



RESEARCH ARTICLE

Growth enhancement of agricultural crops using seaweed liquid fertilizer

Akshada Parab* & Sunil Shankhadarwar

Department of Botany, Ramnarain Ruia Autonomous College, Mumbai 400 019, India

*Email: parabakshada10@gmail.com



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Abstract

Extensive use of chemical fertilizer is leading to infertility of soil and various hazardous effect on the ecosystem. Using biofertilizer reduces these harmful effects. Many are commercially available, being a poor match to chemical fertilizer they are not used on a large scale. Near coastal regions seaweeds are used as biofertilizers. Recent studies show seaweeds have multiple growth regulators, macro and micronutrients, polysaccharides which are necessary for plant growth. The study is carried out to check the bioactivity of Seaweed liquid fertilizer (SLF) as a growth stimulant. Sargassum cinereum, Ulva rigida and Ahnfeltia plicata found on the Indian coastline were used. SLFs were prepared from each of three species in varying concentration (0.02%, 0.04%, 0.06%, 0.08% and 0.1% v/v) with water and Urea as control. The test plant selected was Vigna radiata. Agronomic characters like germination rate, shoot length, flowering and fruiting period of test plants were studied. Phyto-hormones from SLF were detected by thin-layer chromatography. Elemental analysis of micronutrients in SLF was carried by ICP-AES. Along with Chlorophyll and Protein estimation of grown plants were checked. SLF shelf life was studied using chemical and biological preservatives. The effect of 0.06% concentration of all SLFs showed enhancement in growth and phytochemicals in plants. Plants treated with SLF showed flowering earlier than control plants. Thus, using SLF bio-fertilizer can become an alternative method to reduce the use of harmful chemical fertilizers.

Keywords

Seaweeds, Bio-fertilizer, Phytohormones, ICP-AES, Shelf-life

Introduction

Agriculture is the backbone of India; various schemes, modifications, hybrid varieties etc. are developed to enhance the yield of crops. The use of fertilizers, insecticides, herbicides etc. has increased after the green revolution. Overuse of chemical fertilizers has increased the crop yield and production but also there is excessive chemical leaching into the soil, causing bioaccumulation and other harmful effects. The health impact is increased in several cancers and other diseases. As a result, farmers are opting to reuse traditional methods. The use of organic fertilizers like manures, biostimulants is increasing. Production of organic fertilizers is increasing since 2012 according to reports from the Department of Fertilizers Ministry of Chemicals and Fertilizers Government of India (1). Organic fertilizers include the ones made from living organisms like plants, animals, and microbes (2).

Organic fertilizers are also prepared from algae, both micro and macro. Algae biofertilizers are effective as they are rich in proteins, lipids, polysaccharides, nutrients. Cyanobacteria like Nostoc and Anabaena are already naturally enhancing agriculture crops, there are emerging studies in the use of microalgae fertilizers but the time taken to grow algae is more (3). Talking about seaweeds, which are grown naturally in coastal areas, are equally rich in all the contents. Seaweeds are marine macroscopic algae that are used as biofertilizers from ancient times i.e., Romans in the 1st century AD as well as by the Chinese (4). Commercial use of seaweeds in agriculture is carried out for the last seven decades (5). Seaweed provides nutrition as it contains macro and micronutrients, amino acids, vitamins, phytohormones, polysaccharides etc (6). These help in increase in growth and development, tolerate environmental stress (7), enhance nutrient uptake from soil (8) and also increase nutrients in the plants itself (9).

Seaweeds are used as biofertilizers, manures as well as biostimulants. The application of foliar spray of the liquid extracts has shown a tremendous effect on the growth and flowering of tomato plants (10). Seaweed fertilizers are more beneficial than chemical and other organic fertilizers as they are biodegradable, non-polluting, non-toxic and as mentioned earlier have various growth-promoting organic compounds (11). SLF was prepared from *Codium decorticatum*, with varying concentrations and tested on capsicum plants (12). SLF was also tested on cucumber plants (13), for growth enhancement. There are studies on using a combination of seaweeds in preparing SLF (14) and all these studies have a common concentration of SLF used.

In India, seaweeds are commercially grown for pharmaceuticals and cosmetics. Agricultural applications of seaweeds are less explored. Seaweed fertilizer available in markets is made from Ascophyllum nodusum a brown alga imported from European countries which makes it expensive. As a result, very few people know the use and buy it. Hence, the present work follows the use of indigenous seaweeds i.e. the ones found in our country, like Sargassum cinereum, Ulva rigida and Ahnfeltia plicata as biofertilizers and biostimulants for growth enhancement crops. Concentration of each SLF prepared is 0.02%, 0.04% 0.06%, 0.08% and 0.1%, which is unlike other studies published as more concentrated SLFs are used (3, 12, 13). The selected crop in this study, Moong - Vigna radiata has been selected and grown using SLF and with water and urea as control. Agronomic and biochemical tests were conducted to compare the effects of SLF and chemical fertilizer which is further discussed in the study.

Thus, the study states the preparation of seaweed extracts which are economically cheap and easily available in coastal regions rather than imported ones. The concentrations used are low and have an impact on crops. Also, preparation of SLF is discussed which opens up start-up opportunities for farmers and fishermen, also reducing the use of chemical fertilizer.

Materials and Methods

Sample collection

Seaweed samples were collected from the west coast of Maharashtra, India. Three species from each of the classes i.e., Chlorophyceae, Rhodophyceae and Phaeophyceae were selected. *Ulva rigida* C. Agardh was collected from Malvan, *Sargasussum cinereum* J. Agardh was collected from Devgad while, *Ahnfeltia plicata* (Hudson) E. M. Fries was collected from Alibaug. The seaweed samples were authenticated from the Botanical Survey of India, Southern Regional Circle T.N.A.U. campus, Coimbatore in November 2019. These seaweeds were identified up to species level.

Collected seaweeds were washed thoroughly to remove the salt and sand attached, along with living impurities like marine organisms along with seashells were separated. These washed seaweeds were then shade dried at room temperature. Further, packed airtight and stored at 37 °C in an incubator to keep the biochemical properties intact. Samples were used in dried form for the study.

Preparation of seaweed liquid fertilizer

Seaweed liquid fertilizer of each sample was prepared by modifying the method (15). One gm of dried seaweed was taken in 100 ml of distilled water which was further autoclaved at 15 Psi for 30 mins. It was then cooled and filtered through a muslin cloth. The solution was then centrifuged for 10 mins at 3000 rpm. The supernatant was collected and stored in the refrigerator for further use. The extract from was 0.1% extract which was then diluted into different concentrations using distilled water and made into 0.02%, 0.04%, 0.06%, 0.08% and 0.1%. The same concentration was used for germination and further the plants were given SLF every week as a foliar spray and direct application in coco peat.

Seed selection and treatment

The crop selected for testing the SLF was Moong beans ($Vigna\ radiata$). The seeds were washed with distilled water two times than with 70% alcohol again with D/W. Further, they were washed with 0.1% HgCl₂ and then with sterile distilled water two to three times to avoid infection. At last, they were dried on filter paper and were further maintained.

Seeds are further soaked in each of the three SLF of different concentrations (0.02%, 0.04%, 0.06%, 0.08% and 0.1%) which were kept overnight. Distilled water was used as control and chemical control as urea (0.05 g). Ten seeds are sown in pots containing coco peat to avoid interactions with soil minerals. Data was noted for 10 days. There was a total of 7 pots with each type of concentration of SLF along with 2 control. Initially, pots were kept in a greenhouse and maintained for temperature and humidity. Further, the study was carried out in direct sunlight. For the rate of germination, the plants were noted for 10 day and agronomic characters were under observation for 10 weeks. As mentioned earlier, SLF was applied at week intervals - 10 ml/week of which 5 ml was applied as a foliar spray and the remaining 5 ml was directly applied in cocopeat.

Estimation of the plant hormone

Endogenously present plant hormones i.e., Auxin - Indole acetic acid (IAA) and Cytokinin - kinetin were detected using thin-layer chromatography. Merk Silica gel 60 F254 TLC plates were used the aqueous liquid extracts were used for both the detection. Initially, for IAA 15 ml of the sample was taken and evaporated to 5 ml. which was then adjusted to pH 2.8 using 7N HCl. Further to this, 15 ml ethyl acetate was added and the aqueous fraction was separated which was again adjusted to pH 7.0 using 7N NaOH. To this watersaturated butanol was added and this aqueous layer was again taken are evaporated. Methanol was used for reconstitution and the sample preparation was stored at 4 °C in dark. The Mobile phase used was n-Butanol: acetic acid: Ethanol (12:3:5) (V: V: V) and Salkowski's reagent was used for spraying and treated at 80 °C. Plates were visualized under visible light at 554 nm. For cytokinin, the aqueous 15 ml extract was taken centrifuged at 40 °C for 20 mins. It was mixed with 15 ml of ethyl acetate of which an aqueous layer was separated and evaporated which was reconstituted with methanol. For this, the mobile phase was n-Butanol: Rate of germination Acetic acid: water (12:3:5) (V: V: V). Plates were visualized at 366 nm (16).

Elemental analysis

Seaweeds already consist of various minerals. Plants need minerals in major and minor quantities. In the following work, plant minor elements were detected from the aqueous extracts using the Induced Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) method. The following was carried out at SAIF-IIT Bombay in November 2019. The

phyll a and b and total chlorophylls were estimated. All the plants including control and urea treated were also tested

Protein estimation

Proteins were also calculated from the leaves of Moong to check enhancement. Control and urea-treated leaves were tested. Lowry's method of estimation was carried out (18). Leaves were extracted into aqueous extracts.

The shelf life of SLF

It was seen at SLF if stored outside gets contaminated by fungus and bacteria. Hence to avoid it, they were stored in spray bottles in the refrigerator. For storing outside at room temperature was also tested. Sodium benzoate was used in varying concentrations along with natural preservation using clove oil. Clove oil was also used in varying concentrations. The test was carried out for one month.

Results

The Rate of germination was observed in the three SLF compared with control and Urea. It was observed after 48 hrs of treatment that, *U. rigida* treated plants showed maximum germination followed by A. plicata and S. cinereum (Table 1-3). Maximum growth that was 80% which is 8 out of 10 seeds germinated was in *U. rigida* treated seeds with 0.06% concentrations. While in S. cinereum it was 10% of the total in 0.06% concentration. Water treated seeds showed a 40% rate while urea treated ones showed 0% germination (Fig. 6).



Fig. 1. Plants grown in *U. rigida* SLF along with water as control

elements selected were Copper-Cu, Nickel-Ni, Molybdenum -Mb, Zinc-Zn and Manganese-Mn. The following elements are useful in various metabolic pathways in plants. For control, distilled water was tested. 5 ml of each SLF of three seaweeds i.e., *U. rigida*, *S. cinereum* and *A. plicata* were tested.

Biochemical parameters of plants treated with SLFs Chlorophyll estimation

Chlorophyll content of the Moong plants, when started flowering at 5th week, were tested. Only leaves were taken. Chlorophyll was estimated by Aron's method using 80% acetone extracts at 645 nm, 652 nm and 663 nm. Chloro-

Agronomic characters

Height of plants

The height of shoots was recorded in the initial stages of growth after the seeds were sown in cocopeat. The average height of all plants in each concentration of SLF was recorded. In the first week, 0.2% S. cinereum treated plants were tallest followed by 0.8% S. cinereum SLF with a height of 8.5 cm and 7.8 cm respectively. It was recorded that plants treated with 0.6% U. rigida SLF and 0.6% A. plicata SLF showed 6.3 cm plantlets. Urea treated plants were the least of 2.4 cm and water treated was 4.5 cm (Table 1-3).

Table 1. Rate of germination, agronomic characters -the height of plants, number of leaves and number of flowers along with biochemical parameters like chlorophyll content and protein content for *U. rigida* treated plants along with control as water and urea

Sl. No.	SLF concentration (%)	Rate of germination (48hours)	Shoot height (cm)	Number of leaves	Number of flowers	Chlorophyll A (mg/g) ± SE	Chlorophyll B (mg/g) ± SE	Total chlorophyll (mg/g) ± SE	Protein (mg/g) ± SE
1	0.02%	40%	3.6	6	0	3.14 ±0.03	3.58±0.007	3.94±0.06	196.3±0.25
2	0.04%	70%	4.8	12	1	6.63 ±0.01	7.748±0.02	10.26±0.02	237.1± 0.48
3	0.06%	80%	6.3	10	0	7.17 ±0.04	7.35±0.01	9.08±0.004	235.8±0.74
4	0.08%	70%	4.2	8	0	3.76 ± 0.01	4.51±0.003	5.93±0.007	190.7±0.56
5	0.1%	70%	5.8	12	0	6.75±0.05	6.85±0.004	8.41±0.004	200.0±0.28
6	Urea	0%	2.4	6	0	5.82 ± 0.03	3.68±0.02	5.33±0.009	231.5±0.87
7	water	40%	4.5	6	0	4.06 ± 0.01	4.26±0.03	6.72±0.006	263.6±0.98



Fig. 2. Plants grown in S. cinereum SLF along with water as control.

Table 2. Rate of germination, agronomic characters -the height of plants, number of leaves and number of flowers along with biochemical parameters like chlorophyll content and protein content for *S. cinereum* treated plants along with control as water and urea

Sr.no	SLF concentration (%)	Rate of germina- tion (48hours)	Shoot height (cm)	Number of leaves	Number of flowers	Chlorophyll A (mg/g) ± SE	Chlorophyll B (mg/g) ± SE	Total chlorophyll (mg/g) ± SE	Protein (mg/g) ± SE
1	0.02%	60%	5.2	8	1	4.40±0.004	5.05±0.04	5.10±0.03	191.1±0.23
2	0.04%	60%	5	11	1	5.25±0.01	7.91±0.003	9.74±0.02	194.4±0.3
3	0.06%	60%	6.3	12	0	4.31±0.08	4.75±0.001	6.41±0.004	190.7±0.81
4	0.08%	50%	4.2	7	0	6.39±0.008	4.84±0.006	7.50±0.064	183.3±0.03
5	0.1%	30%	5.3	8	0	6.55±0.041	4.34±0.002	7.12±0.02	201.9±0.09
6	Urea	0%	2.4	6	0	5.88±0.01	3.68±0.03	5.33±0.04	231.5±0.23
7	water	40%	4.5	6	0	4.08±0.003	4.26±0.02	6.72±0.12	263.6±0.41



Fig. 3. Plants grown in A. plicata SLF along with water as control

Number of leaves

As an agronomic character, several leaves were recorded after five weeks of germination. Water and urea treated Number of flowers were used as control. In the fifth week, U. rigida and S. cinereum were with 12 leaves while A. plicata treated leaves were similar to S. cinereum with a maximum number of 12

leaves in 0.6% SLF. Water and urea were treated where with 6 leaves each (Table 1-3).

Flowering and fruiting were seen in the plants treated with SLF after five weeks after germination. In the fifth week, S. cinereum treated plants were with the greatest number of

Table 3. Rate of germination, agronomic characters -the height of plants, number of leaves and number of flowers along with biochemical parameters like chlorophyll content and protein content for A. plicata treated plants along with control as water and urea

Sr.no	SLF concentration (%)	Rate of germination (48hours)	Shoot height (cm)	Number of leaves	Number of flowers	Chlorophyll A (mg/g) ± SE	Chlorophyll B (mg/g) ± SE	Total chlorophyll (mg/g) ± SE	Protein (mg/g) ± SE
1	0.02%	20%	8.5	12	2	5.92±0.08	7.71±0.06	9.89±0.007	201.2±0.56
2	0.04%	20%	4.6	11	0	8.44±0.005	11.32±0.007	14.11±0.001	184.9±0.03
3	0.06%	10%	5.1	7	1	6.62±0.008	9.69±0.02	12.03±0.003	241.5±0.25
4	0.08%	40%	7.8	8	2	4.85±0.007	6.23±0.009	7.74±0.006	254.7±0.52
5	0.1%	40%	6.2	8	0	4.16±0.004	4.73±0.006	6.275±0.02	281.4±0.49
6	Urea	0%	2.4	6	0	5.8±0.03	3.68±0.004	5.33±0.01	231.5±0.69
7	water	40%	4.5	6	0	4.08±0.06	4.26±0.02	6.724±0.006	263.6±0.30

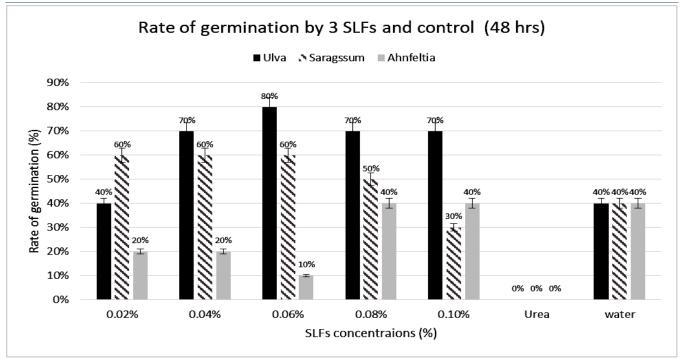


Fig. 6. Rate of germination by SLFs and compared with urea and water.

flowers and buds. It was 2 buds per concentration of 0.2% water and urea treated were 263.6 mg/g and 231.5 mg/g and 0.6% SLF. While A. plicata SLF treated were with 1 flow-respectively (Table 1-3). er in 0.2% and 0.4% concentration of SLF. *U. rigida* had only one flower in 0.4% concentration. Urea and water showed no flowering (Table 1-3).

Biochemical parameters

Chlorophyll

The chlorophyll content of plants was checked which were Induced coupled plasma-atomic emission spectroscopy treated with SLF and control as water and urea. The Maximum total chlorophyll content was in plants treated with S. cinereum SLF with 14.11 mg/g with maximum chlorophyll an 8.44 mg/g and chlorophyll b as 11.32 mg/g. least was in A. plicata with chlorophyll a 4.40 mg/g and chlorophyll b with 5.05 mg/g. Water treated showed the lowest of chlorophyll an of 4.07 mg/g and 3.688 mg/g chlorophyll b. while urea treated showed 5.88 mg/g of chlorophyll a and 4.26 mg/g chlorophyll b (Table 1-3) and (Fig. 7).

Proteins

The protein content of plants treated with SLF was estimated along with urea and water as a control. It was observed that S. cinereum was with the maximum protein content of 281.4 mg/g while A. plicata was lowest with 162.9 mg/g.

Plant hormones

Auxin TLC was carried out to check the presence, it was seen in all the seaweed extracts. While for cytokinin, it was only detected in *U. rigida* and *S. cinereum* (Fig. 4 &

(ICP-AES)

ICP-AES was carried out to check the presence of microelements. It was seen that the maximum content of Copper was in Rhodophyceae, Manganese content was maximum in Phaeophyceae members. Nickel was about 0.053 ppm in Chlorophyceae and Zinc was in the highest amounts in Rhodophyceae. Molybdenum was not detected in any of the seaweed (Table 4).

Shelf life

As per the conditions and quantity of sodium benzoate added as well as clove oil, no bacterial or fungal growth was seen in clove oil-treated extracts while there was bacterial growth in all the sodium benzoate treated extracts (Table SLF Saragssum (%)

Fig. 7. Total chlorophyll content in moong treated with SLFs and compared with urea and water.

■ SLF Ulva (%)

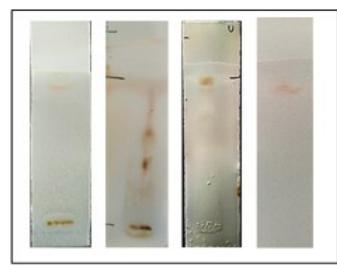


Fig. 4. Presence of Auxin by TLC; from left to right (*Sargassum* SLF, *Ahnfeltia* SLF, *Ulva* SLF and Standard Auxin IAA)

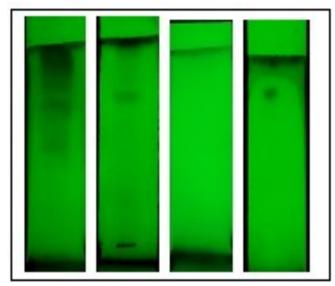


Fig. 5. Presence of Cytokinin by TLC; from left to right (*Sargassum* SLF, *Ulva* SLF, *Ahnfeltia* SLF and Standard kinetin).

Table 4. ICP-AES carried out on SLFs along with microelements. ND means less than 0.01ppm

■ SLF Ahnfeltia (%)

Sample	Cu	Mn	Ni	Zn	Мо
Concentration	ppm	ppm	ppm	ppm	ppm
Distilled water	ND	ND	ND	0.016	ND
A. plicata	0.038	0.099	0.019	0.147	ND
S. cinereum	0.022	0.229	0.019	0.066	ND
U. rigida	0.038	0.25	0.053	0.113	ND

 $\textbf{Table 5.} \ Shelf \ life \ comparison \ of \ different \ concentration \ of \ Sodium \ benzoate \ and \ Clove \ oil \ to \ fungal \ growth$

Sr.no	Concentration	Observations
	Sodium benzoate:	
	0.01g	+
	0.02g	+
1	0.03g	-
	0.04g	-
	0.05g	-
	Clove oil	
2	0.01ml	-
2	0.02ml	-
	0.03ml	-

Discussion

Seaweeds are used as biofertilizers for many centuries. Research on Seaweeds as fertilizers is carried out for the last three decades. Seaweed liquid fertilizer (SLF) is applied as a biostimulant. Many seaweeds are worked upon for the same some are also commercially available as of products. (19) Ascophyllum nodosum found in Scandinavian countries is commercially available as of SLF. And also, these countries are the main suppliers of these biostimulants. The Effects of these biostimulants are much ranging from enhancement in flowering and fruiting, increase in protein, antioxidant content, increased in yield and as well as re-

duced in infections and pests (20).

In the present work, three indigenous seaweeds found at the western coast are tested. All three seaweeds are authenticated and used in optimum quantities. Work is carried on *Vigna radiata* as the plant has a short cycle. The three seaweeds are used in five different concentrations each from 0.2% to 1% along with water and urea as control. Seaweed extracts were prepared by autoclaving the seaweed with distilled water. There are many other methods of preparation, but comparatively, this method is easy and cost-effective (21). Method was modified and concentrations were reduced, which also shows the minimum quantity of seaweed can also show enhancement in crops.

The Rate of germination was maximum in *U. rigida* treated seeds i.e. 80% germination at 0.06% concentration. While in *A. plicata* in lower concentrates showed maximum growth. Similar results were seen in pearl millets treated with *Gracilaria dura* lower concentrates showed a maximum rate of germination, as well as maximum chlorophyll and protein content (22), thus for Red algae lower concentration of SLF is profitable.

Agronomic characters were recorded, the height of shoots was recorded after a week, it was observed the plants treated with SLF were tall compared to plants treated with urea and water. Due to the presence of various endogenous auxins like indole-3-carboxylic acid, indole-3acetic acid etc (23). Number of leaves was counted as a part of growth parameters, plants were treated with seaweeds had a greater number of leaves compared to control. The number of leaves is proportional to the rate of photosynthesis (24). Several flowers were also counted in the fifth week when it was just started to bloom, the rate was also calculated in the seventh week. Urea and water-treated plants were without flowers. Seaweed extracts contain plant hormones, micro and macronutrients as well as polysaccharides which enhance flowering. Similar results were observed in Tagetes erecta with Sargassum wightii (25). The effect of seaweed commercial extracts like Kelpak was tested on three varieties of capsicum along with chemical fertilizer as control. It was observed seaweed fertilizer had better results compared to chemical fertilizers (26).

Plant hormones and growth regulators are present in seaweeds (27). Seaweed liquid extracts were tested for the presence of cytokinin and auxins. Auxins were present in all three extracts while cytokinin was present in *U. rigida* and *S. cinereum*. Although there are reports on the presence of cytokinins endogenously present in various red algae species (28). Changes can be due to temperature and storage variations, as mentioned in a work carried out with *Ecklonia maxima* stored at various temperatures thus varying in concentrations of plant hormones were observed (29). Also, extraction of phytohormones from seaweed is carried out earlier, but extraction of auxins and cytokinins from prepared SLF is not reported. Thus, the study becomes unique as actual concentration of growth hormones is been detected.

Trace nutrients were detected using the ICP-AES method. Micronutrients are essential in plant metabolic

processes. Copper, Zinc, Nickel and Manganese were present in all three extracts while Molybdenum was not detected. There was an earlier report on *Vaucheria sessilis* for the presence of various micro and macronutrients (30). These nutrients play role in various enzymatic activities, thus increasing plants responses to the environment.

Thus, from this study, it was observed that seaweed extracts show enhancement compared to the chemical fertilizer

Conclusion

Seaweed liquid fertilizer thus enhances the growth of plants. It was observed from the first treatment to seeds till flowering and fruiting. The Rate of germination was increased in plants treated with SLF. It was observed that in U. rigida treated plants, 0.06% was optimum for growth. While in A. plicata lower concentration has a maximum germination rate, while for S. cinereum treated plants initially, there was less rate followed in 100% germination in the next 24 hrs. Due to the increase in the rate of germination, further, these plants showed enhancement in flowering. Enhancement is observed due to various growthstimulating hormones, micronutrients and macronutrients (31). There are many reports on the bio stimulating activity of seaweeds on various plants. Moong is a fast-growing crop and an essential legume. It was seen that plants treated only with water and urea had no flowering and fruiting, thus proving that SLF contains bio-stimulating properties. Based on the detection of plant hormones it is clear that there is the presence of auxins in extracts and cytokinins in S. cinereum and U. rigida. Reports are on the presence of indigenous plant hormones in seaweeds (32). But in aqueous extracts, there are fewer reports and ICP-AES reports confirm the presence of micronutrients. Estimating biochemical parameters help in confirming enhancement due to SLF. From the study, it was concluded that three indigenous present seaweeds can be used for preparing SLF. S. cinereum with the best results followed by U. rigida and A. plicata. Thus, seaweed liquid fertilizer shows significant enhancement in growth and as a source of biostimulant for agriculture crops

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Authors contributions

The current study was initiated and planned by SS. AP collected samples, carried out experiments, analysed data and wrote the manuscript. SS guided and edited the manuscript followed by resolving the reviewers' edits.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interests to declare.

Ethical issues: None.

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