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RED SEAWEED EXTRACT AS A BIOSTIMULANT ON GROWTH AND BIOCHEMICAL PARAMETERS OF MICROALGAE *CHLORELLA VULGARIS*

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ABSTRACT:

Biostimulants and biofertilizers are considered environmentally friendly and cost-effective alternatives to synthetic products such as fertilizers, crop protection products and plant growth regulators. In the present study, the potential of seaweed liquid fertilizer (SLF) of marine red algae Gracilariacorticata and Grateloupialithophila which were collected from Kovalong coast, Tamil Nadu was evaluated for its effect on the growth, biochemical parameters and pigment content of Chlorella vulgaris.The BBM medium for Chlorella was amended with different concentrations of this SLF viz., 0.5M, 1.0M, 1.5M, 2.0M concentrations per Liter of the medium. It was estimated from our studies that the 2.0M and 1.5M concentrations of both Gracilariacorticata and Grateloupialithophila as SLF were more effective for the alga's growth and showed highest amount of pigment accumulation at these concentrations. The SLF showed increase in carotenoid content of Chlorella day by day with increase in biomass concentration. There were minimal changes in Protein accumulation thus proving that the SLF enhanced the alga's growth and the Protein content was not affected. Thus this alga can be grown efficiently with SLF in a shorter time period and supplied to the people without any side-effects. We also estimated the SLF's Auxin content in Gracilariacorticata and had highest Auxin content than Grateloupialithophila. By this we conclude that, the increase in growth of Chlorella vulgaris is influenced by presence of growth hormones in the seaweed.

KEYWORDS: *Chlorella vulgaris, BBM, Seaweed Liquid Ferilizer, Gracilariacorticata, Grateloupialithophila, Biostimulant.*

INTRODUCTION

Microalgae contribute to a balanced diet because of their composition. Beside numerous essential nutrients, carotenoids are in the focus for food applications. *Chlorella vulgaris* represents a good carotenoid source for potential use in foods (Gille*et al*., 2015). *Chlorella* is non-toxic and naturally wrapped in amino acid which can be easily uptake by our body instantly. It is a unicellular, non-motile, spherical green algae and its size varies from 7µm – 10µm in diameter. Biochemical composition of *Parachlorella* had 30% lipid, 5% carbohydrate, 15% protein and 8% starch and 40% of the extracted lipid was composed of PUFAs through GC-MS analysis (L. Chin Ming *et al.*, 2012). They contain chlorophyll a and b as main photosynthetic pigment. It contains vitamin B, C, and E, and

also contains essential amino acids, enzymes and trace elements (Raja *et al.*, 2008). *Chlorella* serves as essential nutrients, natural colorant, cosmetic additive and health food which present in a higher quantity in *Chlorella* (Apt and Behrens, 1999). β-carotene act as potent antioxidant, immunomodulatory and used for preventing human cancer. It stimulates immunity in treatment of all degenerative diseases by secreting *Chlorella* growth factor (CGF) (Raja and Hemaiswariya, 2010).

For centuries seaweed has been used as fertilizer. Liquid seaweed fertilizer is not only organic, but comes from a sustainable source and can be harvested without damaging the environment. Seaweed extract act as a potent biostimulant of various plant and microalgae growth. They contain phytohormones and organic or inorganic nutrients essential for growth of plants (Sridhar and Rengasamy 2010a: Sridhar and Rengasamy, 2010b: Sridhar and Rengasmay, 2011. It also enhances the content of pigments, proteins and other biochemical composition of microalgae (Thirumaran*et al*., 2009; Erulan*et al.*, 2009). They are used as a Biofertilizer or seaweed liquid fertilizer (Rajkumar and Subramanian, 1999). Seaweeds have been proven as a source of antioxidants, plant growth hormones, osmoprotectants, mineral nutrients and many other organic compounds including novel bioactive molecules (Akila and Jeyadoss 2010; Ramarajan*et al.*, 2013; Pacholczak*et al.*, 2016). The utilization of seaweeds as biofertilizer was considered to compensate for the lack and deficiency of N, P and K in soils (Anantharaman*et al.*, 2010, Srijaya*et al.*, 2010; Sunarpi*et al.*, 2010; *Tuhy et al.*, 2015; Vyomendra and kumar 2016).

The objective of the present study was to estimate the efficacy of the red algae *Gracilariacorticata* and *Grateloupialithophila* as SLF for the growth, Biochemical and pigment contents of the economically important microalga *Chlorella.vulgaris.* The Growth hormone auxin will also be isolated and estimated for the red alga.

MATERIALS AND METHODS

Chlorella vulgaris was obtained from the culture collection of CAS in Botany, University of Madras, Guindy campus, Ch-25 and maintained in Bold Basal medium (BBM) at 24±1°C in a thermostatically controlled room, illuminated with fluorescent tubes (Philips 40W) providing 30µEm2/s and 12:12hrs light/dark cycle.

Preparation of Seaweed Liquid Fertilizer (SLF)

Seaweeds or macroalgae belonging to Rhodophyceae members' viz., Gracilariacorticata. and Grateloupialithophila were collected from Kovalong coast, Tamil Nadu in the month of January 2012 and it was brought to the laboratory, washed initially with seawater and finally with freshwater to

remove sand particles and macroscopic epiphytes. They were shade dried first for four days followed by oven drying at 50°C. Then the material was hand crushed and made as coarse powder using mixer grinder and it was taken for the preparation of seaweed liquid fertilizer.

The BBM medium for Chlorella was amended with different concentrations of this SLF powder prepared from Gracilariacorticata and Grateloupialithophila viz., 0.5M, 1.0M, 1.5M, 2.0M concentrations per Liter of the medium. Then the algal cultures were grown under laboratory conditions at 24±1°C in a thermostatically controlled room, illuminated with fluorescent tubes (Philips 40W) providing 30µEm2/s and 12:12 h light/dark cycle.

Growth study

The growth study was carried out for a period of 16 days for the two organisms under laboratory conditions. At every alternate day 5ml of the sample was withdrawn and centrifuged at 5000 rpm for 10 minutes and the amounts of pigments namely, Chlorophyll-a, Chlorophyll-b, β-carotene, Protein and carbohydrates were estimated.

Estimation of chlorophyll-a, chlorophyll-b (Mackinney, 1941) and β-carotene (Arnon, 1949)

Five milliliters of culture sample was taken and centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and the algal pellets were suspended in 5 ml of 80% acetone and incubated at 4°C for overnight. Then the sample was centrifuged at 5000 rpm for 15 minutes and collected both pellet and supernatant. The optical density of the supernatant was read at 663 nm for chlorophyll-a, 645 nm for chlorophyll-b and at 640nm for β-carotene in the Spectrophotometer. The amount of chlorophyll content was calculated by the following formula:

Chl-a (µg/ml) = $O.D_{663}$ *12.63*Volume of acetone / Volume of water.

Chl-b (µg/ml) = $(0.0229\times OD_{645})-(0.00488\times OD_{663})$

Carotenoids (µg/ml) = $(4.1\times OD_{450})-(0.0435\times$ Chl-a)$-(0.0367\times$Chl-b)

Extraction of total protein (Bradford, 1978)

Five milliliters of culture sample was taken and centrifuged at 5000 rpm for 10 minutes and discard the supernatant. The algal pellets were suspended with 50 M Tris-HCl buffer. The cells were ruptured by adding Lysozyme and kept aside for an hour, and then the sample was centrifuged at 15,000 rpm for 10 minutes.

Estimation of total carbohydrates (Dubois *et al*., 1956)

Five milliliters of the sample was taken and centrifuged at 5000rpm for 15 minutes and discarded the supernatant. The algal pellet was suspended with 0.1M, pH 6.8 phosphate buffer. The cells were ruptured by sonication for 2 minutes and centrifuged at 15,000rpm for 15 minutes. Total carbohydrate was determined with a standard graph prepared by using different concentrations of D-Glucose (10-100 µg/mL).

Extraction and estimation of auxin

Extraction of IAA was carried by Gordon and Paleg method (1957).2% p-dimethyl aminobenzaldehyde in 2N HCl in 80% ethanol is used to spray the chromatogram and dry in an oven at 100°C for 8-10 minutes. Examine the development of graycolor which is typical of indole.

Quantitative measurement of IAA (Gordon and Paleg, 1957)

Removed the spot containing IAA from the chromatogram corresponding to the authentic IAA and eluted in 2 mL of methanol. To 1mL of extract containing IAA, add 2mL of Salper reagent (1 mL of 0.5M $FeCl_2$ in 50 mL of 35% $HClO_4$, prepared afresh). Add the reagent drop wise but rapidly with continuous agitation. The samples were incubated in the dark for 60 minutes. The pink color was found stable and measured its absorbance at 565 nm against a solvent reagent blank. From a standard graph drawn from the concentrations of IAA 10-100 µg/mL, quantified the amount of IAA in the sample. The values were expressed µg/mL of 1.0% SLF.

RESULTS AND DISCUSSION

In the present study, we have emphasized on the usage of SLF as it contains growth hormones like auxin, cytokinin and gibberllins as they can enhance the growth (without any harm to the environment) of the nutritionally important Single Cell Protein *Chlorella*(Plate 1) as it is a highly nutritive microalgae used in various industries worldwide as it contain high amount of proteins, vitamins, carbohydrates and pigments.

The pigment analysis of *Chlorella* in our studies showed that it contained high amount of chlorophylls and carotenoids when treated with Seaweed fertilizer prepared by red algae (*Gracilariacorticata*. and *Grateloupialithophila*sp.(Plate 2) at various concentrations when compared to the control (Plate 3).

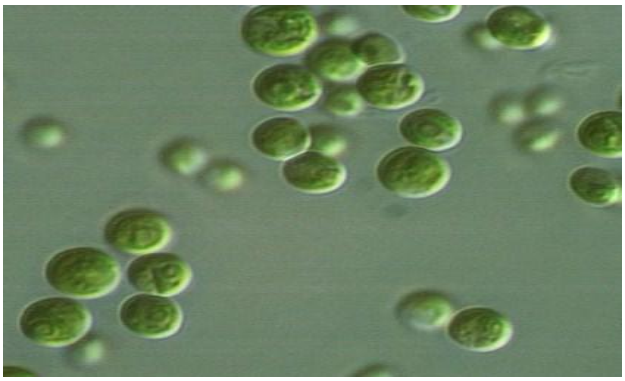

PLATE 1*CHLORELLA VULGARIS*

PLATE 2*GRACILARIA CORTICATA* ***GRATELOUPIA LITHOPHILA***

PLATE 3 *CHLORELLA VULGARIS* (A) CONTROL, (B) 1.5M AND (C) 2.0M SLF CONCENTRATION OF *GRACILARIA CORTICATA*

Chlorophyll-a (Growth)

The growth of the organism is depicted by chlorophyll-a content deposition. *Chlorellavulgaris* treated with *Gracillariaedulis* as SLF showed the highest accumulation of chlorophyll-a content to be found highest in 2.0M with the value of 1.029µg/ml followed by 1.5M (0.997 µg/ml), 1.0M (0.896 µg/ml) and so on.*Chlorella* treated with *Grateloupialithophila* as SLF showed the same trend with 2.0M (0.802 µg/ml) followed by 1.5M (0.757 µg/ml), 1.0M (0.562) respectively (Fig. 1).

Chlorophyll-b

SLF treated *Chlorellavulgaris* showed gradual increase in pigment concentration. Chlorophyll-b is one of the photosynthetic pigments necessary for photosynthesis of *Chlorella*. Periodic measurement of chlorophyll-b in SLF treated *Chlorella* reveals that it has an increasing effect on chlorophyll-b concentration. Higher concentration (2.0M) of SLF showed high chlorophyll-b content in 16th day of growth (0.0256 µg/ml) in *Gracillariaedulis*while at 1.5M of SLF concentration (0.0178 µg/ml) *in Grateloupialithophila*on the 14th day of growth (Fig.2).

β-Carotene

β-Carotene serves as an essential nutrient and has high demand in the market as a natural food coloring agent, as an additive to cosmetics and also as a health food. Besides its physiological function, β-carotene has a wide range of applications. It has been reported that carotenoids extracted from *Chlorella ellipsoidea* and *Chlorella vulgaris* inhibited colon cancer development (Cha 2008). Daily consumption of *Chlorella* supplements provided the potential of health benefits reducing serum lipid risk factors, mainly triglycerides and total cholesterol, in mildly hypercholesterolemic subjects. The effect was related to carotenoid consumption Ryu*et al.*, 2014. The present study showed that SLF not only increases the growth rate, it also enhanced the β-carotene content in *Chlorella* at 2.0M concentration of SLF (1.484 in *Gracilariacorticata* and 1.200 µg/ml in *Grateloupialithophila*) followed by the lower concentrations in the order of 1.5M, 1.0M , 0.5M and control (Fig.3).

Total Protein

Chlorella is a protein rich green algae also used as a SCP for food and feed. In our studies we did not find much difference in the protein content of the alga when grown in SLF treated medium. It only alters or enhances the growth of the organism and thus increases the yield. The protein is much unaltered and its nutritional value in retained as such without any side effects. Protein concentration increased slightly at 2.0M concentration of both *Gracilariacorticata* and *Grateloupialithophila*. The highest concentration of protein was found from 10th day onwards in *Gracilariacorticata*(83 µg/ml) treated algae while *Grateloupialithophila* treated algae show highest protein content (84 µg/ml) in 1.0M and 1.5M concentrations from 2nd day onwards (Fig. 4).

Total Carbohydrates

The carbohydrate content showed a totally different graph from that of protein graphs. The 2.0M concentration of *Gracilariacorticata* as SLF showed the highest carbohydrate accumulation on 10th day

(115 µg/ml) onwards, while *Grateloupialithophila* as SLF showed the highest content from 14th day onwards (113 µg/ml). Thus we can say that we can increase the content of carbohydrates by supplementing SLF as growth regulators (Fig. 5).The graphical representation of pigments, protein, and carbohydrate and growth rate of seaweed extract treated *Chlorella* has shown that it has a positive regulation and seaweed fertilizer prepared by red algae is useful for industrial applications to get more profit.

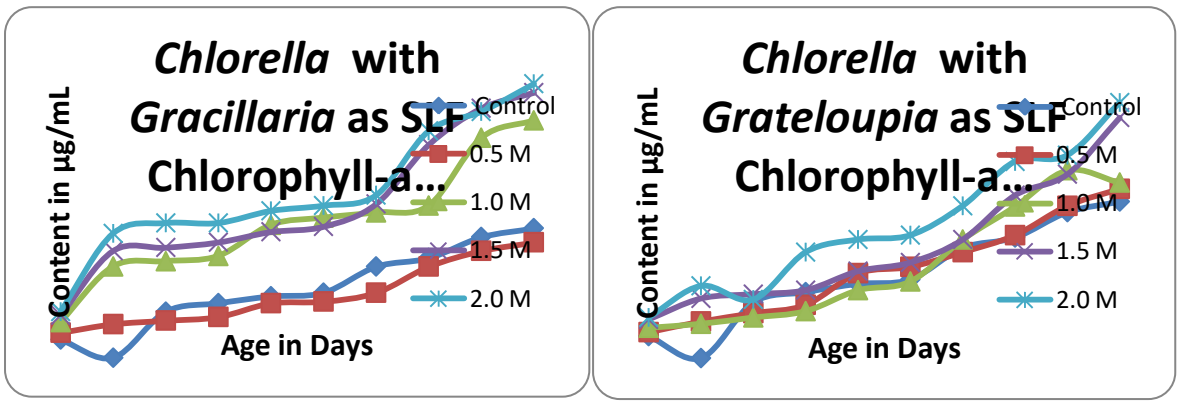

FIG. 1 CHLOROPHYLL – A CONTENT OF *CHLORELLA* TREATED WITH *GRACILARIA* AND *GRATELOUPIA* AS SLF

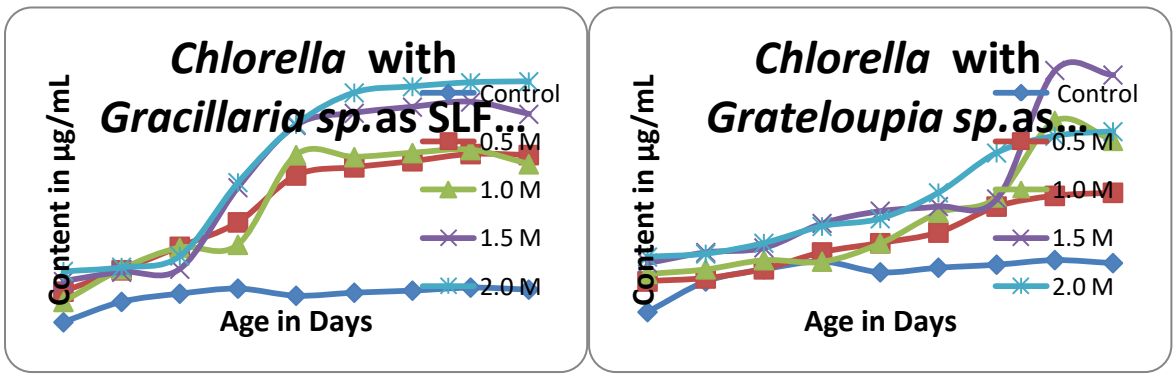

FIG. 2CHLOROPHYLL – B CONTENT OF *CHLORELLA* TREATED WITH *GRACILARIA* AND *GRATELOUPIA* AS SLF

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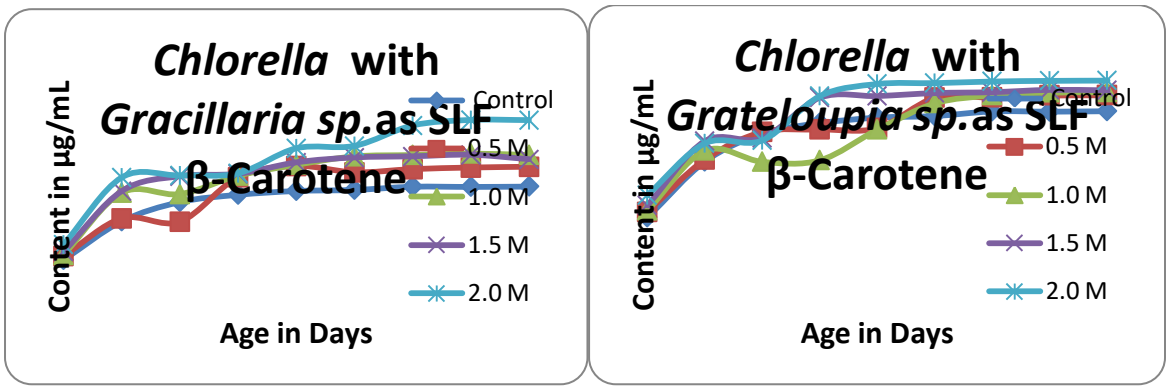

FIG. 3 B-CAROTENECONTENT OF *CHLORELLA* TREATED WITH *GRACILARIA* AND *GRATELOUPIA* AS SLF

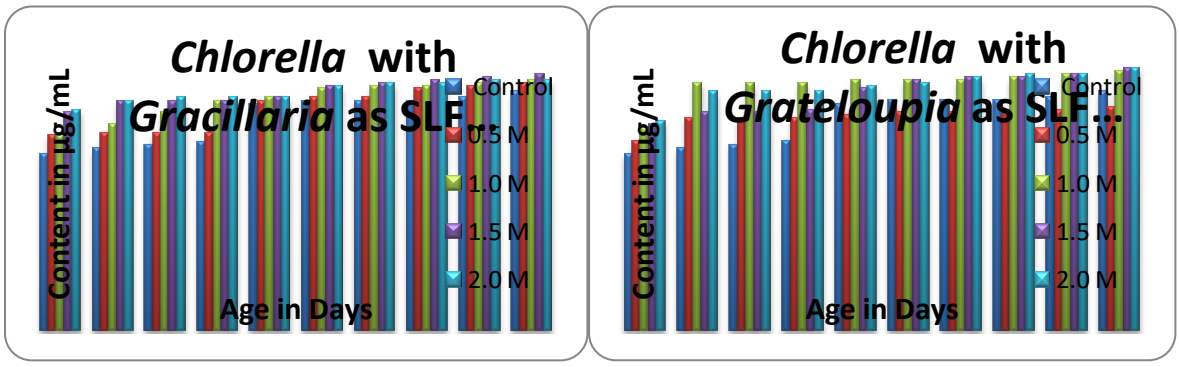

FIG. 4TOTAL PROTEIN CONTENT OF *CHLORELLA* TREATED WITH *GRACILARIA* AND *GRATELOUPIA* AS SLF

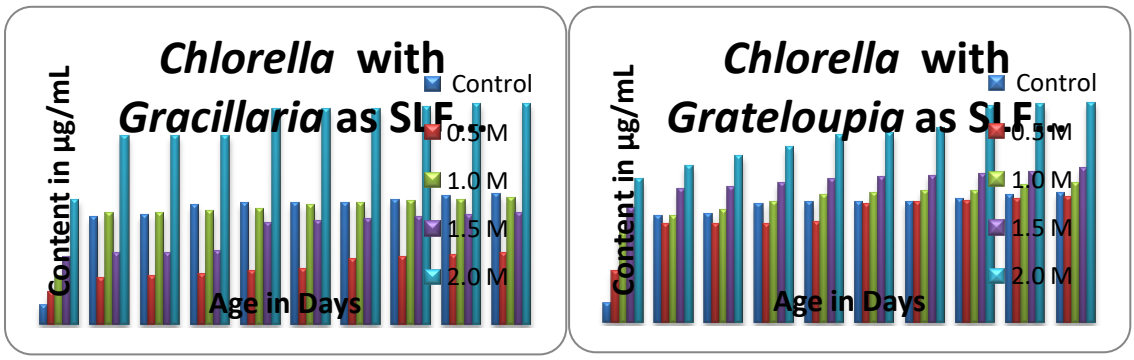

FIG. 5 TOTAL CARBOHYDRATE CONTENT OF *CHLORELLA* TREATED WITH *GRACILARIA* AND *GRATELOUPIA* AS SLF

TABLE 1. AUXIN CONTENT IN *GRACILARIA CORTICATA* AND IN *GRATELOUPIA LITHOPHILA*

Fractions	Standard Auxinµg/L	*Gracilariacorticata.*µg/L	*Grateloupialithophila*µg/L
$NaHCO_3$ Fraction **Alkaline Auxin**	57	33	46
Diethyl ether Upper layer **Acidic Auxins**	60	19	30
Diethyl ether Lower layer **Acidic Auxins**	52	73	47
Total Auxins	-	125	123

AUXIN

The effect of *Chlorella* growth by seaweed extract treatment is mainly due to the presence of plant growth regulators, minerals and trace elements. Auxin was first discovered phytohormones in higher plants and some algae. It is useful for cell division and development. In microalgae,auxin facilitates the increase in cell division there by the biomass concentration of microalgae also increases. The biochemical analysis of red algae showed that it contains growth inducing hormone auxins in appreciable amount, 125µg/L in *Gracilariacorticata*and 123µg/L in *Grateloupialithophila.* (Table 1).

CONCLUSION

From this study, it could be concluded that the 2.0M and 1.5M concentrations of both *Gracilariacorticata*and *Grateloupialithophila* as SLF were more effective for the alga's growth and showed highest amount of pigment accumulation at these concentrations.The SLF also showed increase in carotenoid content of *Chlorella* day by day with increase in biomass concentration. There were minimal changes in Protein accumulation thus proving that the SLF enhanced the alga's growth and the Protein content was not affected. Thus this alga can be grown efficiently with SLF in a shorter time period and supplied to the people without any side-effects.This study showed that the SLF's Auxin content in*Gracilariacorticata, which* hadhighest Auxin content than *Grateloupialithophila.*By this we conclude that, the increase in growth of *Chlorella* is influenced by presence of growth hormones in seaweed.

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