Asian Journal of Biological and Life Sciences Original Research

Effect of *ULVA LACTUCA* linn and *PADINA TETRASTROMATICA* HAUCH concentrate on growth and yield of *LABLAB PUPUREUS* L.

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Submitted : 19.01.2017 Accepted : 28.03.2017 Published : 30.04.2017

Abstract

A pot experiment was conducted to investigate the effect of seaweed concentrate of *Ulva lactuca* Linn and *Padina tetrastromatica* HAUCH on the growth and biochemical characteristics of *Lablab purpureus* L. The growth parameters such as root length, shoot length, no of leaves and no. of tendrils were recorded during the period of 30th day and 40th day after treatment. The highest shoot length, root length, no of leaves, no of tendrils were recorded in the *L.purpureus* treated with *P.tetrastromatica* concentrate than the control and *U.lactuca* concentrate treated plants. The seaweeds enhanced the vitamin-c content, the maximum (1.502 mg/g fw) was recorded in *L. purpureus* on the 40th day treated with *P.tetrastromatica* extract. Biochemical constituent in leaves treated with *P.tetrastromatica* concentrate was enhanced in comparison with *U. lactuca* concentrate.

Key words : Seaweed Liquid Fertilizer; Marine seaweed *U. lactuca (LINN), P. tetrastromatica (HAUCH); Growth parameters,* Antioxidant; FT-IR; Vegetable plant- *L. purpureus*. L.

INTRODUCTION

India is an agricultural country; nearly 70% of the population thrives in rural areas, engaged in agriculture 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 causing health problems in human by biomagnifications.

The growing agricultural practices need more fertilizers for higher yield to satisfy food for human beings. There are many growth hormones, regulators and promoters available to enhance yield attributes. The developed countries utilized such growth hormones 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.

The use of seaweeds as manure in farming practice is very ancient and common practice among the Romans and also practiced in Britain, France, Spain, Japan and China. The use of marine macro algae as fertilizer in crop production has a long tradition in coastal areas all over the world. Seaweed cast continued to be so valuable to farmers, even in the early 1900s. In many countries seaweed and beach cast are still used in both agriculture and horticulture. Many studies in the past three decades have found wide application in modern agriculture for the use of seaweeds as fertilizer. They are used as whole or finely chopped powdered algal manure or aqueous extracts. In recent years the use of these seaweeds in modern agriculture has been investigated by many.

Seaweeds contain all the trace elements and plant growth hormones required by plants. It was also reported that seaweed manure is rich in potassium but poor in nitrogen and phosphorus than the farm manure. There are many plant growth hormones, 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. [1] also 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. 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.

In the present day world, the seaweed fertilizers are often found to be more successful than the chemical fertilizers. In India, large quantity of macroscopic marine algae has been utilized 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 interest as foliar spray for inducing faster growth and yield in cereal crops, vegetables, fruits, orchards and horticultural plants. [2] observed that the soil application of SLF of *Enteromorpha clathrata* and *Hypnea musciformis* increased the growth characteristics of green gram, black gram and rice. Seaweed concentrate (Kelpak) increased the growth and yield significantly in potassium stressed wheat. These result suggested that the mineral elements within Kelpak are partly responsible for its effect. Aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increased yield and quality of fruits. [3] observed that the application of SLF of *Ascophyllum nodosum* increased the chlorophyll levels of Cucumber cotyledons and tomato plants.

The present study intends to investigate the effect of Seaweed

PLATE.1

***U.LACTUCA* LINN**

***P.TETRASTROMATICA* HAUCK**

concentrate prepared from *Ulva lactuca* Linn and *Padina tetrastromatica* HAUCH on the growth, yield and biochemical constituents of *Lablab purpureus* L.

MATERIALS AND METHODS

COLLECTION OF SEAWEED

Seaweeds 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 2000m from northeast to south east direction. The coast is formed with sand stones and beach rocks and located 14km. north of Kanyakumari. Dominated species of algae representing the classes Chloropyceae-(*Ulva lactuca),* Phaeophceae-(*Padina tetrastromatica)* were chosen for the present study. Samples were collected during low tide in the forenoon.

The handpicked seaweeds from the intertidal waters were carefully sorted, cleaned to remove extraneous matter such as epiphytes, shells 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 powered using electric grinder.

QUANTITATIVE BIOCHEMICAL ANALYSIS OF SEAWEEDS:

The powdered seaweeds were analyzed for quantifications of protein, carbohydrate, lipid, tannin and 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 65ºC for 48 hrs and the concentrated seaweed sample was considered as 100% seaweed concentrate [4].

EFFECT OF *ULVA LACTUCA* LINN AND *PADINA TETRASTROMATICA* HAUCK CONCENTRATE ON THE GROWTH PARAMETERS AND BIOCHEMICAL CONSTITUENTS OF *LABLAB PURPUREUS* LINN

The seeds of *Lablab purpureus* Linn. (hyacinth bean) is potted carefully with soil (2kg soil/pot) .After germination the seedlings were irrigated with water for 5 days. Thereafter, pots with seedlings were categorized into control and experimental. The seaweed extract was sprayed over the experimental plants for 10 days once 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 analyzed. The morphological attributes such as stem length, root length, number of leaves, number of tendrils, colour were recorded. Total chlorophyll, chlorophyll a, chlorophyll b, carotenoids [5] and biochemical constituents such as protein [6], carbohydrates [7], vitamin-C [8], flavonoids [9], phenol [10], tannins and lipids [11] were analyzed as per the standard methods.

FT-IR SPECTROSCOPIC ANALYSIS [12]

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* ,*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}.

RESULT

EFFECT OF SEAWEED EXTRACT ON *L.PURPUREUS* LINN. GROWTH UNDER POT CONDITION

Experiments were initiated using *L.purpureus* Linn. (30days

Table 1: Effect of *Ulva lactuca* extract on growth parameters of *Lablab purpureus*

Growth attributes	Control		Experimental	
	30th day	40th day	30th day	40th day
Stem length (cm)	31.33±2.05	36.29±16.82	41.19±18.88	40.81±17.03
Root length (cm)	43.33±2.86	43.9±17.09	36.09±17.81	38.22±17.41
No.of leaves	36.33±4.49	63.3±20.38	48.27±20.38	43.51±19.56
No.of tendrils	28±0.81	39.72±12.65	28.01±10.90	34.61±10.02

Table 2: Effect of *Padina tetrastomatica* extract on morphology of *Lablab purpureus*

Growth attributes	Control		Experimental	
	30th day	40th day	30th day	40th day
Stem length (cm)	47.33±0.47	37.35±14.87	43.23±19.79	47.72±19.06
Root length (cm)	15±0.816	25.85±18.44	23.23±15	16.6±1.247
No.of leaves	37±0.817	32.47±12.01	41.25±22.56	84±4.32
No.of tendrils	37.66±2.05	49.46±26.94	55.24±26.39	85.66±8.24

old) seedlings burlapped from the field at Mela Eral, Thoothukudi, Tamil nadu and were grown in earthern 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.

The influence of seaweed extract concentrates (SWC) obtain from *U.lactca* Linn. and *P.tetrastromatica* Hauck. treated on the growth *of L.purpureus* Linn were studied. The growth parameters such as root length shoot length, No.of. Leaves, No.of.tendrials, and colour of the leaves were recorded, during the period of 30th day and 40th day after treatment.

The highest shoot length, root length, no of leaves, no of tendrils were recorded in the *L. purpureus* treated with *P. tetrastromatica* concentrate than the control and *U. lactuca* concentrate treated plants. The seaweed concentrate treatment increased the growth parameters significantly when compared to the control. The plants (*L. purpureus*) treated with seaweed concentrate of *P. tetrastromatica* 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.

EFFECT OF FOLIAR APPLICATION OF SEAWEED EXTRACTS ON CHLOROPHYLL PIGMENTS

Pigmented plant compounds are important anti-inflammatory and antioxidant substances and people who eat more of them have a decreased risk of cancer. Green plants contain particularly large amounts of chlorophyll, which is a detoxifier and possibly anticancer agent . Estimation of chlorophylls revealed that seaweeds extracts were predominantly enhanced the production of Total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoid (Table 3 and 4).

Table-5 showed the impact of SWC on chlorophyll pigments in *P. tetrastromatica, U. lactca* on 30th and 40th day of estimation there was little variation in total chlorophyll, chlorophyll-a, chlorophyll-b, carotenoid. Among control and seaweed concentrate treated plants of *P. tetrastromatica*, enhanced the chlorophyll pigments in the experimental plants. In the present study high Mg and Fe content in *P.tetrastromatica* might have influenced the synthesis of cholorophyll. Both the seaweeds extract prepared from *U. Lactuca* and *P. tetrastormatica* were found to have fertilizing ability. The highest protein (29.4 mg/g fw) was recorded at *U. lactuca* concentrate treated *L. purpureus*. 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, carbohydrates, lipids, in the experimental plants than control. Biochemical constituent in leaves treated with *P.tetrastromatica* concentrate was enhanced in comparison with *U. lactuca* concentrate. Utilization of seaweed concentrate as organic fertilizer to improve the growth of crop plants were quite exiting and promising and it needs a thorough characterization of seaweeds. Hence the present investigation was undertaken to determine the biochemical component such as protein, carbohydrate, vitamin-c, lipid, phenol, flavonoid, 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 were noticed in *U. lactuca* and *P. tetrastromatica* . (table5)

FTIR SPECTRAL ANALYSIS

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 to 2535.16cm^1 (Plate 2).

Table 3: Effect of *U. lactuca* extract on photosynthetic pigments of *Lablab purpureus*

Photosynthetic pigment	Control		Experimental	
	30th day Mg/g fw	40th day Mg/g fw	30th day Mg/g fw	40th day Mg/g fw
Total chlorophyll	0.021±0.002	0.320±0.005	0.008±0.008	0.022±0.002
Chlorophyll-a	0.011±0.004	0.021±0.001	0.019±0.019	0.075±0.060
Chlorophyll-b	0.018±0.002	0.019±0.004	0.011±0.011	0.013±0.002
Carotenoid	0.328±0.007	0.392±0.0023	0.012±0.012	0.375±0.001

Table 4: Effect of *P. tetrastomatica* extract on photosynthetic pigments of *Lablab purpureus*

Photosynthetic pigment	Control		Experimental	
	30th day Mg/g fw	40th day Mg/g fw	30th day Mg/g fw	40th day Mg/g fw
Total chlorophyll	0.026±0.006	0.035±0.005	0.0008±0.008	0.425±0.003
Chlorophyll-a	0.013±0.008	0.016±0.009	0.0075±0.060	0.019±0.002
Chlorophyll-b	0.020±0.005	0.029±0.008	0.0004±0.004	0.023±0.002
Carotenoid	0.423±0.012	0.4353±0.01	0.0125±0.001	0.856±0.050

Table 5 **Bio constituents of *U. lactuca* and *P. tetrastromatica***

Seaweed	protein mg/g dw	Lipid mg/g dw	Carbohydrates mg/g dw	flavonoid mg(QE)/ g dw	tannin mg/g dw	vitamin –C mg/g dw	phenol mg GAE Equ/g
U. lactuca	27.2±1.5	0.3±0.0	61.5±2.3	0.02±0.0	0.12±0.001	0.24±0.02	5.8±09.006
P.tetrastromatica	29.4±0.3	0.8±0.1	58.4±1.2	0.07±0.0	0.24±0.001	0.81±0.03	21.80±0.50

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

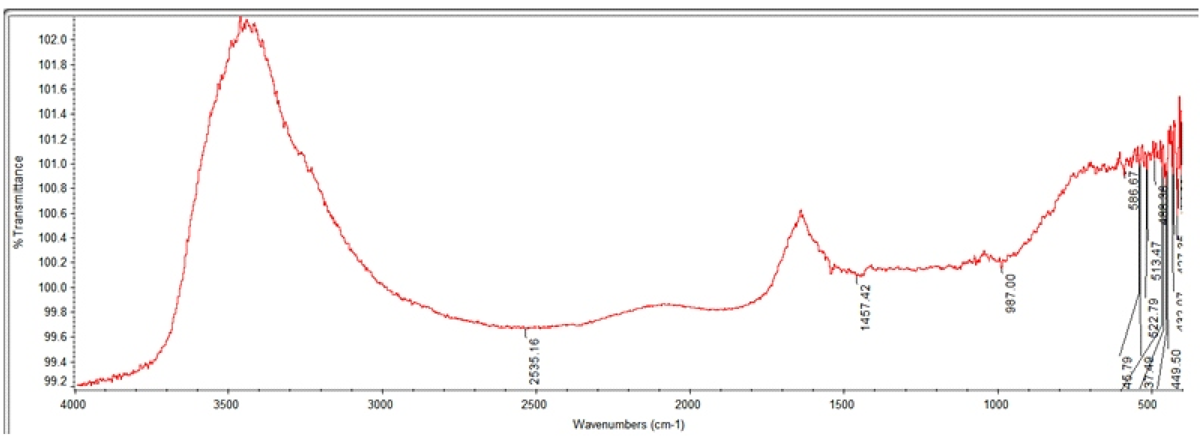
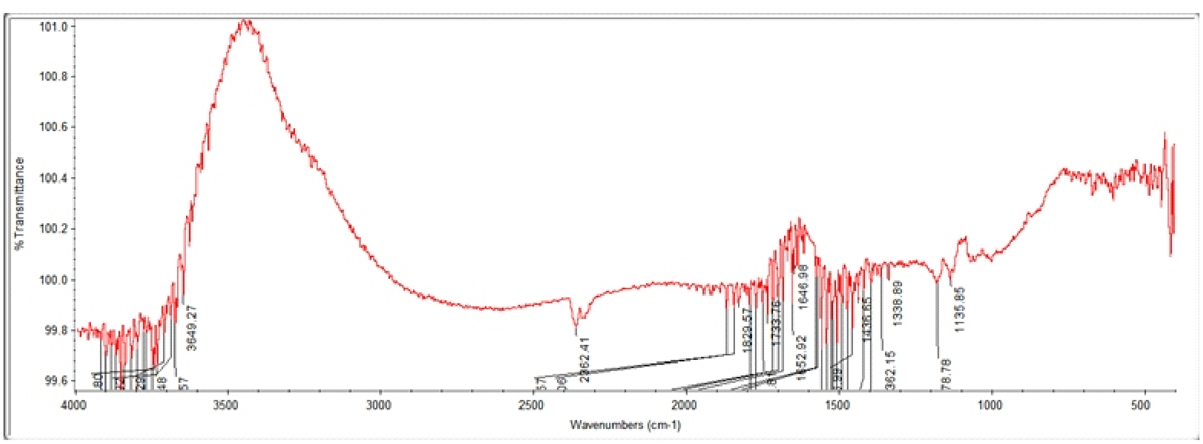


Plate 3 FTIR Spectrum of *Ulva lactuca* Linn.



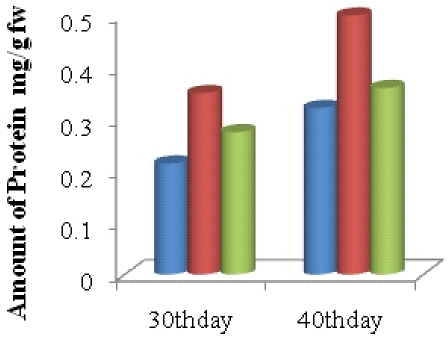


Fig. 1 Effect of Seaweed concentrate on protein content in *L. purpureus*

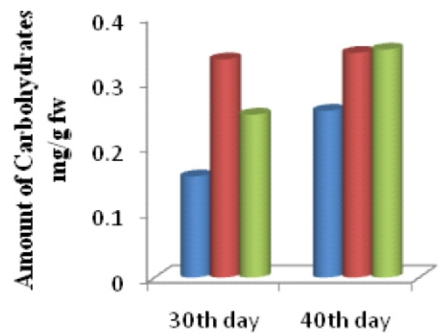


Fig. 2 Effect of Seaweed concentrate on carbhohydrate content in *L. purpureus*

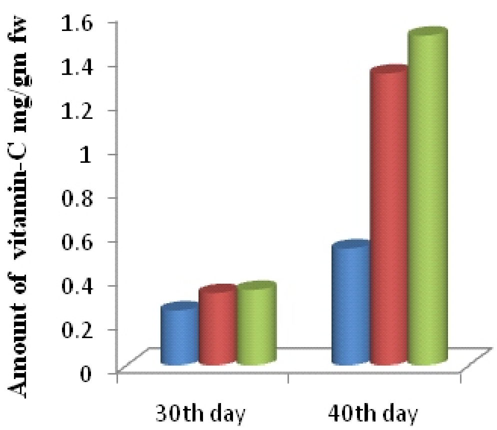


Fig. 3 Effect of Seaweed concentrate on Vitamin-C content in L. purpureus

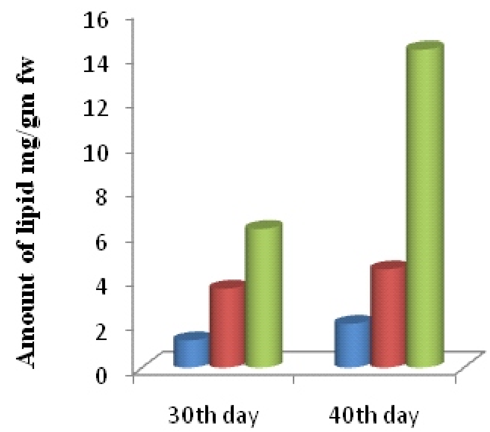


Fig. 4 Effect of Seaweed concentrate on Lipid content in L. purpureus

Control U.lactuca P.tetrastromatica

Aqueous concentrate obtained from seaweed was applied as foliar spray on 30 days old plants .
Plants were grown in earthern pots under natural condition.
Control- Plants retained without any treatment

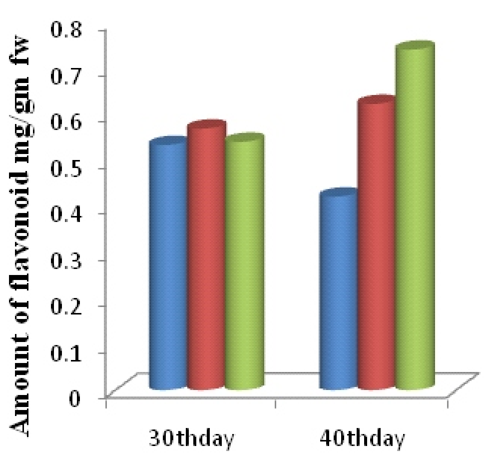


Fig. 5 Effect of Seaweed concentrate on flavonoid content in L. purpureus

FTIR Spectrum of *U. lactuca* showed major peaks at range from 1135.35cm^{-1} to 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.

DISCUSSION

The responses of plants to seaweed application are varied. These include higher yield, increased nutrient uptake, changes in plant tissue composition, increased resistance to frost, fungal disease and insect attack, longer shelf life of fruit and better seed germination. The present study was undertaken to investigate the effect of seaweed concentrate of *U. lactuca* and *P. tetrastromatica* on the growth and biochemical characteristics of *L. purpureus*.

The highest shoot length, root length, no of leaves, no of tendrils were recorded in the *L. purpureus* treated with *P. tetrastromatica* concentrate than the control and *U. lactuca* concentrate treated plants.The seaweed concentrate treatment increased the growth parameters significantly when compared to

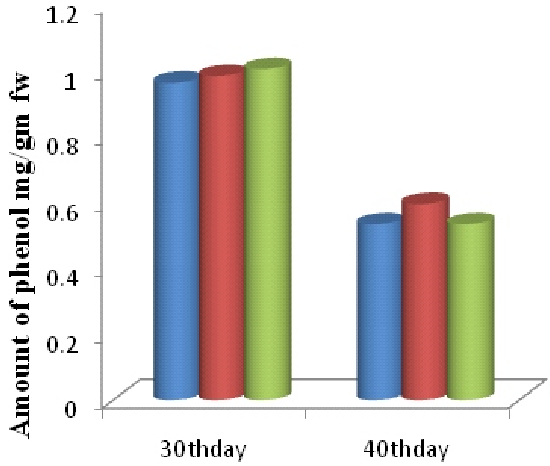


Fig. 6 Effect of Seaweed concentrate on Phenol content in *L. purpureus*

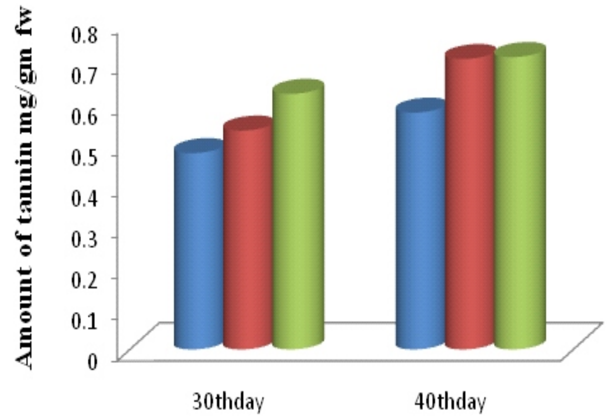


Fig. 7 Effect of Seaweed concentrate on tannin content in *L. purpureus*

Control U.lactuca P.tetrastromatica

Aqueous concentrate obtained from seaweed was applied as foliar spray on 30 days old plants .
Plants were grown in earthern pots under natural condition.
Control- Plants retained without any treatment.

the control, similar results were obtained in green algae *Ulva fenestrate* and *Codium fragile*, red algae *Trichocarpus crinitus*, brown algae *Sargassum pallidium* on Buck wheat and Soya bean [13-14]. [15] reported that lower concentration of SLF from *Stoechospermum marginatum* promoted the growth of brinjal and [16] also reported similar effect in cowpea. Similarly *Ascophylum* and *Laminaria* accelerated the growth of maize[17]. Promotive effects of seaweed concentrate application might 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 [18] .The increased shoot growth, 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 the growth parameters [19-21].

Our findings coincide with some earlier findings [22]. [3] 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 [23] is in accordance with the present study. In the present study high Mg and Fe content in *P. tetrastromatica* might have influenced the synthesis of cholorophyll. Both the seaweeds extract prepared from *U. Lactuca* and *P. tetrastormatica* 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 [24].

Biochemical constituent in leaves treated with *P.tetrastromatica* concentrate was enhanced in comparison with *U. lactuca* concentrate. The same trend was observed in *Trigonella foenum* [25] and in *Solanum melongenea* [26]. Utilization of seaweed concentrate as organic fertilizer to improve the growth of crop plants were quite exiting and promising and it needs a thorough characterization of seaweeds. Hence the present investigation was undertaken to determine the biochemical component such as protein, carbohydrate, vitamin-c, lipid, phenol, flavonoid, 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 were noticed in *U. lactuca* and *P. tetrastromatica*. (Table.4).

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 6). FTIR Spectrum of *U. lactuca* showed major peaks at range from 1135.35cm^{-1}, 3917.76cm^{-1} (Plate 7). 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 [27]. CH_3 and CH_2 groups [28-29] 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 [30]. The peak around 1662-1653 cm^{-1} of the spectrum of both species is due to the C-O stretching and N-O asymmetric stretching indicative ester group [31]. The weak bands around 1558 cm^{-1} represent C=C stretching vibration indicative of the lignin[32-33]. The absorption band at 1246 cm^{-1} and 1258 cm^{-1} are due to S=O (sulfate esters) [34-35]. 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 [36] and polysaccharides [37]. 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[38]. The weak absorption band observed near 600 670 cm^{-1} due to C-S and C=S stretching vibrations (sulfides). The brown algae *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*.

CONCLUSION

The present investigations revealed that the seaweed species of *U. lactuca* and *P. tetrasromatica* were observed to be a potential source of fertilizer to the *L. purpureus.* Present findings encourage the application of such seaweeds as natural fertilizer in agricultural sector. Hence, this simple practice of application of ecofriendly seaweed liquid fertilizers to crops may be useful for the growers for attaining better germination, growth and yield. Findings of this work are useful to further research to evaluation by isolation, characterization and identifications of growth hormones.

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