Indian Journal of Natural Products and Resources
Vol. 12(2), June 2021, pp 300-306

Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using different methods extract on growth and pigments of black gram (*Vigna mungo* L.)

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Received 07 July 2019; Revised 25 February 2021

To investigate the growth, percentage of germination, shoot and root length, fresh and dry weight along with photosynthetic pigments (chlorophyll a, b, and total chlorophyll) of *Vigna mungo* was analyzed by using seaweed (*Padina tetrastromatica*) SLF and seagrass (*Cymodocea serrulata*) SGLF extracts of seven days old seedlings. The seaweed and seagrass extracts were prepared by five different methods such as low temperature, boiling, autoclave, alcohol, and alcohol aqueous extracts with different concentration (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0%). The results revealed that significant effect showed low temperature, autoclave, and alcohol aqueous methods of seaweed and seagrass extract preparation, the study suggested that field trial to assess the seaweed and seagrass fertilizer can be used as biofertilizer for better cultivation of *Vigna mungo*.

Keywords: Black gram, *Cymodocea serrulata*, *Padina tetrastromatica*, Seagrass, Seaweed.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/02

Introduction

The seaweeds and seagrasses are important marine living resources among other natural resources of the marine ecosystem. These resources can act as bio factories of various commercial application including pharmaceutical, nutraceutical, and agriculture worldwide. Fertilizer is one of the most important inputs in agricultural production. Different chemical fertilizers may lead to different environmental pollution as well as diseases for humans. Earlier studies have proved that the hazardous effect of chemical fertilizers on the environment and human health by directly or indirectly[1]. The seaweed suspensions can be alternative management, especially for organic farming. Many of them are used as food preparation and also raw materials for fertilizer or compost for increasing productivity[2]. More than 15 million tons of seaweeds are produced annually[3]. Seagrasses are the only flowering plants growing in marine ecosystems. There are 13 genera and 58 species available in the entire world, most of the seagrasses are used extensively as soil fertilizer (SGLF) for coconut and other plantations in Tamil Nadu and Kerala. Seagrasses are being used as animal feed and raw material for many industries[4]. Seaweeds are rich in micro and macronutrients and their extracts contain polysaccharides (e.g., galactan, fucoidan, alginate, and laminarin), proteins (e.g., lectins), polyunsaturated fatty acids (PUFAs), pigments (e.g., chlorophylls, carotenoids, and phycobiliproteins), polyphenols (e.g., phenolic acids, flavonoids, cinnamic acid, isoflavones, benzoic acid, and lignans, quercetin), minerals (e.g., K, Mg, Ca, and Na), and plant growth hormones (e.g., cytokinins, auxins, gibberellins, and abscisic acid)[5,6]. Seaweed contains all the trace elements and growth hormones required by plants. Recently there is a growing concern over the use of seaweed liquid fertilizer[7]. The use of seaweed fertilizer as a biostimulant, when applied to leaves or roots, improves crop development and consequently enhances the yield[8,9]. The combined seaweed and seagrass extracts contain plant growth hormones, regulators, carbohydrates, amino acids, auxins, gibberellins and vitamins which enhance the yield and quality of crops, seed germination, resistance to frost, fungal and insect attacks[10]. Seaweed fertilizer was found to be superior to chemical fertilizer. The presence of a high level of organic matter assists to retain the moisture and minerals in the upper soil which facilitate roots[11]. In the present day world, seaweed fertilizers are often found to be more successful than chemical fertilizers[12]. In view of the above facts,

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the present study has been attempted to prepare seaweed (*Padina tetrastromatica*) and seagrass (*Cymodocea serrulata*) extracts by using different methods and evaluated their growth and pigments enhancing potential.

Materials and Methods

Collection and identification of seaweed and seagrass

The selected seaweed *Padina tetrastromatica* Hauck belongs to *Phaeophyceae* family Seagrass *Cymodocea serrulata* (R. Brown) Ascherson & Magnus belongs to the family *Cymodoceae* were collected from the intertidal and subtidal regions up to 1 m depth from Hare Island, Tuticorin coast, Gulf of Mannar Biosphere Reserve, Tamil Nadu. Samples were collected during low tide in the forenoon. The sample was quickly washed in freshwater for 1-3 minutes to remove all unwanted impurities and salt. The cleaned sample was placed in new polythene bags and kept in an icebox containing slush ice and transport to the laboratory. The collected seaweeds were identified by using the standard algal flora[13].

Preparation of different methods of seaweed (SLF) & seagrasses liquid fertilizer (SGLF)

The washed and cleaned seaweed and seagrass were shade dried for five days at room temperature then the sample was chopped into fine fragment and powder with the help of a mixer grinder. The powder was preserved in vials to prepare seaweed and seagrass liquid fertilizer. The preparation methods of seaweed and seagrass liquid fertilizer are as follows.

Low-temperature heating method

Exactly 100 g of powder (50 g of seaweed and 50 g of seagrass) was weighed then 1 L of deionized water was added. It was heated to 60 ^{o}C and maintained for 24 hours in a hot air oven. The extracts were filtered through a muslin cloth[14].

Boiling method

Exactly 100 g of powder (50 g of seaweed and 50 g of seagrass) was weighed then 100 mL of distilled water was added. It was heated to 100 ^{o}C in water for 1 hour. The extracts were filtered and stored in a refrigerator till use[15].

Autoclave method

Exactly 100 g of powder (50 g of seaweed and 50 g of seagrass) was weighed then distilled water was added in the ratio of 1:10. And it was autoclaved at 15 Ibs pressure for 1 hour. The extracts were filtered and stored in a refrigerator till use[16].

Alcoholic extraction method

Exactly 100 g of powder (50 g of seaweed and 50 g of seagrass) were mixed with 100 mL of alcohol and stirred well and then filtered. The filter was considered as 100% concentration of extract[17].

Alcoholic-aqueous method

Exactly 100 g of powder (50 g of seaweed and 50 g of seagrass) was soaked in 40 mL alcohol for 12 hours and shaken vigorously to dissolve the alcohol-soluble constituents. The supernatant was saved and the residue was boiled in 50 mL of distilled water for 30 minutes, then cooled and filtered. Alcohol and water-soluble supernatants were mixed and the volume was made up to 100 mL with distilled water to get 100% extract[17].

Selection of crop plant

The crop plant selected for the present study was *Vigna mungo* (L) Hepper VBN4 (black gram) which belongs to the family *Fabaceae*. It is an important pulse cultivated in the Indian subcontinent. The seeds were collected from the Tamil Nadu Agricultural Research Centre in Kaveripattinam, Krishnagiri, Tamil Nadu, India. Healthy seeds free from visible infection with uniform size and colour. Seeds were sterile with 0.1% Mercury chloride then washed with distilled water.

The sterile seeds were soaked in each concentration of different methods of SLF & SGLF liquid fertilizer for 12 hours. Control was maintained as distilled water.

Germination percentage

After the soaking period, the seeds were rolled in germination towels. The growth parameters were estimated after 7 days. The germination percentage was calculated by the following formula.

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds sown}} \times 100$$

Fresh and dry weight

The seedlings were blotted in blotting paper and weighed. They were dried in a hot air oven at 80^{o}C for 24 hours. The dry weight was taken by using an electrical single pan balance[18].

Estimation of chlorophyll

Chlorophyll content was estimated spectrophotometrically according to the method of Arnon[19].

Results

Low-temperature method

The effect of the combined extracts of SLF and SGLF in the Low-temperature method (growth parameters: germination %, shoot and root length, fresh and dry weight and chlorophyll pigments) are presented in Table 1 and Fig 1, respectively. About 99% of germination was observed at 0.5% of combined extract when compared to all other concentration except control. The results revealed that the maximum shoot and root length (25.55 cm and 15.15 cm) was 3% of the combined extract, respectively. The highest fresh weight (0.4 g) was observed in 1% and dry weight (0.306 g) was shown in 4% of the combined extract. The chlorophyll a (3.71 mg/g), b (0.405 mg/g) and total chlorophyll (4.11 mg/g) maximum level were observed in 0.5% of combined extract in 7 days of old seedlings of *V. mungo.*

Boiling method

The effect of the combined extract of SLF and SGLF in the boiling method (growth parameters: germination %, shoot and root length, fresh and dry weight and chlorophyll pigments) were presented in Table 2 and Fig. 2, respectively. The 95% of germination was observed at 0.5% of the combined extract. The results of maximum shoot length

Table 1 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using low-temperature method extract on growth parameters of black gram (*Vigna mungo* L)

Parameters	Control	Concentration (%)					
		0.5	1.0	2.0	3.0	4.0	5.0
% of germination	80	99	98	95	95	93	93
Shoot length (cm)	12.8±1.818	23.8±1.39	20.85±1.49	24.7±1.20	25.5±1	24.15±0.86	23.75±1
Root length (cm)	7.9±0.737	10.6±1.62	14.2±2.03	15.1±1.44	15.15±1.90	14.5±1.53	14±1.63
Fresh weight (g)	0.201±0.041	0.369±0.06	0.4±0.97	0.35±0.07	0.348±0.95	0.353±0.43	0.025±0.02
Dry weight (g)	0.020±1.02	0.03±0.03	0.027±0.043	0.03±0.65	0.024±0.012	0.306±0.312	0.024±0.15

The value represents the mean of 10 samples with their Standard Deviation (±)

Table 2 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using boiling method extract on growth parameters of black gram (*Vigna mungo* L)

Parameters	Control	Concentration (%)					
		0.5	1.0	2.0	3.0	4.0	5.0
% of germination	86	95	96	98	99	96	94
Shoot length (cm)	12.8±1.818	21.65±1.90	21.95±1.64	24±1.61	23.55±	21.2±1.29	24.4±1.53
Root length (cm)	7.9±0.737	11.25±1.66	14.55±1.25	13.9±1.46	13.2±1.01	13.62±1.65	11±1.78
Fresh weight (g)	0.201±0.041	0.298±0.23	0.295±0.67	0.299±0.45	0.302±1.06	0.336±0.98	0.397±1.21
Dry weight (g)	0.020±1.02	0.023±0.34	0.023±0.11	0.022±0.312	0.021±0.05	0.022±0.044	0.028±0.02

The value represents the mean of 10 samples with their Standard Deviation (±)

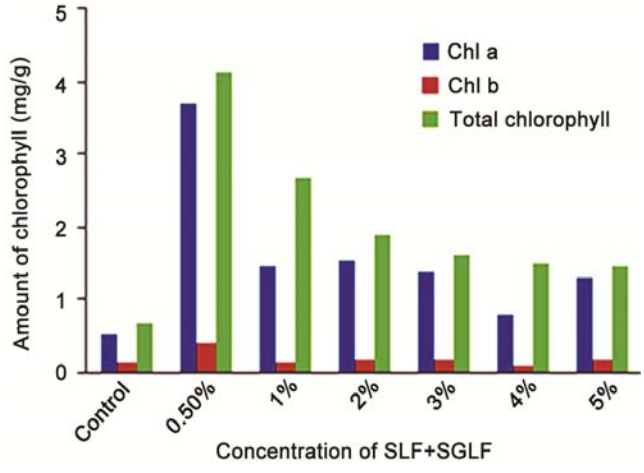

Fig. 1 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using low-temperature method extract on pigments of black gram (*Vigna mungo* L).

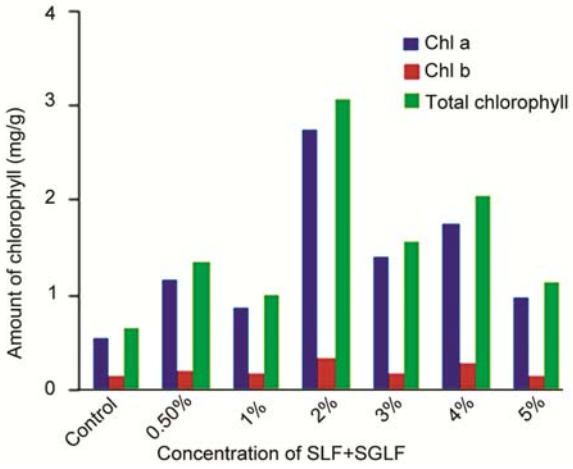

Fig. 2 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using boiling method extract on pigments of black gram (*Vigna mungo* L).

(24.4 cm) were 5% of combined extract and root length (14.55 cm) in 1%. The highest fresh weight (0.397 g) dry weight (0.028 g) was shown in 5% of the combined extract. The chlorophyll a (2.72 mg/g), b (0.32 mg/g) and total chlorophyll (3.05 mg/g) maximum level (Fig. 2) were observed in 2% of combined extract in 7 days old seedlings of *V. mungo.*

Autoclave method

The effect of the combined extract of SLF and SGLF in the autoclave method (growth parameters: germination %, shoot and root length, fresh and dry weight and chlorophyll pigments) were presented in Table 3 and Fig. 3, respectively. The 100% of maximum germination was observed at 0.5% of the combined extract. The results of maximum shoot length (28.8 cm) were observed at 3% of combined extract and root length (31.3 cm) in 2%. The highest fresh weight (0.332 g) and dry weight (0.036 g) were exhibited in 0.5% and 1% of the combined extract. The chlorophyll a (1.53 mg/g), b (0.23 mg/g) and total chlorophyll (1.76 mg/g) maximum level (Fig. 3) were observed in 0.5% of extract in 7 days old seedlings of *V. mungo*.

Alcohol extraction method

The effect of the combined extract of SLF and SGLF in the Alcohol extraction method (growth parameters: germination %, shoot and root length, fresh and dry weight, and chlorophyll pigments) were presented in Table 4 and Fig. 4, respectively. The 98% of maximum germination was observed at 0.5% of the combined extract. The results of maximum shoot length (23.5 cm) and root length (13.8 cm) were observed at 0.5% of the combined extract. The highest fresh weight (0.376 g) in 4% and dry weight (0.021 g) were exhibited in 2% and 5% of the combined extract. The chlorophyll a (1.86 mg/g), b (0.17 mg/g) and total chlorophyll (2.04 mg/g) maximum level (Fig. 4) were seen in 0.5% of a combined extract of 7 days old seedlings of *V.mungo*.

Alcohol-aqueous method

The effect of the combined extract of SLF and SGLF in the Alcohol-aqueous method (growth

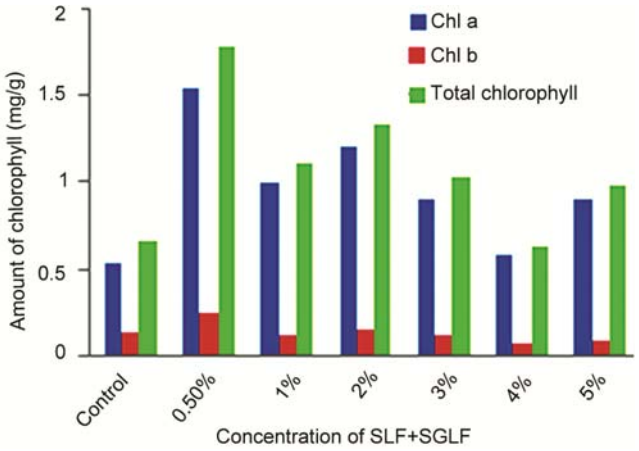

Fig. 3 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using autoclave method extract on pigments of black gram (*Vigna mungo* L).

Table 3 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using autoclave method extract on growth parameters of black gram (*Vigna mungo* L)

Parameters	Control	Concentration (%)					
		0.5	1.0	2.0	3.0	4.0	5.0
% of germination	88	100	99	99	96	90	93
Shoot length (cm)	12.8±1.818	23.6±0.80	25.55±0.82	25.05±1.55	28.8±1.73	25.1±1.21	25.3±1.37
Root length (cm)	7.9±0.737	11.6±1.09	16.7±1.20	31.3±1.03	12.34±0.89	15.1±1.15	12.8±1.12
Fresh weight (g)	0.201±0.041	0.332±0.98	0.299±0.26	0.309±0.22	0.269±0.02	0.303±0.21	0.325±0.74
Dry weight (g)	0.020±1.02	0.026±0.82	0.036±0.77	0.028±0.32	0.02±0.23	0.026±0.13	0.025±0.30

The value represents the mean of 10 samples with their Standard Deviation (±)

Table 4 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using alcohol extraction method on growth parameters of black gram (*Vigna mungo* L)

Parameters	Control	Concentration (%)					
		0.5	1.0	2.0	3.0	4.0	5.0
% of germination	88	98	99	100	100	96	95
Shoot length (cm)	12.8±1.818	23.5±0.80	17.5±0.82	21.1±51.55	15.5±1.55	5.7±1.73	7.9±1.21
Root length (cm)	7.9±0.737	13.8±1.09	10.9±1.20	12.25±1.20	10.65±1.03	4.8±0.89	7.8±1.15
Fresh weight (g)	0.201±0.041	0.286±0.02	0.308±0.134	0.291±0.21	0.202±0.43	0.376±0.32	0.254±0.65
Dry weight (g)	0.020±1.02	0.021±0.31	0.02±0.64	0.021±0.132	0.015±1.02	0.15±0.08	0.021±0.06

The value represents the mean of 10 samples with their Standard Deviation (±)

parameters: germination %, shoot and root length, fresh and dry weight, and chlorophyll pigments) were presented in Table 5 and Fig. 5, respectively. The 98% of maximum germination was observed at 0.5% in the combined extract. The results of maximum shoot length (26.5 cm) were observed at 4% and root length (15.8 cm) in 3% of the combined extract. The highest fresh weight (0.377 g) in 1% and dry weight (0.026 g) were exhibited in 2% of the combined extract. The chlorophyll a (2.03 mg/g), b (0.25 mg/g) and total chlorophyll (2.29 mg/g) maximum level (Fig. 5) were observed in 0.5% of combined extract in 7 days old seedlings of *V.mungo*.

Discussion

In the present study, the shoot and root length were seen significantly highest in the autoclave method (28.8 cm) and (31.3 cm) followed by the alcohol aqueous method. When considering fresh and dry weight, the highest value was found in the low-temperature method. Also, photosynthetic pigments amount was highest in the low-temperature method of combined SLF and SGLF. Among the five methods of extraction, the low temperature, autoclave, and alcohol aqueous methods are very useful to follow the SLF and SGLF combined extraction making procedure. Autoclave method, alcohol extraction method was found to be more active in a low percentage of concentration (0.5 %). The results showed a significant increase in the percentage of germination from 0.5 to 3.0% of the combined extract. Then gradual decrease in germination percentage was found in 4.0 and 5.0% of the combined extract of SLF and SGLF. Among the different methods, the results showed the maximum shoot and root length in 2% of combined extract as 25.5 and 15.15 cm, respectively, the highest fresh weight and dry weight were recorded in 0.5, 1.0, and 2.0% of the combined extract. The 100% of seed germination was observed in 2.0 and 3.0% of the combined extract, the highest pigments content was recorded in 0.5% of the combined extract of SLF and SGLF and also similar to the seeds treated with a low concentration of all Seaweed Liquid Fertilizer showed better results in growth parameters as compared to

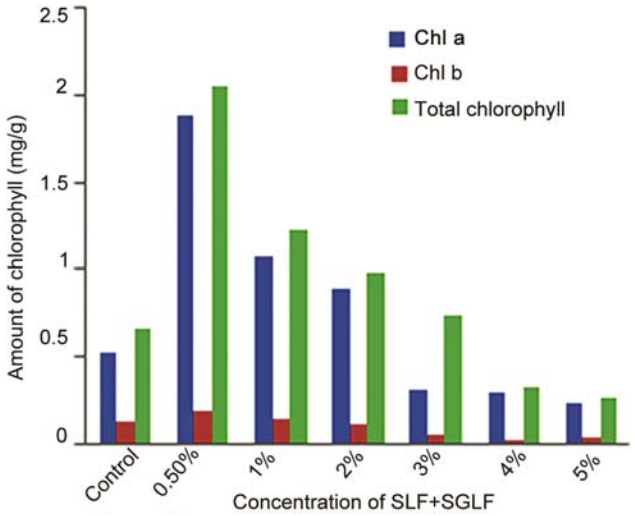

Fig. 4 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using alcohol extraction method extract on pigments of black gram (*Vigna mungo* L).

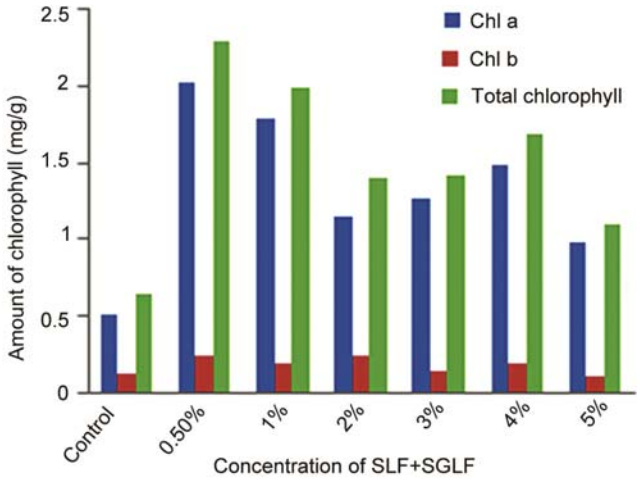

Fig. 5 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using alcohol-aqueous method extract on pigments of black gram (*Vigna mungo* L).

Table 5 — Effect of *Padina tetrastromatica* and *Cymodocea serrulata* using alcohol-aqueous method extract on growth parameters of black gram (*Vigna mungo* L)

Parameters	Control	Concentration (%)					
		0.5	1.0	2.0	3.0	4.0	5.0
% of germination	88	98	100	100	100	97	94
Shoot length (cm)	12.8±1.818	26.3±1.23	24.35±2	26.1±0.84	24.55±0.97	26.5±1.57	24.2±1.14
Root length (cm)	7.9±0.737	13.45±1.25	14.9±1.53	14.95±2.23	15.85±1.53	15.3±0.93	13.95±1.12
Fresh weight (g)	0.201±0.041	0.3117±0.07	0.377±0.43	0.3±0.87	0.291±0.08	0.286±0.76	0.294±0.43
Dry weight (g)	0.020±1.02	0.024±1.03	0.022±0.96	0.026±0.99	0.023±0.05	0.023±0.66	0.02±0.43

The value represents the mean of 10 samples with their Standard Deviation (±)

other concentration of Seaweed Liquid Fertilizer treatment[20]. Similar results were found in their study of *Stoechospermum* SLF and *Syringodium* SGLF by the method of autoclave and alcohol extraction[21]. In addition, comparable observation was made by some earlier workers[22,23]. The chlorophyll a and b also increased by the combined bio fertilizer[24]. This application also increases the protein and carbohydrate content in the seedling of *Helianthus annuus*[25]. The seaweed extract is found effective in increasing the growth parameters[26]. The higher concentration of the seaweed and seagrass extracts showed decreasing percentage of germination. Another study proved important information about the optimization of seaweed liquid extracts on onion crop[27]. Seaweeds can be used as a bio-fertilizer in organic agriculture[28]. Seaweed extracts explored for better seed germination, plant growth and which act as a potential biocide and enhance the yields of standing crop[29,30]. Marine resource such as seaweeds and seagrass has been documented for several decades of their innumerable application. The increased seedling growth may be due to the presence of growth-promoting substances such as IAA and IBA, gibberellins, cytokinins, auxins and amino acids[31,32]. Earlier reports suggested that the reason for growth and yields of crops due to seaweeds and seagrasses have been comprised growth of inorganic compounds like vitamins, micro and macronutrients and organic compounds like betaines, polysaccharides, phenolic compounds[33].

Conclusion

Considering the results, the combined extract derived from (seaweed) *Padina tetrastromatica*, and (seagrass) *Cymodocea serrulata* can be utilized as a biofertilizer for better cultivation of *Vigna mungo* seedlings. By using the fertilizers obtained from these kind of marine natural resources may avoid consequences of chemical fertilizers on earth and environment. The present study have been explain the significant role of seaweed and seagrass biofertilizer to avoid hazardous chemical fertilizers. Further field trials are needed for more growth and developmental analysis of different crops with different soil.

Acknowledgement

The author Mr. C. Santhosh gratefully acknowledges University Research Fellowship providing by the Department of Botany, Periyar University, Salem, Tamil Nadu (Ref. No. PU/AD-3/URF/007253/2019- dt 25.04.2019). University Research Fellowship for this study.

Conflict of interest

The authors declare that they have no conflict of interest.

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