Natural Resources for Human Health

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Original Research

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Received 03 August 2021
Revised 12 August 2021
Accepted 16 August 2021
Available online 16 August 2021

Edited by Barbara Sawicka

KEYWORDS:
seaweed extract
rhizobium
crop improvement
biochemical content
plant biostimulants

Natr Resour Human Health 2021; 1 (1): 43-50
https://doi.org/10.53365/nrfhh/141292
eISSN: 2583-1194

Synergetic effects of seaweed extract and Rhizobium on cowpea

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ABSTRACT: Seaweed extracts have been used as fertilizer for crops to enhance the yield and quality of crop products. Only very few studies have been carried out on the effects of seaweed extracts and beneficial soil microbes on the growth of crop plants. Hence, the present study was conducted to investigate the synergistic effect of seaweed extract (SE) prepared from *Sargassum wightii* Greville, with and without applying Rhizobium biofertilizer, on seed germination, seedling growth, biochemical constituents and yield of cowpea (*Vigna unguiculata* Walp Var. pusa 151 (family Fabaceae). Seaweed extract (1% concentration), with or without Rhizobium (biofertilizer) treatment, compared with the control, significantly ($p \leq 0.05$) enhanced vegetative growth (dry weight of shoot and root, number of lateral roots and total leaf area), biochemical parameters (total chlorophyll, carotenoids, proteins, lipids, total sugar and amino acids) and yield and yield components (pod number, length, weight, number of seeds per pod and 100 seeds weight). Seaweed extract application, along with Rhizobium biofertilizer, exhibited better results in vegetative growth, biochemical and yield than the seaweed extract alone in enhancing the growth and yield of cowpea.

1. INTRODUCTION

Cowpea is one of the legumes cultivated throughout the world for their nutritional, agricultural and economic value and as a source of food for humans and animals. Economic agriculture of legumes is based on the efficient symbiotic nitrogen fixation in their nodules, which depends on the rhizobial populations of the soil (Postgate, 1972). The undesirable effects of inorganic fertilizers on the environment have stimulated studies on new natural resources of fertilizers, bio-stimulants and soil amendments. Seaweed biomass represents a substitute for conventional inorganic fertilizers. The favourable effects of SE applications on seed germination and establishment, enhanced crop response and yield have been investigated in cowpea (Sivasankari et al., 2006), soybean (Rathore et al., 2009) and maize (Rengasamy et al., 2015a, b) and extensively reviewed by Khan et al. (2009) and Craigie (2011). The beneficial outcome of SE application on crop plants is due to various components that may work synergistically at different concentrations. Moreover, SE is readily biodegradable, free from toxins, non-polluting and non-hazardous. Plant growth regulators, such as cytokinins (W. Stirk et al., 2003), auxins (W.A. Stirk et al., 2004), gibberellins (W.A. Stirk et al., 2014), betaines (Blunden, 1991; Whapham et al., 1993), micro and macronutrients, relatively low molecular weight compounds, such as polyamines and brassinosteroids (W.A. Stirk et al., 2004, 2014) and major components polysaccharides (lez et al., 2013), polyphenols (Rengasamy et al., 2015b) essential for the plant growth and development, have been reported from seaweeds.

Our literature survey could ascertain that recent works on the effects of SE on beneficial soil microbes. The beneficial effect of SE on Rhizobium (CP-1 and MB-1 of cowpea miscellaneous group) in culture has been studied (Thevanathan et al., 2005). Ishii et al. (2000) observed that alginate oligosaccharides, obtained from brown seaweed, significantly enhanced AM fungi' hyphal growth and prompted their establishment on trifoliate orange seedlings roots. Extracts of various green, red and brown seaweeds could be used as an AM fungi growth enhancer (Kuwada et al., 1999, 2000, 2006). So far, no study deals with the synergetic effect of seaweed extract and Rhizobium on crop plants, and the present study was initiated to fill the lacuna. Therefore the objective of this study was to determine the effects of a seaweed extract alone or with the application of Rhizobium biofertilizer on the growth, yield and biochemical parameters of *Vigna unguiculata*.

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2. MATERIALS AND METHODS

2.1. Collection of seaweeds

In June 2014, the seaweed [*Sargassum wightii* Greville (Phaeophyceae)] was taken from the Rameswaram shore (9° 25' N and 79° 15' E), Tamil Nadu, India. The fronds were handpicked and cleaned thoroughly by washing with seawater followed by freshwater. They were then transported to the laboratory using an icebox.

2.2. Preparation of seaweed extract

SE was prepared using the approach of Rao (1990). Seaweed that had been washed and cleaned was shade-dried for five days and then oven-dried at 60-65 ^{o}C for 24 hours before being ground coarsely in a mixer-grinder. The powder was soaked in distilled water at a 1: 20 (w/v) ratio and autoclaved for 60 minutes at 121 ^{o}C, 20 pressure. The hot seaweed extract is filtered and cooled with Whatman No.1 filter paper. The filtrate was then centrifuged for 30 minutes at 1500 x g at 4 ^{o}C. It was then kept at 60 °C for 48 hours.

2.3. Selection of crop plant and Rhizobium biofertilizer

Vigna unguiculata (L.) Walp Var. pusa 151 (family Fabaceae), commonly known as cowpea was study plant. Seeds were obtained from Tamil Nadu's Regional Pulses Research Station in Vamban, Pudukottai District. Healthy seeds were separated and stored until further experiments. The Rhizobium (Pon-rhizobium) biofertilizer was obtained from Pasic Biofertilizers, Puducherry (India).

2.4. Experimental procedures and biochemical analysis

The seeds were immersed in different concentrations of SE (0.5, 1, 1.5 , and 2 %) for 24 hours; the control seeds were steeped in distilled water for the same amount of time. The experiment was carried out in earthen pots (30 cm in diameter and 40 cm in height), which were filled with a mixture of sterilised garden soil and sand in a 2:1 (volume/volume) proportion. A total of 10 seeds were sown in each pot, with three pots per concentration remaining after the seeds had been soaked overnight. Two parallel sets were prepared for the two treatments, one set contained the SE-soaked seeds, and the other set included SE-soaked seeds inoculated with Rhizobium biofertilizer (applied as layering on the soil surface). The pots were arranged in three separate units for sampling on different days (viz., 15^{th}, 30^{th} and 45^{th} days) to ensure uniform environmental conditions for the growth of the plants. Pots were watered once every two days. A respective concentration of SE (10 ml) was applied to the rhizosphere at 10-day intervals. A random sample of plants from each treatment was chosen for analysis. The length of shoots and roots, the fresh weight of shoots and roots, the number of lateral roots, the total leaf area, and other characteristics are measured. Plant pigments (Arnon, 1949; Kirk & Allen, 1965) were measured at 15^{th}, 30^{th} and 45^{th} days after the emergence of the 1^{st} set of leaves. Total protein (Lowry et al., 1951), lipids (Folch & Standby, 1956), sugars (Willis & Yemm, 1954) and amino acids (S. Moore & Stein, 1948) and yield parameters were recorded on the 45^{th} day.

2.5. Physico-chemical analyses of SE

The SE color was observed visually, and its pH was measured using an Elico pH meter. Magnesium, iron, zinc, boron, copper, cobalt, calcium, sodium, potassium, manganese, chloride, sulfate, and nitrate contents were analyzed in the extracts following previous method (Apha et al., 1995), using ICP - OES (Perkin Elmer Mayer, Optima 2100 DV, and Flame Photometer – Systronics.

2.6. Statistical analysis

The data were analysed using SPSS 16.1 statistical software and Tukey's HSD multiple comparisons test at a 5% threshold of significance to distinguish treatment means.

3. RESULTS AND DISCUSSION

The *Sargassum wightii* extract was brown, and its pH was 6.6. The elemental compositions for micro and macro elements are presented in **Table 1**.

Table 1
Elemental composition of *Sargassum wightii* extract (g kg^{-1})

Element	Concentration
Copper	0.8
Zinc	1.1
Boron	3.8
Iron	6.9
Sulfate	39.50
Phosphorus	54.20
Nitrate	114.10
Magnesium	167.1
Calcium	170.0
Potassium	310.0
Sodium	490.0
Chloride	1180.0

Seed germination was 100% at 1% and 1.5% SE with or without biofertilizer and was 90% in other concentrations (0.5% and 2%) and control. The SE alone and with biofertilizer at 1% increased the growth parameters than control. (**Figure 1**).

The highest total chlorophyll and carotenoids concentrations were recorded in both the treatments (SE and SE+RB) at 1% seaweed extract on the 30^{th} day (**Figure 2**).

Application of 1% SE alone or rhizobium increased protein, lipids, sugars, and amino acid content significantly ($p \leq 0.05$) over the control (**Figure 3**).

The highest number of pods, number of seeds per pod and seed weight were recorded at 1 % seaweed extract in both the treatments (**Figure 4**).

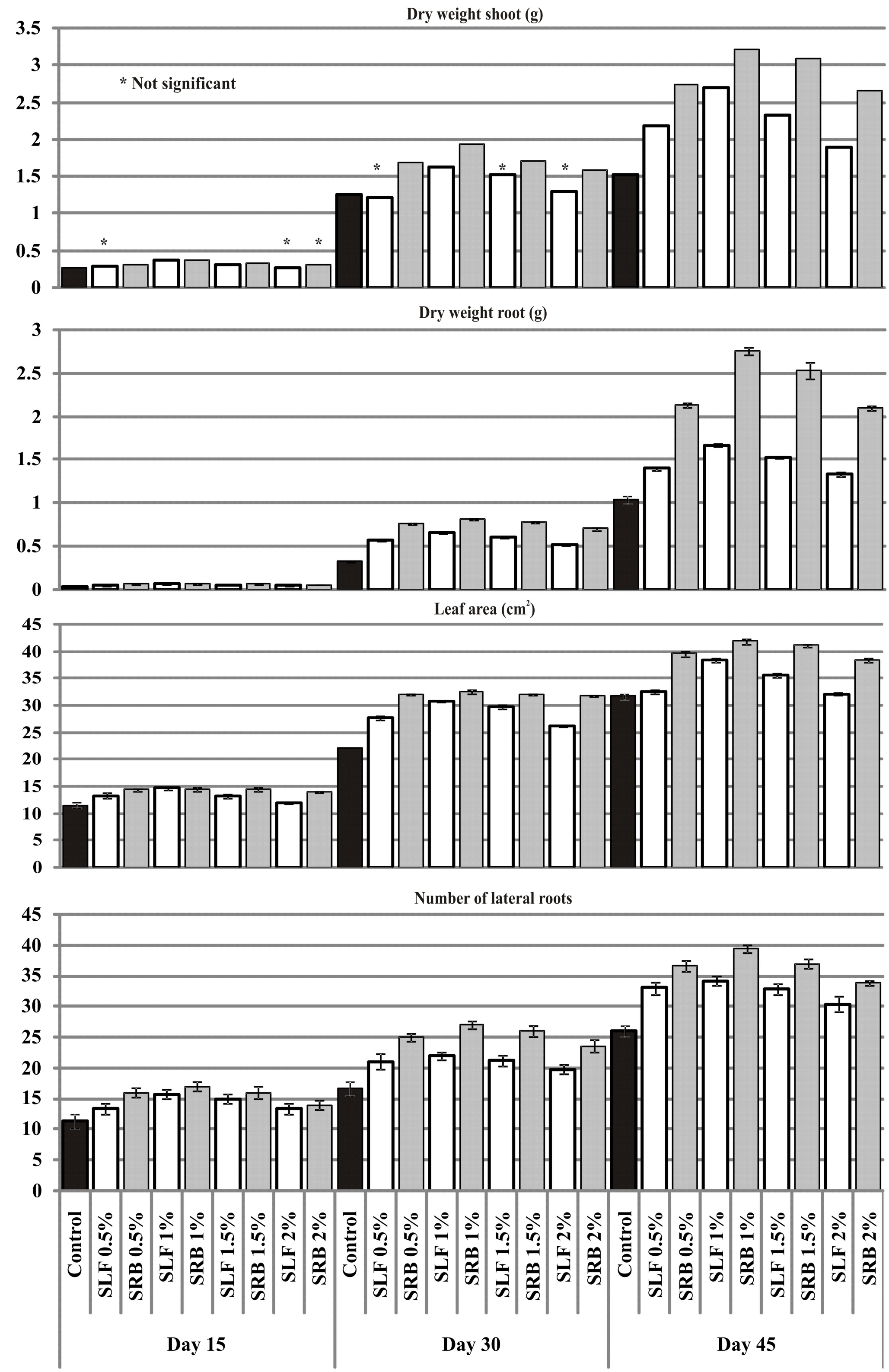

Figure 1. The effect of SE and Rhizobium biofertilizer treatments on growth of V. unguiculata

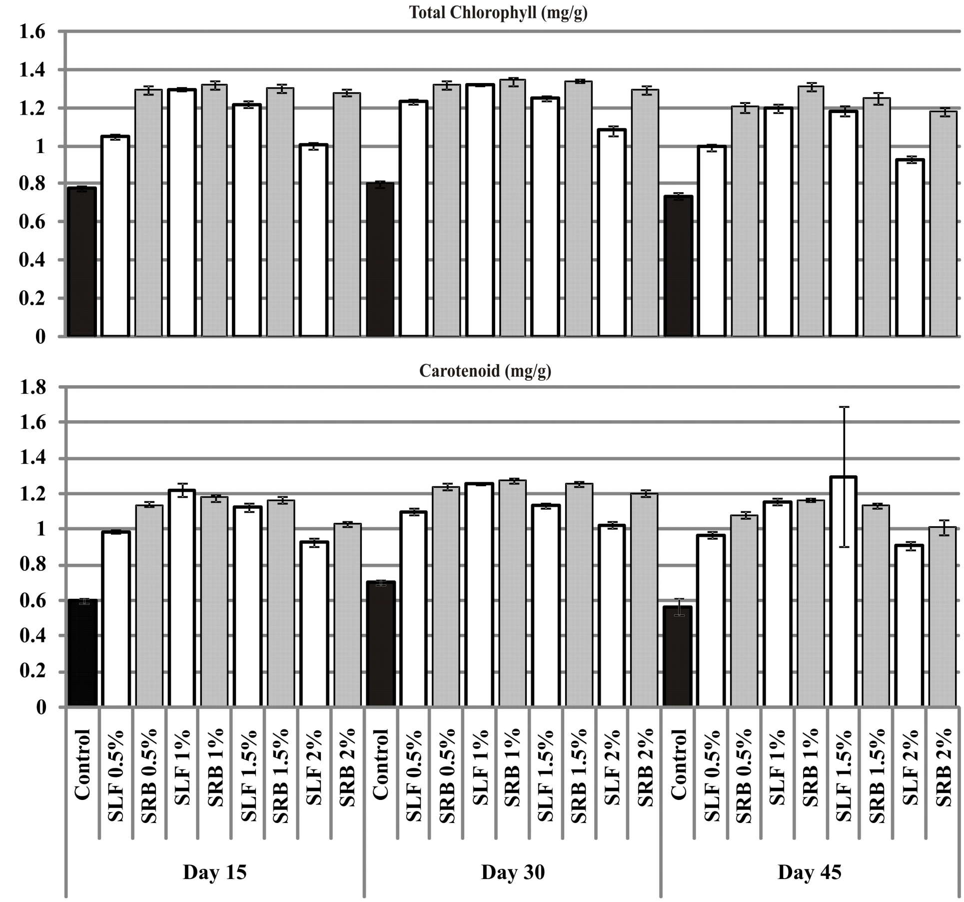

Figure 2. The effect of SE and Rhizobium biofertilizer treatments on photosynthetic pigments (mg/g) of *V.unguiculata*

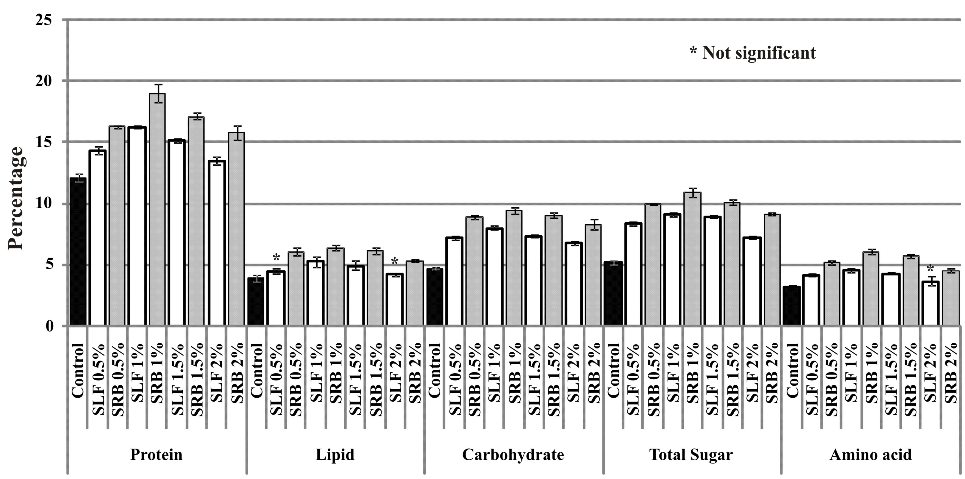

Figure 3. The effect of SE and Rhizobium biofertilizer treatment on biochemical parameters (µg/g) of *V. unguiculata*

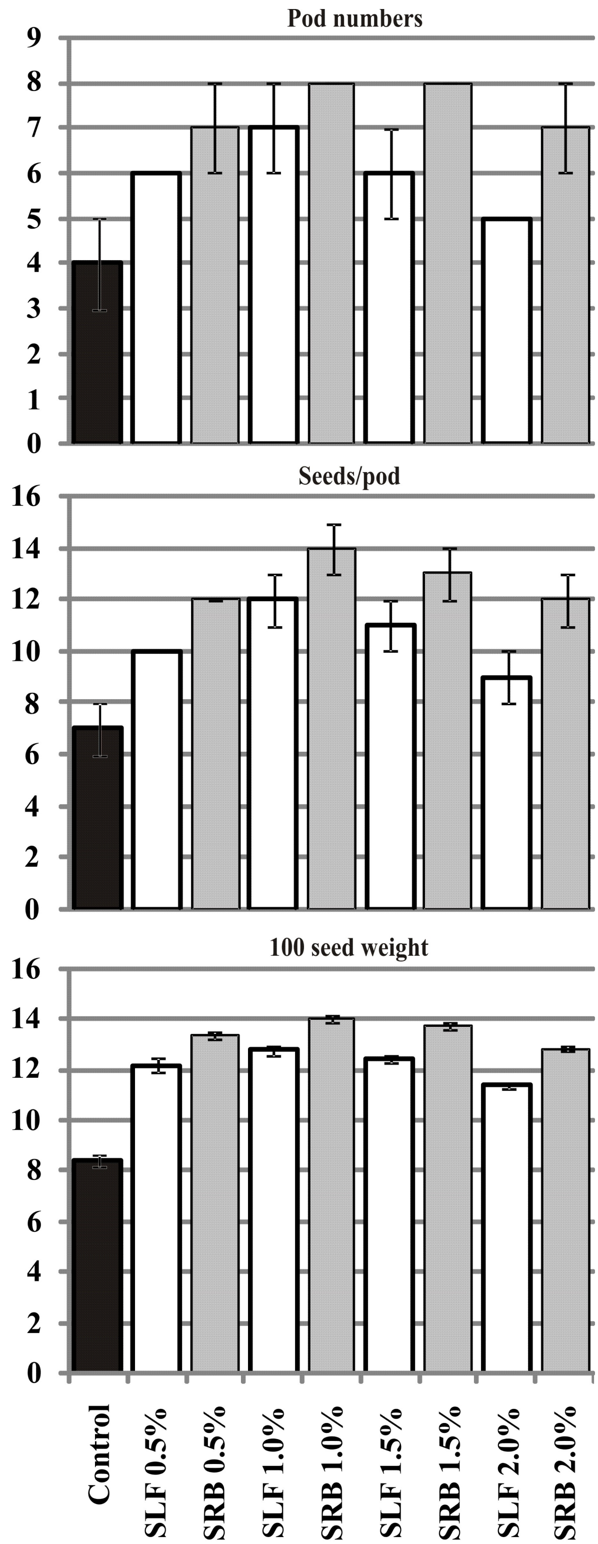

Figure 4. The effect of SE and Rhizobium biofertilizer treatment on yield of *V. unguiculata*

The yield was significantly increased in all concentrations of seaweed extract compared with the control.

3.1. Seed germination

The seeds of *V. unguiculata* treated with lower concentrations (1% and 1.5%) of SE with or without biofertilizer application showed the highest germination rate. Similar observations were made in *V. sinensis* (Sivasankari et al., 2006), vegetables and fruit crops (Hong et al., 2007), *Triticum aestivum* (Kumar & Sahoo, 2011) and *V. mungo* (Kalaivanan & Venkatesalu, 2012). The observed plant stimulant activity of seaweed extract was much better than previous reports.

3.2. Seedling growth

The seeds treated with the lower concentrations of SE, i.e., 1% and 1.5%, with or without rhizobium, resulted in increased dry weight of shoot and roots, lateral roots and leaf area relative to higher SE concentrations and the control (**Figure 1**). Vasantharaja et al. (2019) reported that treatment with a lower concentration (3%) of *S. swartzii* extract significantly improved the growth characteristics of *V.unguiculata*, which is consistent with the current study. Similarly, Battacharyya et al. (2015) also find seaweed extract at lower concentrations enhanced the growth of many plants. In support of the findings of the present study, seaweed extract treatment increased the plant vegetative growth, leaf area, plant biomass and total volume of the root system (Elansary et al., 2016; Hernández-Herrera et al., 2014; Lola-Luz et al., 2013; Mancuso et al., 2006). In addition to growth hormones, nutrients like nitrogen, phosphorus and potash are essential for cell division, cell enlargement and plant growth. The enhanced growth of *V. unigiculata* may be attributable to such essential elements as nitrogen, phosphorous and potassium in the seaweed extract. Furthermore, calcium and sulfur stimulate the microbial (Rhizobium) activity and formation of nodules, respectively, which contribute to plant growth. These observations support Rathore et al. (2009) 's statement that the micro and macronutrients in the seaweed extract promote plant growth (Singh et al., 2016). Similar increased seedling growth from the application of *S. wightii* extract have been reported for *V. sinensis* (Sivasankari et al., 2006), *Gossypium hirsutum* (Ragavendra et al., 2007) and *Triticum aestivum* (Kumar & Sahoo, 2011). Researchers found that *Ecklonia maxima* eckol enhanced the growth of maize seedlings, according to (Rengasamy et al., 2015a, 2015b) .

3.3. Biochemical contents

Photosynthesis is the process by which plants capture solar energy and store nutrients. The amount of plant productivity is determined directly by the photosynthetic ability of leaves. Seaweed liquid fertilizer applied at 1% with or without the Rhizobium biofertilizer produced higher amounts of pigments, such as chlorophyll and carotenoid, in *V. unigiculata* compared with higher concentrations of SE and control (**Figure 2**). The chlorophyll content of leaves was raised by the application of seaweed extract in similar investigations (Whapham et al., 1993; Spinelli et al., 2010; Krainc et al., 2012; Jannin et al., 2013; Yao et al., 2020) (Jannin et al., 2013; Spinelli et al., 2010; Whapham et al., 1993; Yao et al., 2020). The elements, such as nitrogen, copper, iron, magnesium, sulfur, zinc and manganese, are involved in the photosynthesis and production of chlorophyll. At lower concentrations of SE, the seaweed extract had appropriate levels of nutrients, which may have contributed to the rise in pigment content (Clárk, 1983).

Similarly, Paul and Nongkynrih (1996) reported that the catalytic activities, synthesis and maintenance of chlorophyll in seaweed extract-treated plants were attributable to the presence of iron, copper and magnesium in the SE concentrates. This could be attributable to the presence of essential minerals, such as iron, nickel, copper, and magnesium, in the seaweed extract (Fanero et al., 1996). The foliar application of seaweed extract significantly stimulated photosynthesis, carbon, nitrogen and sulphur metabolism (Jannin et al., 2013). The improved growth response of crop plants supplemented with seaweed extract is due to the presence of micro and macronutrients (Singh et al., 2016), polysaccharides (Rolland et al., 2002), plant growth hormones (Khan et al., 2009).

Seaweed extract applied at 1% with or without the Rhizobium biofertilizer enhanced protein, lipid, sugar and amino acid contents in *V. unigiculata* relative to other concentrations of SE and the control (**Figure 3**). The increased biochemical constituents at the lower concentrations of SE might be on account of the absorption, by the seedlings, of most of the necessary elements, such as nitrate, potassium, copper, zinc and boron. These elements are necessary for the synthesis and translocation of various biochemicals and their metabolism. This is supported by the observations of K Kannan and Selvan (1990) in Vigna radiata. Tamil Selvan reported similar trends and Kannan (1994) in Vigna mungo treated with *Hypnea musciformis* extract and by Sivasankari et al. (2006) in *V. sinensis* and Vasantharaja et al. (2019) in *V.unguiculata* treated with Sargassum extract.

3.4. Yield

The favourable effects on yield parameters at the lower concentration (1%) of SE alone and with Rhizobium (**Figure 4**). Similarly, encouraging effects on the yield of 'Thompson seedless' grape (*Vitis vinifera* L.) (Norrie & Keathley, 2005), *Capsicum annuum* (Arthur et al., 2003), *T. aestivum* (Kumar & Sahoo, 2011), *Vigna radiata* and *Vigna mungo* (Pramanick et al., 2013, 2016) and *Solanum lycopersicum* (Yao et al., 2020) have been reported with the use of seaweed extract. The presence of hormones, particularly cytokinins, in the extracts of seaweed-treated plants is thought to be associated to increased production (Featonby-Smith, 1984; Featonby-Smith & Staden, 1983a, 1983b). According to Singh et al. (2016), micro- and macronutrients in seaweed extract may activate signaling pathways, synergising plant physiological processes, thereby increasing crop productivity.

The SE application alone at 1% and with Rhizobium showed the most prominent effects in all the tested parameters (vegetative growth, biochemical and yield), whereas 0.5% and 1.5% seaweed extract in both treatments produced more or less similar effects but lower than those at the 1% concentration of tested parameters. The effects were further reduced at 2% compared with the other three concentrations, but they remained higher than the controls. Among the two treatments, the effect was comparatively higher in SE with Rhizobium treatment in all the tested parameters than that of SE treatment alone. According to Eyras et al. (1998) and K.K. Moore (2004) , this may be due to the application of SE, which may have increased soil aeration and capillary activity of soil pores, which stimulated plant root development and soil microbial activity. The results revealed that the difference in the effects between the two treatments and three different sampling days was highly significant for all the growth and biochemical parameters observed.

4. CONCLUSIONS

We concluded that the seed-soaking treatments using the extract from *Sargassum wightii* alone and in combination with Rhizobium biofertilizer increased biochemical parameters, which resulted in improved germination, growth, pigment content and yield characteristics of *Vigna unguiculata*. The growth-promoting properties of the seaweed extract and the cumulative effect with Rhizobium were confirmed. Seed treatment of SE with Rhizobium biofertilizer was more effective than the SE alone in enhancing cowpea's growth, yield, and biochemicals. These findings suggest that combining seaweed extract with rhizobium to promote cowpea growth and yield in sustainable agricultural systems could be a viable option. Furthermore, biological-based alternatives reduce the use of synthetic fertilizer and its negative impacts on the biosphere.

ABBREVIATIONS

SE or SLF- Seaweed extract
SRB- Seaweed extract + *Rhizobium* biofertilizer

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

ACKNOWLEDGMENTS

Thanks to Annamalai University's Faculty of Marine Sciences for the use of their facilities to perform this research.

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FUNDING

No grant was received from any agencies.

AUTHOR CONTRIBUTIONS

RA was involved in all phases of the study, including the conception and design of the experiments, the gathering and analysis of the data, and the writing of the manuscript. PA was engaged in the research design, manuscript writing, and manuscript revision.

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