



RESEARCH ARTICLE

Unleashing the power of seaweed biostimulants to improve seedling vigour in green gram (*Vigna radiata*)

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Abstract

Seaweed extracts enhance plant growth and yield by improving physiological and biochemical traits in plants. This study was conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, to evaluate the effects of seaweed derived liquid extracts (SLEs) derived from *Ulva lactuca*, *Kappaphycus alvarezii* and *Sargassum wightii* on seedling vigour in green gram (*Vigna radiata*). Seeds were treated with 2 % and 5 % SLEs, prepared as aqueous and alkaline extracts and various parameters associated with seed germination and seedling growth were assessed. Among the treatments, 2 % SLEs from *Ulva lactuca* and *Kappaphycus alvarezii* were effective in terms of higher germination percentage, reduced mean germination time, improved germination index and superior seedling vigour. In addition, SLE treated seeds displayed improved root and shoot growth, with higher fresh and dry weights, indicating the positive effects of the seaweed extracts. Further, 5 % aqueous and alkaline extracts treated seeds showed reduced growth compared to the 2 % SLEs. These findings underscore the importance of optimized seaweed biostimulants in enhancing early seedling growth of green gram, advancing sustainable agricultural practices with reduced reliance on chemical fertilizers. Subsequently, the most effective treatments were selected for further experiments involving seedlings treated with the same seaweed species and additional combinations. Among these, 3 % SLE combinations of both aqueous and alkaline extracts and 2 % aqueous SLEs particularly from *Kappaphycus alvarezii* and *Ulva lactuca*, demonstrated improved seedling establishment.

Keywords: biofertilizer; concentration; green gram; SLEs; seaweed; vigour

Introduction

Agricultural intensification mostly depends on inorganic fertilizers to increase crop production and meet rising global food demand. However, over a time an accelerated use of inorganic fertilizers, is devastating the environment viz, soil, water, climate and increased pest outbreaks (1). Many studies were conducted to improve productivity through organic inputs without compromising environmental safety and global bodies including UNEP, FAO and World Bank demanded a profound perspective change in the growth of agriculture and promote the expansion of agroecological knowledge and application (2). One such strategy is "green farming" which has developed as a viable alternative that prioritizes environmental health while maintaining agricultural output, tackling present agricultural difficulties concurrently. In this context, incorporating biostimulants, a substance, organism, or organic byproduct used to boost plant or crop quality, yield, stress tolerance and protective qualities is regarded promising approach (3). Numerous categories of biostimulants, seaweed extracts derived from marine macroalgae (4) which contain auxins, gibberellins, cytokinins, trace minerals and antioxidants

(5) are used for improving plant productivity in biotic and abiotic stresses (6). Seaweed-based bioproducts are increasingly used in crop production systems due to their unique bioactive components and phytostimulatory properties that promote plant growth and yield in a different crop species including pulses (7). The response of crops to biostimulants has been encouraging in terms of improvement of yield and quality especially in pulses.

Pulses, including mung beans are vital source of proteins and essential amino acids and play a crucial role in enhancing soil fertility through biological nitrogen fixation in symbiosis with *Rhizobium* bacteria (8). Mung beans, in particular, offer substantial nutritional benefits, providing large amount of protein, fat and dietary fibre and an essential component of both human nutrition and sustainable agricultural practices (3). Mung bean is primarily cultivated as a kharif crop across Bihar, Uttar Pradesh, Madhya Pradesh and various regions of peninsular India, under resource-limited and challenging environments, often susceptible to drought and various biotic and abiotic stresses (9). Renowned for their exceptional virtues and numerous benefits, pulses are

constitute part towards attaining agenda for International Year of Pulses 2030 (10). The use of biostimulants in pulses is paramount to enhance productivity and Nutrient use efficiency (11). Crop productivity is largely influenced by the successful establishment of seedlings, which in turn depends on seedling vigour (12). Precise regulation of seedling vigour, germination timing and seedling establishment is crucial for uniform plant development, directly influencing marketable yield potential and maximizing profitability (12). With the increasing emphasis on sustainable farming practices, this study explores the role of biostimulants in improving the seedling establishment and growth of mung bean. This study aimed to examine the effect of seaweed-derived liquid extracts on seed germination and physiological traits associated with seedling growth of green gram.

Material and Methods

Extraction of liquid seaweed extracts

Three types of seaweed viz., *Ulva lactuca* (green algae), *Sargassum wightii* (brown algae) and *Kappaphycus alvarezii* (red algae) were collected from Mandapam, Ramanathapuram district, Tamil Nadu. The samples were transferred to the laboratory in paper bags, dried in an oven at 60 °C for 72 hrs and processed into a fine powder with an electric grinder. For each sample, 20 g of powdered seaweed was swirled constantly with 1 L of distilled water or 1N KOH for 15 min and placed in a water bath at 90 °C for 3 hrs. The heated mixtures were filtered through whatman Filter paper 52 filter paper and the extracts were stored as stock solutions. Based on their genus and species, the liquid seaweed extracts were coded as *Kappaphycus alvarezii* (KA), *Sargassum wightii* (SW) and *Ulva lactuca* (UL) and the pH of extracts was adjusted to between 6.2 and 6.5 (Fig. 1).

Measurement of seed germination associated traits

Green gram (*Vigna radiata*) variety Co 6 (used in this experiment was obtained from Department of Pulses, Tamil

Nadu Agricultural University, Coimbatore, Tamil Nadu, India. A total of 100 seeds per treatment was used with a completely randomized block design. Before treating with seaweed derived liquid extracts (SLEs), green gram seeds were sterilized with 80 % ethanol solution for 10 min, followed by rinsing with sterile distilled water. Two blotting sheets per petri dish were placed (Fig. 2). The treated seeds were placed on blotting paper within sterile 90mm petri plates. Each plate received 5 mL of either distilled water (control) or SLEs at varying concentrations (2.0 % and 5.0 %). The detailed treatment list is provided in Table 2. The dishes were incubated at 25 ± 1 °C with a 16 hrs light and 8 hrs dark cycle. Germination was considered complete when the radicle reached greater than 2 mm. The parameters such as mean germination percentage (GP), germination index (GI), germination rate index (GRI), seedling vigour index (SVI), plumule length, radicle length, total seedling length and seedling dry weight were measured as per standard calculations (Table. 1). The effect of SLEs on seedling development and germination was assessed after 12 days.

Assessing seedling growth traits under greenhouse condition

Green gram seedlings were grown in a controlled greenhouse condition at 25 °C with 16 hrs light and 8 hrs dark period in portraits filled with perlite enriched soil mix (Fig. 3). The plants were divided into 13 treatments and 6 replications. These treatments contained both individual and blended SLEs at lower doses, which were chosen based on the most effective treatments from previous experiment. The detailed treatment structure is provided in Table 3. After sowing, seeds were watered daily for the first 12 days, based on water requirements until germination. From the 13th day onwards, the treatments were provided with 10 mL of seaweed derived liquid extracts (SLEs) and control 10 mL of distilled water on weekly interval. The plants were grown for 30 days in the greenhouse at $\sim 25 \pm 2$ °C. Morphological traits such as leaf area, fresh and dry weight were measured. Physiological parameters such as chlorophyll content using SPAD, NDVI, photosynthetic efficiency (Fv/Fm) and quantum yield were recorded using fluorescence meter.



Fig. 1. Extracted seaweed based liquid extracts from various seaweed species.

Seaweed-based biostimulants following extraction, prepared at 2 % and 5 % concentrations. The extracts were derived from three seaweed species: *Kappaphycus alvarezii*, *Sargassum wightii* and *Ulva lactuca*

Table 1. K Formulas used to evaluate seed germination and growth

Parameters	Formula	Reference
Germination Percentage (GP)	$GP = \frac{\text{Total no of seeds}}{\text{No. of germinated seeds}} \times 100$	(19)
Germination Index (GI)	$GI = \frac{\sum Gt}{Tt}$ Gt - No. of germinated seeds on a specific day (day t) Tt - total duration of the germination period in days.	(20)
Seedling Vigour Index (SVI)	$SVI = (\text{Seedling length in cm} \times \text{Germination percentage})$	(21)

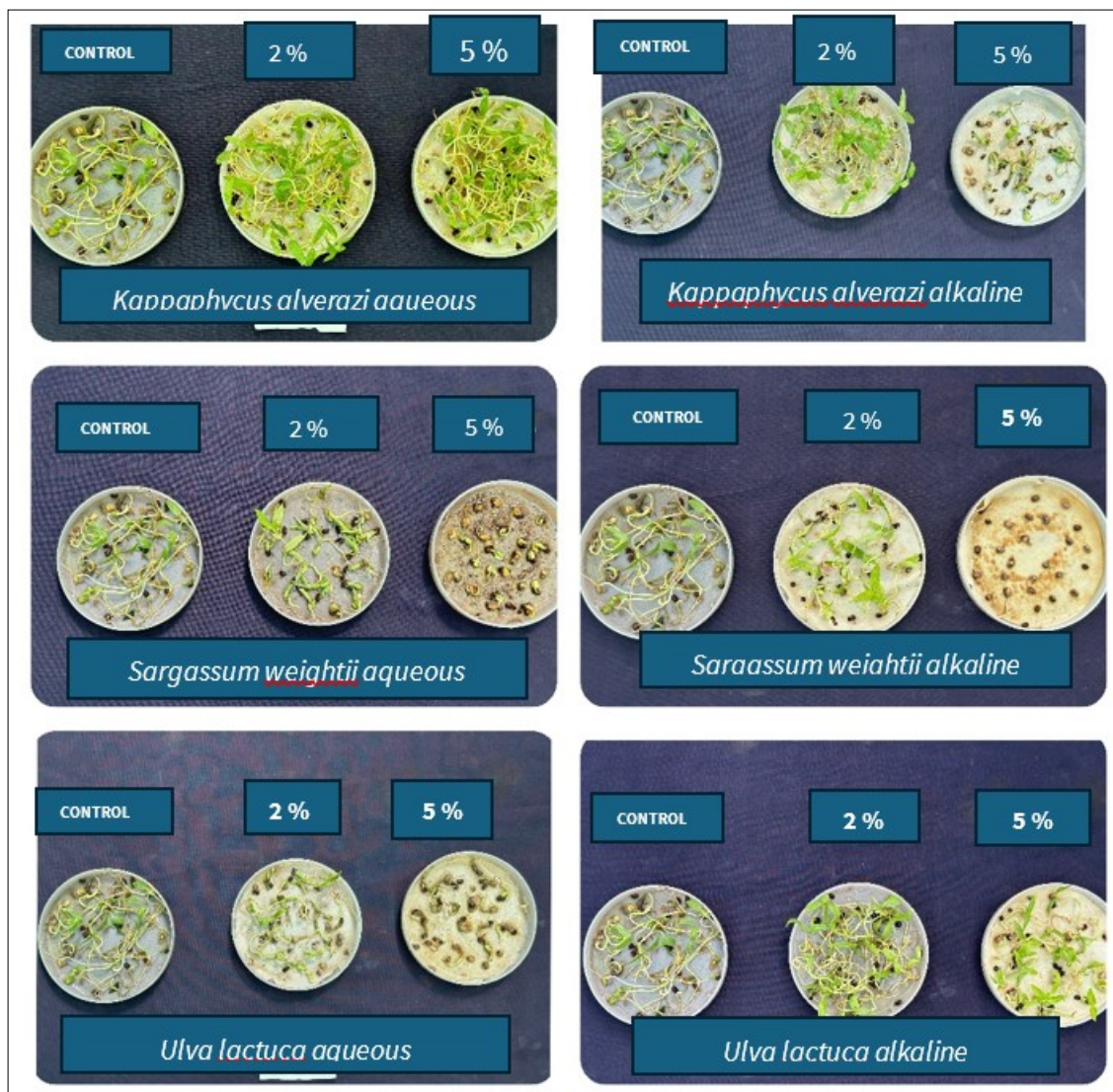


Fig. 2. Green gram germination assessment set up.

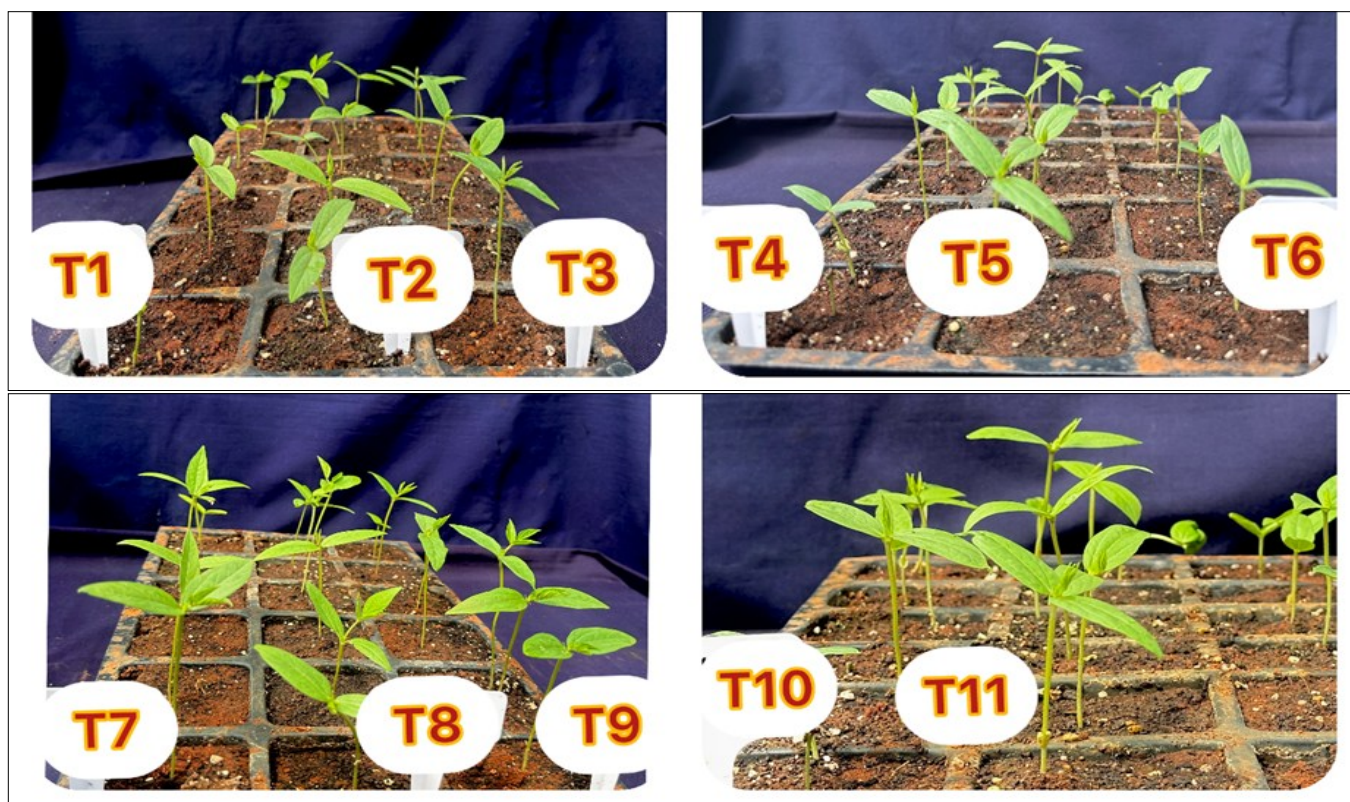


Fig. 3. Visual representation of portray set up of green gram plants.

Table 2. Treatments followed for the germination assessment using seaweed derived liquid extract (SLE)

Treatment code	Description of treatment
T ₁	: 2 % aqueous <i>Kappaphycus alvarezii</i>
T ₂	: 5 % aqueous <i>Kappaphycus alvarezii</i>
T ₃	: 2 % alkaline <i>Kappaphycus alvarezii</i>
T ₄	: 5 % alkaline <i>Kappaphycus alvarezii</i>
T ₅	: 2 % aqueous <i>Sargassum wightii</i>
T ₆	: 5 % aqueous <i>Sargassum wightii</i>
T ₇	: 2 % alkaline <i>Sargassum wightii</i>
T ₈	: 5 % alkaline <i>Sargassum wightii</i>
T ₉	: 2 % aqueous <i>Ulva lactuca</i>
T ₁₀	: 5 % aqueous <i>Ulva lactuca</i>
T ₁₁	: 2 % alkaline <i>Ulva lactuca</i>
T ₁₂	: 5 % alkaline <i>Ulva lactuca</i>
T ₁₃	: Control

Table 3. Treatments followed for the greenhouse assessment using seaweed derived liquid extract (SLE)

Treatment code	Description of treatment
T ₁	: Control
T ₂	: 2 % aqueous <i>Kappaphycus alvarezii</i>
T ₃	: 2 % aqueous <i>Sargassum wightii</i>
T ₄	: 2 % aqueous <i>Ulva lactuca</i>
T ₅	: 2 % alkaline <i>Kappaphycus alvarezii</i>
T ₆	: 2 % alkaline <i>Sargassum wightii</i>
T ₇	: 2 % alkaline <i>Ulva lactuca</i>
T ₈	: 3 % aqueous combination
T ₉	: 3 % alkaline combination
T ₁₀	: 2 % aqueous combination
T ₁₁	: 2 % alkaline combination

The morphological traits such as Specific Leaf Area (SLA), Specific Leaf Weight (SLW), leaf dry weight were recorded. The first treatment served as control, with plants cultivated without any SLEs. For the other treatments, each experimental unit received either distilled water (control) or SLEs. The study followed a completely randomized design.

Statistical analysis

The data were examined for significant differences using analysis of variance (ANOVA) and the mean separation was performed using the least significant difference (LSD) method. In both the studies, one-way ANOVA at 5 % significance differences is followed. All statistical analyses were performed using the Statistical package SPSS.

Results and Discussion

Seaweed-based biostimulants are becoming an important component of crop production due to their potential to enhance growth and productivity through improvements in key physiological and morphological traits. These extracts, derived from diverse seaweed species, offer an eco-friendly and sustainable alternative to conventional growth promoters, playing a pivotal role in improving plant resilience, growth and

yield. These compounds target critical parameters such as chlorophyll content, photosynthetic efficiency, canopy development and address fundamental aspects of plant health, leading to enhanced growth under various conditions (13). In the context of green gram, the application of these seaweed-based extracts reveals improvements in specific traits that collectively promote plant growth.

The first experiment investigated the impact of SLEs on the germination and seedling growth of green gram. Results demonstrated that SLE treatments, particularly at lower concentrations, significantly enhanced germination traits such as germination percentage and germination index while reducing mean germination time (Fig. 7) (14). In case of GP, aqueous *K. alvarezii* showed 89 % (Fig. 2), which was markedly higher than the control (54-72 %). Similarly, *U. lactuca* extracts showed GP values ranging between 70-88 %, while *S. wightii* had slightly lower GP (~72 %) however, better than the control (~69 %). The GRI was notably higher for the 2 % treatments, with *K. alvarezii* between 8600-8800, implying faster and more uniform germination (Fig. 4) compared to the control (7000-7500). SVI, *K. alvarezii* and *U. lactuca* extracts exhibited robust performances ranging from 1093 to 2728 (Fig. 5), reflecting enhanced early growth and seedling development, while *S. wightii* showed moderate SVI improvements. Seedling lengths were significantly higher for 2 % treatments, with *K. alvarezii* producing lengths of 1.8-6.1 cm and *U. lactuca* achieving lengths of 4.4-7.4 cm, compared to 3.3-4.0 cm in the control. Survival rates were consistently superior, ranging from ~74-89 % across 2 % treatments, whereas the control showed lower rates 33-72 % (Fig. 8).

Furthermore, the promptness index indicated better synchronization of germination in the 2 % treatments compared to the control (Fig. 7). The 2 % seaweed extract treatments provided substantial enhancements in germination percentage, seedling vigour (Fig. 5) and growth parameters, while 5 % treatments are slightly less effective, which may be due to the interference of these compounds in the chlorophyll biosynthesis enzymes production. The second experiment conducted in portrays, assessed various treatments with different combinations of seaweed derived liquid extracts to evaluate their effects on key physiological parameters viz., chlorophyll content, photosynthetic efficiency (Fv/Fm), leaf area, leaf dry weight and morphological traits such as Specific Leaf Area (SLA), Specific Leaf Weight (SLW). The data reveals insights into the overall performance of each treatment. The leaf area represents the canopy's capacity to intercept sunlight, a key driver of photosynthesis and energy generation. Additionally, the earlier research provides strong evidence that tracking the changes in Leaf Area (LA) values is crucial for predicting crop yields and gaining insights into the overall growth patterns of crops (15). Treatments that boost LA ensures that the plant has a larger photosynthetic surface area, which directly enhances energy capture and utilization. Similarly, the leaf dry weight (Fig. 6) reflects the plant's ability to convert absorbed light into biomass, a critical indicator of overall vigour and structural development (16).

Chlorophyll content, directly correlates with photosynthetic activity and energy production, while Fv/Fm is the maximum quantum efficiency of photosystem II, which

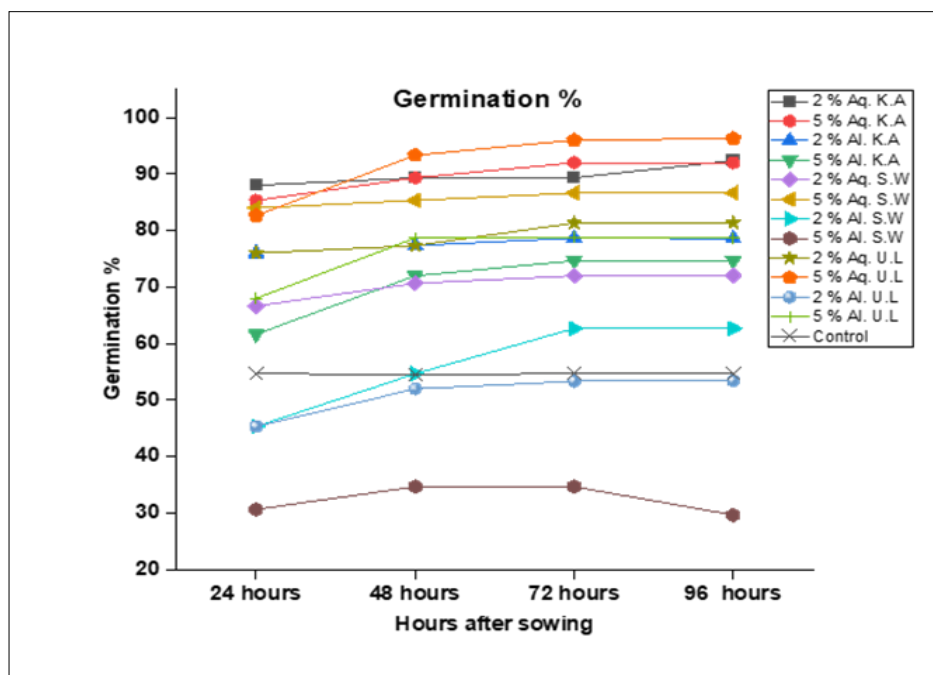


Fig. 4. Impact of different SLEs on hourly germination percentage.

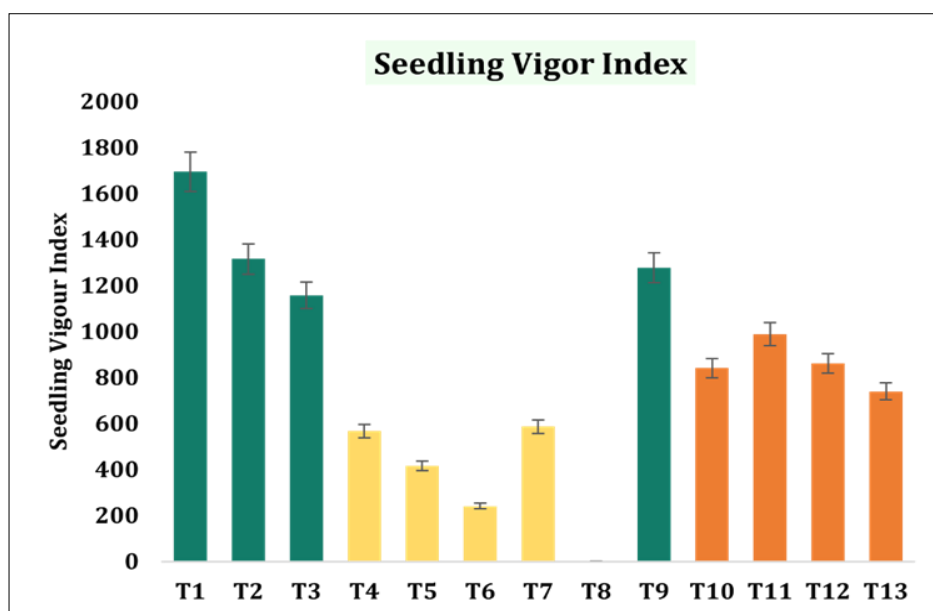


Fig. 5. Impact of different SLEs on seedling vigor index.

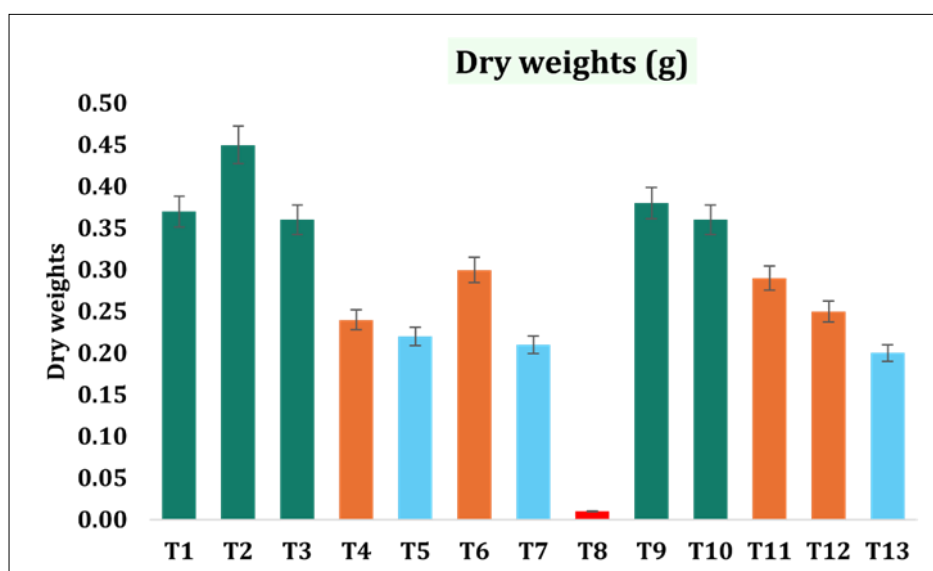


Fig. 6. Impact of different SLEs on dry weight.

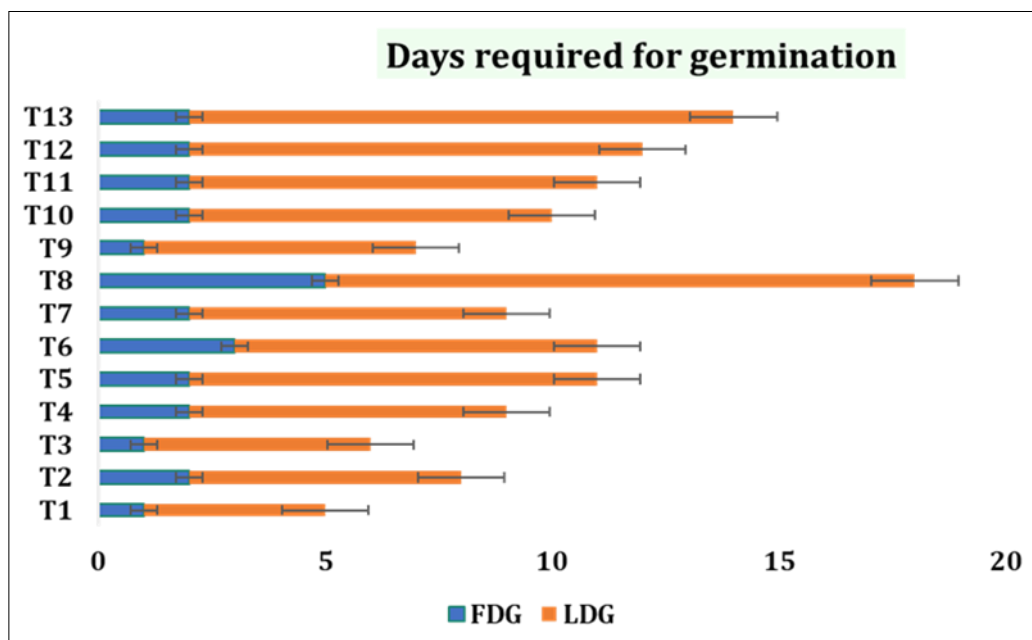


Fig. 7. Impact of different SLEs on time taken for first and last germination.

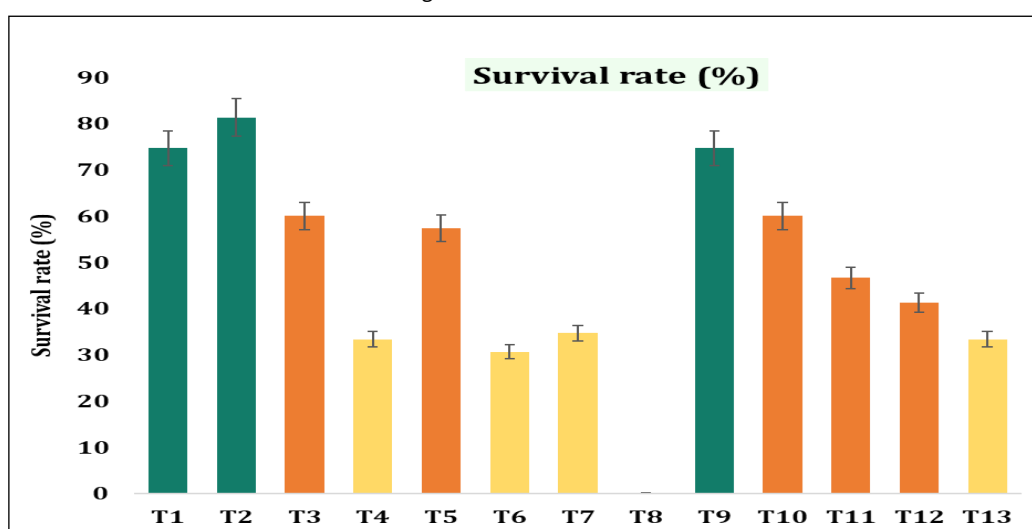


Fig. 8. Impact of different SLEs on survival of seedlings.

signifies the plant's ability to sustain efficient photosynthesis under both optimal and stress conditions (17). Therefore, these traits contribute to a healthier and more productive plant, reflected in parameters viz., Normalized Difference Vegetation Index (NDVI), indicating overall vegetation health and canopy greenness, similar to the earlier studies (18), these improvements promote to a plant with a robust structure, increased photosynthetic capacity and better stress resilience. The aqueous formulations appear to be more effective than alkaline extracts, likely due to better absorption and assimilation by the plant. The aqueous 2 % *K. alvarezii* treatment (SLW: 3.6 mg/cm², LA: 28.19, SLA 164.28 cm²/g) shown in Table 5.

Superior performance in terms of leaf dry weight reflecting efficient resource utilization and high biomass partitioning. Additionally, exhibited favourable photosynthetic efficiency, as indicated by SPAD values (Table 4.), suggesting a robust growth response. Similarly, the Aqueous 2 % *U. lactuca* treatment (Table 5). LA: 27.16, SLW: 3.9 mg/cm², SLA: 215.61 cm²/g, with slightly lower leaf dry weight, photosynthetic activity and LA, indicating efficient biomass accumulation and effective use of available resources. The aqueous 3 %

combination SLEs, SLW: 3.9 g/cm², LA: 35.08, SLA: 146.79 cm²/g and alkaline 3 % combination SLEs, SLW: 4.5 mg/cm², LA: 43.71, SLA: 106.88 cm²/g (Table 5.) treatments exhibited moderate leaf area development, with their respective photosynthetic efficiency suggesting areas for improvement. Although these treatments did not reach the highest performance levels, they still demonstrated favourable performance compared to other treatments, indicating potential for enhanced efficacy under optimized conditions.

The aqueous 2 % *K. alvarezii* formulation is the most effective for promoting root growth, showing high seedling vigour and efficient resource use, including SLW and SLA. For shoot growth, the alkaline 3 % combination SLEs performed best, with the highest leaf area and strong canopy development. However, the aqueous 2 % *K. alvarezii* also showed excellent results for shoot growth while being eco-friendly. Overall, the aqueous 2 % *K. alvarezii* is the best formulation, supporting both strong root development and shoot growth, making it an ideal choice for balanced and sustainable plant growth.

Table 4. Influence of SLEs on physiological parameters of green gram in greenhouse study

Treatments	SPAD	Fv/Fm	NDVI
Alkaline 2 % Combination	30.450 ± 4.687	0.811 ± 0.020	0.417 ± 0.100
Alkaline 3 % Combination	35.267 ± 2.556	0.794 ± 0.030	0.423 ± 0.174
Alkaline 2 % <i>kappaphycus alverazi</i>	30.000 ± 3.389	0.781 ± 0.021	0.492 ± 0.080
Alkaline 2 % <i>Sargassum weightii</i>	30.167 ± 8.082	0.816 ± 0.009	0.350 ± 0.103
Alkaline 2 % <i>Ulva lactuca</i>	27.633 ± 5.768	0.822 ± 0.003	0.393 ± 0.088
Aqueous 2 % Combination	30.550 ± 4.641	0.814 ± 0.025	0.497 ± 0.075
Aqueous 3 % Combination	26.533 ± 3.817	0.788 ± 0.010	0.515 ± 0.090
Aqueous 2 % <i>Kappaphycus alverazi</i>	30.083 ± 4.178	0.784 ± 0.038	0.553 ± 0.018
Aqueous 2 % <i>Sargassum weightii</i>	31.433 ± 7.108	0.822 ± 0.004	0.438 ± 0.111
Aqueous <i>Ulva lactuca</i>	26.200 ± 5.582	0.891 ± 0.029	0.480 ± 0.101
Control	28.583 ± 5.474	0.848 ± 0.009	0.447 ± 0.109

Fv/Fm - Fluorescence; NDVI - Normalized difference vegetation index

Table 5. Effect of SLEs on SLA, SLW and LA in greenhouse study

Treatments	LA	SLW (mg/cm ²)	SLA (cm ² /g)
Aqueous 2 % <i>Kappaphycus alverazi</i>	28.19	3.6	164.28
Aqueous <i>Ulva lactuca</i>	27.16	3.9	215.61
Aqueous 3 % combination	35.08	3.9	146.79
Alkaline 3 % combination	43.71	4.5	106.88
Alkaline 2 % <i>Kappaphycus alverazi</i>	34.06	3.5	236.54
Alkaline 2 % <i>Sargassum weightii</i>	12.39	5.7	167.47
Alkaline 2 % <i>Ulva Lactuca</i>	12.72	7.2	84.83
Aqueous 2 % combination	12.97	5.0	106.38
Alkaline 2 % combination	19.86	4.2	73.74
Aqueous 2 % <i>Sargassum weightii</i>	21.66	4.6	80.55
Control	12.00	2.5	44.67

LA - Leaf Area; SLW - Specific Leaf Weight; SLA - Specific Leaf Area

Conclusion

The study highlights the remarkable potential of seaweed-based extracts in promoting sustainable crop production. The 2 % *K. alvarezii* and *U. lactuca* treatments notably improved key parameters such as germination rate, seedling vigour and early growth, outperforming the control. In greenhouse conditions, aqueous treatments shown better in photosynthetic efficiency and leaf dry weight. The superior performance of aqueous 2 % *K. alvarezii* and combination SLEs stresses the importance of selecting the right combination and concentration to maximize plant growth and productivity to improve plant health, making it a promising solution for modern agriculture.

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Authors' contributions

HV carried out the laboratory and greenhouse studies, participated in the sequence alignment, data collection and drafted the manuscript. BP participated in the study proposal, design of the study supervision and manuscript corrections. SR participated in the sequence alignment, manuscript correction and design coordination. MV participated in suggestions for the study design. GS conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

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Ethical issues: None

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

The authors used ChatGPT to improve language clarity and readability. The final content was reviewed and edited to ensure accuracy and the author(s) take full responsibility for the publication.

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