



Effect of *Ulva lactuca* and *Padina tetrastromatica* concentrate on growth and yield of *Momordica charantia*

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ABSTRACT

The effect of Seaweed Liquid Fertilizer (SLF) of *Ulva lactuca* and *Padina tetrastromatica* on biochemical constituents, antioxidants, growth and yield parameters such as number of leaves, weight of fruits in *Momordica charantia* was examined. The Seaweed Liquid Fertilizer was found to be effective in increasing the growth and yield in low doses (1:4 and 1:6 conc.) than the control, 1:2 and higher concentrations of seaweed Liquid fertilizer.

Introduction

India is an agricultural country, Nearly 70% of the population living in rural areas are engaged in agriculture and making the backbone of our economy. The fast growing population is mounting tremendous pressure in food production in the country. To meet out this increasing demand, farmers use chemical fertilizers to enhance the crop production. Continuous use of these chemical fertilizers depletes essential soil nutrients and minerals that are naturally found in fertile soil. The toxic chemicals (arsenic and cadmium) from the chemical fertilizers accumulate in plant products and cause health problems in human by biomagnifications. The growing agricultural practices need more fertilizers for higher yield to meet out the need of food for human. There are many growth inducers, regulators and promoters available to enhance yield attributes. In developed countries such growth inducers are utilised in cultivation of crops. In recent years, the use of natural seaweed products as substitutes to the conventional synthetic fertilizers has assumed importance. In agriculture, the application of seaweeds are so many, as soil conditioners, fertilizers and green manure due to the presence of high amount of potassium salts, micronutrients and growth substances (Thirumaran *et al.*, 2009).

Seaweed contains essential minerals such as Ca, K, Mg, Po₄, S, N, Fe, Cu, Mn, Bo and Zn and has been found to

increase the sugar contents because of high content of potash. Seaweed fertilizer is suitable for root crops and cabbage. Zia (1990) reported that seaweed fertilizer is beneficial for plant growth, because of the presence of organic and inorganic constituents which increase the nutrients uptake and help in the assimilation of carbohydrates and protein contents in plants. The application of seaweed manure increases the growth, yield, flowering and fruiting period of plants. Wahab, (1991) found that different concentrations of seaweed effect differently on different plants. Seaweed fertilizer is beneficial for plant growth because of presence of organic, inorganic and constituents which increase the nutrient uptake and help in assimilation of carbohydrate and protein contents of the plant and hence increase in the plant yield. Marine algae contain good quantities of minerals. Hence, in USA, UK, France and Norway seaweed fertilizer is used as liquid spray to supplement the horticultural plants (Bokil *et al.*, 1972). It is noticeable that seaweed fertilizer increases the resistibility against diseases and reduces the chance of insect attack. The nature of seaweed fertilizer is alkaline (Phool B. Zahid, 1999). There are many plant growth inducers, regulators and promoters available to enhance yield attributes. Seaweed liquid fertilizers will be useful for achieving higher agricultural production, because the extract contains growth promoting hormones (IAA and IBA), Cytokinins, Gibberellins, trace elements, vitamins,

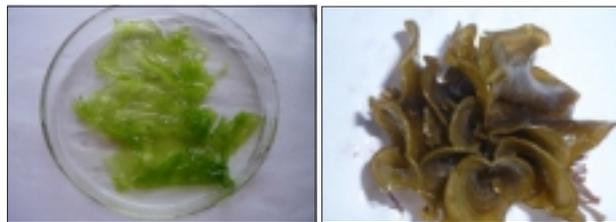
aminoacids, antibiotics and micronutrients. Booth (1969) also reported that seaweeds as fertilizers was not only due to nitrogen, phosphorus and potash content, but also due to trace elements and metabolites. The higher amount of water soluble potash, other minerals and trace elements are present in seaweeds and are readily absorbed by plants and they control deficiency diseases. The carbohydrates and other organic matter present in seaweeds alter the nature of soil and improve its moisture retaining capacity.

Recently, global attraction is on the seaweed fertilizers are often found to be more successful than the chemical fertilizers. In India, large quantity of macroscopic marine algae has been utilised directly as manure or in the form of compost by coastal peoples. Besides their application as Farm Yard Manure (FYM), Liquid extract obtained from seaweeds popularly known as SLF/LSF has recently gained much importance as foliar spray for inducing faster growth and yield in cereal crops, vegetables, fruits, orchards and horticultural plants. Kannan and Tamilselvan (1990) reported that the soil application of SLF of *Enteromorpha clathrata* and *Hypnea musciformis* increased the growth characteristics of green gram, black gram and rice. Genus *Momordica* of Cucurbitaceae has a wide range of distributions in India and in South Asia. A large quantity of the *Momordica charantia* is exported to Delhi, Agra Gwalior and other area of the country. Some quantity is also sold in the local markets. This plant is of great economic and commercial value in Thoothukudi. Perusal of literature shows that there is no study pertaining the growth of *Momordica*. Study related to growth and development of commercially important *M. charantia* will be of high value and may infuse better cultivation practices to obtain a better yield. Hence, the present study was made to investigate the effect of seaweed concentrate prepared from *Ulva lactuca* Linn and *Padina tetraströmatica* HAUCH on the growth, yield and biochemical constituents of *Momordica charantia* Linn.

Materials and Methods

Collection of seaweed

The green seaweed *Ulva lactuca* Linnaeus and brown seaweed *Padina tetraströmatica* Hauck were collected from the intertidal and subtidal regions up to 1m depth from Panchal, a small coastal village in Thirunelveli district. The coast extends about 2000 m from northeast to south east direction. The coast is formed with sand stones and beach rocks and located 14 km north of Kanyakumari. The materials were collected during low tide in the forenoon in January and February, 2014. The collected seaweeds were identified on the basis of pigmentation, morphology and authenticated by Dr. P. Anantharaman, Associate Professor, CAS of Marine Biology, Annamalai University in Parangipettai, India. The handpicked seaweeds from the intertidal waters were carefully sorted, cleaned to remove extraneous matter such as epiphytes, shells



U.lactuca Linn

P.tetraströmatica Hauck

Plate-1. Morphological features of *U.lactuca* and *P.tetraströmatica*

and associated fauna, washed with seawater followed by fresh water to remove sand particles. After draining off the water, the seaweeds were shade dried. Samples were coarsely powdered using electric grinder.

Quantitative Biochemical analysis of seaweeds

The powdered samples were analyzed for quantifications of protein, carbohydrate, lipid, tannin, phenol.

Preparations of seaweed extract for foliar application

Seaweeds were cooked in a pressure cooker for 30 minutes. The ratio of material and water was 1:10 (w/v). The extract was removed from the pressure cooker and then centrifuged at 5000-10000 rpm to remove most of the suspended impurities. The filtrate was dried in a hot air-oven at 65°C for 48 hrs and the concentrated seaweed sample was considered as 100% seaweed concentrate (Rama Rao, 1990).

Effect of *Ulva lactuca* and *Padina tetraströmatica* concentrate on the growth parameters and biochemical constituents of *Momordica charantia*

The seeds of *Momordica charantia* Linnaeus (Bitter melon) were potted carefully with soil (2kg soil/pot). After germination the seedlings were irrigated with water for 5 days. Thereafter, pots with seedlings were categorised into control and experimental. The seaweed extract was sprayed on to experimental plants for 10 days once in a day during early morning. Control plant was sprinkled only with water. Growth pattern was observed regularly after the treatment and recorded.

After every 10 days, the morphological attributes and biochemical constituents of both plants were analysed. The morphological attributes such as stem length, root length, number of leaves, number of tendrils and colour were recorded. Samples from each treatment were randomly drawn on 30 and 40th day after treatment. Leaves were used to estimate Total chlorophyll, chlorophyll a, chlorophyll b, carotenoids and biochemical constituents such as protein, carbohydrate, vitamin-C, flavonoid, phenol, tannin and lipid were analysed as per the standard procedures. Triplicate samples were used and the mean values with standard deviation are presented. Yield potential was recorded on 40th day based on the number of barren and filled seeds.

FTIR

Fourier Transform Infrared Spectroscopy (FT-IR) by KBr-pellet method was adopted for identification of functional groups present in the bioactive compounds of banana peel extracts mediated AgNPs. One milligram of peel powder (*P.tetrastromatica* and *U. lactuca*) was mixed with 100mg of dry potassium bromide (FT-IR grade) and then compared into a pellet using hydraulic press (5000-10000 psi). The pellet was immediately put into the sample holder and FT-IR (systronics 166) spectra were recorded in the range of 400-40000cm⁻¹.

Statistical analysis

Results obtained in this study have been subjected to the following statistical analysis by using computer software. i. Standard deviation ii. Simple correlation.

Results

Effect of seaweed extract on the growth of *M. charantia* condition

Experiments were initiated during December, 2013 using *M.charantia* (30 days old) seedlings burlapped from the field at Mela eral, Thoothukudi, Tamil nadu and were grown in earthen pots containing native soil to maintain the physical and chemical parameters of soil. Next day onwards application of SWC was started. A set without SWC application was maintained as control. In *M. charantia* 100% seed germination was found to be at 1:4 and 1:6 SLF without chemical fertilizer (Table-1). The influence of seaweed extract concentrates (SWC) obtained from *U. lactuca* and *P. tetrastromatica* was treated on the growth of *M. charantia*. The growth parameters

such as root length shoot length, number of leaves, number of tendrils, number of vegetables and colour of the leaves were recorded during the period of 30th day and 40th day after treatment. The shoot length of the *M. charantia* varied from 31 to 78cm. The maximum shoot length was noted in the plants received *P. tetrastromatica* (1:4) extract. The values of shoot length of *M. charantia* are depicted in Table 2 and 3. The root length of the *M. charantia* varied from 9.2 to 18cm. The maximum root length was noted in the plants received *P. tetrastromatica* (1:6) extract. The values of root length of *M. charantia* are depicted in Table 2 and 3. Number of leaves and Number of tendrils were influenced by the seaweed extracts and maximum was recorded for *P. tetrastromatica* extracts.

The seaweed concentrate treatment increased the growth parameters significantly when compared to the control. Similar results were obtained with green algae *Ulva fenestrata* and *Codium fragile*, red alga *Trichocarpus crinitus*, brown alga *Sargassum pallidum* on Buck wheat and Soya bean (Anisimov *et.al.*, 2013; Anisimov and Chaikina, 2014). Vijanand (2004) reported that lower concentration of SLF from *Stoechospermum marginatum* promoted the growth of brinjal and Sivasankari *et.al.* (2006) also reported similar effect in cowpea. Similarly *Ascophyllum* and *Laminaria* accelerated the growth of maize (Stephenson,1974). In the present study *M. charantia* treated with seaweed concentrate of and *U. lactuca* showed better results in growth parameters which may be directly attributed by the presence of essential macro, micro nutrients phenyl acetic acid and other closely related compounds like growth regulates in optimum level (Table-1). Promotive effects of seaweed concentrate application might

Table-1. Effect of *Ulva lactuca* and *Padina tetrastromatica* extract on the yield of *Momordica charantia*

Attributes		Control	1:2	1:4	1:6	1:8
<i>Ulva lactuca</i> extract	Seed germination	80%	90%	100%	100%	85%
	No. of leaves	21.43±0.4	33.66±2.4	36.52±15.4	34.31±13.4	25.01±12.3
	No. of tendrils	24.66±1.6	43.33±1.24	47.69±21.2	51.26±21.3	28.57±12.9
	No of vegetables	8±0.002	11±0.009	13±0.004	15±0.003	10±0.0043
<i>Padina tetrastromatica</i> extract	Semination	80%	90%	100%	100%	85%
	No. of leaves	36.3±4.49	63.33±20.4	48.27±20.3	43.27±19.5	38.08±23.6
	No. of tendrils	28±0.816	29.72±12.6	28.01±10.9	34.61±20	30.63±19.8
	No of vegetables	8±0.0054	10±0.0032	18±0.0045	16±0.0067	11±0.0058

Table-2. Growth and pigment concentration of *U. lactuca* extract on *Momordica charantia*

Parameters	Control	1:2SLF	1:4 SLF	1:6 SLF	1:8SLF
Shoot length	35.33±0.94	36.31±0.83	43±0.81	37.97±14.06	35.29±12.75
Root length	12.3±0.06	12.50±0.08	21.9±1.13	22.57±12.91	19.01±12.33
Total chlorophyll	0.01±0.04	0.01±0.03	0.02±0.04	0.02±0.09	0.024±0.02
Chlorophyll ^a	0.09±0.04	0.30±0.02	0.03±0.04	0.07±0.01	0.05±0.04
Chlorophyll ^b	0.12±0.03	0.13±0.03	0.15±0.04	0.17±0.02	0.01±0.06
Carotenoids	0.38±0.01	0.39±0.01	0.43±0.03	0.39±0.02	0.54±0.01
Length of vegetables	3.85±0.05	6.22±0.07	6.76±0.05	5.79±0.06	4.92±0.04
Weight of vegetables	12.66±0.05	14.26±0.06	14.83±0.08	13.43±0.03	11.16±0.04

Table-3. Growth and pigment concentration of *P. tetrastromatica* extract on *Momordica charantia*

Parameters	Control	1:2SLF	1:4 SLF	1:6 SLF	1:8SLF
Shoot length	31.3±2.05	36.29±16.82	43±0.81	37.97±14.06	35.29±12.75
Root length	12.3±0.06	12.5±0.08	43.3±2.86	43.90±17.90	40.22±17.41
Total chlorophyll	0.06±0.02	0.02±0.05	0.02±0.02	0.02±0.02	0.02±0.02
Chlorophyll" a"	0.07±0.04	0.01±0.05	0.01±0.07	0.05±0.01	0.03±0.01
Chlorophyll" b"	0.01±0.065	0.01±0.01	0.02±0.07	0.01±0.05	0.01±0.03
Carotenoids	0.01±0.013	0.09±0.03	0.12±0.05	0.54±0.07	0.38±0.01
Length of vegetables	4.85±0.06	4.93±0.05	6.11±0.08	6.46±0.07	5.91±-0.06
Weight of vegetables	14.63 ±0.07	15.63±0.05	13.23±0.02	10.66±0.03	8.59±0.02

Table-4. Bio chemical constituents of *U. lactuca* and *P. tetrastromatica*

Seaweed	Protein mg/g dw	Lipid mg/g dw	Carbohydrate mg/g dw	Flavonoid mg(QE)/g dw	Tannin mg/g dw	Viamin -C mg/g dw	Phenol mg GAE Equ/g
<i>Ulva lactuca</i>	27.2±1.5	0.3±0.0	61.5±2.3	0.02±0.0	0.12±0.01	0.24±0.02	5.8±09.06
<i>Padina tetrastromatica</i>	29.4±0.3	0.8±0.1	58.4±1.2	0.07±0.0	0.24±0.01	0.81±0.03	21.80±0.5

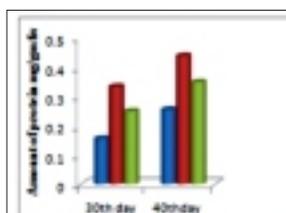
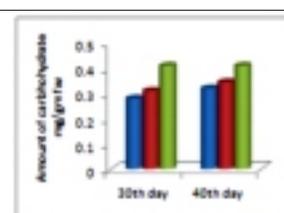
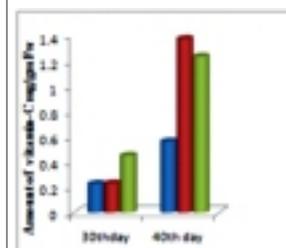
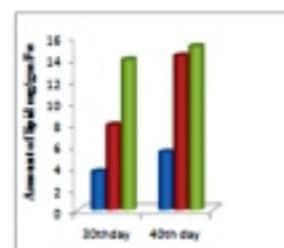
also be because of increased root proliferation and establishment. There by, plants were able to mine nutrients even from distant places and deeper soil horizon, in balanced proportions. Besides foliar applications of seaweed concentrate regulates bio-physical activities, which effectively resulted in maintaining higher photosynthetic activity. (Wedad, 2015). They increased shoot growth and root growth in the seaweed concentrate treated plants observed in the present study would possibly be due to the seaweed components such as minerals, nutrients, aminoacids, vitamins, cytokinins, auxins and ascorbic acid. These components when observed through cellular metabolism leading to enhancement of all the growth parameters (Crouch and Vanstaden, 1993; Strick *et al.*, 2003, Maria Victorial Rani and Revathy, 2009).

The present findings coincide with some earlier findings (Blunden *et al.*, 1977). Whapham *et al.* (1993) observed that the application of SLF of *Ascophyllum nodosum* increased the chlorophyll of cucumber and tomato plants. Foliar applications of Seaweed Control (SWC) improved chlorophyll production in rice crop (Flora and Maria Victorial Rani, 2012) is in accordance with the present study. In the present investigation high Mg and Fe content in *P. tetrastromatica* might have influenced the synthesis of chlorophyll. Both the seaweeds extract prepared from *U. lactuca* and *P. tetrastromatica* were found to have fertilizing ability. This may be due to increasing of chlorophyll which can improve photosynthesis. So it would increase production of carbohydrate and alternative explanation is that organic molecules such as organic acids, methionine thereby increasing their absorbance and so can increase carbohydrates (Papenfus *et al.*, 2013).

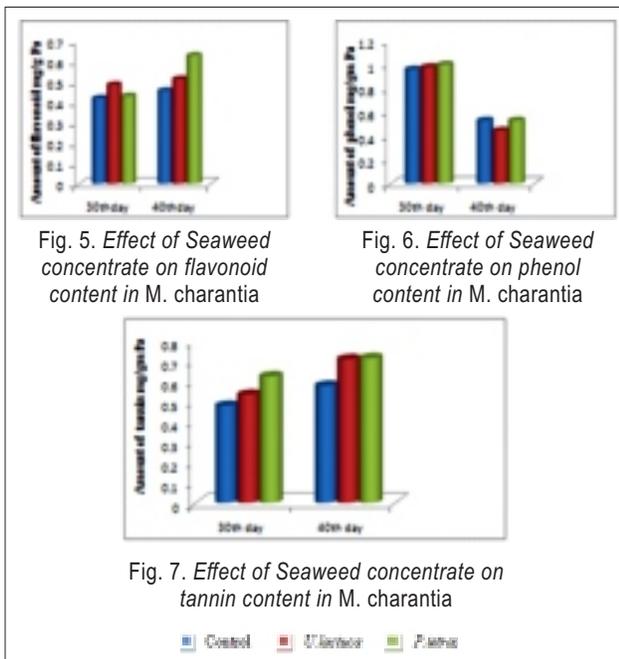
Effect seaweed extracts on the biochemical compositions of leaves in experimental plants

In order to understand the role of SWC on the

synthesis of biochemical composition in the control and experimental plants, estimation of soluble protein, carbohydrate, vitamin-C, lipid, phenol, flavonoid and tannin were carried out and the data are presented in Table-4 and figure 1-7. The highest protein (29.4 mg/g fw) was recorded at *U. lactuca* concentrate treated *Momordica charantia*. The increase in the protein content at seaweed concentrate might be due to the absorption of most necessary elements by the experimental plants. Application of SWC induced the formation of biochemical constituents like soluble protein, carbohydrate, lipid in the experimental plants than control. It is also confirmed by the statistical analysis in which all the growth parameters and biochemical constituents positively correlated with weight

Fig. 1. Effect of Seaweed concentrate on protein content in *M. charantia*Fig. 2. Effect of Seaweed concentrate on carbohydrate content in *M. charantia*Fig. 3. Effect of Seaweed concentrate on vitamin-C content in *M. charantia*Fig. 4. Effect of Seaweed concentrate on lipid content in *M. charantia*

■ Control ■ *U. lactuca* ■ *Padina*



of *M.charantia* treated by *U.lactuca* and *P.tetrastromatica* (Table-5).

Biochemical constituent in leaves treated with *P.tetrastromatica* concentrate enhanced in comparison with *U. lactuca* concentrate. The same trend was observed in *Trigonella foenum* (Pise and Sabale, 2010) and in *Solanum melongenea* (Ramya et al., 2015). Utilization of seaweed concentrate as organic fertilizer to improve the growth of crop plants was quite exiting and promising and it needs a thorough characterization of seaweeds. Hence, the present investigation was made to determine the biochemical component such as protein, carbohydrate, vitamin-c, lipid, phenol, flavonoid and tannin, in *U. lactuca* and *P.tetrastromatica* belonging to Chlorophyceae and Phaeophyceae. The present study revealed the highest level of protein, carbohydrate, lipids, vitamin-c, flavonoid, phenol and tannin content in *U. lactuca* and *P. tetrastromatica* (Table-4).

FT-IR

FT-IR spectrum is able to predict the main chemical constituents in FTIR Spectrum of *P. tetrastromatica* showed major peaks at range from 403.72 cm⁻¹, 2535.16cm⁻¹ (Plate-2). FTIR Spectrum of *U. lactuca* showed major peaks at range from 1135.35cm⁻¹, 3917.76cm⁻¹ (Plate-3). The strong absorption bands at 3371 and 3408 cm⁻¹ in both algae C-H, O-H and NH stretching vibrations, characteristic of the presence of amino acids (Rao, 1963). CH₃ and CH₂ groups (Socrates, 1994, Bellamy, 1975) indicative of the chlorophyll groups at 2924 and 2854 cm⁻¹. The weak band 2344-2365 cm⁻¹ observed in the samples of both species may correspond to the C-O stretching band which is a characteristic peak for carboxylic group

Table-5. Correlation co-efficient obtained by relating different variables with weight of *M.charantia* treated with *U.lactuca* and *P.tetrastromatica* extracts

Variables	r value			
	<i>U. lactuca</i>		<i>P. tetrastromatica</i>	
	30 days	40 days	30 days	40 days
No. of leaves	0.420	0.610	0.670	0.750
No. of tendrils	0.720	0.100	0.250	0.640
No of vegetables	0.560	0.820	0.530	0.770
Shoot length	0.727	0.660	0.752	0.765
Root length	0.095	0.145	0.487	0.652
Total chlorophyll	0.690	0.630	0.550	0.650
Chlorophyll "a"	0.580	0.350	0.490	0.590
Chlorophyll "b"	0.770	0.790	0.660	0.590
Carotenoids	0.532	0.673	0.457	0.673
Length of Vegetable	0.329	0.543	0.681	0.732
Soluble protein	0.660	0.850	0.730	0.820
Carbohydrate	0.784	0.350	0.760	0.810
Vitamin-C	0.583	0.492	0.687	0.642
Lipid	0.700	0.610	0.590	0.370
Phenol	0.320	0.460	0.650	0.750
Flavonoid	0.630	0.450	0.680	0.840
Tannin	0.610	0.600	0.790	0.740

(Rajasekar et al.,2013).The peak around 1662-1653 cm⁻¹of the spectrum of both species is due to the C-O stretching and N-O asymmetric stretching indicative ester group (Stewart,1995). The weak bands around 1558 cm⁻¹ represent C=C stretching vibration indicative of the lignin (Kubo and Kadla,2005; Chatjigakis,1998). The absorption band at 1246 cm⁻¹and 1258 cm⁻¹are due to S=O (sulfate esters) (Silva et al.,2005; Boeriu et

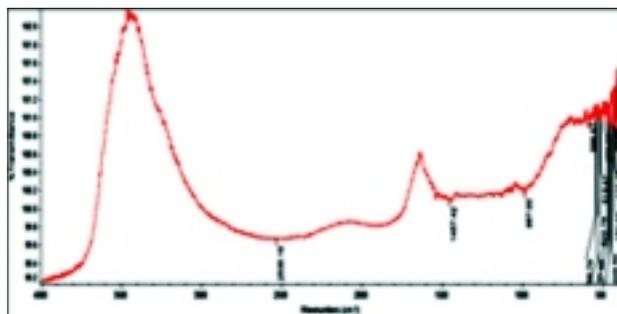


Plate-2. FTIR Spectrum of *P. tetrastromatica* Hauch

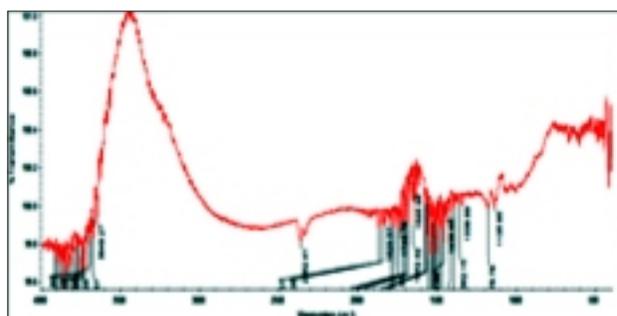


Plate-3. FTIR Spectrum of *Ulva lactuca* Linn.

al., 2004). The absorption bands at 1100-1000 cm^{-1} in the fingerprint region indicate several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates (Li *et al.*, 2004) and polysaccharides (Nakamoto, 1986). The seaweeds both *P. tetrastromatica* and *U. lactuca* contain a strong absorption band at 1034 cm^{-1} due to S=O stretching vibration also indicates the starch and polysaccharides content in the sample. The absorption peak around 800 -860 cm^{-1} may correspond to the S=O, which indicates the presence of the sulfonate group, observed generally in seaweeds (Figueira, 1999). The weak absorption band observed near 600 – 670 cm^{-1} due to C-S and C=S stretching vibrations (sulfides). The brown alga *P. tetrastromatica* contains weak absorption band at 531 cm^{-1} and 504 cm^{-1} due to brominated and iodo components present in it. But this absorption band is not present in *U. lactuca*.

It may be concluded that the growth and biochemical characteristics of vegetable crop *M. charantia* could be promoted by the presence of Total chlorophyll, chlorophyll a, chlorophyll b, carotenoids and biochemical constituents such as protein, carbohydrate, vitamin-C, flavonoid, phenol, tannin and lipid etc in the SLF of *U. lactuca* and *P. tetrastromatica*. All the parameters have contributed positively towards yield and revealed by their r values. Cytokinin and magnesium, which are considered as essential growth promoting constituents in chlorophyll biosynthesis might have played a key role in enhancement of growth and physiology of Bitter guard. However, optimum concentration of seaweed liquid extracts is necessary as in this study 1:4 1st 1:6 SLF had better influence on growth and productivity of Bitter guard plants. The present 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 farming.

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