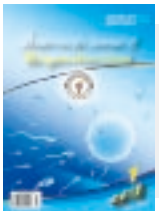

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Biofertilizing efficiency of *Sargassum polycystum* extract on growth and biochemical composition of *Vigna radiata* and *Vigna mungo*

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ABSTRACT

Objective: To evaluate the effect of marine brown alga *Sargassum polycystum* extract on growth and biochemical parameters of *Vigna radiata* and *Vigna mungo*. **Methods:** Different concentrations of algal extracts (0.5%, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0%) were prepared and applied to the crops at every 10-day intervals under natural conditions. After 30 d, the plants were harvested to evaluate the growth and biochemical parameters. **Results:** Seaweed liquid fertilizers treated seedlings showed maximum growth in 3.0% concentration when compared to the untreated seedlings. Similarly, biochemical parameters such as photosynthetic pigments, protein, reducing sugar, total sugar and amino acids exhibited increases in 3.0% concentration seaweed extract. Decreases in growth and biochemical parameters were noticed in concentrations higher than 3.0%. **Conclusions:** Presence of micronutrients and growth regulating substances in the liquid extract help healthier and faster productivity of the crop.

1. Introduction

In the current global scenario, increasing pollution and contamination of farm lands is a growing concern of the farmers engaged in increasing food production. Indian economy is agriculture based particularly in the rural areas where 70% of the population live with agriculture and allied activities as its main occupation[1]. Currently, most agricultural lands are polluted and degraded to varying extents by persistent use of chemical fertilizers and pesticides, and alternative approaches are needed to safeguard the situation. Use of environment friendly biofertilizers as a sustainable resolution for eco-friendly agricultural practices is gaining momentum in many countries. India also intends to achieve second green revolution based on biodegradable, non-toxic, non-polluting and environmentally safe technology[2]. Natural seaweeds, the macrophytes are emerging as one of the sought-after biofertilizers amenable for fractional replacement of predictable chemical fertilizer[3–5]. At present, seaweed extract products are used in agriculture practice and are commercialized. Seaweed liquid fertilizers (SLF) are available as manure, foliar spray, granular powder for soil conditioners and soil drench[6].

SLF contains nutrients to promote the growth of the host plant, when applied to plant surfaces, seeds, soil and interior part of the plant. SLF has nutrients to enhance the plant growth through solubilising phosphorus, nitrogen fixation and the production of growth regulating substances. Moreover, plant growth nutrients such

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as copper, molybdenum, potassium, selenium, magnesium, iodine, iron, nitrogen, cobalt, zinc, manganese and nickel are components of SLF[7]. Seaweed extracts improve seed germination, early seedling vigor, stimulate the extra buds, root growth and increase the validity of vegetables and fruits[8]. In addition to crop yield, others including nutrient uptake, protein content, resistance of pathogens and stress conditions, the quality of the crop improve[9-11]. Seaweed manure is common and a very ancient farming practice. SLF contains abundant presence of plant growth regulators such as auxin[12,13], cytokinin[14], indole-3-acetic acid[15] and gibberellins[16]. Hence, marine resources, particularly brown algae play a major role in agriculture. The present study was subjected to evaluate the growth efficiency of seaweed liquid fertilizer of brown macro algae *Sargassum polycystum* C. Agardh (*S. polycystum* C. Agardh) on legume plants of *Vigna radiata* (*V. radiata*) and *Vigna mungo* (*V. mungo*).

2. Materials and methods

2.1. Collection of seaweed

Fresh specimens of brown seaweed, *S. polycystum* C. Agardh collected from Gulf of Mannar region (09° 19′ N, 79° 03′ E), Rameswaram, Tamil Nadu, India. The specimens were thoroughly washed with seawater to remove epiphytes, then exhaustively washed with tap water twice, and followed by a wash with distilled water to remove salt content and other contaminants. The seaweed materials subsequently dried in shade for 7 d, then they were stored at 4 ℃ for future use after finely ground in an electric mixer.

2.2. Preparation of seaweed liquid fertilizer

A total of 500 g dried seaweed powder was mixed with 1 L of distilled water and then boiled for 1 h. After the hot water extraction, the extract was filtered through muslin cloth. The obtained filtrate was taken as 100% concentrated seaweed extract and it was made up to different concentrations (0.5%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%) using distilled water[17].

2.3. Physio-chemical and growth promoting substances of S. polycystum extract

Physical characteristics such as color and pH of *S. polycystum* extract were noticed. Chemical elements presented in the seaweed extract such as magnesium, iron, chloride, sulphate, copper, sodium, calcium, zinc, nitrate, cobalt, phosphate, potassium, and manganese were estimated using the procedures outlined by the American Public Health Association[18]. Furthermore, the liquid extract of *S. polycystum* was subjected for estimation of plant growth regulators such as auxin, gibberellin and cytokinin[19].

2.3.1. Selection of crop plant

Plants selected for the present study were of *V. radiata* and *V. mungo* seeds belonging to the family, Fabaceae. The seeds were purchased from a commercial market, Chennai, Tamil Nadu. Dry healthy seeds, with standardized size and weight were selected for the experimental study.

2.3.2. Preparation of pot study

Selected healthy seeds were surface sterilized with 0.1% mercury chloride for 1 min and followed by rinsing with distilled water for several times. Then the seeds were sown in pots (24 cm × 18 cm) with a mixture of red soil and sand. The pots were watered regularly. After every ten day interval 50 mL of different concentrations (0.5%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%) of *S. polycystum* extract was treated for the tested plants *V. radiata* and *V. mungo*. Extract alone water irrigated plants were considered as control. All the experimental pots were repeated thrice.

2.3.3. Growth promoting efficiency of V. radiata and V. mungo

Plants were uprooted carefully from each treatment at the end of 30 d after seed sowing. All the uprooted plants from each triplicates were analyzed for growth parameters of shoot and root lengths (cm). The uprooted plants were washed and blotted on blotting paper before weighing fresh weight for calculation. Then the plants were dried in a hot air oven at 60 ℃ for 24 h for determining dry weight (mg/g fr.wt) by Infradigi digital weighing balance 1N201L, India. The number of root nodules was also calculated and expressed in nos. Leaf area (mm^2) was calculated by using of systeronic Leaf Area Meter 211, India.

2.4. Biochemical profile of V. radiata and V. mungo

2.4.1. Estimation of photosynthetic pigments

The amount of photosynthetic pigments was determined by the standard method[20]. One hundred milligram of fresh leaves were homogenized with 10 mL of acetone using mortar and pestle. The filtrate was centrifuged at 10 000 r/min for 20 min, and the resultant supernatant was diluted with acetone. The absorbance was read at 663 nm for chl a and 645 nm for chl b and the total chlorophyll was calculated.

2.4.2. Estimation of protein

Fifty milligrams of fresh leaves were homogenized with 10% cold Trichloro acetic acid and centrifuged at 10 000 r/min for 15 min in 4 ℃. The reaction system contained 4.5 mL of 2% Na_2CO_3 in 0.1 N NaOH, 0.5 mL of 2% $CuSo_4$, 2% $KNaC_4H_4O_6 \cdot 4H_2O$ and 0.1 mL of sample incubated at room temperature for 30 min. The absorbance was read at 660 nm using Elico double beam UV-VIS spectrophotometer SL 210. The calibration curve was prepared by using bovine serum albumin[21].

2.4.3. Estimation of total sugar

Total sugar was determined by phenol-sulfuric acid method[22]. A reaction mixture contained 1 mL sample, 1 mL of 5% phenol, 5 mL of concentrated sulphuric acid and incubated at room temperature for 30 min. The presence of total sugar was detected by recording the absorbance at 490 nm. Glucose was used as a standard.

2.4.4. Amino acid analysis

A test tube contains 1 mL sample neutralized with 0.1 N NaOH using methyl red indicator, and 1 mL ninhydrin reagent was boiled for 20 min. The test tube was added with 5 mL of dilution solution contains n-propanol and distilled water at 1:1 ratio. The absorbance was read at 570 nm and the concentration of amino acids (mg/g fr.wt) were calculated from the standard graph of leucine[23].

2.4.5. Determination of reducing sugar

Reducing sugar was determined according to the method described by Nelson[24]. Briefly, 1 mL sample was mixed with 1 mL of copper reagent (2.5 g of sodium potassium tartarate, 20 g of anhydrous sodium sulphate, 2 g of sodium bicarbonate), and 1 mL of arsenomolybdate reagent (2.5 g of ammonium molybdate, 0.3 g of sodium arsenate). The reaction mixture was incubated for 15 min at 30 ℃. Absorbance was read at 500 nm.

2.5. Statistical analysis

Statistical analysis was carried out by one way ANOVA followed by Duncan's Multiple Range Test (P<0.05). Data were calculated using Software Package of Social Sciences (SPSS) version 18.0 for Windows[25].

3. Results

Physiochemical properties of the brown alga *S. polycystum* are presented in Table 1. The physical appearance of the extract was brown colored and the pH of the extract was 6.8. Similarly, in the micro elemental analysis sodium (85.30 mg/L), chloride (75.02 mg/L) and calcium (69.37 mg/L) accounted for large quantity and other elements were present in substantial level. In growth hormone analysis, cytokinin (7.35 mg/L) was present higher than gibberellin and auxin.

Table 1
Phsio-chemical parameters and growth hormones of *S. polycystum* liquid extract.

Parameters	Values
Physical parameters	
Colour	Brown syrup
pH	6.80
Chemical parameters or micro elements (mg/L)	
Magnesium	21.60
Sodium	85.30
Potassium	6.81
Iron	3.57
Phosphate	35.16
Calcium	69.37
Chloride	75.02
Sulphate	46.62
Copper	4.70
Zinc	1.86
Nitrate	56.00
Manganese	2.00
Growth hormones (mg/L)	
Auxin	3.20
Gibberellin	4.15
Cytokinin	7.35

The effects of various concentrations of *S. polycystum* extracts on *V. radiata* and *V. mungo* were studied and their growth parameters and biochemical parameters were analyzed. The results showed in Table 2 and 3 indicated that seaweed extract enhanced the growth rate and various other physiological parameters of *V. radiata* and *V. mungo*. Growth parameters of *V. radiata* such as shoot length (142%), root length (149%), leaf area (219%), root nodules (249%), fresh weight (249%) and dry weight (250%); whereas in *V. mungo*, it showed that shoot length (170%), root length (139%), root nodules (172%), leaf area (170%), fresh weight (236%) and dry weight (271%) got a tremendous boost, respectively. All the above parameters of *V. radiata* and *V. mungo* were increased potential growth up to 3.0% and decreased up to 5.0%. The control plants showed lowest growth parameters when compared to the treated plants respectively.

Various biochemical ingredients such as photosynthetic pigments (chl a, chl b, and total chlorophyll), protein, amino acid, reducing sugar and total sugar were estimated in *V. radiata* and *V. mungo* (Table 4 and 5). It was evident that lower concentrations of 3% *S. polycystum* extract increased biochemical parameters in *V. radiata*

Table 2
Potential of *S. polycystum* liquid extract on growth parameters of *V. radiata* (n=6).

Concentration	Shoot length (cm)	Root length (cm)	Leaf area (mm^2)	Root nodules (no.)	Fresh weight (mg)	Dry weight (mg)
Control	17.213±0.190^a	12.380±0.220^a	22.116±0.140^a	5.33±0.28^a	2.170±0.170^a	0.553±0.060^a
0.5%	18.106±0.100^a	13.383±0.140^a	25.433±0.370^b	5.66±0.28^a	2.373±0.240^a	0.763±0.020^a
1.0%	19.520±0.150^b	14.506±0.200^a	30.253±0.170^c	6.66±0.28^a	3.323±0.280^a	0.873±0.030^a
2.0%	21.967±0.370^c	16.346±0.170^a	39.740±0.410^d	9.33±0.28^b	4.013±0.240^a	1.096±0.040^a
3.0%	24.603±0.160^d	18.563±0.200^b	48.550±0.270^e	13.33±0.28^c	5.410±0.290^b	1.386±0.030^a
4.0%	21.266±0.170^c	15.413±0.150^a	38.423±0.570^d	9.66±0.28^b	3.386±0.250^a	0.870±0.030^a
5.0%	19.443±0.100^b	13.460±0.190^a	23.866±0.350^a	7.66±0.28^a	2.230±0.180^a	0.773±0.030^a

Values are expressed as mean ± SD. Means sharing different letter correspond to a different level of significance between different treatments (P<0.05).

Table 3

Potential of *S. polycystum* liquid extract on growth parameters of *V. mungo* (*n*=6).

Concentration	Shoot length (cm)	Root length (cm)	Leaf area (mm^2)	Root nodules (no.)	Fresh weight (mg)	Dry weight (mg)
Control	15.936±0.230^a	11.880±0.340^a	27.773±0.490^a	8.333±0.280^a	2.423±0.110^a	0.583±0.040^a
0.5%	16.943±0.380^a	12.713±0.260^a	30.030±0.430^b	10.666±0.280^b	2.916±0.140^a	0.796±0.030^a
1.0%	19.023±0.340^b	13.406±0.170^a	36.363±0.600^c	11.666±0.280^b	3.620±0.130^b	0.910±0.040^a
2.0%	24.890±0.440^c	15.386±0.300^a	42.636±0.420^d	12.333±0.280^b	4.593±0.130^b	1.053±0.030^a
3.0%	27.200±0.170^d	16.566±0.400^b	47.250±0.130^e	14.333±0.280^c	5.733±0.110^c	1.583±0.010^a
4.0%	20.883±0.210^b	14.343±0.340^a	40.510±0.540^d	10.666±0.280^b	3.426±0.230^b	0.780±0.020^a
5.0%	17.663±0.170^a	12.100±0.330^a	31.783±0.480^b	7.666±0.570^a	1.840±0.120^a	0.690±0.010^a

Values are expressed as mean ± SD. Means sharing different letter correspond to a different level of significance between different treatments (P<0.05).

plants including chl a (155%), chl b (196%), total chlorophyll (171%), protein (233%), amino acid (492%), reducing sugar (201%) and total sugar (130%). Similarly, the amount of biochemical parameters in treated plants of *V. mungo* including chl a (179%), chl b (202%), total chlorophyll (188%), protein (217%), amino acid (439%), reducing sugar (228%) and total sugar (135%) was found to be high at 3% concentration of the extract. The other treated concentrations (0.5%, 1%, 2%, 4% and 5%) showed reduced levels of biochemical contents in both treated plants.

4. Discussion

Higher concentrations of cytokinin are also reported in earlier findings of brown algae *Sargassum wightii* (*S. wightii*)[26] and *Stoechospermum marginatum*[27] where cytokinin is reported in much higher concentration than auxin and gibberellins. The growth stimulation potential of SLF may be due to the presence of micro and macro elements, vitamins and more important plant growth hormones like the cytokinins[28,29]. In addition, chelating nutrients reportedly help the enhancement of nutrient uptake as in *Ascophyllum nodosum* because of the presence of some organic acids[11].

Previous investigations and reviews have documented the responses of the different concentrations of several seaweed extracts against many other plants. Surprisingly, the extract of same seaweed *S. polycystum* on seed germination and growth rate of *Cajanus cajan* (*C. cajan*) was studied and it promoted the seed germination and growth rate in lower concentrations of 1.5%[30]. Earlier studies of seaweed extracts were found to be effective in *V. radiata*[31,32], *V. mungo*[33], and *Vigna ungiculata*[34]. Ganapathy and Sivakumar[35] reported seaweed extract of *Ulva reticulata* (*U. reticulata*) to increase crop productivity of *Arachis hypogea* by spraying 2% concentration. In particular, *V. mungo* plant treated with seaweed extracts like *Caulerpa scalpelliformis* (*C. scalpelliformis*)[36] and *U. reticulata*[37] responded very favorably. Furthermore, data were obtained from the commercial seaweed extract and extract of *Sargassum plagiophyllum* treated *V. radiata* and *V. mungo*. Both the extracts enhanced seed germination and seedling growth up to 0.75% in *V. mungo*; whereas, 1.0% commercial seaweed extract and 1.5 % SLF showed enhanced seedling growth in *V. radiata*[38].

In previous findings, 10% of SLF from brown seaweed *Colpomenia sinuosa* increased the chlorophyll content, protein, total sugars,

Table 4

Potential of *S. polycystum* liquid extract on biochemical parameters of *V. radiata* (mg/g fr.wt) (*n*=6).

Concentration	Chl a	Chl b	Total chl	Protein	Reducing sugar	Total sugar	Amino acid
Control	0.691±0.030^a	0.427±0.070^a	1.118±0.030^a	9.846±0.260^a	2.666±0.270^a	22.770±0.310^a	0.833±0.180^a
0.5%	0.762±0.040^a	0.452±0.040^a	1.214±0.060^a	12.800±0.380^b	3.156±0.160^a	24.826±0.140^b	1.263±0.340^a
1.0%	0.828±0.030^a	0.552±0.030^a	1.380±0.020^a	16.026±0.130^c	3.990±0.230^b	25.906±0.290^b	2.120±0.340^a
2.0%	0.942±0.050^a	0.626±0.030^a	1.569±0.050^a	19.030±0.190^d	4.573±0.190^c	27.806±0.370^c	3.060±0.240^b
3.0%	1.074±0.050^a	0.840±0.030^a	1.914±0.030^b	22.943±0.330^e	5.366±0.140^c	29.710±0.460^d	4.103±0.110^c
4.0%	0.959±0.050^a	0.726±0.030^a	1.686±0.070^a	20.180±0.210^d	3.996±0.390^b	27.060±0.150^c	2.533±0.420^b
5.0%	0.862±0.050^a	0.622±0.050^a	1.484±0.010^a	17.246±0.240^c	3.153±0.360^b	25.256±0.290^b	1.410±0.320^a

Values are expressed as mean ± SD. Means sharing different letter correspond to a different level of significance between different treatments (P<0.05). Values in parenthesis are percent over control.

Table 5

Potential of *S. polycystum* liquid extract on biochemical parameters of *V. mungo* (mg/g fr.wt) (*n*=6).

Concentration	Chl a	Chl b	Total chl	Protein	Reducing sugar	Total sugar	Amino acid
Control	0.596±0.080^a	0.388±0.020^a	0.984±0.040^a	11.060±0.310^a	2.343±0.280^a	21.960±0.160^a	0.983±0.180^a
0.5%	0.676±0.090^a	0.466±0.030^a	1.142±0.070^a	14.976±0.150^b	3.066±0.130^a	22.886±0.240^a	1.566±0.210^a
1.0%	0.784±0.030^a	0.538±0.020^a	1.322±0.030^a	16.680±0.370^b	3.636±0.190^b	24.956±0.310^b	2.226±0.190^b
2.0%	0.899±0.070^a	0.640±0.050^a	1.540±0.040^a	20.313±0.340^c	4.403±0.130^b	27.230±0.340^c	3.313±0.200^c
3.0%	1.073±0.040^a	0.784±0.060^a	1.857±0.090^a	24.103±0.390^d	5.360±0.180^c	29.750±0.380^d	4.320±0.370^d
4.0%	0.850±0.050^a	0.621±0.030^a	1.471±0.030^a	19.036±0.400^c	3.893±0.170^b	25.406±0.380^b	2.716±0.310^a
5.0%	0.684±0.030^a	0.477±0.050^a	1.161±0.050^a	15.616±0.280^b	2.770±0.260^a	22.710±0.370^a	1.843±0.400^a

Values are expressed as mean ± SD. Means sharing different letter correspond to a different level of significance between different treatments (P<0.05).

lipids, phenols and carotenoids in *V. radiata*[2]. However, Blunden *et al*[39] have reported that the increase of photosynthetic pigments may be due to the presence of betaines. It helps a better grana development and increase the chloroplast number and size[40]. Jebasingh *et al*[41] examined the seaweed liquid fertilizer from green seaweed *C. scalpelliformis*, brown seaweed *Sargassum duplicatum* and red seaweed *Lamminaria pinnatifida* treated against *V. mungo*. All the three extracts elevated the growth and chlorophyll content at lower concentrations of 10%. It also reported that lower concentration of *U. reticulata* (2%) treatmen on *V. mungo* resulted in increased photosynthetic pigments, protein, starch, reducing and non-reducing sugars[37]. The above results are comparable with the results of the present study. A support to this study comes from the observation that 20% of *C. scalpelliformis* SLF stimulates the photosynthetic pigments, amino acid, reducing sugar and total sugar content of *V. mungo*[36]. Moreover, when *C. cajan* was treated with SLF of *Chaetomorpha linum*, *Grateloupia lithophila* and *S. wightii*, the SLF of *S. wightii* enhanced the chlorophyll, carotenoid, amino acid, protein, lipid and total sugar of *C. cajan* at lower concentration of 20% when compared to *Chaetomorpha linum* and *Grateloupia lithophila*[42]. However, the effect *S. polycystum* on *C. cajan* increased the biochemical constituents that were chl a, chl b, ascorbic acid, sugars, starch, protein, nitrate reductase activity at lower concentrations of 1.5%[30]. The total sugar content was high in lower concentrations of all three extracts *Laurencia obtusa*, *Corallina elongata* and *Jania rubens* in *Abelmoschus esculentu*[43]. The presence of N, P, K in seaweed extract apparently helped to enhance the growth rate and increase the chlorophyll content as well as increase the protein and amino acid content of treated plants *V. radiata* and *V. mungo*. Lower concentration of 3% increases the crop productivity while decline is observed in higher concentrations may be due to the presence of high levels of nutrients like Mg, Ca, Cu, I, K, Zn, and Na that restrains the cell division[44]. Moreover, the presence of different levels of minerals and biostimulants fails to supply all the nutrients required by a plant in necessary quantities[45]. All these results make it clear that seaweed extracts have potential growth promoting substances which enhances the growth and biochemical constituents of the plants.

In the current scenario of population increase, especially in the developing world, there is a need for increased food production. The farmers need to adapt to a fertilizer to improve the health of the crops free from chemical fertilizers and pesticides. Seaweed liquid fertilizer is the answer as it increases various crop productions as evidenced from this study. The extract of *S. polycystum* no doubt, improves the growth and biochemical parameters of *V. radiata* and *V. mungo* due to the presence of growth promoting hormones, micro and macro elements. Lower concentration of 3% increases the crop productivity while decline is observed in higher concentrations when compared to control respectively. The study also reveals that the *S. polycystum* extract is suitable to make a cost effective and eco-friendly organic farming for sustainable crop production.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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