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Effect of liquid seaweed fertilizer of *Sargassum wightii* grev. on the growth and biochemical content of green gram (*Vigna radiata* (L.) R. wilczek)

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Abstract
Intensive investigations were made on the efficiency of Liquid Seaweed Fertilizer (LSF) obtained from the brown seaweed *Sargassum wightii* Grev. on the germination, growth and biochemical constituents of green gram (*Vigna radiata* (L.) R. Wilczek) under laboratory conditions and in pots. The seeds that are soaked for 6 hrs duration at lower concentrations such as 0.5% and 1.0% showed faster germination compared with the seeds that are soaked at higher concentration (2.0%) for long durations (12 hrs and 24 hrs). In pot studies, both the shoot length and root length were found to be increased as the concentration of LSF increases, but at high concentration i.e. at 2.0% concentration the growth was slightly reduced. Of the two different types of applications i.e. foliar and root applications, the foliar application was found to be more effective in determining the growth of the plant than the root application. Plants received with 1.0% concentration of LSF as foliar spray showed more shoot and root length, early flowering and more number of pods. The biochemical analysis of the experimental plants showed that the foliar treated plants showed more photosynthetic pigments compared to root treated plants, whereas the accumulation of total protein, total carbohydrate and total lipid content was found maximum in root treated plants than the foliar treated plants.

Keywords: Liquid Seaweed Fertilizer, foliar application, *Sargassum wightii*, *Vigna radiata*, green gram

INTRODUCTION

Seaweeds or marine macro algae are the primitive group of organisms with no true roots, stems and leaves, and they are one of the import marine living resources with tremendous commercial importance (Kaliaperumal *et al.*, 1987). Seaweeds are useful to man as food, feed, fodder, biofertilizer, phytochemicals (Agar-agar, alginates and carragennan) and as a sources of bioactive compounds. Nearly 221 species (32 Chlorophytes, 125 Rhodophytes and 64 Phaeophytes) of seaweeds are being used for commercial exploitation worldwide. From this 145 species (66%) are used as food (79 Rhodophytes, 28 Chlorphytes and 38 Phaeophytes); over half of the Rhodophytes and Phaeophytes are used for phycocolliod production (41 species for alginates, 33 species for agar and 27 species for carrageenan); 24 species are used in traditional medicines; 25 species are used in agriculture, including animal feed and fertilizer, while two species are used in the production of paper in Italy (Zemke-White and Ohno, 1999).

The use of seaweed as manure is important in the present day world as the seaweed fertilizers are often found to be more successful than the chemical fertilizers (Bokil *et al*., 1972). The value of marine algae as agriculture fertilizer was recognized since Fourth century as a partial substitute for manure (Chapman, 1950). The utilization of seaweed as manure is a common practice in coastal areas throughout the world. Ancient Romans were practicing the art of using whole and chopped seaweeds as manure (Newton, 1951). Two main forms in which the seaweeds are at present used Agriculturally and horticulturally in many countries as seaweed meal and liquid extract. While, seaweed meal takes months to become fully effective in soil as plant nutrients have to be broken down by bacteria and it can be used by the plant. Liquid fertilizer, obtained from seaweed contains polysaccharide content, which is already broken down, becomes effective at once (Stephenson, 1974).

Coastal farmers applied seaweed manure to many corps as seaweeds contain good amount of nitrogen, potassium and other minerals and trace elements, and also the carbohydrates and other organic matters present in seaweeds helps in altering the nature of soil and improving its moisture retaining capacity (Simpson and Hayes, 1958). Apart from macro and micro nutrients seaweeds contain many growth promoting hormones like cytokinin, gibberellins, auxins (Tay *et al*., 1987). Seaweed extracts are known to enhance seed germination and growth, increased uptake of nutrients, impart a degree of frost resistance and make plants to withstand better towards phytopathological fungi and insect pests (Bhosle *et al*., 1975).

The Gulf of Mannar region harbors a variety of seaweeds with a maximum potential in the Indian Ocean region. The brown seaweed *Sargassum wightii* Grev. is among the most abundant species. In the present investigation, the effect of Liquid Seaweed Fertilizer (LSF) obtained from *Sargassum wightii* was tested on green gram (*Vigna radiata* (L.) R. Wilczek) to find out its effect on seed germination, early growth of seedlings and its biochemical

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composition under laboratory and in pot studies.

MATERIALS AND METHODS

The specimen of brown seaweed *Sargassum wightii* Grev. was collected from Mandapam coast, Tamil Nadu. The collected seaweed was washed with seawater initially to remove macroscopic epiphytes and sand particles and finally with fresh water to remove adhering salt. They were shade dried for four days followed by oven dry at 60°C for 12h. Then the materials were hand crushed and made as coarse powder using a mixer grinder. This was added with distilled water in a ratio of 1 : 20 (w/v) and autoclaved at 121°C, 15lbs/sq.inch for 30 minutes. The hot extract was filtered through cheese cloth and allowed to cool at room temperature Rama Rao (1990).

Preparation of different concentrations of LSF

Different concentrations of LSF viz., 0.5%, 1.0%, and 2.0% (v/v) were prepared from the known concentration of LSF stock by adding distilled water for laboratory experiments and tap water for pot studies.

Laboratory studies

The effect of LSF on the seed germination and early growth of seedlings on test plant was made under the laboratory conditions at 30 μE m^{-2} s^{-1} light intensity, 12h/12h light dark cycle, at room temperature.

Germination and early growth studies

About one gram of seeds of *Vigna radiata* (L.) R. Wilczek was taken and surface sterilized with 0.1% mercuric chloride for one minute and washed thoroughly in sterilized distilled water. Then they were soaked in different concentrations (10 seeds per concentration) of LSF: 0.5%, 1.0% and 2.0% for 6h, 12h and 24h of durations. After the treatment the seeds were placed on acid washed coarse sand. The coarse sand was initially washed thoroughly twice with tap water followed by treating with conc. HCl and washed thoroughly in running tap water for 30 minutes in order to remove the acid and nutrients present in the sand. It was then dried at room temperature. Three hundred gram of the sand was taken in a plastic cup (7.5 cm diameter and 9.5 cm height) and a pinhole was made at the bottom in order to avoid stagnant of water. To each cup five LSF treated seeds were placed just 2.0 cm below the surface of sand. Then the sand was moistened with distilled water and kept under laboratory condition. The growth characteristics namely: the length of radicle and plumule and fresh weight of seedlings (average of triplicates) were recorded for seedling at the end of 5th day.

Pot studies

Detailed studies were conducted in pots (19.5 cm diameter and 20 cm height) for about 50 days in partial shade. Pot studies were divided in two sets; one set for Foliar Application (FA) and other set for Root Application (RA), and one pot is kept as control which received water alone. Freshly prepared different concentrations of LSF (100 ml) were applied at an interval of 7 days for about 7 weeks, for foliar application of LSF, water sprayer was used. The date of Flowering and fruiting were recorded in different experimental plants, and on 50th day the experimental plants were uprooted for the observation of different parameters namely, shoot length, root length, number of leaves. Leaves were also taken for the analysis of various biochemical parameters like total chlorophyll, chlorophyll *a*, chlorophyll *b* (Mackinney, 1941), total protein (Bradford, 1976), total carbohydrate (Dubois *et al*., 1956), and total lipid content (Folch *et al*., 1957). The values were expressed as mg/g fresh weight.

RESULTS

Studies made on effect of Liquid Seaweed Fertilizer (LSF) obtained from the brown seaweed *Sargassum wightii* on germination and early growth of seedlings under laboratory conditions and pot studies made on green gram *Vigna radiata*(L.) R. Wilczek revealed the following observations:

Laboratory studies
Seed germination and early growth

The seeds that are soaked for 6 hrs duration at lower concentrations such as 0.5% and 1.0% showed faster germination compared with the seeds that are soaked at higher concentration (2.0%) for long durations (12 hrs and 24 hrs). Similarly the length of the plumule and radicle, of seedlings were more in 6hrs soaked seeds at 0.5% concentration compared to 12 hrs and 24 hrs soaked seeds. The fresh weight of the seedlings was also more in the seeds that are soaked at 6 hrs duration than other two higher durations (12 hrs and 24 hrs). A maximum fresh weight of 0.437 g was recorded in the seedling of seeds that are soaked at 0.5% concentration of 6 hrs duration (Table 1).

Table 1.Effect of *Sargassum wightii* LSF on the seedling growth of green gram

Parameters	Duration of soaking	LSF Concentrations			
		Control	0.5%	1.0%	2.0%
Length of plumule (cm)	6h	1.5±0.4	4.1±0.5	2.5±0.4	2.1±0.5
	12h		1.7±0.4	2.2±0.3	3.4±0.6
	24h		0.7±0.4	1.5±0.1	2.3±0.4
Length of radical (cm)	6h	4.9±0.4	7.2±0.7	6.6±0.7	6.2±0.5
	12h		6.0±0.6	6.5±0.5	8.3±0.3
	24h		8.5±0.4	7.2±0.4	4.2±0.5
Fresh weight (g)	6h	0.245±0.1	0.437±0.1	0.346±0.1	0.305±0.1
	12h		0.281±0.1	0.315±0.1	0.393±0.1
	24h		0.294±0.1	0.217±0.1	0.210±0.1

Pot studies

In the pot studies, plants treated with different concentrations of LSF viz., 0.5%, 1.0% and 2.0% showed that LSF enhanced the growth of the plants compared to control. Both the shoot length and root length were found to be increased as the concentration of LSF increases, but at high concentration i.e. at 2.0% concentration the growth was slightly reduced.

Of the two different types of applications i.e. foliar and root applications, the foliar application is found to be more effective in determining the growth of the plant than the root application. A maximum shoot and root length of 17.2 cm and 6.9 cm respectively were obtained in the plants received 1.0% LSF as foliar spray and also the number of leaves was more in plants received with 1.0% LSF in both foliar treated plants as well as in root treated plants (Plate 4).

Early flowering was also observed in plants treated with foliar spray of 1.0% LSF concentration, the flowering was started on the 30th day after the germination, followed by the plants received with 2.0% LSF of foliar spray on 33 days. Whereas, the plants received root applications, as well as control and 0.5% concentrations of LSF showed delayed flowering; they started producing flowers only after 37 days. And also the number of pods per plant was also more in 1.0% foliar spray treated plants (15-18 pods) followed by 1.0% root treated plants (11-14), but 2.0% of LSF treated plants showed less number of pods as in control and 0.5% concentration (Table 2).

Table 2. Effect of *Sargassum wightii* LSF on the growth and yield of green gram

Parameters	Control	LSF concentrations					
		0.5%		1.0%		2.0%	
		FA	RA	FA	RA	FA	RA
Shoot length (cm)	13.9±1.3	15.7±1.9	11.8±1.7	17.2±1.8	16.6±0.9	15.2±1.4	12.2±1.6
Root length (cm)	3.1±0.8	4.6±0.9	4.1±0.5	6.2±0.8	6.4±0.8	4.5±0.7	3.7±0.7
Number of leaves per plant	4-5	5-6	5-6	7-9	7-8	5-7	4-6
Number of pods per plant	5-7	5-7	6-7	15-18	11-14	7-8	5-8

FA – Foliar Application: RA – Root Application

Biochemical parameters

The biochemical parameters recorded on the total Chlorophyll, Chlorophyll *a*, Chlorophyll *b*, total protein, total carbohydrate and total lipid content of the leaves of the experimental plants revealed the following findings:

The concentration of total Chlorophyll and Chlorophyll *a* were found increased as the concentration of LSF increases. The foliar treated plants showed more Chlorophyll and Chlorophyll *a* as compared to root treated plants which showed almost similar values in various percentages of LSF treated plants. The plant received with 1.0% LSF as foliar spray showed a maximum total chlorophyll content of 5.5mg/g Fresh weight compared to control which is 2.3mg/g. Whereas, a maximum value of chlorophyll *a* i.e. 4.7mg/g fresh weight was recorded in the plants received 2.0% LSF as foliar spray. In contrary, Chlorophyll *b* was found to be more in plants received LSF as root application at 0.5% and 1.0% concentrations; however, a maximum value of 1.8mg/g fresh weight of Chlorophyll b was recorded in the plants received 2.0% of LSF as foliar application (Fig. 1a,2a).

The accumulation of total protein, total carbohydrate and total lipid content was found maximum in root treated plants than the foliar treated plants. In the case of protein, maximum protein content 3.5mg/g fresh weight was recorded in the plants received 1.0% LSF similarly the total lipid content was also more at 1.0% concentration (14.3mg/g fresh weight), whereas the total carbohydrate content was more i.e. 6.3mg/g fresh weight at 2.0% concentration (Fig. 1b, 2b).

Fig.1a Effect of *Sargassum wightii* LSF on the pigments of *Vigna radiata* at foliar application

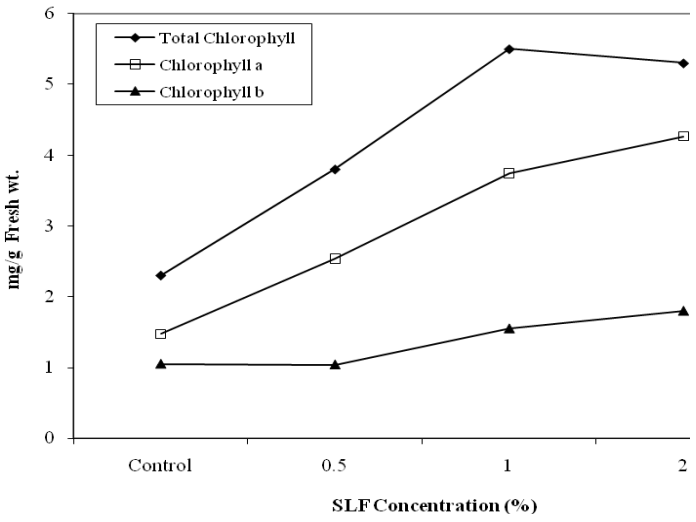


Fig.1b Effect of *Sargassum wightii* LSF on the total protein, total carbohydrate and total lipid content of *Vigna radiata* at foliar application

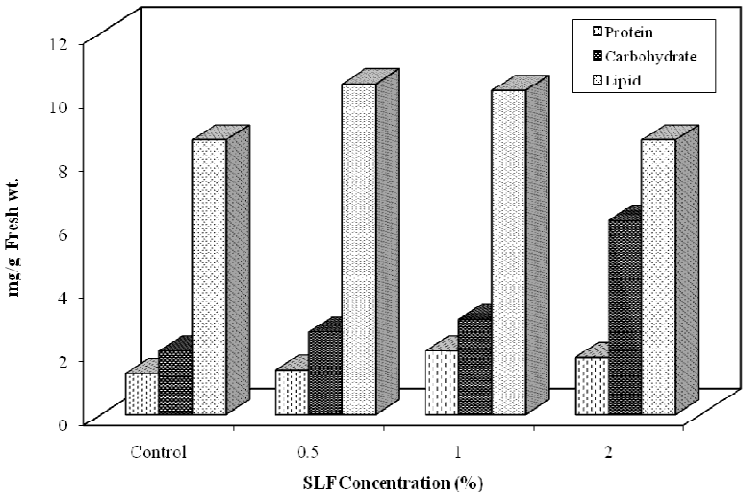


Fig.2a Effect of *Sargassum wightii* LSF on the pigments of *Vigna radiata* at root application

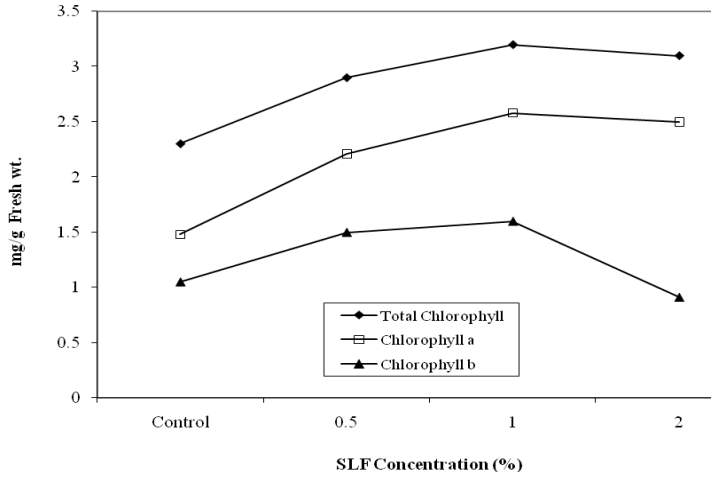
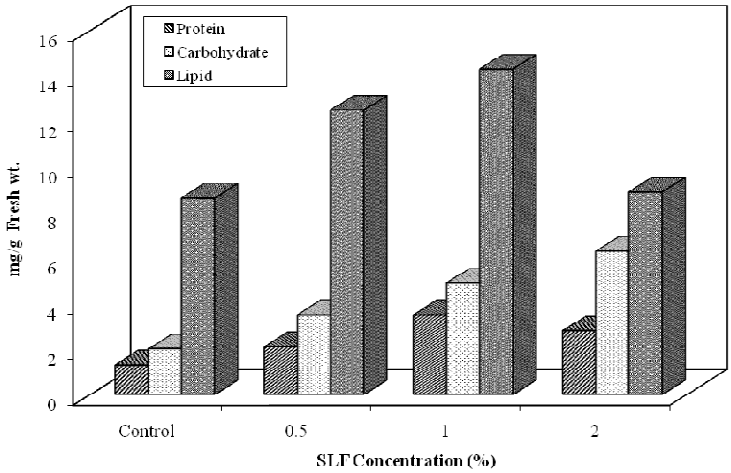


Fig.2b Effect of *Sargassum wightii* LSF on the total protein, total carbohydrate and total lipid content of *Vigna radiata* at root application



DISCUSSION

Seaweeds are known to concentrate several folds of nutrients available in their habitats. From time memorial, the nutrients in the land are being leached in to the ocean through rains, rivers etc. Utilization of seaweed as Liquid Seaweed Fertilizer (LSF) is one of the excellent means to get the lost nutrients back to the land. Application of LSF plays a significant role in improving the yield of crop plants by about 20-30%. In the present study the LSF obtained from the brown seaweed *Sargassum wightii* on *Vigna radiata* elucidated certain interesting findings.

Venkataraman Kumar *et al*., (1993) reported that Liquid Seaweed Fertilizer promoted seed germination and early seedling growth up to a concentration of 0.75% in black gram and up to 1.5% in green gram. Dilute extracts were found to be more effective than the concentrated extract (Bukhari and Untawale, 1978) and the seedling growth appeared to be poor at higher concentration (15 and 20%) and showed symptoms of gradual delay (Jayachandran and Ramasamy, 1999). In the present study also the seaweed extract of *Sargassum wightii* accelerated the seed germination and early growth of the green gram in terms of length in both radicle and plumule when soaked in 0.5% concentration for 6 hrs duration compared to high concentration (2.0%). But when Bhosle *et al*. (1975) studied the effect of different concentrations of extracts obtained from *Padina tetrastromatica* and *Sragassum tenerrimum* on the growth of *Phaseolus vulgaris* under laboratory condition, they found that 10% concentration of the extracts promoted growth indicating that different plants require a different concentration of LSF for their growth.

In pot studies, both the shoot length and root length were found to be increased as the concentration of LSF increases, but at high concentration i.e. at 2.0% concentration the growth was slightly reduced, similar observation was made by Jayachandran and Ramasamy (1999) when they treated *Arachis hypogea* with different concentrations of LSF obtained from *Hypnea musciformis* were the length of root and shoot became progressively decreased with increasing concentration of the extract, a maximum number of seven leaves were recorded in plants applied with 2.5% concentration. However, more number of lateral roots was noticed in 10% concentration. But Sylvia *et al*. (2005) reported that higher dilutions of LSFs are more effective than lower dilutions. In the present study number of leaves was more only at 1.0% concentration in both foliar treated and root treated plants, whereas at 2.0% concentration the number of leaves was reduced. Sekar *et al*., (1995) also reported similar observation in *Vigna unguiculata* when treated with *Ulva* lactuca LSF showed good growth at low concentration (0.25%).

Seaweed extract also known to promote hormonal activity resulting in the initiation of flowers at an early stage. Taylor and Wilkinson (1977) reported early flowering in plants received lower dosages of seaweed extract. In the present study also the plants received 1.0% concentration of LSF showed early flowering followed by 2.0% concentration. Chaudhary and Loneregan (1972) suggested that high dose LSF with excess organic content will delay the flower production due to toxicity.

The number of pods was also more in 1.0% concentration both in foliar well as root treated plants. Rama Rao (1991) showed that the aqueous extract obtained from *Sargassum wightii* when applied as foliar spray on cultivated *Zizyphus mauritiana* increased the yield and quality of fruits. The foliar application increased the fresh weight of the fruits by about 25% over control. Similarly the seaweed extracts of members of Laminariaceae and Fucaceae when applied as foliar application on banana showed that the time of fruiting decreased while the average bunch weight of the fruits increased (Blunden, 1972)

The biochemical analysis of the experimental plants showed that the foliar treated plants showed more photosynthetic pigments compared to root treated plants, whereas the accumulation of total protein, total carbohydrate and total lipid content was found maximum in root treated plants than in the foliar treated plants. The total protein and total lipid content was more at 1.0% concentration whereas the carbohydrate content was more at 2.0% concentration. Anantharaj and Venkatesalu (2001) reported that the LSF of *Caulerpa recemosa* and *Gracilaria edulis* on *Vigna catajung* showed that the low concentrations of aqueous extracts promoted the seedling growth, fresh and dry weight, Chlorophyll content, protein, aminoacids and total sugar than higher concentration of LSF. Similarly the crude extracts obtained from three green seaweeds namely *Cladophora dalmatica, Enteromorpha intestinalis* and *Ulva lactuca* and red seaweed *Pterocladia pinnata* enhanced the protein content, soluble sugars and chlorophyll content in leaves of *Vicia faba* at low concentrations (El-Sheekh and El-Saied, 1999).

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