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 synergetic effect of seaweed extract (SE) prepared from Sargassum weightii Greville, with and without applying Rhizobium biofertilizer, on seed germination, seedling growth, biochemical constituents and yield of cowpea (Vigna unguiculata) Wälp Var. pura 151 (family Fabaceae). Seaweed extract (1% concentration), with or without Rhizobium (biofertilizer) treatment, compared with the control, significantly (p ≤ 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.A. 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), macronutrients (Ca, K, P) and micronutrients (Fe, Cu, Zn, B, Mn, Co, and Mo), relatively low molecular weight compounds, such as polyamines and brassinosteroids (W.A. Stirk et al., 2004, 2014) and major components polysaccharides (Iez 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 very few research works have been carried out 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.
2. MATERIALS AND METHODS

2.1. Collection of seaweeds

The seaweed [Sargassum wightii Greville (Phaeophyceae)] was collected from the intertidal region of the Rameswaram coast (9° 25' N and 79° 15' E), Tamil Nadu, India, in June 2014. 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

The method of Rao (1990) was followed for the preparation of SE. Washed and cleaned seaweed was shade-dried for five days and then oven-dried at 60-65°C for 24 h and were made into a coarse powder using a mixer-grinder. The powder was soaked in distilled water in a ratio of 1:20 (w/v) and autoclaved at 121°C, 20 psi for 60 min. The hot seaweed extract was filtered through Whatman No.1 filter paper and allowed to cool at room temperature. The filtrate was then centrifuged at 1500 x g for 30 min at 4°C. The supernatant was collected and kept at 60°C in an oven for 48 h. The dried extract was considered 100% seaweed extract, and from this, concentrations of 0.5%, 1%, 1.5% and 2% were prepared with distilled water. As the SE contained organic matter, they were refrigerated at 4°C to prevent spoilage.

2.3. Selection of crop plant and Rhizobium biofertilizer

The crop plant selected for this study was Vigna unguiculata (L.) Walp Var. pusa 151 (family Fabaceae), commonly known as cowpea. Seeds were purchased from the Regional Pulses Research Station, Vamban, Pudukottai District, Tamil Nadu, India. Healthy seeds free from visible defects were segregated and stored in a metal container until needed for the experiments. The Rhizobium (Pon-rhizobium) biofertilizer was obtained from Pasic Biofertilizers, Puducherry (India).

2.4. Experimental procedures and biochemical analysis

The seeds were soaked in different concentrations (0.5%, 1.0%, 1.5% and 2%) of SE for 24 h; the control was soaked in distilled water. The experiment was conducted in earthen pots (30 cm diameter and 40 cm height) filled with a mixture of sterilized garden soil and sand at the ratio of 2:1(v/v). The soaked seeds were planted at the rate of 10 seeds per pot, and three pots per concentration were maintained. 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., 15th, 30th and 45th 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. Plants from each treatment were randomly drawn for analysis. The length of shoots and roots, fresh weight of shoots and roots, number of lateral roots, total leaf area, chlorophyll-a, chlorophyll-b, total chlorophyll (Arnon, 1949) and carotenoid (Kirk & Allen, 1965) were measured at 15th, 30th and 45th days after the emergence of the 1st 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, such as number of pods, number of seeds/pod, pod length, the weight of pods and 100-seed weight were recorded on the 45th 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 the American Public Health Association methods (Apha et al., 1995), using ICP - Perkin Elmer Mayer Optical Emission Spectrophotometer, Optima 2100 DV, and Flame Photometer – Systronics.

2.6. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) with SPSS 16.1 statistical package, and Tukey’s HSD multiple comparisons test was employed at a 5 % level of significance to separate 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>
<thead>
<tr>
<th>Element</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>0.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.1</td>
</tr>
<tr>
<td>Boron</td>
<td>3.8</td>
</tr>
<tr>
<td>Iron</td>
<td>6.9</td>
</tr>
<tr>
<td>Sulfate</td>
<td>39.50</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>54.20</td>
</tr>
<tr>
<td>Nitrate</td>
<td>114.10</td>
</tr>
<tr>
<td>Magnesium</td>
<td>167.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>170.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>310.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>490.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>1180.0</td>
</tr>
</tbody>
</table>

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% significantly (p ≤ 0.05) increased the growth parameters (dry weight of shoots and roots, number of lateral roots and total leaf areas) compared with the control (Figure 1).
Figure 1. The effect of SE and Rhizobium biofertilizer treatments on growth of V. unguiculata
The highest total chlorophyll and carotenoids concentrations were recorded in both the treatments (SE and SE+RB) at 1% seaweed extract on the 30th day (Figure 2).

Application of 1% SE alone or rhizobium increased protein, lipids, sugars, and amino acid content significantly (p ≤ 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).

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 *Vigna sinensis* (Sivasankari et al., 2006), vegetables and fruit crops (Hong et al., 2007), *Triticum aestivum* (Kumar & Sahoo, 2011) and *Vigna 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, the number of lateral roots and total 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 *Sargassum swartzii* extract significantly improved the growth characteristics of *V. unguiculata*, which is consistent with the results of the current study. Similarly, Battacharyya et al. (2015) also reported use of 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. unguiculata* 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 presence of 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 *Vigna sinensis* (Sivasankari et al., 2006), *Gossypium hirsutum* (Ragavendra et al., 2007) and *Triticum aestivum* (Kumar & Sahoo, 2011). Rengasamy et al. (2015a & b) reported that eckol, a phenolic compound from *Ecklonia maxima*, stimulated the seedling growth of maize.

### 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. unguiculata* compared with higher concentrations of SE and control (Figure 2). Like the present study, several studies have also documented that the application of seaweed extract increased the chlorophyll content in leaves (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. In the present study, the increased pigment content at lower concentrations of SE might be directly attributed to the presence of such nutrients at an optimal level in the seaweed extract (Clarke, 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. Increased photosynthetic pigments in SE-treated plants could be attributable 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. unguiculata* 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 and Keathley, 2006), *Capsicum annum* (Arthur et al., 2003), *T. aestivum* (Kumar
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*
et al., 2013, 2016) and Solanum lycopersicum (Yao et al., 2020) have been reported due to the application of seaweed extract. Increased yield in seaweed-treated plants is thought to be linked with the presence of the hormone in the extracts, especially cytokinins (Featonby-Smith and van Staden 1983a; b; 1984). 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. This might be attributable to the application of SE, which might have enhanced soil aeration and capillary activity of soil pores, which stimulated the growth of plant root system and soil microbial activity, as suggested by Eyras et al. (1998) and K.K. Moore (2004). 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.

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AUTHOR CONTRIBUTIONS

RA involved with the experimental design, conducted the work, data collection, conducted the experiments, statistical analysis and manuscript preparation of the research. PA involved in experimental design of the research, manuscript preparation and reviewed the manuscript

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ABBREVIATIONS

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

CONFLICTS OF INTEREST

The authors declare no competing interests.


