Fig. 2C). A strong positive correlation was found between germination percentage and α-amylase activity (Fig. 2D).

GC-MS analyses of metabolite compounds in presoaked seeds

GC-MS analysis of metabolite compounds from seeds presoaked in 0.5% *Sargassum myricocystum* methanol ex-

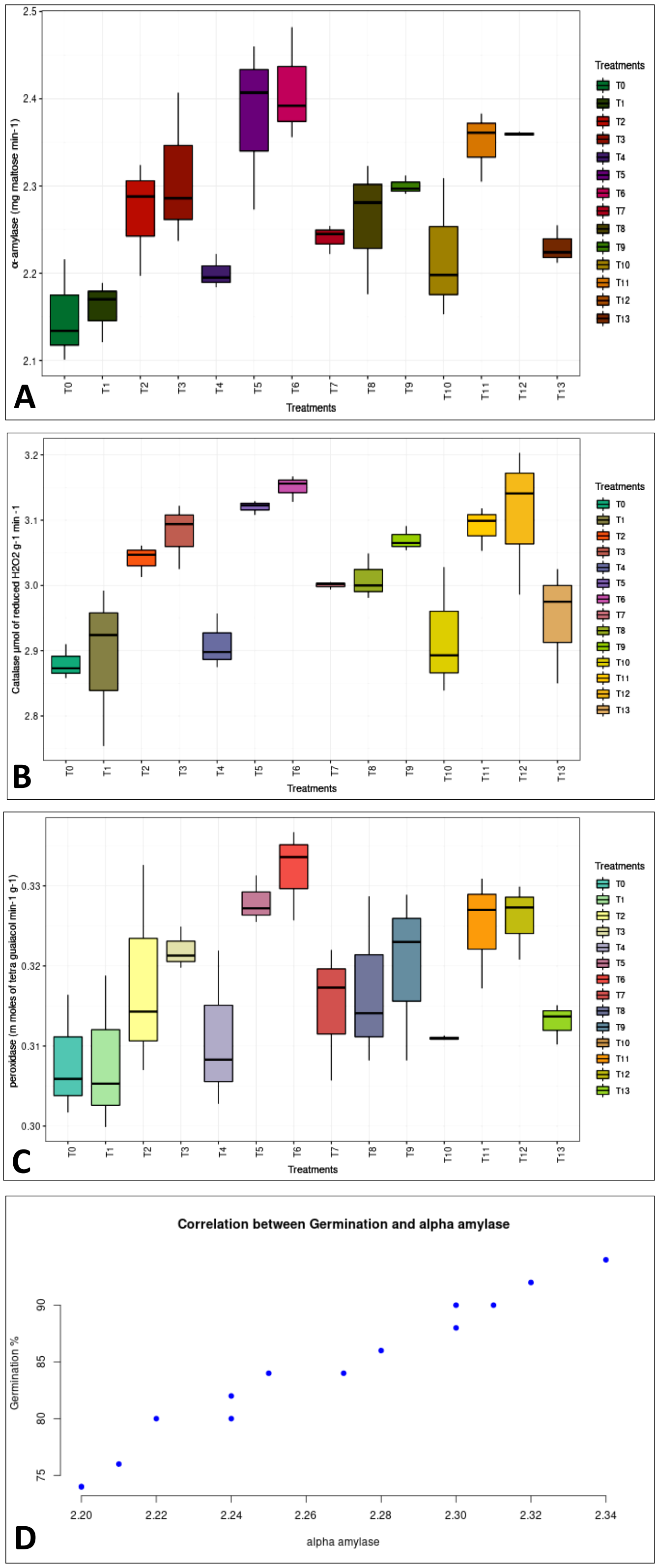

Fig. 2. The effect of seed presoaking on (**A**) α-amylase (**B**) catalase (**C**) peroxi-

tract (T6) revealed distinct differences in the composition of metabolite blends released by the seaweed extract compared to those released by the water-soaked control.

Sixty compounds identified from the water-soaked seeds included 2-Cyclopenten-1-one, 2-hydroxy; oxirane; oxalic acid, monoamide; n-propyl tetradecyl ester; 2-propenoic acid, 3-(4-methoxyphenyl); hexadecanoic acid, methyl ester; n-hexadecanoic acid; and benzene. These identified compounds exhibit antimicrobial, antiviral, antibacterial, and antifungal activities. The primary chemical constituents were hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, with a peak area of 21.14% and a retention time of 16.507 minutes, and octadecanoic acid, methyl ester, with a peak area of 10.86% and a retention time of 14.285 minutes (Fig. 3).

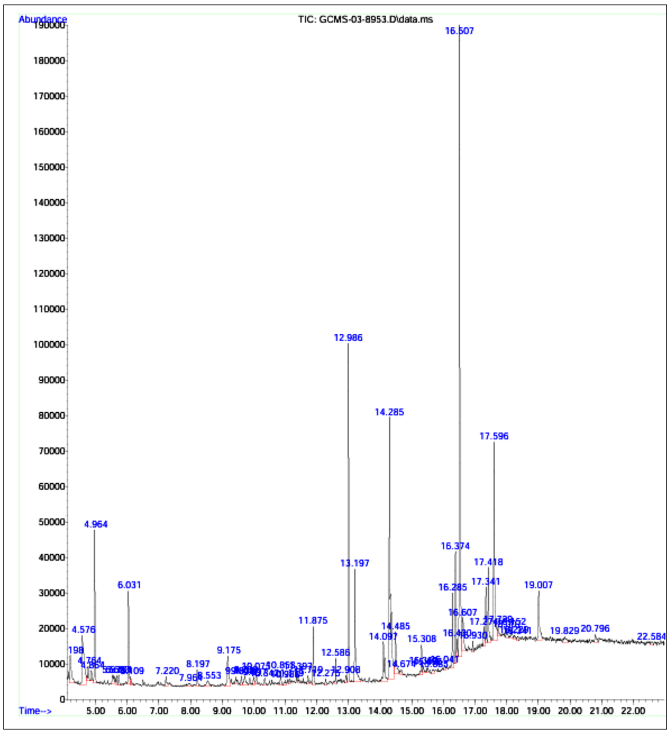

Fig. 3. GC-MS chromatogram of metabolite profiling for water-soaked seeds of the improved kavuni CO 57.

In contrast, 60 chemical constituents were identified from paddy seeds presoaked with the seaweed extract, including decane, 2-pyrrolidine methanol, rhodopin, acetamide, hexanedioic acid, 1,2-benzene dicarboxylic acid, bis (2-methylpropyl) and mono (2-ethylhexyl) ester, campesterol, stigmasterol, and gamma-sitosterol. The

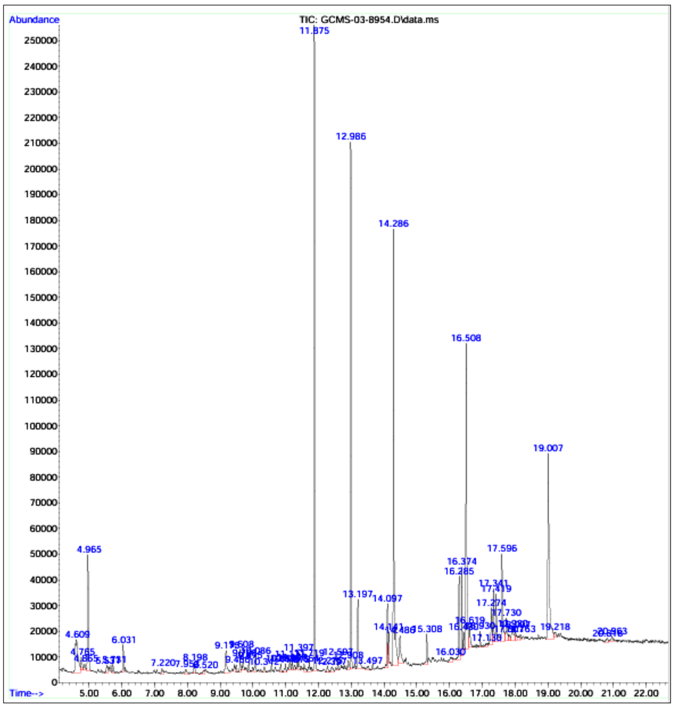

Fig. 4. GC-MS chromatogram of metabolite profiling for 0.5 % *Sargassum myricocystum* methanol extract-soaked seeds of the improved kavuni CO 57.

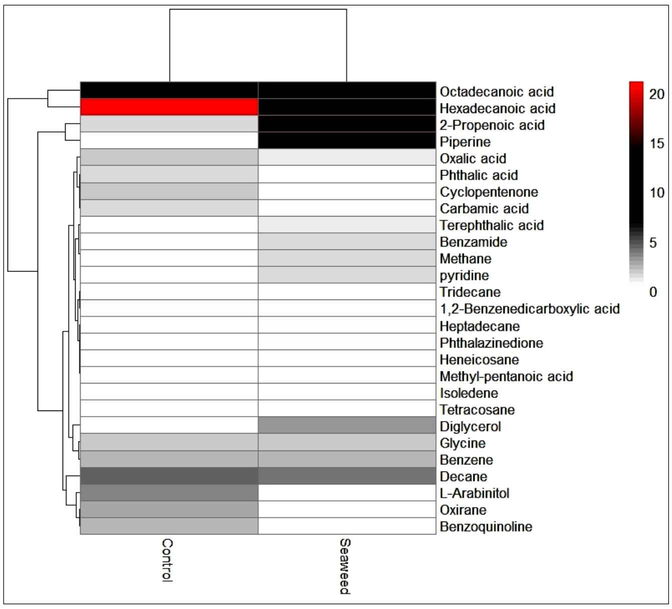

Fig. 5. Heatmap visualization - the colour gradient from yellow to blue represents the range of metabolite expression levels, with yellow indicating lower levels and blue indicating higher levels. Treatments are **T_2** - control (Water-soaked seeds); **T_6** - seed presoaked with 0.5 % Sargassum *myricocystum* methanol extract.

identified compounds possess antimicrobial, antiviral, antibacterial, antifungal, antioxidant, and insecticidal activities. The primary chemical constituents were 2-propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester, with a peak area of 13.51% and a retention time of 11.875 minutes, and 2-propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester, with a peak area of 12.13% and a retention time of 12.986 minutes (Fig. 4). Additionally, heatmap analysis revealed significant differences in metabolite expression patterns between the two groups (Fig. 5).

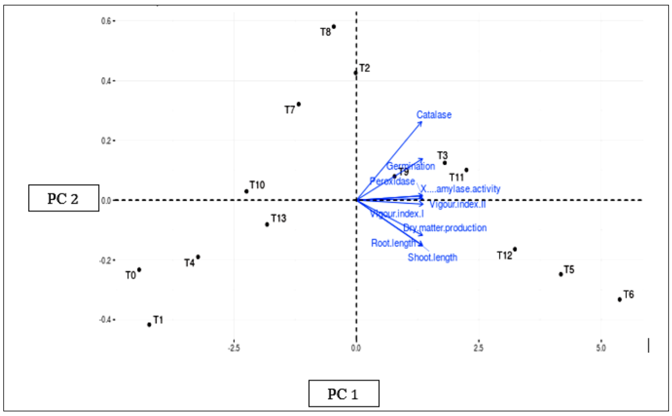

Fig. 6. The principal component analysis shows treatments' impact on variables such as seed quality and biochemical parameters of improved kavuni CO 57.

Principal component analysis

Principal Component Analysis (PCA) was used to examine the relationships among the measured seedling growth parameters (Fig. 6). This statistical method is particularly useful for simplifying datasets with many correlated variables by reducing them to a smaller number of principal components. In this analysis, the first principal component (PC1) accounts for 98.2% of the total variance, while the second principal component (PC2) captures 0.9%. Together, PC1 and PC2 explain 99.1% of the variance in the data.

Germination, α-amylase, catalase, and peroxidase showed strong positive contributions to both PC1 and PC2, indicating that these factors played a major role in the variability captured by these components. Additionally, vigor indices I and II, root length, shoot length, and dry matter production exhibited weaker associations with PC1 but still contributed to the overall variation.

The points labeled T0, T1–T13 represent different treatment groups, positioned according to their similarity in variable response. Treatment T2, located at the top near the germination vector, suggests a high germination rate and a positive association with catalase activity.

Treatments T6 and T5, positioned further down and to the right, exhibit a distinctive response pattern with a stronger influence from variables like root length and shoot length.

Treatment T1, positioned in the bottom left quadrant, is negatively associated with most variables shown in the PCA plot, indicating it may have lower overall performance for the measured parameters.

This PCA visualization helps identify which treatments (T6, T5) may be optimal for specific growth parameters. Treatments that align closely with growth-promoting variables, such as seedling vigour index or germination rate, may be favourable for enhancing overall seedling development. Conversely, treatments with distinct separations, like T1, could indicate unique or less effective responses, warranting further investigation or exclusion from recommended practices.

Discussion

Seaweeds, or macroalgae, are multicellular marine organisms that play a crucial role in coastal marine ecosystems. They are classified into three primary groups based on their pigmentation: Phaeophyta (brown), Rhodophyta (red), and Chlorophyta (green). Extracts obtained from these seaweeds are rich in a variety of bioactive compounds (26), including polysaccharides, pigments, phenolic compounds, proteins and bioactive peptides, phytohormones, as well as both micro- and macronutrients (26–29). Numerous studies have highlighted the potential benefits of using SE as biostimulants under both normal and stressed environmental conditions (26–30).

SE are abundant in phytohormones, sterols such as fucosterol, carbohydrates, polysaccharides, sugars, polyphenols (including flavonoids), macro- and micronutrients, vitamins, lipids, amino acids, and proteins, including enzymes (26–30).

Soluble alginates and protein hydrolysates derived from seaweeds have been shown to enhance the aggregation of soil particles, thereby improving nutrient availability, aeration, and water retention in the soil (26–31). Beyond the direct benefits to plants, SE positively influence the soil microbiome. Studies indicate that microbes can absorb free amino acids more effectively than plants; in some crops, only 6% to 25% of the flagged amino acids were taken up by the roots, with the remainder absorbed by soil microorganisms (32).

Various phenolic compounds have been identified in seaweeds, with brown seaweeds primarily containing phlorotannins, while red and green species are richer in bromophenols, flavonoids, and phenolic acids (33). Phlorotannins, in particular, contain a higher number of phenolic rings compared to other phenolic compounds, which is associated with enhanced antioxidant activity (34).

Micronutrients are also present in seaweed products, whether in their fresh, dried, or extracted forms (35). Another group of bioactive molecules found in SE is plant hormones. The hormonal composition of seaweeds is similar to that of terrestrial plants. Although the mechanisms of action for these hormones in seaweeds are not fully understood, various phytohormones, including bioactive forms of auxins, cytokinins (CK), abscisic acid (ABA), and gibberellins, have been identified (36). Additionally, ethylene, brassinosteroids, salicylic acid, jasmonates (JA), and strigolactones have also been detected in SE (28).

The compounds present in SE may function as signaling molecules that regulate key pathways at both the transcriptional and post-translational levels (via microRNAs), leading to the differential expression of essential genes in crops. This regulation can enhance plant growth by affecting genes related to cell metabolism, including those involved in lipid, amino acid, and nucleotide metabolism, glycolysis, and transport, as well as cell and cell wall development (37).

SE have shown significant positive effects on crop growth, yield, and quality in various studies. Foliar application of SE has enhanced growth parameters and yield components in different field crops (38). In bean plants, lower concentrations of *Fucus spiralis* and *Ulva rigida* extracts improved shoot and root length, chlorophyll content, and protein levels (39). Similarly, wheat plants irrigated with *Ascophyllum nodosum* extract exhibited increased height, dry mass, and spike number. SE contain multiple growth regulators, as well as macro- and micronutrients essential for plant development (40). These biostimulants can serve as an eco-friendly alternative to inorganic fertilizers, promoting early seed germination, improving crop performance, and enhancing resistance to biotic and abiotic stresses (38–40). However, high concentrations of SE may have negative effects on plant growth (39).

In the present study, presoaked seeds (T6) showed improvements in average quality parameters compared to the control. These findings align with earlier studies, which indicated that enhancing shoot and root length in bean plants led to significant improvements in seed germination, seedling growth, and biochemical parameters (39).

Seeds treated with seaweed nanopowder showed an increase in germination percentage in pigeon pea (41). In soybean, the application of seaweed extract at a 15% concentration resulted in taller plants compared to the control group (42). Similarly, foliar spraying of 0.4% SE led to increased plant height in green gram (43).

A combination of Kappaphycus sap at 10% with the recommended dose of fertilizer (RDF) significantly increased the leaf area index in maize compared to the control group (44). In rice, soil application of seaweed extract gel at 12.5 kg/ha, combined with a foliar spray of 0.5% seaweed extract at the tillering and panicle initiation stages, resulted in a higher leaf area index (45).

In green gram, applying 15% Kappaphycus sap along with the recommended fertilizer dose led to higher dry matter production (46), while seeds treated with seaweed nanopowder produced greater dry matter in pigeon pea (41). The use of biostimulants, such as Algex derived from *Ascophyllum nodosum*, significantly enhanced dry matter production in red clover compared to untreated plants (47).

SE have demonstrated positive effects on crop growth and biochemical parameters when applied at optimal concentrations. Studies on various crops, including wheat, beans, and legumes, have shown enhanced growth metrics such as shoot and root length, as well as increased dry weight (48). Biochemical parameters, such as chlorophyll content, protein levels, and enzyme activities, were also improved. Notably, catalase activity increased with higher seaweed extract concentrations in wheat (48), while peroxidase activity was enhanced at moderate concentrations. Alpha-amylase activity increased in cowpea treated with *Ulva lactuca* extract. The optimal concentration for most beneficial effects was generally found to be between 3-10% of seaweed extract, with higher concentrations sometimes showing inhibitory effects (49). These studies suggest that SE can serve as effective biofertilizers, promoting crop growth and enhancing biochemical parameters.

In rice, soil application of seaweed extract gel at 12.5 kg/ha, combined with foliar spraying of 0.5% extract at critical growth stages (tillering and panicle initiation), resulted in a significantly higher 1000-grain weight (45). Furthermore, the same treatment led to a substantial improvement in the harvest index, reaching 44%. Additionally, the application of seaweed extract gel at 25 kg/ha increased the uptake of nutrients such as nitrogen, phosphorus, potassium, iron, zinc, copper, and manganese in rice compared to the control group (45).

Based on GC-MS analysis results, 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester plays a significant role in seed germination and plant growth by acting as a growth promoter and disease inhibitor. Research indicates that derivatives of 4-methoxyphenyl compounds enhance germination energy and biometric parameters in various seeds, suggesting their effectiveness in improving seed viability and growth parameters (50). Additionally, compounds such as 2-(4-methoxyphenyl) propionic acid can stimulate stem growth while inhibiting root development, optimizing the edible yield of sprouts. Furthermore, these compounds exhibit potential in preventing plant diseases by inhibiting phytopathogen virulence without adversely affecting plant growth. Overall, integrating such compounds into agricultural practices could lead to improved crop yields and healthier plants (51).

Octadecanoic acid, methyl ester, is a significant compound found in various plant sources, including seaweeds. Research indicates that seaweed species like *Cladophora rupestris* contain novel octadecadienoic fatty acids, which can be analyzed through oxidative ozonolysis techniques to identify their structures (52). Furthermore, octadecanoic acid, methyl ester has demonstrated antiviral properties, particularly when combined with ribavirin against the measles virus, showing enhanced efficacy at lower concentrations (53). Additionally, the photo-oxidation of lipids in dried seaweed can influence the volatile compounds produced, which may include derivatives of octadecanoic acid. Overall, the incorporation of octadecanoic acid, methyl ester in seaweed extract presoaked seeds presents potential for both nutritional and therapeutic applications.

Conclusion

The study demonstrated that presoaking seeds with seaweed extract significantly enhanced seed growth parameters in Improved Kavuni CO 57. Seeds soaked in a 0.5% methanol extract of *Sargassum myricocystum* for 28 hours exhibited higher germination rates, longer root and shoot lengths, and a greater seedling vigor index compared to untreated seeds. Biochemical analysis revealed elevated levels of α-amylase, catalase, peroxidase, and other key enzymes in the treated seeds. These results suggest that seaweed extract serves as an effective and eco-friendly alternative to chemical fertilizers, promoting sustainable agriculture while improving crop quality and yield. Further research should explore the long-term impacts of seaweed presoaking on overall crop yield and investigate the method's applicability across different crops. Such studies would help validate these findings and provide valuable insights for broader agricultural applications.

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Authors' contributions

RE carried out the experiments and prepared the original draft of the writing. KS Conceptualization. AA and TS supervised the work and drafted and reviewed the manuscript. AG participated in the sequence alignment and editing. MLM visualization. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

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

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