Original Article

Influence of Seaweed Liquid Fertilizer on the Growth of *Trigonellafoenum-graecum* L.

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ABSTRACT

The effect of seaweed liquid fertilizer (SLF) of *Sargassum wightii* on germination, growth and biochemical constituents of *Trigonellafoenum-graecum*L. (Fenugreek) was studied. *Sargassum wightii* was collected from Kanyakumari (longitude 8.0883° N, latitude 77.5385° E) for the present study. Different concentrations (2%, 4%, 6%, 8% and 10%) of SLF were prepared using distilled water. Maximum germination percentage (100 ± 0.00%), fresh weight (3.63±0.18 g), dry weight (0.22±0.04 g), shoot length (6.83 ± 1.04 cm), root length (3.26 ± 0.20 cm), recorded at 6% concentration of *Sargassum wightii* liquid fertilizer treated fenugreek. Pigments such as total chlorophyll (11.95±0.22 mg.g/fr.wt) and carotenoids (4.9±0.1 mg.g/fr.wt) contents in leaves recorded at 6% SLF were the highest. The 6% SLF treated seedling showed the highest content of protein (6.8±0.1mg.g/fr.wt), aminoacid (3.63 ± 0.05mg.g/fr.wt), carbohydrate (2.6 ± 0.1 mg.g/fr.wt), ascorbic acid (0.36±0.015 mg.g/fr.wt), phenol (0.8±0.01 mg.g/fr.wt), nitrate (9.96± 1.35 mg.g/fr.wt) and nitrate reductase (14.96±0.85 mg.g/fr.wt) activity of shoots and roots respectively. The SLF showed better responses at lower concentration when compared to control and higher concentrations showed declining trend. The present study demonstrated that *Sargassum wightii* liquid extract could serve as an alternative biofertilizer as it is eco-friendly, cheaper, deliver substantial economic and environmental benefits to farmers.

Key words: *Sargassum wightii*, Seaweed liquid fertilizer, *Trigonellafoenum-graecum*, Germination and Growth, Brown seaweeds.

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INTRODUCTION

Agriculture confronted multiple demanding situations to provide more food for a growing population.[1] The expected agricultural production has to be doubled so as to cater the needs of estimated 9 billion people during 2050. More than 870 million people are reportedly chronically hungry, many of them small farmers.[2] Organic farming is proving as a remedy to cure ills of modern chemical agriculture.[3] Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity.[4] The toxic chemicals (arsenic and cadmium) from the chemical fertilizers accumulate in plant products causing health problems in human by biomagnifications.[5] The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites. In recent years, research on marine sources has attracted a lot of attention globally.[6] In recent years, many bioactive compounds have been extracted from various marine plants, marine animals and marine organisms.[7] Algae are the amazing sustainable resources in the marine ecology which have been used as a source of foodstuff and drug.[8] Marine algae express plant protection against phytopathogens, and also endorse the production of bioactive molecules proficient to induce resistance in plants.[9] Marine microalgae or seaweed are found in the coastal region between high

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tide to low tide and in the sub-tidal region up to a depth where 0.01% photosynthetic light.[10] Commercially available varieties of marine macroalgae are commonly referred to as seaweeds.[11] Seaweeds, especially brown seaweeds such as *Sargassum* species, have been used in farming systems in coastal areas of the world since the twelfth century. In India people consume seaweeds indirectly in the form of phycocolloids added in chocolate, ice cream, jellies and as stabilizers in food products.[12] As primary producers they produce oxygen and organic compounds which serve as the basic tropic level or food for many other living being.[13] Seaweeds contain more than 60 elements, macro and micro-nutrients, proteins, carbohydrates, vitamins, and aminoacids.[14] Seaweeds are significant viable resources because of their industrial potential; particularly, their extracts contain polysaccharides, proteins, polyphenols, nutrient elements and important plant growth hormones or regulators, that make them as an ample substitutes of biofertilizers and they are actually named biostimulants.[15] The biomedical applications of seaweed are mainly due to its functional groups.[16] Over the past several decades seaweeds have generated an enormous amount of interest in the pharmacological industry with enormous medicinal properties.[17] Algal biomass has also been composted and then used for growing crops on various types of soil.[18] Due to their potential usage in organic and sustainable agriculture, in particular in rain-fed crops, the use of seaweed extracts has gained popularity in recent years as a means of avoiding excessive fertilizer application and enhancing mineral absorption.[19] Seaweeds extracts are marketed as liquid fertilizers and biostimulants since they contain many growth regulators such as cytokinins, auxins, gibberellins, betaines, macronutrients such as Ca, K, P, and micronutrients like Fe, Cu, Zn, B, Mn, Co, and Mo, necessary for the development and growth of plant.[20] Seaweed fertilizer was found to be superior to chemical fertilizer because of high level of organic matter which aids in retaining moisture and minerals in the upper soil level available to the root.[21] Products with functional properties containing organic compounds derived from natural sources, rather than being a product of heavy organic synthesis are increasingly demanded by consumers.[22] Some products, such as Kelpak, Seaspray, Seasol, SM3, Cytex and Seacrop, are commercially available. Seaweed fertilizers are better than other fertilizers and are very economical.[23] The long term application of inorganic fertilizers is to increase the productivity of crop and they lead to the ill-effect of the ecology of the agricultural systems.[24] A number of commercial seaweed extract products are available for use in agriculture and horticulture.[25] Seaweeds are known to aid and stimulate growth of vegetables, fruits and other crops. They include major and minor plant nutrients and organic compounds such as auxins, gibberellins, ethylene and betaine precursors that influence plant growth.[26] Seaweed fertilizer was considered to be effective to chemical fertilizer due to increase level of organic matter helps in retaining moisture and minerals in the upper soil level accessible to the roots. Recently researchers proved that seaweed fertilizers are better than other fertilizers and are very economical.[27] The genus name Trigonella, due to the triangular shape of its flowers, has the Latin meaning 'little triangle,'Thespecies '*foenum-graecum'* means 'Greek hay'

It is also called 'ox horn' or 'goat horn' as its two seed pods extend from the nodes of the stem base in opposite directions and mimic an ox or goat horns.[28] Fenugreek is one of the condiments known to mankind and has been cultivated for a very long time. It has medical properties such as role of phyto-estrogens, diosgenins to fight breast cancer, reduction of serum cholesterol.[29] Fenugreek is a herb commonly found in many Asian, European and African countries. India is the largest producer of fenugreek in the world. Rajasthan, Gujarat, Uttaranchal, Uttar Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states of India.[30] The beneficial effect of seaweed extract application is as a result of many components that may work synergistically at different concentrations, although the mode of action still remains unknown.[31] Hence the present study was conducted tofind out the effect of seaweed (*Sargassum wightii*) on the growth and biochemical parameters of *Trigonellafoenum-graecum* L.

MATERIALS AND METHODS

Sample collection

Brown seaweed *Sargassum wightii* was collected from Id inthakarai, Kanyakumari District, (longitude 8.0883° N, latitude77.5385° E) Tamil Nadu. To extract all the impurities, sand particles and epiphytes, the algal sample was handpicked and completely cleaned with sea water. It was placed in an ice box with slush ice, shipped to the laboratory and carefully washed to remove the salt on the surface of the sample using tap water. The water was drained off and the algal material was spread on blotting paper to remove excess water. They were shade dried. The dried seaweeds are finally pulverized in the commercial grinder and the powdered seaweed samples are used for further analysis.

Preparation of Seaweed Liquid Extracts (SLE)

One kilograms of seaweed was taken and boiled with 1 L of distilled water for an hour and then extract was filtered through muslin cloth .The filtrate was allowed to cool at room temperature and thereafter, through Whatmann No. 41 (pore size 20-25 μm) filter paper.[32] The filtrate was 100% seaweed extract. Now, the extract was made up into 100ml with distilled water (10%).[33] From this, different concentrations of seaweed liquid extract (2, 4, 6, 8 and 10%) were prepared by diluting with distilled water. For further research, the seaweed extract was kept at 4°C.

Physico-chemical analysis of SLE

The color and pH of the SLE was recorded. The composition of elements such as Cu, Mn, Fe, Zn, and Mg were estimated using Atomic Absorption Spectrophotometer.

Selection of crop plant

The crop plant, selected for the present study was *Trigonellafoenum-graecum* L. belonging to the family Fabaceae. The seeds were collected from the local market. For the experimental purposes, seeds of uniform scale, colour and weight were selected.

Germination and early growth studies

About ten gram of seeds of *Trigonellafoenum-graecum* L was taken and surface sterilized with 0.1% mercuric chloride for one minute and washed thoroughly in sterilized distilled water. 20 numbers of each seeds were placed in sterilized tissue paper which placed on petriplates. About 5ml of various concentrated seaweed liquid fertilizer was added at 3 days intervals. Then it was incubated under room temperature of 37°C. Seed germination was occurred 10 days.[1] After germination, the percentages of germination data was recorded by the following formula, [Germination % = Number of Seeds Germinated/ Total Number of Seeds X 100].

Growth parameters

After seed germinated fresh weight measured immediately. They were dried in a hot air oven at 80°C for 24 hr and then dry weight was taken. The mean values were expressed in mg/g.[34] Then the shoot length and root length were calculated.

Preparation of Pot study

Red Soil and sand was chosen for the pot study which was collected in Sri Kaliswari College campus, Sivakasi, Tamil Nadu, India. Soil was collected and it was allowed to dry under shadow for removal of moisture content. Then the soil was sterilized by autoclave under 121°C and 15Lbs pressure. Then both red and sand soil was put on to the various pots at 1:1 ratio. Seeds of Fenugreek were purchased from local market. The seed to seed distance in pot was maintained as 3-5cm and pots were maintained regularly. Potted plants were drenched with different concentrations of liquid extracts. About 20 ml of different concentrations of extracts were given at interval of 4 days. After 30 days, yield parameters and Biochemical constituents were observed. All pot experiments were done in triplicates each under natural uniform conditions. Then the soil containing chemical parameters were analyzed in Agriculture Department, Aruppukottai, Tamil Nadu, India.

Biochemical estimation

The biochemical constituents such as Chlorophyll a, b, totalchlorophyll content,[35] Carotenoid content,[36] Carbohydrate,[37] Aminoacid,[38] Protein,[39] Ascorbic acid,[40] Phenolic compound, Nitrate reductase[41] and Leaf Nitrate were estimated.

RESULTS

The brown seaweeds were collected from Idinthakarai, Kanyakumari Dist (longitude 8.0883° N, latitude 77.5385° E). The species was identified as *Sargassum wightii* by its morphological structure (Figure 1).

The FT-IR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. FTIR spectrum of the methanol extract of *S. wightii* was analyzed by FT-IR (Figure 2).

The peak at 3442.94cm^{-1} shows the presence of Hydroxyl group, 1840.09cm^{-1} due to the presence of O-H, 1796.73cm^{-1}, 1741.78cm^{-1} due to the presence of

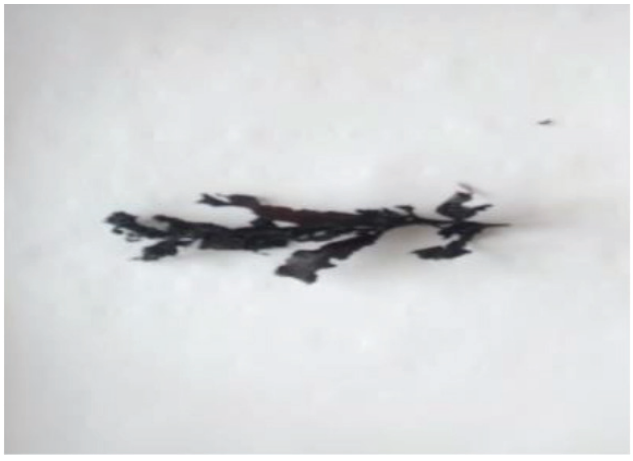

Figure 1: Brown seaweeds collected for the study.

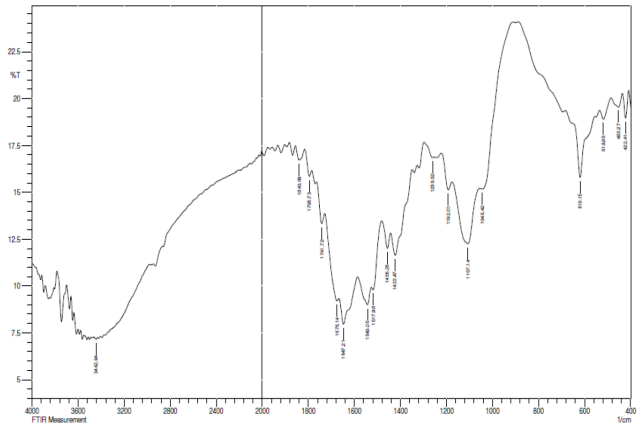

Figure 2: FTIR spectrum of the methanolic extract of *S. wightii.*

Table 1: Physico-chemical analysis of liquid extract of *Sargassum wightii.*

Chemical Parameters(ppm)	Fe	Mg	Mn	Zn	Cu
	2.91	1.69	0.09	0.18	0.05
Physical Parameters	**Colour**	**pH**			
	Brown	6.3			

C=O,1678cm^{-1}, 1647.21cm^{-1} due to the presence of acetyl lactone group, 1543.05cm^{-1} the presence of C=N, 1517.98cm^{-1} due to the presence of N=N, 1456.26cm^{-1} shows the presence of thiazole group, 1259cm^{-1} due to the presence of C=S group, 1192cm^{-1} due to the presence of C-O-C group,1107.14cm^{-1} shows the presence of carbon-nitrogen covalent bonding, 619.15cm^{-1} shows the presence of bromine and 422.41cm^{-1} shows the presence of iodine. Further the extract of *Sargassum wightii* was prepared. From this extract various concentrated liquid fertilizer were prepared.

The physico-chemical analysis of liquid extract of brown marine alga revealed the presence of Iron (2.9 ppm), Magnesium (0.9ppm), Manganese (1.6ppm), Zinc (1.8ppm), Copper (0.5ppm). Among the elements Iron (2.9ppm) was found to be abundant in the extract (Table 1).

The effect of seaweed extract of *S. wightii* on germination percentage and growth of *Trigonellafoenum-graecum* L. is presented in Figure 3. In 6% concentration of 3rd day and 6th day of *S. wightii* also exhibited highest germination percentage of 95±0.57, 100±0.0. The control plant recorded a growth of 98±0.88 percentage of germination (Figure 4). A reduction in biological activity resulting to sickly brown cotyledons which was caused by higher concentrations of seaweed extract, possibly either due to the lack of chloroplast integrity or due to the interaction of other compounds in the seaweed extract.

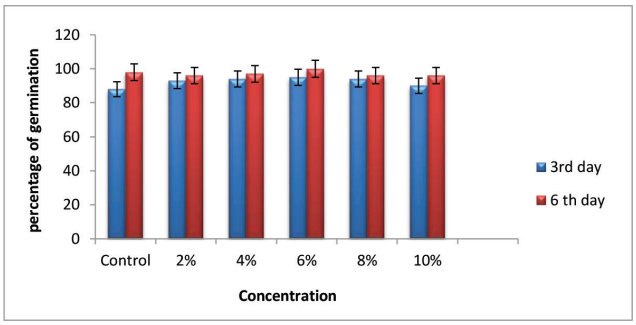

Figure 3: Influence of Seaweed liquid fertilizer (SLF) of *Sargassum wightii* on germination percentage of *Trigonellafoenum-graecum* L.

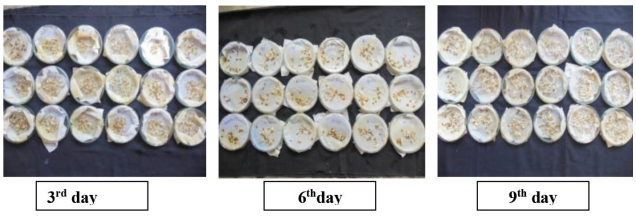

Figure 4: Seed Germination of *Trigonellafoenum-graecum* L.

Table 2: Influence of Seaweed liquid fertilizer (SLF) of *Sargassum wightii* on shoot, root length, fresh and dry weight of *Trigonellafoenum-graecum* L.

Concentration	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control	5.16 ± 1.04	1.5 ± 0.5	3.07±0.18	0.22±0.02
2%	5.66 ± 2.30	2 ± 0.2	2.76±0.71	0.16±0.01
4%	6 ± 0.5	2.36 ± 0.11	2.54±0.55	0.17±0.01
6%	6.83 ± 1.04	3.26 ± 0.20	3.63±0.18	0.22±0.04
8%	4.83 ± 0.76	1.73 ± 0.25	3.46± 0.07	0.18±0.02
10%	3.83 ± 0.76	1.43 ± 0.37	3.47±0.19	0.15±0.04

The various growth parameters were analyzed and highest root length (3.26±0.20cm), shoot length (6.83± 1.04cm) fresh (3.63±0.18 g) and dry weight (0.22±0.04 g) were observed at 6% concentration extract of *S. wightii* (Table 2).

There was a significant difference in biochemical status of different concentration levels. The biochemical constituents increased with concentration levels upto 6% and thereafter it was declined. The highest values of Chlorophyll 'a' (4.05 ± 0.10), Chlorophyll 'a' (7.89 ± 0.17), total Chlorophyll (11.95 ± 0.22), Carotenoid (4.9 ± 0.1) was observed as shown in

Table 3, Protein (6.8±0.05), Aminoacid (3.63 ± 0.05), Carbohydrate (2.6 ± 0.1), Phenol (0.8 ± 0.005), Ascorbic acid(0.36 ± 0.008) were shown in Table 4, Nitrate (9.96 ± 1.35) and Nitrate reductase (14.96 ± 0.85) were recorded at 6% *S. wightii* liquid extract in Table 5.

Table 3: Influence of Seaweed liquid fertilizer (SLF) of *Sargassum wightii* on photosynthetic pigments of *Trigonellafoenum-graecum* L.

Concentration	Chlorophyll 'a' (mg.g/fr.wt)	Chlorophyll 'b' (mg.g/fr.wt)	Total Chlorophyll (mg.g/fr.wt)	Carotenoid (mg.g/fr.wt)
Control	3.17±0.14	3.38±0.23	6.55±0.09	3±0.1
2%	3.35±0.13	4.56±0.20	7.91±0.13	3.53±0.15
4%	3.6±0.06	5.01±0.18	8.62±0.16	3.63±0.15
6%	4.05±0.10	7.89±0.17	11.95±0.22	4.9±0.1
8%	3.7±0.20	7.48±0.24	11.17±0.05	4.43±0.15
10%	3.63±0.20	6.25±0.24	9.88±0.04	3.9±0.1

Table 4: Protein, Amino acid, Carbohydrate, Ascorbic acid and Phenol content of *Trigonellafoenum-graecum* L.

Concentration	Protein (mg.g/fr.wt)	Amino acid (mg.g/fr.wt)	Carbohydrate (mg.g/fr.wt)	Ascorbicacid (mg.g/fr.wt)	Phenol (mg.g/fr.w)
Control	1±0.1	0.96 ± 0.05	1.2 ± 0.1	0.15±0.01	0.57±0.02
2%	2.6±0.1	2.26 ± 0.05	1.6 ± 0.05	0.25±0.01	0.58±0.01
4%	4.5± 0.1	2.43 ± 0.05	2.1 ± 0.1	0.35±0.01	0.62±0.01
6%	6.8±0.1	3.63 ± 0.05	2.6 ± 0.1	0.36±0.015	0.8±0.01
8%	5.3±0.15	2.3 ± 0.17	1.7 ± 0.1	0.32±0.01	0.63±0.01
10%	4.5±0.1	2.26 ± 0.05	1.9 ± 0.1	0.23±0.01	0.53±0.01

Table 5: Influence of Seaweed liquid fertilizer (SLF) of *Sargassum wightii* on Nitrate and Nitrate reductase content of *Trigonellafoenum-graecum* L.

Concentration	Nitrate (mg.g/fr.wt)	Nitrate reductase (mg.g/fr.wt)
Control	4.2± 1.01	6.63±0.85
2%	5.1± 1.01	6.93±0.49
4%	8.63±0.65	8.3±0.8
6%	9.96± 1.35	14.96±0.85
8%	5.96±0.65	14.13±0.85
10%	6.4± 1.01	11.63±0.85

DISCUSSION

Seaweeds show great potential as a source of seaweed liquid fertilizer for raising food crops. The present study highlights the efficacy of seaweed liquid fertilizer obtained from the brown seaweed. The time has come to produce a substantial quantity of food to the rapidly growing world population. It is an expected that there will be great deficiency of food materials in future. It is necessary to avoid this fast approaching disaster; also the production of various type of crop production is to be increased adequately.[5] The best candidate to be used as a bio-fertilizer to enhance canola growth, yield, and salt stress tolerance was found to be *Ulvalactuca*.[42] Seaweed liquid extracts of *S. wightii* substantially accelerated the growth rate and physiology of cluster bean. There was a noticeable increase in growth and biochemical parameters when 1.5%of seaweed liquid extracts of *S. wightii* applied to cluster bean plant. Higher concentrations (2.0%) were found to show inhibiting effect on all the above parameters studied. Total plant height (33%), total fresh weight (155%), dry weight (140%), leaf area (61%) and moisture content (55%) were enhanced when 1.5% concentrations of liquid extracts was applied. Further, the retarding effect (reduction by 2% to 27%) in growth parameters was corresponding to increase in the concentrations (2.0%, 2.5% and 5.0%). When treated with varying concentrations of *S. wightii* extracts with Cluster bean plants, it showed differential responses in biochemical parameters. The amount of photosynthetic pigments (by 78%), protein (by 73%), sugar (by 101%) and the activity of nitrate reductase (by 159%) were found to be enhanced in cluster bean plants when 1.5% concentrations of extract were given. Other treatments such as 2.0%, 2.5% and 5.0% showed reduced levels of these parameters in the treated plants.[43] The present study showed when the concentration increased (8% and 10%) that inhibiting the growth of fenugreek. Application of seaweed as biofertilizer will be useful in enriching the soil and achieving higher production in the place of costly chemical fertilizer. Significant differences were observed in the germination rate of fenugreek at lower concentrations than higher concentrations of the tested brown seaweeds and this is may be due to the presence of growth promoting substances such asindole butyric acid (IBA), gibberellins A and B, cytokinins, micronutrients (Fe, Cu, Zn, Co, Mo, Mn and Ni) and indole-3-acetic acid (IAA).[44] The present results are in agreement with that the low concentration of aqueous extracts of *S. wightii* promoted the seedling growth of *V. sinensis*.[27] The increased growth parameters of *V. mungo* at lower concentration may be due to the

presence of higher levels of N, P, K in the seaweed extract of *S. wightii*. The dilute seaweed extracts more effective than the concentrated extract.[45] The seed germination, shoot and root length of *V. radiate* (L.) were maximum at 10% SLF of *C. sinuosa*.[46] The effect of SLF of *C. recemosa* and *G. edulis*on growth and biochemical constituents of *V. catajung* was also evidence for this study.[47] *C. cajan* seaweed extract of the present study seaweed extracts treated on the vegetable crops of *Abelmoschus esculentus* and *Lycopersicon lycopericum*. Different concentrations (10, 15, 20, 25 and 30%) of SLF were used and better results were obtained in lower doses (10, 15, 20).[48] Protein content of fenugreek seeds was increased by decrease the concentration of seaweed extracts. The negative responses with increase the concentration of seaweed extracts on seed germination and protein content can be attributed to presence of regulator hormones or high levels of minerals that inhibited the growth.[34] In the present study, *S. vulgare* was the effective seaweed extract on fenugreek plant which showed higher germination rate and protein content and this may be due to that *S. vulgare* contains low amount of phenol and high amount of carbohydrate and nitrogen which play a significant role in crop quality[49] and also that the activation of nitrate reductase, key enzyme of nitrogen metabolism which enhanced the growth.[50] The highest protein, amino acid, reducing and total sugar contents of *V. mungo* were observed at lower concentration of SLE of *C. scalpelliformis* (25%). Such a rise in protein, amino acid, reducing and total sugar contents may be attributed to the increased availability and absorption of necessary elements (Ca, Na, K, Mg, N and Zn) present in the seaweed extracts.[4] The beneficial effect of seaweed extracts on seed germination and plant growth promotion may be due to the presence of plant growth promoting substances or hormones present in the seaweed extracts. From the results, the work also suggested that, development of natural seaweed liquid fertilizer (SLF) by using these potential seaweeds (*S. wightii, U. fasciata*and *P. boergesenii*) in agriculture for crop plants will definitely enhance the crop production.[1] The Seed germination, shoot, root length, fresh, dry weight, chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids were found to be the maximum at 6% SLF. So it may be concluded from present study that, lower dose (6%) of SLF leads to higher growth, yield and soil profile compared to other concentrations. So the present study revealed that lower concentration of SLF increased the growth of fenugreek. When the concentration increased that leads to inhibit the fenugreek growth.

CONCLUSION

In the present study algal samples were collected from Idinthakarai, Kanyakumari district. Methanolic extract of *S.wightii* was prepared and it was used for GC-MS and FT-IR analysis for identification of functional group mass value. Seaweed liquid fertilizer was prepared. Then various concentrated SLF was prepared, such as 2%, 4%, 6%, 8% and 10%. Various concentrations of SLE of *S. wightii* applied to fenugreek plant. Seed germination and pot study was carried out. From the seed germination study root length, shoot length, dry weight and fresh weight was measured. In pot study Fenugreek containing photosynthetic pigments, protein, aminoacid, carbohydrate, phenol, ascorbic acid, nitrate and nitrate reductase content was measured. It may be concluded that the growth and biochemical characteristics of *Trigonellafoenum-graecum* L. could be promoted by the presence of micro and macro elements, growth hormones, vitamins etc. in the SLF of *S. wightii*. However, optimum concentration of seaweed liquid extracts in necessary as in this study 6% SLF had better influence on growth and productivity of fenugreek plants. The study also emphasizes that seaweed extracts can be effectively used as organic biostimulants to the agricultural crops and also much useful in the practices of organic forming. The present study is additional information for an alternate to synthetic fertilizers and further study is necessary to recompense the constraints.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SLF: Seaweed Liquid Fertilizer; **SLE:** Seaweed Liquid Extract.

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