INFLUENCE OF SEAWEED LIQUID FERTILIZER ON GROWTH AND BIOCHEMICAL PARAMETERS OF VIGNA MUNGO AND VIGNA RADIATA

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Abstract: Plants have to deal with a lot of environmental stressors. Biofertilizer has been identified as an alternative way for these problems. Kelp is a wonderful soil amendment and a big advantage of these that we can spray them directly onto plant leaves for direct leaf uptake. The main benefit of Seaweed Liquid Fertilizer (SLF) is the natural plant growth regulators and hormones it contains that help plants grow faster, healthier and stronger. One of the main things they do is help plants deal with those environmental stressors such as heat, cold, wind, drought and disease. The bio-fertilizing efficiency of Seaweed Liquid Fertilizers from brown marine alga Sargassum myriocystum on growth and biochemical parameters of Vigna mungo (Black gram) and Vigna radiata (Green gram) was studied. Seaweeds were made to coarse powder and stock solution was prepared. Different concentrations such as 1%, 2%, 3%, 4% and 5% were prepared and used as foliar spray. Seaweed Liquid Fertilizer (SLF) at low concentration (2%) exhibited promoting effect.

Keywords: Sargassum myriocystum, Seaweed Liquid Fertilizer, Biofertilizer, *Vigna mungo, Vigna radiata*.

1. Introduction

Agriculture is an art, science and industry of managing the growth of plants and animals for human use. India is mainly an agricultural country. Agricultural product of significant economic value includes rice, wheat, potato, tomato, onion, mangoes, sugarcane, beans, cotton, etc. Agriculture is the backbone of Indian economy. It continues to play a dominant part in the overall economic scenario of India. We depend on agricultural outputs for our food requirements. India produces large quantity of food grains such as millets, cereals, pulses, etc., to feed a population of over 1.21 billion. Agriculture includes preparation of soil for cultivation of crops, harvesting crops, breeding and raising livestock, dairying and forestry. The two major types of agriculture are: Traditional agriculture and Modern or Industrialized agriculture. Modern agriculture makes use of hybrid seeds of single crop variety, technologically advanced equipment, chemical fertilizers, pesticides and water to produce large amounts of single crop. Excess use of fertilizers in fields causes micronutrient imbalance, leaching deep into the soil contaminating the groundwater, and eutrophication. The pesticides used to increase crop yield, have several side effects that are harmful to humans, enhancing the risk of cancer.

Seaweeds have been used as manure, cattle feed, food for human consumption and as a source of phycocolloids such as agar, alginic acid and carrageenan (Chapman, 1970). Besides their application as farmyard manure (FYM), liquid extracts obtained from seaweeds (LSF/ SLF) have recently gained importance as foliar sprays for several crops (Thivy, 1961; Metha et al., 1967; Bokil et al., 1974) because the extract contains growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, Ni etc.), vitamins and amino acids (Challen and Hemingway, 1965). Thus, when these are applied to seeds or soil, stimulate growth of the plants (Blunden, 1971).

Booth (1969) observed that the value of seaweeds as fertilizers was not only due to nitrogen, phosphorus and potash content, but also because of the presence of trace elements and metabolites. Aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increased yield and quality of fruits (Rama Rao, 1991). Seaweed fertilizer was found to be superior to chemical fertilizer because of the high level of organic matter and aids in retaining moisture and minerals in the upper soil level available to the roots (Wallen Kemp, 1955). Growth promoting effect of seaweed liquid fertilizer (*Enteromorpha intestinalis*) on the sesame crop plant was reported by Gandhiyappan and Perumal (2001). Application of SLF is one of the excellent means to get the lost nutrients back to the land and improve the yield of crop plants by about 20-30%.

Earlier reports showed that the *U. lactuca* SLF improved accumulation of total carbohydrate, protein and lipid contents (more than 37.0%, 58.0% and 60.0% respectively) when the marigold plants received 1.0% SLF (Sridhar and Rengasamy, et.al., 2010). The enhancement of growth, biochemical and yield characteristics of vegetable crop *Cyamposis tetragonoloba* was found to be due to the presence of micro and macro elements, growth hormones, trace elements, vitamins etc. in algal extracts. Cytokinin and magnesium which are considered as an essential growth promoting factors in chlorophyll biosynthesis played a vital role in enhancement of growth and physiology of cluster bean. Further, the study also emphasized that seaweed extracts can be effectively used as organic biostimulants to the plants and an eco-friendly approach to organic farming (Sivasangari Ramya, S. Nagaraj and N. Vijayanand et. al., 2011).

Some commercial products available in market are Maxicrop, Algifert, Goemar, Kelpak, Seaspray, Seasol, SM3, Cytex and Seacrop. Seaweed fertilizers are better than other fertilizers and are very economical. The present study was undertaken to investigate the effect of Seaweed Liquid Fertilizers (SLF) on the growth and biochemical characteristics of green gram and black gram.

2. Material and Methods

2.1 Collection of Seaweeds

The seaweed used in the present study was *S. myriocystum* belonging to the family Phaeophyceae (brown algae). Fresh thallus of the brown alga was collected from Gulf of Mannar in the southern part of the peninsular India, Rameshwaram (latitude 8° 47' N and 9° 15' N; longitude 78° 12' E and 79° 14' E).The alga was handpicked (low tide periods 0.5 to 1.0 meter), washed initially with sea water to remove the unwanted impurities, adhering sand particles, epiphytes and associated fauna. The alga was brought to the laboratory and washed thoroughly in tap water 3 to 4 times to remove excess salt. The washed and cleaned seaweeds were shade dried (27ºC to 30ºC) followed by oven drying for 48h at 60°C (Rathore et al., 2009). The oven dried material was then ground with the help of Mixer grinder (Preethi Eco-chef, India). The powdered seaweed samples were stored in airtight container for future use.

2.2 Preparation of Seaweed Liquid Fertilizer (SLF):

The seaweed liquid fertilizer was prepared by the method of Rama Rao (1990). The coarse powder of seaweeds was mixed with distilled water in the ratio of 1: 20 (w/v) and the mixture was autoclaved at 120°C, 15 lbs/sq for 30 minutes. Then, the mixture was allowed to cool at room temperature. The extract was filtered through double-layered cheese cloth and the filtrate was collected. The obtained filtrate was treated as 100% SLF concentration and from this, different concentrations (1%, 2%, 3%, 4%, and 5%) (**Table 1**) were prepared using distilled water (Bhosle et al., 1975). As the seaweed liquid fertilizers contained organic matter, the seaweed liquid fertilizers were refrigerated between zero and 4 °C.

2.3. Physico-Chemical Analyses of SLF:

Seaweed Liquid Fertilizer was also subjected to elemental analysis for the concentration of following elements viz., calcium, magnesium, sodium, potassium, iron, phosphate, chloride, sulphur, silicon, zinc, copper and nitrate content (**Table 2**). Estimations were carried out using Atomic Absorption Spectrophotometer (Humpshires, 1956).

Table 1: Preparation of SLF at different concentrations

Percentage Concentration (%)	Volume of Extract (mL)	Volume of Distilled Water (mL)
1%	10	90
2%	20	80
3%	30	70
4%	40	60
5%	50	50

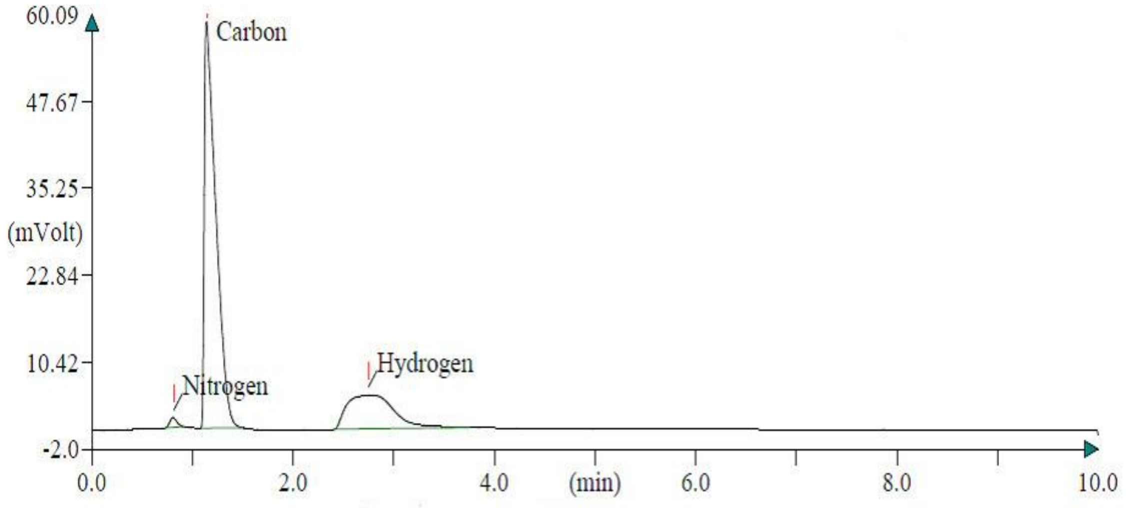
Table 2: Physio-chemical analysis of liquid extract of *S. myriocystum*

Elemental Analysis	*S. myriocystum*
Colour	Brown
pH	6.9
Zinc	0.119
Lead	0.008
Iron	2.633
Manganese	5.227
Cobalt	0.081
Nickel	0.002
Chromium	0.303
Copper	0.064
Potassium	2.07
Magnesium	18.13
Sodium	5.69

All the parameters given are in mg/L except colour and pH.

2.4 Analysis of Carbon, Hydrogen, and Nitrogen from S. myriocystum liquid extract

The CHN Analyzer (Thermo finnigan, CHN, Italy, Model FLASH EA 1112 Series) find utility in determining the percentages of Carbon, Hydrogen, and Nitrogen of organic compounds, based on the principle of "Dumas method" which involves the complete and instantaneous oxidation of the sample by "flash combustion". The combustion products are separated by a chromatographic column and detected by the thermal conductivity detector (T.C.D.), which gives an output signal proportional to the concentration of the individual components of the mixture. The observed results of CHN analysis are shown in **Fig 1**.



Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Compone
1	0.817	68905	1.212	Nitroge
2	1.150	4608772	33.746	Carbon
3	2.750	1569287	6.297	Hydroge
		6246964	41.255	

Figure 1: CHN content of *S. myriocystum*

2.5 Selection of Crop Plant

The experimental crop plant selected for the present study was *Vigna mungo* and *Vigna radiata* belonging to the family *Fabaceae*. The certified seeds were procured from National Seed Corporation, Chennai, Tamil Nadu, India. The healthy seeds having uniform size, shape, colour, weight and free from visible infection were selected for the study. The selected seeds were stored in a metallic tin until usage (Rao, 1976).

2.5.1 Pre-treatment

The selected seeds were surface sterilized with 0.1% mercuric chloride for 1 to 2 minutes and the surface sterilized seeds were repeatedly rinsed with sterile distilled water 5 times for 5 minutes to remove any trace of mercuric chloride. The seeds were soaked in different concentrations of SLF *i.e.*, 1%, 2%, 3%, 4%, 5%, while the control was soaked in distilled water for a period of 24 hours. The seeds were sown in grow bags measuring 13cmx18cm size and was used for further study.

2.5.2 Soil preparation and plant maintenance

The farm yard manure soil was prepared using red soil, sand and manure (1:1:1 ratio), free from pebbles and stones and was filled in grow bags. The experiment was conducted in the green house. The plants were irrigated regularly and the treatments were given to the plants as foliar application in different concentrations of SLF (1% to 5%). The first foliar spray treatment was given to 7 days old seedlings. Thereafter, sprays at 7 days interval were given up to 30 days. The control plants were treated only with distilled water as foliar spray. Plants from the grow bags were uprooted carefully, washed in tap water. Plants from each concentration and control were drawn for various analyses. Fifteen day old plants and 30- day- old plants were taken for the following parameters total plant height (cm), shoot length (cm), root length (cm), total fresh weight (g), total dry weight (g) and moisture content (%).

The biochemical constituents such as total proteins (Bradford, et al., 1976), carbohydrates (Dubois et al 1956), lipid content (Folch et.al., 1956), and chlorophyll a, b and carotenoids (Lichtenthaler, et.al., 1987) were estimated in *V. mungo and V. radiata.* Triplicate samples were used for all the parameters and the mean values were represented.

3. RESULTS

In the greenhouse studies, plants treated with different concentrations of SLF viz., 1%, 2%, 3%, 4% and 5% showed enhanced growth compared to control. Better result was observed in the plants treated with 2% SLF of seaweed (**Table 3**).

3.1 Effect of SLF treatments on growth parameters of *Vigna radiata*

The values were recorded in 15 days intervals up to 30th day. On the 15th day, 2% SLF treated plants showed shoot length of 11.5±0.28cm and root length of 7±0.17cm, followed by 3% SLF treated plant (shoot length 10.33±0.27cm; root length 6.9±0.15cm). The root length of the plant varied from 1.85±0.05 to 5.03±0.01 cm/seedlings. The maximum root length was recorded as 7.03±0.01 cm/seedling in the plant that received 2% SLF (**Table 3 and 4**).

3.2 Effect of SLF treatments on growth parameters of *Vigna mungo*

The values of shoot length, root length and number of lateral roots of *Vigna mungo* showed increment over the control at all the concentrations of SLF except 4% and 5%. Shoot length, root length and other growth parameters were gradually decreased as the concentration of SLF increased. The 2% SLF treatment increased the growth parameters significantly when compared to control and other concentrations (**Table 5 and 6**).

Table 3: Effect of different percentage of seaweed liquid fertilizer on growth parameters of *Vigna radiata* on 15th day

Concentrations of SLF	Root Length (cm)	Shoot Length (cm)	Total plant height (cm)	Fresh weight (g)	Dry weight (g)	Moisture content (%)
Control	**4.83**	**8.5**	**13.33**	**0.383**	**0.13**	**63.14**
1%	**5.83**	**10.17**	**16**	**0.46**	**0.16**	**65.74**
2%	**7**	**11.5**	**18.5**	**0.54**	**0.15**	**72.3**
3%	**6.83**	**11.33**	**18.17**	**0.52**	**0.18**	**64.01**
4%	**4**	**6.83**	**10.83**	**0.27**	**0.15**	**35.48**
5%	**3.667**	**9.5**	**13.17**	**0.453**	**0.31**	**29.33**

Table 4: Effect of different percentage of seaweed liquid fertilizer on growth parameters of Vigna radiata on 30th day.

Concentrations of SLF	Root Length (cm)	Shoot Length (cm)	Total plant height (cm)	Fresh weight (g)	Dry weight (g)	Moisture content (%)
Control	**13.67**	**20.33**	**34**	**0.953**	**0.393**	**57.34**
1%	**17.6**	**24.6**	**42.2**	**1.25**	**0.52**	**58.11**
2%	**18.67**	**27.67**	**46.33**	**1.393**	**0.437**	**68.86**
3%	**18**	**26.67**	**44.67**	**1.141**	**0.567**	**49.64**
4%	**10.33**	**14.33**	**24.67**	**0.73**	**0.273**	**63.15**
5%	**8.667**	**8.333**	**20**	**0.453**	**0.317**	**29.33**

Table 5: Effect of different percentage of seaweed liquid fertilizer on growth parameters of Vigna mungo on 15th day.

Concentrations of SLF	Root Length (cm)	Shoot Length (cm)	Total plant height (cm)	Fresh weight (g)	Dry weight (g)	Moisture content (%)
Control	**12.57**	**7.833**	**20.4**	**0.093**	**0.02**	**74.22**
1%	**16.1**	**9.81**	**25.91**	**0.18**	**0.09**	**51.38**
2%	**17.33**	**11.23**	**28.57**	**0.26**	**0.063**	**75.85**
3%	**15.67**	**9.367**	**25.03**	**0.103**	**0.05**	**54.92**
4%	**8.01**	**9.417**	**17.43**	**0.133**	**0.07**	**46.2**
5%	**5.8**	**8.333**	**14.13**	**0.167**	**0.073**	**55.32**

Table 6: Effect of different percentage of seaweed liquid fertilizer on growth parameters of Vigna mungo on 30th day.

Concentrations of SLF	Root Length (cm)	Shoot Length (cm)	Total plant height (cm)	Fresh weight (g)	Dry weight (g)	Moisture content (%)
Control	**20.45**	**16.77**	**37.21**	**1.23**	**0.45**	**63.36**
1%	**30.33**	**21.93**	**52.27**	**1.463**	**0.5**	**63.43**
2%	**34.96**	**23.33**	**58.3**	**1.573**	**0.36**	**77.48**
3%	**26.47**	**19**	**45.47**	**1.083**	**0.5**	**53.62**
4%	**21.67**	**18**	**39.67**	**0.533**	**0.19**	**54.64**
5%	**17**	**7.067**	**24.07**	**0.3**	**0.183**	**27.78**

3.3 Biochemical studies in SLF treated plants

The biochemical parameters of the experimental plants viz., total protein, carbohydrates, lipids and chlorophyll contents were recorded. A noticeable increase in the biochemical parameters was recorded on 30th day in *V. radiata and V. mungo* with 2% and 3% SLF treatment. Higher concentrations (3%, 4% and 5%) of SLF were found to have inhibiting effect on biochemical parameters. The accumulation of total protein and total carbohydrate was found maximum in 3% SLF treated plants. Maximum chlorophyll content was recorded in 2% SLF on 30th day in treated plants. Increment in the total chlorophyll was recorded in 3% SLF treated plants and decreased content of chlorophyll was recorded in 5% SLF treated plants. Maximum protein content was recorded in 2% SLF treated plants of V. radiata and V. mungo. Similarly, total carbohydrate content was also more in 2% SLF treated plants. Better result was observed in 2% and 3% SLF concentrations when compared to higher concentrations.

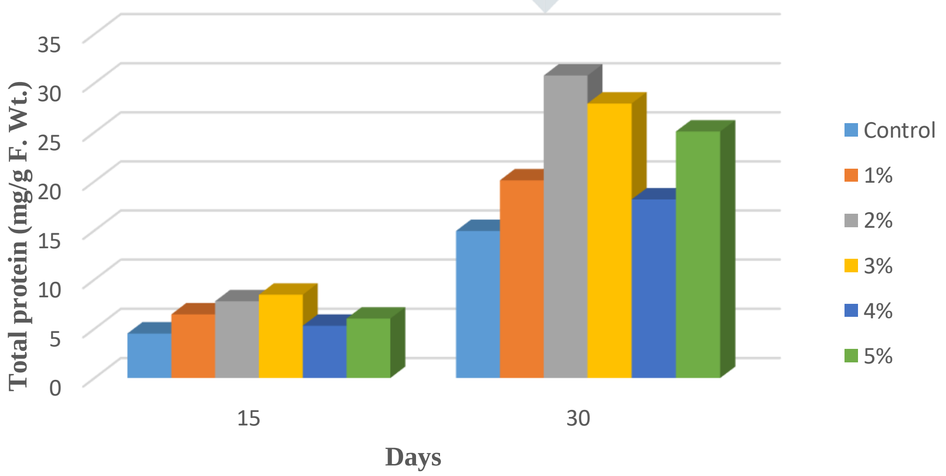


Fig 2. Effect of SLF on protein content of Vigna radiata

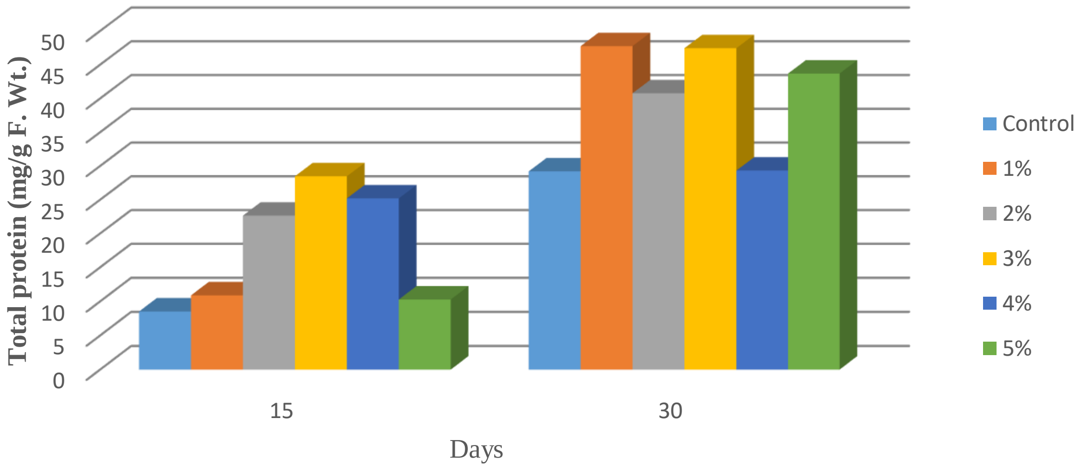


Fig 3. Effect of SLF on protein content of Vigna mungo

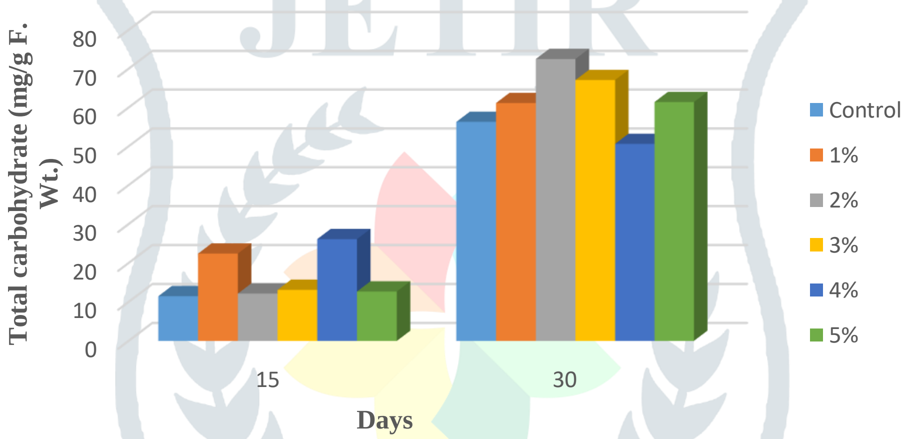


Fig 4. Effect of different percentage of seaweed extract on carbohydrate content of *Vigna radiata*

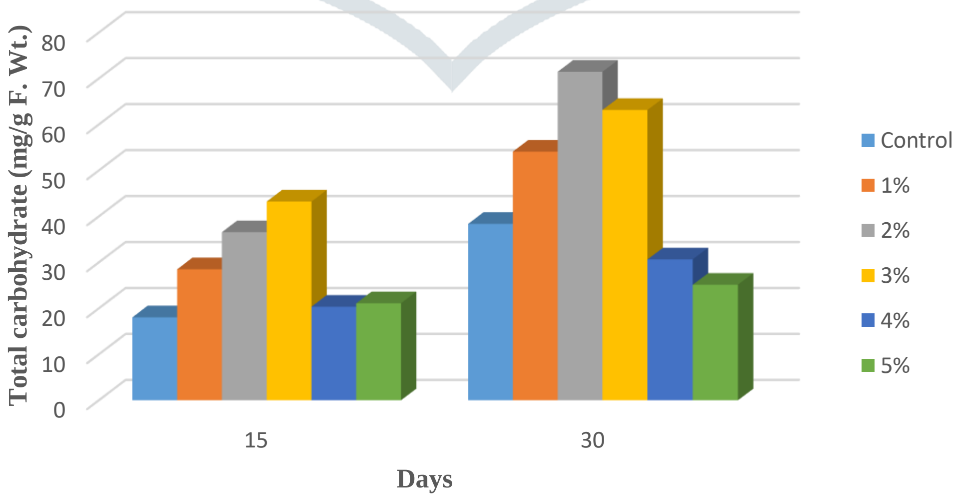


Fig 5. Effect of different percentage of seaweed extract on carbohydrate content of *Vigna mungo*

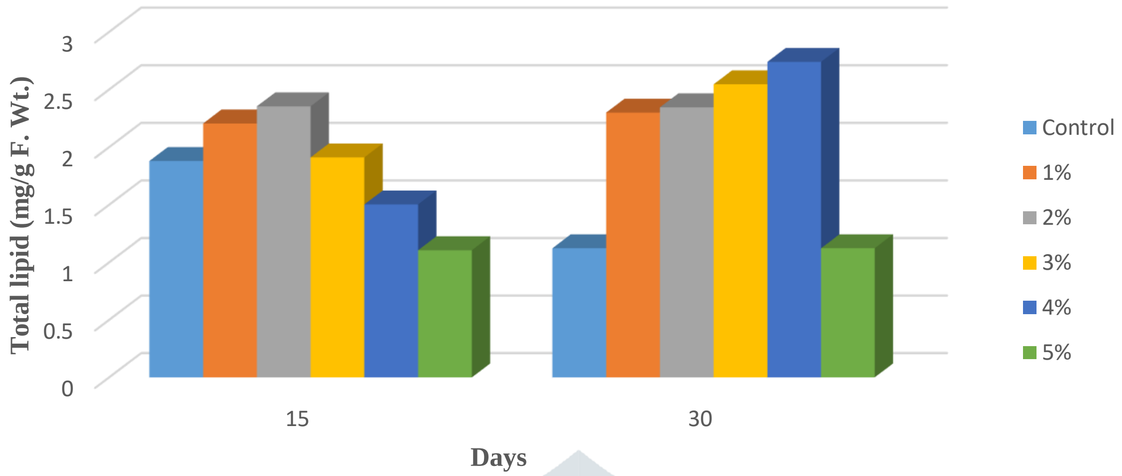


Fig 6. Effect of different percentage of seaweed extract on lipid content of *Vigna radiata*

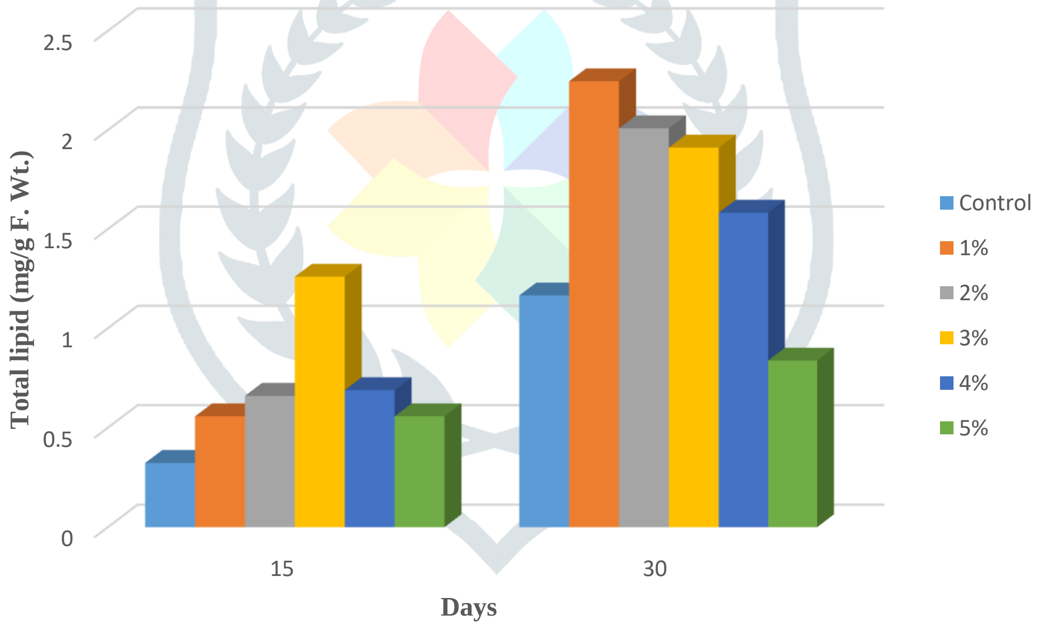


Fig 7. Effect of different percentage of seaweed extract on lipid content of *Vigna mungo*

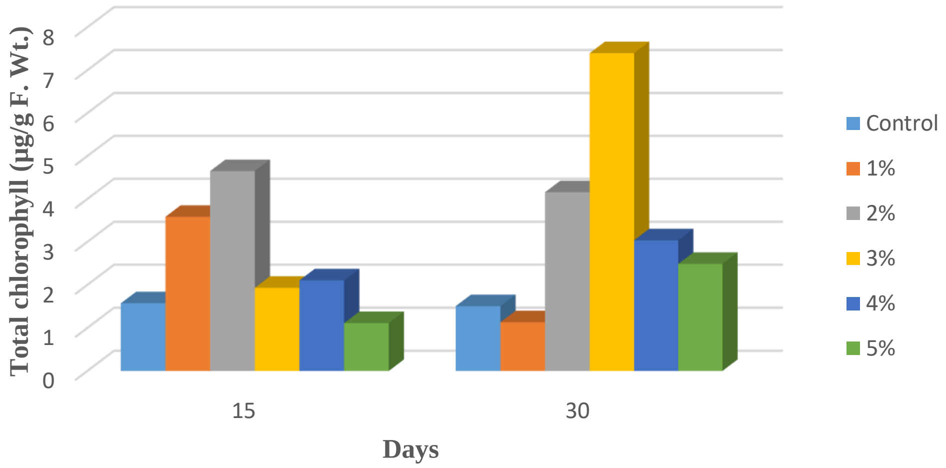


Fig 8. Effect of SLF on total chlorophyll content of *Vigna radiata*

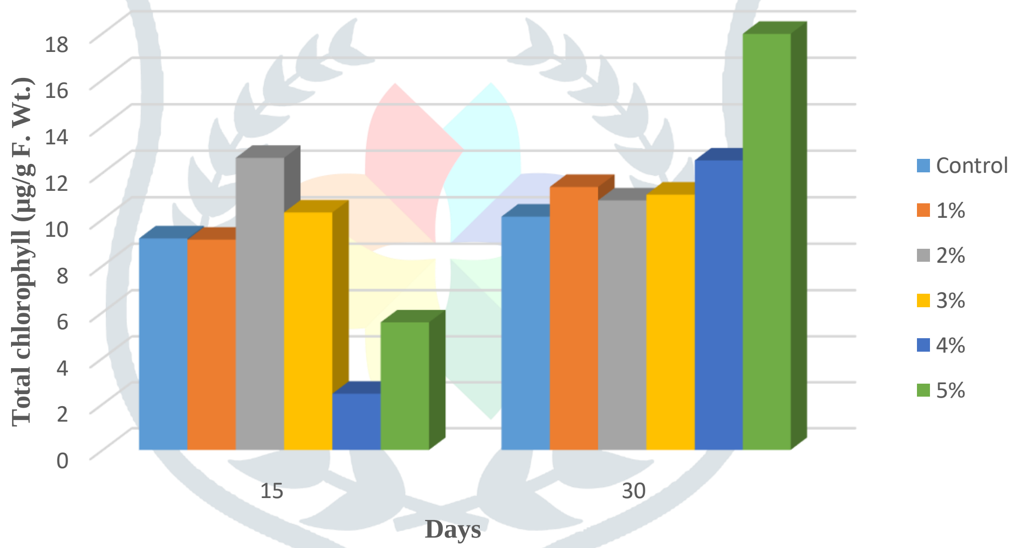


Fig 9. Effect of SLF on total chlorophyll content of Vigna mungo

4. DISCUSSION

The SLF of *S. myriocystum* holds higher content of calcium, sodium, potassium, iron, phosphate, chloride, silica, sulphate, copper and nitrate. Similar observations have been noted in earlier studies by Sivasankari et al., (2006b) who reported the physico-chemical properties of SLF of *S. wightii* and *Caulerpa chemnitzia*.

Similar observations have also been made in earlier studies, where *Padina* induced maximum seedling growth at lower concentrations in *C. cajan* (Mohan et al., 1994) and *Vigna radiata* (Venkataraman Kumar et al., 1993). Statistically significant differences were observed for shoot length, fresh and dry weight. The growth enhancing potential of seaweeds might be attributed to the presence of macro and micronutrients (Challen and Hemingway, 1965).

In the present study, seeds of *V. radiata* and *V. mungo* soaked in different concentrations (1% to 5%) of *S. myriocystum* extracts showed higher rates of germination, while the higher concentrations inhibited the germination. The germination percentage decreased with the increased concentration of SLF. This finding coincided with the reports in *Cajanus cajan* (Erulan et al., 2009), *Arabidopsis thaliana* (Kalidass et al., 2010), *Vigna radiata* (Renuka Bai et al., 2011), *Triticum aestivum* (Kumar and Sahoo, 2011), maize, ragi and kambu (Rajkumar Immanuel and Subramanian, 1999) and *Vigna catajung* and *Dolichos biflorus* (Anantharaj and Venkatesalu, 2001, 2002).

Thirumaran et al., (2009) studied the effect of *Chaetomorpha antennina* and *Rosenvingea intricata* on seed germination, fruit setting and vegetable weight of *Abelmoschus esculentus*. They got better results at lower concentration than higher concentration. The growth rate was increased up to 2% concentration and thereafter it showed a decreasing trend, thus better growth was when *S. myriocystum* extract was used especially in lower concentrations.

Ganapathyselvam et al., (2013) reported that the *Vigna mungo* seeds soaked in lower concentrations of the SLF of *Ulva reticulata* showed higher rates of germination, whereas it was inhibited by the higher concentrations. Thirumaran et al., (2009) reported that the *Sargassum wightii* SLF treatment increased total chlorophyll and carotenoids content of both the test plants at lower concentration (20%) of SLF with or without chemical fertilizer. Blunden et al., (1996) reported that the seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in plants. The present study revealed that seaweed extract prepared from *S. myriocystum* have fertilizing ability. The higher fertilizing ability of *S. myriocystum* may be due to the presence of high Mg and Fe content which has influenced the synthesis of chlorophyll content in foliar treated *V. mungo*.

In the current study, the lower concentrations (1% and 2%) of SLF increased the carbohydrate content of all tested plants when compared to control; higher concentrations decreased the carbohydrate content. Higher content of sugar was observed in plants treated with 2% SLF. The same trend was observed by Tamilselvan and Kannan (1994) in *H. musciformis* with NPK application in black gram. Anantharaj and Venkatesalu (2001, 2002) reported the same in *Vigna catajung* and *Dolichos biforus*.

Further, Sethi and Adhikary (2008) reported that 1% foliar spray enhanced the yield parameters such as fruit length and fruit weight of certain vegetable crops. Jayachandran and Ramasamy (1999) reported that root length and shoot length of *Arachis hypogea* became progressively decreased with increasing concentration of Liquid Seaweed Fertilizer obtained from *Hypnea musciformis*, a maximum number of leaves were recorded in plants applied with 2.5% LSF. However, more number of lateral roots was noticed in 10% LSF. Liquid extracts of *Sargassum wightii* (1.5%) and *Ulva lactuca* (1.0%) was found to have maximum influence on growth parameters viz., shoot length, root length, total fresh and dry weight, leaf area and moisture content (Sivasankari Ramya et al., 2010).

Effect of Seaweed Liquid Extract (SLE) of *Caulerpa scalpelliformis* on growth and biochemical constituents were studied in *Vigna mungo*. The lower concentration of SLF of *C. scalpelliformis* (25%) enhanced the percentage of germination, shoot length, root length and biochemical constituents viz., chlorophyll, carotenoid, amino acid, reducing sugar and total sugar contents, and α-amylase activities of shoot and root (Kalaivanan et al., 2012). The increased growth parameters at lower concentration may be due to the presence of higher levels of N, P, and K in the seaweed extract of *C. scalpelliformis*. Similar effect was reported by Sivakumar and Gandhi, (2010) on SLF prepared from *Sargassum wightii* on *V. mungo*. In addition to proper mineral fertilization, biostimulants can enhance the effectiveness of fertilizers as well as nutrient utilization from soil (Frankenberger and Arshad, 1995).

5. CONCLUSION

Soil fertility is diminishing gradually due to soil erosion, loss of nutrients, accumulation of salts and other toxic elements, water logging and unbalanced nutrient compensation. Organic wastes and biofertilizers are the alternate sources to meet the nutrient necessity of crops and to bridge the future gaps. The results of the present study has clearly demonstrated the plant growth promoting effect of Seaweed Liquid Fertilizer (SLF) and suggests to use this algal extract in agriculture to enhance the crop production in eco-friendly manner. They are extremely beneficial in enriching the soil with those microorganisms, which produce organic nutrients for the soil and help in combating diseases. The SLFs are harmless, eco-friendly and low cost agro- input supplementary to chemical fertilizers. They increase the soil fertility, improve soil structure, porosity and water holding capacity and also enhance seed germination and disease resistance.

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