

## Effect of seaweed-based liquid extracts on biomass production and lipid accumulation in *Nannochloropsis oculata* and *Dunaliella salina*

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**Abstract:** Seaweed liquid fertilizers (SLFs) contain macronutrients, micronutrients and trace nutrients essential for microalgal growth. The study aims to evaluate the efficiency of SLFs on the biomass production, biochemical and physiological processes; and the study was carried out at different SLF concentrations of selected seaweeds i.e. *Kappaphycus alvarezii*, *Gelidiella acerosa* and *Turbinaria ornata*. The comparative analysis of the growth performance of two unicellular green algae on three different organic media suggest that the liquid fertilizer from red seaweed was highly efficient than the SLF prepared from brown seaweed. The growth rate of *N. oculata* and *D. salina* in *K. alvarezii* SLF as culture media resulted in 1.25 and 1.21 times higher growth than the control whereas in *G. acerosa* SLF it was 1.17 and 1.1 times higher than the control, respectively. In contrast, in *T. ornata* SLF treated groups, the growth rate of *N. oculata* and *D. salina* was 0.59 and 0.68 times lower than the control, respectively. The maximum biomass concentration values recorded for *N. oculata* in different SLFs were 0.474, 0.422, and 0.194 g/L whereas the maximum productivity obtained was 0.034, 0.030, and 0.014 g/L/d, respectively. *D. salina* grown in three different SLFs of *K. alvarezii*, *G. acerosa* and *T. ornata* resulted in biomass concentration of 0.534, 0.497, and 0.305 g/L and the production values obtained were 0.038, 0.035, and 0.021 g/L/d, respectively. The biochemical analysis also suggests that pigments, protein, carbohydrate and lipid contents were significantly higher than the control. Given the growth performance, physiological and biochemical response of the algae in the SLFs, the best concentration of *K. alvarezii*, *G. acerosa* and *T. ornata* for *N. oculata* growth and biomass and lipid yield were 10%, 8%, and 1%, respectively. However, for *D. salina* it was 10%, 6%, and 2%, respectively. Overall, the findings suggest that SLFs from red seaweeds were more efficient in enhancing the biomass production, pigment, protein, carbohydrate and lipid yield than the SLF prepared from brown seaweed signifying their potential application as an alternative to the commercial culture media.

**Keywords:** Indigenous seaweed resources, cost effective culture media, marine microalgae

### 1. Introduction

Microalgae are potentially nutritious and contain high amount of lipids, proteins, pigments, cellulose, etc., which make them play a vital role in mariculture as the larval stages of aquatic organisms directly depend on microalgae for their food (Cho et al., 1999; Carioca, 2010). They are reserves of lipids and considered a suitable candidate for biofuel production due to their easy cultivation methods and lipid composition (Woodworth et al., 2015). Besides these advantages, the primary constraint in the practical utility is the high production cost mainly subsidized by the price of commercial culture media (Acien et al., 2012).

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The present study aims to contribute in finding a less expensive growth media that has the potential to replace the expensive commercial media.

The microalgae growth and lipid content vary depending on the nutrient composition of the culture media and the environmental parameters (Benavente-Valdés et al., 2016). Hence, selecting suitable culture media that satisfies the nutritional requirement of the microalgae is essential to result in successful biomass production. The extract of seaweeds as a fertilizer is utilized for crops around the globe (Kaliaperumal et al., 1987). They contain several macronutrients, micronutrients and growth-promoting

hormones that make seaweed the best source for fertilizer production (Spinelli et al., 2010). The added benefit is that seaweed extracts are biodegradable, nonpolluting and nonhazardous (Dhargalkar and Pereira, 2005).

For commercial purposes, microalgal cultivation requires considerable amount of nutrients. Utilization of waste materials for culture of microalgae is considered an alternative way that provides solution for cost effective biodiesel production that will lead a sustainable development in industries (Lam and Lee, 2012). Fermentation of biomass waste by enzymatic hydrolysis can be contemplated in deriving an effective fermentable product (Karemore et al., 2013). In this study, to explore the beneficial effects of seaweed liquid fertilizer on the growth, biochemical composition and lipid productivity of two marine microalgae, the enzymatic hydrolysis of three different seaweeds using cellulase enzyme was accomplished. In considering the practical viability and economic viewpoint, the suitable algal species that has a high lipid production potential should be chosen (Del Río et al., 2015). Hence, in this study, two marine microalgae *Nannochloropsis oculata* (eustigmatophyte) and *Dunaliella salina* (chlorophyte) that have the potential of high lipid production were selected.

Seaweed liquid fertilizers (SLFs) are a combination of organic nutrients and plant growth promoting agents that are eco-friendly in nature which lead to sustainable productivity. In India, seaweed extracts are mainly used for coconut plantations especially in the states of Kerala and Tamil Nadu (Kaliaperumal et al., 1987). There are many research findings regarding the effect of SLF as fertilizers for crops such as cluster beans (Vijayanand et al., 2014), brinjal (Satish et al., 2015), ragi (Satish et al. 2016), black gram (Kalaivanan et al., 2012), tomato (Sasikala et al., 2016), etc., but the reports available on utilization of SLFs in microalgal cultivation are few (Gireesh, 2009; Raja et al., 2015). This study report documents a cost-effective methodology and uses locally sourced beach cast seaweeds as organic fertilizer (SLF) for marine microalgae cultivation, which will benefit the mariculture and biofuel industries worldwide. SLF produced from brown (*Turbinaria ornata*) and red seaweeds (*Kappaphycus alvarezii*, *Gelidiella acerosa*) collected from the Gulf of Mannar region, Thoothukudi coast, India proves that these fertilizers were found to be completely effective and suitable alternative to substitute the expensive commercial microalgal culture media to increase the microalgal biomass production and lipid yield.

## 2. Materials and methods

### 2.1. Microalgal species and culture conditions

Two marine microalgae *Nannochloropsis oculata* and *Dunaliella salina* were procured from ICAR-

CMFRI (Central Marine Fisheries Research Institute) Phytoplankton Laboratory, Thoothukudi, India. Both algal species were subcultured in F/2 medium and the temperature was maintained at  $18 \pm 2$  °C with a light intensity of 3000 lux and photoperiod of 18:6 h (light:dark). To retain the algal cells in suspension, the cultures were shaken manually two times a day.

### 2.2. Seaweed liquid fertilizers (SLF) preparation

Beach cast seaweeds were collected from Hare Island, Thoothukudi coast of India and identified as *Kappaphycus alvarezii*, *Gelidiella acerosa*, and *Turbinaria ornata*. The collected seaweeds were separated and washed thoroughly to remove the dirt, salt and associated organisms. After cleaning, it was shade dried for 24 h followed by oven drying at 60 °C for 4 h and then powdered using a mincer. One kg each of the dried seaweed powder was separately mixed with sterilized 3% NaCl in the ratio of 1:9. Then each mixture was supplemented with 10 g of cellulase enzyme (Enzyme Bioscience Pvt. Ltd.) to boost the fermentation process. The procedure followed was reported by Uchida and Murata (2002) for producing single cell detritus to feed the aquatic organism. The procedure was altered by avoiding the microorganisms (yeast and fungi) to boost the fermentation process. Many growth trial experiments standardized the following steps. The mixture was kept undisturbed anaerobically for 15 days. The mixture was whisked daily to hasten the zymotic process. After 15 days, the mixture was filtered using a sieve and then centrifuged (Remi refrigerated centrifuge, C-24 plus) at 5000 rpm for 20 min. The resultant clear supernate was used as microalgal culture media i.e. seaweed liquid fertilizer (SLF).

### 2.3. Nutrient analysis of SLFs

The ammoniacal nitrogen and phosphorous content of the SLFs were analyzed using Nessler method (Crosby, 1968) and Amino Naphthol Sulphonic method (Allen, 1940), respectively. Sodium and potassium were estimated using the Flame photometer (Hald, 1947). Other nutrients such as iron, zinc, calcium, magnesium, manganese, cobalt, copper, and molybdenum were analyzed using atomic absorption spectroscopy (AAS). The nutrient compositions of the prepared SLFs are presented in Table 1.

### 2.4. Experimental setup

All the experiments were conducted in a 100-mL conical flask. Sterilized seawater was inoculated with 5% microalgal inoculum. Different concentrations of SLFs i.e. 1%, 2%, 4%, 6%, 8% and 10% were added as culture media whereas the F/2 medium was kept as control (without SLF). All the experiments were conducted in triplicates.

### 2.5. Determination of algal cell concentration and growth analysis

Growth study was conducted for 14 days. One mL of sample from all the treatments were taken in Haemocytometer

**Table 1.** Nutrient composition of SLFs.

Nutrient composition of SLFs			
Macroelements (mg/L)	<i>K. alvarezii</i>	<i>G. acerosa</i>	<i>T. ornata</i>
N	365	581	304
P	136	119	104
K	14567	8271	6924
Ca	2832	2202	4109
Mg	250	340	352
Na	14166	11191	9787
Trace elements (mg/L)	<i>K. alvarezii</i>	<i>G. acerosa</i>	<i>T. ornata</i>
Fe	160	174	82
Mn	8	16	7
Cu	2.7	2.6	3.9
Zn	81	130	162
Co	NT	NT	NT
Mo	NT	NT	NT

(Improved Neubauer chamber) and the number of cells was counted under light microscope (Nikon Eclipse TS 100). The specific growth rate ( $\mu$ ) of the microalgae was calculated by the formula given by Levasseur (1993):

$$\mu = \ln(x_2) - \ln(x_1) / t_2 - t_1,$$

where  $\mu$  represents the growth rate per unit amount of cell concentration,  $x_1$  and  $x_2$  are cell concentrations at time 1 ( $t_1$ ) and time 2 ( $t_2$ ), respectively.

The doubling time (day) of microalgal cells can be determined from the growth rate using the following the equation given by Fogg and Thake (1987):

$$td = \ln 2 / \mu.$$

## 2.6. Dry weight estimation

The dry weight of algal cells was obtained by filtering 5 mL algal culture from all treatments and control. Ten milliliters of ammonium bicarbonate was added to remove the salts from the algae and the suspension was filtered using an acetate membrane filter of pore size 0.7  $\mu\text{m}$  followed by drying in the oven at 80 °C for 12 h. Then the filter paper with the dry biomass was kept in desiccator followed by weighing both the empty and biomass containing filter paper to obtain the dry algal biomass weight (Rizwan et al., 2017).

## 2.7. Pigment analysis

The algal sample volume of 5 mL was taken from all the treatments and control. It was centrifuged using refrigerated centrifuge (Remi C-24 plus) at 5000 rpm for 5 min at 4 °C. The supernatant was discarded and 5 mL of N, N, dimethylformamide was added to the pellet. It was left undisturbed for 24 h. Following the incubation time, it

was centrifuged at 5000 rpm for 10 min. The supernatant was collected separately and OD value was taken using the spectrophotometer (PerkinElmer, Lambda 25).

The pigments: chlorophyll-*a* (Chamovitz, 1993); chlorophyll-*b* (Chamovitz, 1993); and carotenoid (Moran, 1982) presented in microalgae were calculated as follows:

$$\text{Chlorophyll} - a (\mu\text{g/mL}) = \text{OD}664 \times 11.92$$

$$\text{Chlorophyll} - b (\mu\text{g/mL}) = - 5.6 \times \text{OD}664 + 23.26 \times \text{OD}647$$

$$\text{Carotenoid} (\mu\text{g/mL}) = [\text{OD}461 - (0.046 \times \text{OD}664)] \times 4.$$

## 2.8. Protein analysis

The protein content of algal samples from the treatments and control was analyzed using the standard procedure of Lowry (1951). Bovine serum albumin was used as the standard. Three reagents A (2%  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH), B (1% NaK tartrate in  $\text{H}_2\text{O}$ ), and C (0.5%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ ) were prepared. A mixture of reagent A:B:C was prepared in the ratio of 48:1:1 to obtain reagent I. Reagent II was prepared by mixing Folin phenol with water in the ratio of 1:1. To 1 mL of algal samples, reagent I (4.5 mL) was added and kept for 10 min followed by addition of reagent II (0.5 mL) and kept undisturbed for 30 min. After incubating, the absorbance was measured against the blank with reagents at 660 nm using the spectrophotometer (PerkinElmer, Lambda 25).

## 2.9. Carbohydrate analysis

The carbohydrate content of the algal samples was analyzed by the standard phenol sulfuric acid method by Dubois (1956). Two microliters of algal culture was mixed with 4  $\mu\text{L}$  of distilled water and then 5  $\mu\text{L}$  of 5%

phenol was added to the mixture. To this concentration,  $\text{H}_2\text{SO}_4$  (2.5 mL) was added and vortexed then kept at room temperature to cool down. The absorbance was appraised against the blank (DW with the reagents) at 490 nm using spectrophotometer (PerkinElmer, Lambda 25).

### 2.10. Lipid analysis

The lipid content of the microalgae was analyzed by taking 40 mL of algal culture from the treatments and control. The culture was centrifuged at 3000 rpm for 5 min. Supernatant was discarded and to the pellet, chloroform:methanol: water was added in the ratio of 1:2:0.8 (v/v/v) to acquire an eventual volume of 7.6 mL. This mixture was sonicated at 100 W and 20 kHz for 1 min (Labman Scientific Instruments), vortexed for 30 s and centrifuged for 5 min at 3000 rpm. This ensued in the emergence of three different layers. The upper layer of methanol was removed and the same extraction process was carried out twice to extract if any lipids were present. The bottom layer is chloroform with lipid which was collected in separate tubes. Both the chloroform phases obtained were combined and dried in the oven at 80 °C for 24 h to evaporate the chloroform. After oven drying, the lipid obtained was weighed and expressed as lipid content per ml of algal culture (Rizwan et al., 2017).

### 2.11. Statistical analysis

All the experiments were conducted in triplicates and the average value was taken as an outcome. Mean and the standard deviation of the triplicates were calculated by SPSS 22.0 (IBM Corporation, Armonk, NY, USA). One-way ANOVA was used to find the difference between the treatments and control. Duncan's multiple range test was carried out to find the mean difference at 0.05 significance level.

## 3. Results and discussion

### 3.1. Composition of SLFs

As presented in Table 1, the three different SLFs contained abundant levels of macronutrients, micronutrients, and trace nutrients sufficient for the microalgal growth. The dominant nutrients analyzed were Na, K, Ca, N, P and Mg, whereas other nutrients i.e. Fe and Zn were also present in good amount. Mn and Cu were observed to be at traceable levels, and Co and Mo were at not detectable levels. Nitrogen and Potassium content were significantly high in *G. acerosa* SLF and *K. alvarezii* SLF, respectively. However, iron was present at high levels in both *G. acerosa* and *K. alvarezii* SLFs but lower in *T. ornata* SLF. Generally, potassium and other nutrients are high in seaweed extract, which are water soluble in nature and are readily available for plants to absorb thereby controlling the nutrient deficiency (Mohanty et al., 2013). Seaweeds contain enormous amount of macronutrients, micronutrients,

and trace nutrients (Nasmia et al., 2014; Villares et al., 2007). Karemore et al. (2013) reported that the nutrients such as N, P, K, Ca, Mg, etc., play a paramount part in the biomass and lipid production of microalgae. From this, we can find out that the prepared SLFs contain all the essential nutrients required for growth by microalgae and may act as biostimulant in enhancing the growth and lipid productivity of the microalgae. Higher amount of nutrients was observed in *K. alvarezii* extract when compared to that in *T. conoides* extract (Raja et al., 2015). Hence, the present study in comparison with previous documentation concludes red seaweeds as a very suitable candidate for the preparation of SLF which can satisfy the nutritional prerequisite of microalgae.

### 3.2. Effect of red seaweed SLFs on growth of *N. oculata*

Seaweeds accumulate a high quantity of nutrients from the surrounding water and the extract obtained from them has been used as fertilizer for plant growth (Forsberg et al., 1988; Fornes et al., 2002). The extract not only contains the essential macronutrients but also trace nutrients and growth promoters (Hong et al., 1995). Under different concentrations of SLFs, both the microalgae responded differently. SLFs had a strong stimulation on the growth of both microalgae. The maximum cell density of the green microalgae *N. oculata* was observed as  $28.6 \times 10^6$  cells/mL and  $33.42 \times 10^6$  cells/mL grown under 8% and 10% concentration of *K. alvarezii* SLF on the 10th day whereas in control (F/2 medium) the maximum cell concentration reached up to  $26.8 \times 10^6$  cells/mL on the 12th day (Figure 1). The *K. alvarezii* SLF grown microalgae reached a higher cell density in short period of time when compared with control. The results clearly indicate that growth of microalgae increased with increase in SLF concentrations. *N. oculata* cell density reached a maximum of  $27.88 \times 10^6$  cells/mL and  $29.76 \times 10^6$  cells/mL in 8% and 10% *G. acerosa* SLF on the 12th day, respectively, whereas in F/2 medium it was  $25.41 \times 10^6$  cells/mL. Microalgae grown under various concentration of *G. acerosa* suggest that the growth performance of *N. oculata* varied at different concentrations of SLF and the minimal cell density of  $18.4 \times 10^6$  cells/mL was found in 1% SLF concentration on the 12th day of the experiment (Figure 2). The present study results conclude that both red seaweed SLFs positively influenced the growth rate of *N. oculata*, in which *K. alvarezii* SLF was more efficient than *G. acerosa* SLF in achieving maximum biomass production as well as it has reduced the culture period by achieving the maximum cell density on the 10th day itself (Table 2). Similar results were documented in the study of effect of SLFs prepared from the red seaweeds *Gracilaria corticata* and *Grateloupia lithophila* added as supplement with the Bold's basal medium (BBM) culture medium resulted in increase of growth of the microalgae *Chlorella vulgaris* in comparison

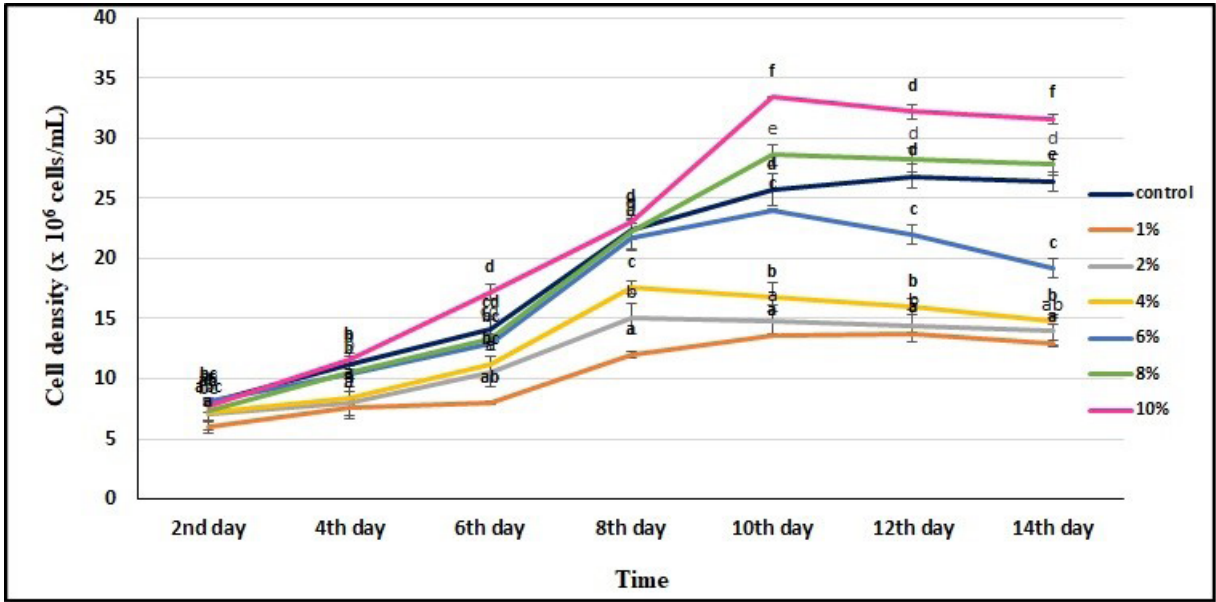


Figure 1. Growth curve of *N. oculata* in different concentrations of *K. alvarezii* SLF. The Bars=SD.

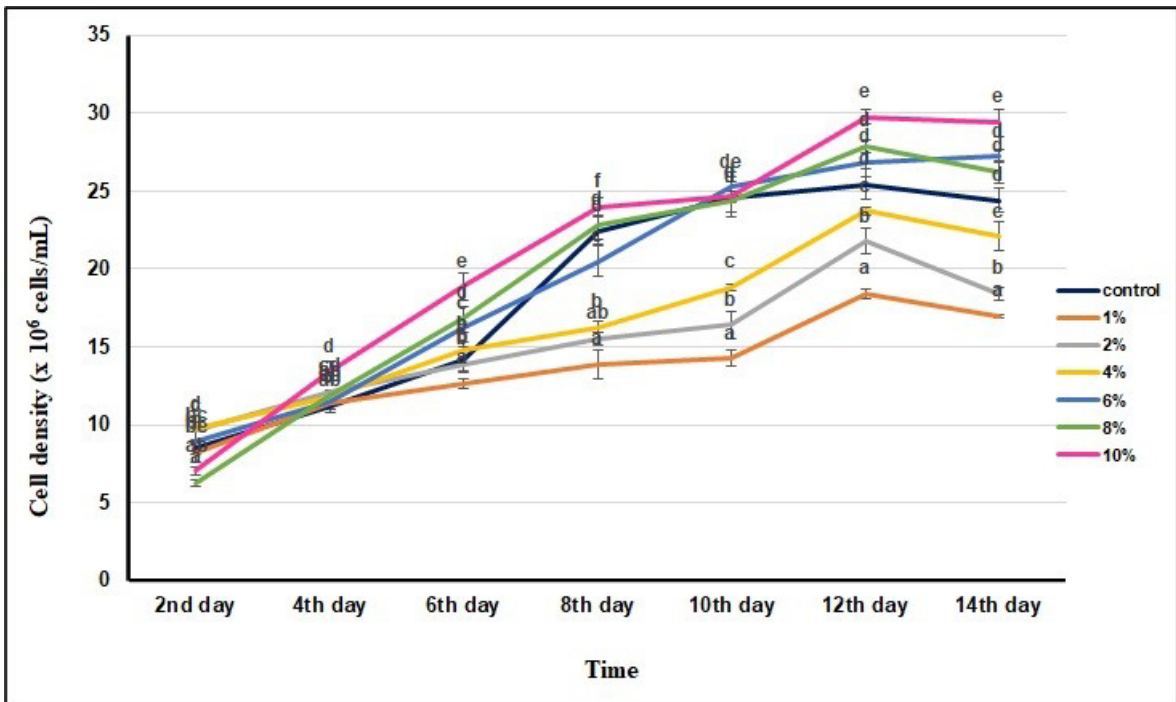


Figure 2. Growth curve of *N. oculata* in different concentrations of *G. acerosa* SLF. The Bars=SD.

to control (BBM without SLF) (Lakshmi and Sheeja, 2021). The results were also in agreement with those given by Zheng et al. (2016) and reported that when two green microalgae *Chlorella-Arc* and *C. sorokiniana* were cultured in kelp waste extract (KWE), the biomass productivity of the algae increased by 5.34 and 31.86 times than the control (Bold's basal medium). This maximum biomass

productivity was obtained at 8% concentration of KWE and the study concluded that the microalgal cell density increases with an increase in seaweed extract concentration. The reason for the higher growth performance may be due to the growth promoting substances present in the SLF which was mentioned in the study conducted by Rosyida et al. (2021) that higher amounts of growth promoting

**Table 2.** Growth parameters of *N. oculata* in three different SLFs.

<i>K. alvarezii</i> SLF	<i>N. oculata</i>			
	SGR (/d)	Doubling time (hours)	Biomass concentration (g/L)	Biomass productivity (g/L/d)
Control	0.1969 ± 0.0015	3.5210	0.3646 ± 0.0292	0.0260 ± 0.0175
1%	0.1261 ± 0.0023	5.4948	0.1929 ± 0.0612	0.0138 ± 0.0036
2%	0.1194 ± 0.0042	5.8048	0.1816 ± 0.0221	0.0130 ± 0.0021
4%	0.1496 ± 0.0028	4.6326	0.2384 ± 0.0106	0.0170 ± 0.0056
6%	0.1893 ± 0.0063	3.6625	0.3405 ± 0.0558	0.0243 ± 0.0071
8%	0.2087 ± 0.002	3.3207	0.4058 ± 0.0273	0.0290 ± 0.0053
10%	0.2260 ± 0.0116	3.0664	0.4741 ± 0.0530	0.0339 ± 0.0045
<i>G. acerosa</i> SLF	SGR (/d)	Doubling time (hours)	Biomass concentration (g/L)	Biomass productivity (g/L/d)
Control	0.1458 ± 0.0036	4.7537	0.3605 ± 0.0108	0.0258 ± 0.0044
1%	0.1165 ± 0.0054	5.9514	0.2611 ± 0.0523	0.0186 ± 0.0028
2%	0.1317 ± 0.0127	5.2624	0.3087 ± 0.0270	0.0221 ± 0.0048
4%	0.1395 ± 0.0088	4.9695	0.3362 ± 0.0833	0.0240 ± 0.0089
6%	0.1509 ± 0.0024	4.5926	0.3814 ± 0.0197	0.0272 ± 0.0034
8%	0.1542 ± 0.0031	4.4938	0.3955 ± 0.0446	0.0283 ± 0.0064
10%	0.1602 ± 0.0243	4.3273	0.4222 ± 0.0021	0.0302 ± 0.0078
<i>T. ornata</i> SLF	SGR (/d)	Doubling time (hours)	Biomass concentration (g/L)	Biomass productivity (g/L/d)
Control	0.1589 ± 0.0206	4.3625	0.3850 ± 0.0347	0.0275 ± 0.0092
1%	0.1065 ± 0.0055	6.5068	0.1949 ± 0.0843	0.0139 ± 0.0043
2%	0.0808 ± 0.0056	8.5738	0.1396 ± 0.0134	0.0100 ± 0.0057
4%	0.0794 ± 0.0085	8.7271	0.1371 ± 0.0251	0.0098 ± 0.0028
6%	0.0670 ± 0.0046	10.3443	0.1166 ± 0.0565	0.0083 ± 0.0035
8%	0.0538 ± 0.0070	12.8922	0.0982 ± 0.0564	0.0070 ± 0.0057
10%	0.0476 ± 0.0148	14.5510	0.0907 ± 0.0452	0.0065 ± 0.0097

substances such as auxin, gibberellin and cytokinins was present in the liquid fertilizer prepared from the seaweed *Ulva* sp.

**3.3. Effect of red seaweed SLFs on growth of *D. salina***

The growth experiment of the dinoflagellate *D. salina* in *K. alvarezii* SLF revealed that the maximum cell density of  $19.04 \times 10^6$  cells/mL and  $20.41 \times 10^6$  cells/mL was obtained in 8% and 10% concentration, respectively, on the 14th day, followed by  $17.6 \times 10^6$  cells/mL in 6% SLF on the 12th day. These three *K. alvarezii* SLF concentration resulted in significantly higher cell density than control ( $16.82 \times 10^6$  cells/mL). Least growth of  $9.81 \times 10^6$  cells/mL was observed in the lowest SLF concentration i.e. 1% SLF (Figure 3). The growth of microalgae was found to be higher with increase in SLF concentration. In *G. acerosa* SLF treated groups, maximum cell density of *D. salina* observed was 19.54 and

$17.76 \times 10^6$  cells/mL in 6% and 8% concentration on the 12th day which was significantly higher in comparison with control ( $17.67$  million cells/mL on the 14th day). A minimal growth of  $11.51 \times 10^6$  cells/mL was found in the algae grown under 1% SLF concentration (Figure 4). *D. salina* grown under various concentration of *G. acerosa* SLF revealed that the best concentration to achieve the highest cell growth was 6% and 8%. These two *G. acerosa* SLF concentration resulted in significantly higher cell density than the control. *D. salina* exhibited maximum cell density when grown under *K. alvarezii* SLF on the 14th day whereas the highest cell density was reached on the 12th day by the microalgae grown using *G. acerosa* SLF (Table 3). The results are in agreement with those reported by Cho et al. (1999) that the growth of the dinoflagellate *Isochrysis galbana* was stimulated when grown in the seaweed extracts of

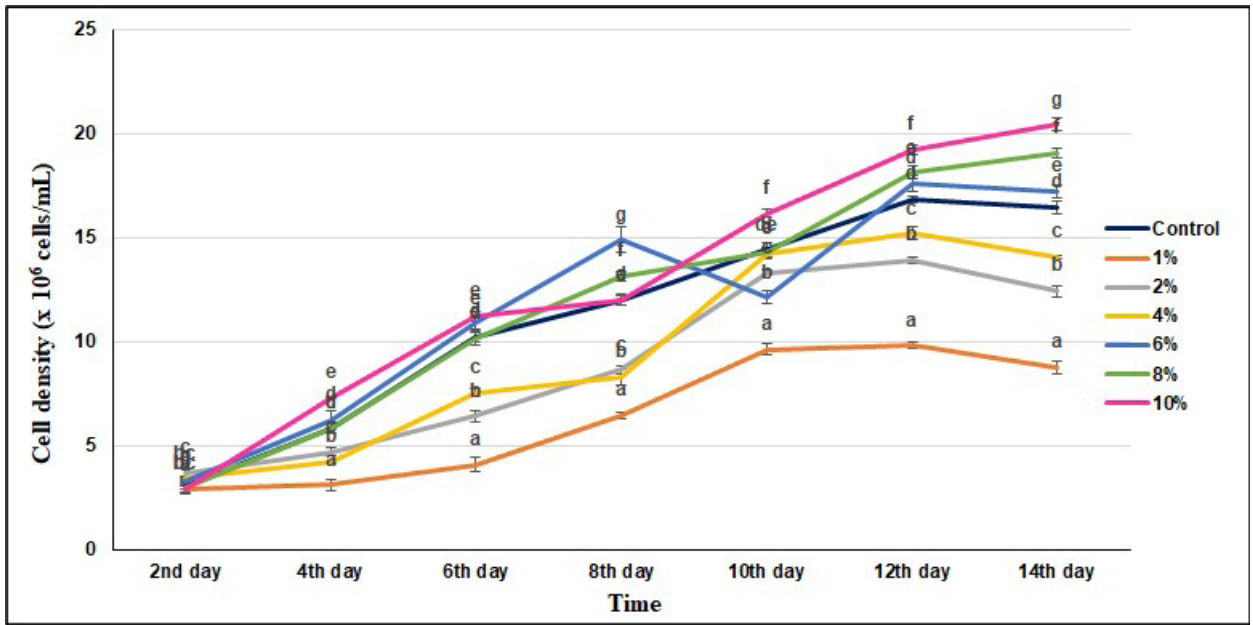


Figure 3. Growth curve of *D. salina* in different concentrations of *K. alvarezii* SLF. The Bars=SD.

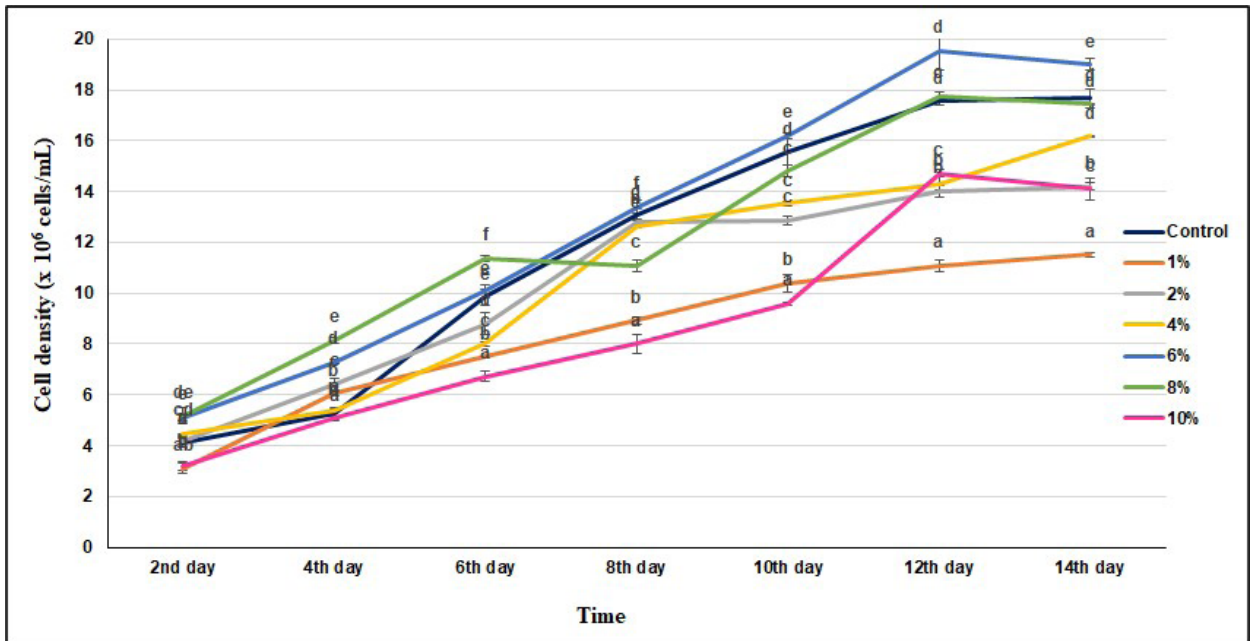


Figure 4. Growth curve of *D. salina* in different concentrations of *G. acerosa* SLF. The Bars=SD.

*Monostroma nitidum*. The research results given by Rohani-Ghadikolaei et al. (2012) also states that the dinoflagellate *Isochrysis galbana* grown in F/2 media with added seaweed extracts of green (*U. lactuca* and *E. intestinalis*) and red (*G. corticate*) seaweed as supplement resulted in higher cell density than control. The reason for this stimulation of growth by seaweed extract (SWE) was provided by Crouch and Van Staden (1993) that the presence of supplementary

nutrients as well as the growth substances such as auxins and cytokinins in SWE make them ideal media for microalgal growth. The microalgae *N. oculata* showed a better growth performance in 10% *G. acerosa* SLF whereas *D. salina* exhibited maximum growth in 6% *G. acerosa* SLF. The reason for this was mentioned by Zhang et al. (2014) that the tolerance level to different organic compounds varies with different microalgal species.

**Table 3.** Growth parameters of *D. salina* in three different SLFs.  $\pm$ : SD.

<i>K. alvarezii</i> SLF	<i>D. salina</i>			
	SGR (/d)	Doubling time (hours)	Biomass concentration (g/L)	Biomass productivity (g/L/d)
Control	0.1582 $\pm$ 0.0026	4.3802	0.4300 $\pm$ 0.0438	0.0307 $\pm$ 0.0106
1%	0.1098 $\pm$ 0.0052	6.3141	0.2290 $\pm$ 0.0247	0.0164 $\pm$ 0.0027
2%	0.1368 $\pm$ 0.0073	5.0675	0.3253 $\pm$ 0.0975	0.0232 $\pm$ 0.0035
4%	0.1462 $\pm$ 0.0034	4.7409	0.3677 $\pm$ 0.0147	0.0263 $\pm$ 0.0039
6%	0.1618 $\pm$ 0.0014	4.2848	0.4501 $\pm$ 0.0169	0.0322 $\pm$ 0.0014
8%	0.1696 $\pm$ 0.0273	4.0873	0.4983 $\pm$ 0.0258	0.0356 $\pm$ 0.0017
10%	0.1749 $\pm$ 0.0139	3.9624	0.5341 $\pm$ 0.0377	0.0382 $\pm$ 0.0028
<i>G. acerosa</i> SLF	<i>D. salina</i>			
	SGR (/d)	Doubling time (hours)	Biomass concentration (g/L)	Biomass productivity (g/L/d)
Control	0.1695 $\pm$ 0.0233	4.0895	0.4624 $\pm$ 0.0851	0.0330 $\pm$ 0.0011
1%	0.1278 $\pm$ 0.0043	5.4252	0.3012 $\pm$ 0.0186	0.0215 $\pm$ 0.0039
2%	0.1489 $\pm$ 0.0075	4.6536	0.3716 $\pm$ 0.0388	0.0265 $\pm$ 0.0028
4%	0.1510 $\pm$ 0.0182	4.5903	0.4234 $\pm$ 0.0483	0.0302 $\pm$ 0.0043
6%	0.1793 $\pm$ 0.0067	3.8668	0.4972 $\pm$ 0.0445	0.0355 $\pm$ 0.0058
8%	0.1706 $\pm$ 0.0047	4.0636	0.4564 $\pm$ 0.0897	0.0326 $\pm$ 0.0039
10%	0.1535 $\pm$ 0.0038	4.5154	0.3690 $\pm$ 0.0487	0.0264 $\pm$ 0.0077
<i>T. ornata</i> SLF	<i>D. salina</i>			
	SGR (/d)	Doubling time (hours)	Biomass concentration (g/L)	Biomass productivity (g/L/d)
Control	0.2524 $\pm$ 0.0062	2.7465	0.4588 $\pm$ 0.0545	0.0328 $\pm$ 0.0073
1%	0.1950 $\pm$ 0.0227	3.5546	0.2311 $\pm$ 0.0874	0.0165 $\pm$ 0.0034
2%	0.2187 $\pm$ 0.0073	3.1696	0.3046 $\pm$ 0.0685	0.0218 $\pm$ 0.0068
4%	0.1822 $\pm$ 0.0024	3.8042	0.1855 $\pm$ 0.0658	0.0133 $\pm$ 0.0075
6%	0.1623 $\pm$ 0.0086	4.2703	0.1623 $\pm$ 0.0258	0.0116 $\pm$ 0.0047
8%	0.1610 $\pm$ 0.0024	4.3045	0.1636 $\pm$ 0.0541	0.0117 $\pm$ 0.0035
10%	0.1460 $\pm$ 0.0022	4.7485	0.1356 $\pm$ 0.4153	0.0097 $\pm$ 0.0178

**3.4. Effect of brown seaweed SLF on growth of both microalgae**

In *T. ornata* SLF grown *N. oculata* the maximum cell density reached was  $13.74 \times 10^6$  cells/mL in 1% concentration on the 14th day which was two times lower in comparison with control ( $27.46 \times 10^6$  cells/mL). The lowest cell density was observed in 10% concentration i.e.  $6.44 \times 10^6$  cells/mL (Figure 5). A reduction in microalgal growth was observed with an increase in the exposed SLF concentration. The growth study of *D. salina* in *T. ornata* SLF revealed that the maximum cell density of 9.14 and  $11.86 \times 10^6$  cells/mL was obtained in 1% and 2% concentration on the 12th day which was significantly lower in comparison with control

( $17.53 \times 10^6$  cells/mL). The least growth of  $5.33 \times 10^6$  cells/mL was found in highest exposed SLF concentration i.e. 10% (Figure 6). Both the microalgae exhibited a very low growth performance when cultured using *T. ornata* SLF. The main reason for the very low growth performance of both microalgae in *T. ornata* SLF can be understood from the nutrient analysis results. The results indicate the low nitrogen and iron concentration in the *T. ornata* SLF may be the major reason for the low growth performance. The reports of Malik et al. (2018) have similar results of decrease in microalgal growth of *Chaetoceros gracilis* when high doses of brown SWE (*Sargassum* sp.) were used as supplement with culture media (prepared with fertilizers).



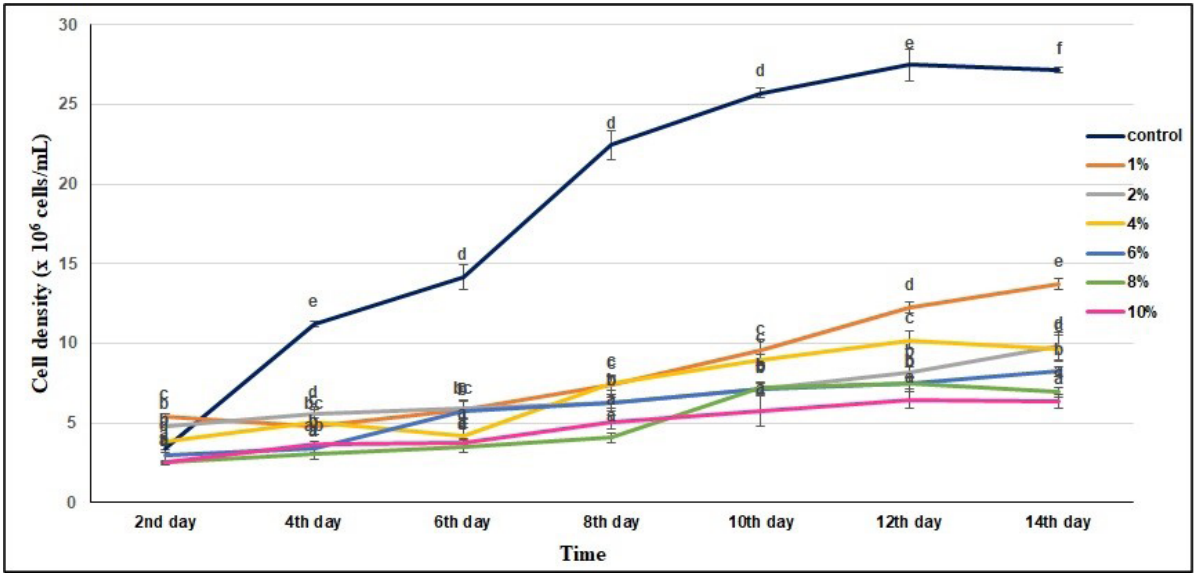


Figure 5. Growth curve of *N. oculata* in different concentrations of *T. ornata* SLF. The Bars=SD.

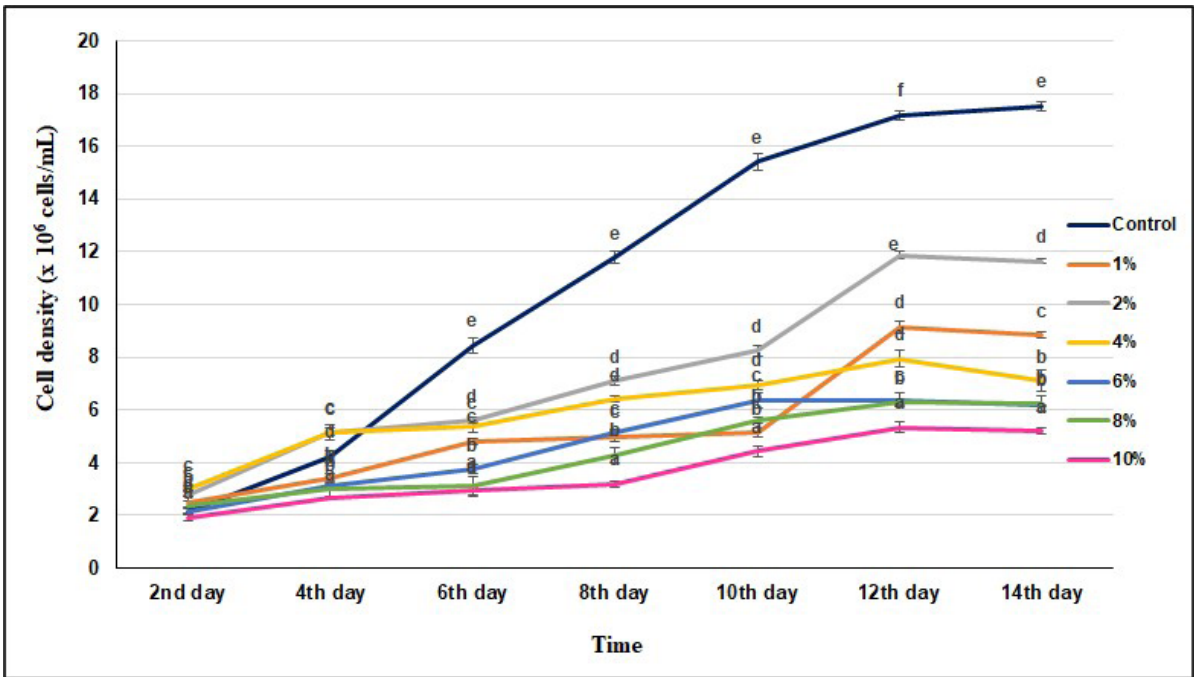


Figure 6. Growth curve of *D. salina* in different concentrations of *T. ornata* SLF. The Bars=SD.

Alvarado et al. (2008) had studied the effect of macroalgae compost amended with disilicate on the growth of the microalgae *Chaetoceros muelleri*. The macroalgal compost was made up of 99% brown seaweed species. The findings suggest that the cell density of the microalgae cultured were more or less similar but not significantly higher than the control (Walne media). From the present study as well as in comparison with the literature, we can conclude that the decrease in growth with increase in SLF concentration

may be due to the low nutrient content and dark brownish nature of the SLF that might have reduced the light penetration thereby inhibiting the photosynthetic process as well as the cell division.

### 3.5. Biochemical composition analysis

#### 3.5.1. Protein content analysis

Based on the nutrient concentration of culture media, the biochemical composition of the microalgal biomass varied

(Herrero et al., 1991). Hence, the biochemical analysis will provide a better understanding about the suitable media to be selected, that enhances the growth rate without affecting the biochemical composition of the species. The commercial media grown *N. oculata* had a protein content of 0.80 mg/mL whereas the maximum protein concentration of 1.17 and 1.08 mg/mL was found in 6% and 8% *G. acerosa* SLF followed by 0.81 and 1 mg/mL in 8% and 10% *K. alvarezii* SLF, respectively, which were significantly higher when compared to control. In 1% *T. ornata* SLF grown green alga the maximum protein concentration of 0.48 mg/mL was observed which was significantly lower than the control (Figure 7). *D. salina* grown in *K. alvarezii* SLF exhibited a maximum protein content of 1.05 and 1.16 mg/mL in the SLF doses of 8% and 10% followed by 0.9 and 0.82 mg/mL in 6% and 8% *G. acerosa* SLF, respectively. Both the SLF grown biomass exhibited maximum protein content than the control (0.937 mg/mL). In *T. ornata* SLF treated group, the highest protein content was observed in 2% i.e. 0.501 mg/mL which was significantly lower in comparison with F/2 media grown dinoflagellate (Figure 8). The study conducted by Lakshmi and Sheeja (2021) also documents the similar of results of slight increase in the protein content of *Chlorella vulgaris* when grown in BBM media with added red seaweed extract such as *G. corticata* and *G. lithophila* in comparison with control (BBM medium). Hence, in comparison of the present study with Lakshmi and Sheeja (2021), we can conclude that the red algal SLF from *K. alvarezii* and *G. acerosa* were more effective media that significantly enhances the growth as well as the protein content of the cultured species. Alvarado et al. (2008) have studied the effect of two different concentration of brown seaweed compost with amended silicate on biochemical composition of the microalgae *Chaetoceros muelleri* and documented that the commercial media grown algae (Walne medium) had a higher protein concentration of 12% whereas in 10% and 20% macroalgal compost grown *C. muelleri* it was only 2% and 5%, respectively. The results of Alvarado et al. (2008) are very similar with the present study that the addition of 99% brown and 1% red-green seaweed extract grown microalgae had a protein content that was significantly lower than the commercial media grown algae. Rohani-Ghadikolaei et al. (2012) have documented that the protein content of microalgae *I. galbana* was found to be increasing when seaweed extract (SWE) was used as supplement with F/2 medium. They have compared efficiency of red (*G. corticata*) and green SWE (*U. lactuca* and *E. intestinalis*) and concluded that the microalgae cultured in green SWE supplemented F/2 media resulted in high protein content in comparison with red SWE and control.

### 3.5.2. Pigment content analysis

Pigments serve as sensitive biomarker to assess the nutritional status of the culture medium. *N. oculata*

treated with 8% and 10% *K. alvarezii* showed a maximum chlorophyll-*a* content of 4.17 and 5.43 µg/mL, respectively, followed by 5 and 4.89 µg/mL in 6% and 8% *G. acerosa* SLF, respectively. SLF treated microalgae resulted in significantly higher chlorophyll accumulation in comparison with control (4.08 µg/mL). The minimal chlorophyll production of 2.84 µg/mL was observed in 1% *T. ornata* SLF treated culture (Figure 9). Similarly, the carotenoid value was also found to be high in 10% *K. alvarezii* and 6% *G. acerosa* SLF grown green alga i.e. 2.05 and 2 µg/mL which were significantly higher than commercial media (F/2) grown alga i.e. 1.34 µg/mL whereas lowest carotenoid pigment was found as 0.95 µg/mL in 1% *T. ornata* SLF grown alga (Figure 10). The pigment value observed from the study clears that the red SLF grown microalga had a high pigment production even higher than the control. Lakshmi and Sheeja (2021) have documented similar results of increase in pigment synthesis of *Chlorella vulgaris* when treated with BBM medium supplemented with red seaweed extracts of *G. corticata* and *G. lithophila*. Chl-*a*, *b* and β-Carotene values of *G. corticata* and *G. lithophila* SWE treated group were 1.029 µg/mL; 0.0256 µg/mL; 1.484 and 0.802 µg/mL; 0.0178 µg/mL; 0.0178 µg/mL, respectively, and these values were reported to be higher than the control group values. The results also conclude that increase in pigment synthesis was directly proportional to the SWE concentration which is in agreement with the present findings. *D. salina* treated with 10% *K. alvarezii* SLF showed the maximum production of Chlorophyll-*a* and *b* value of 5 and 3.92 µg/mL followed by 4.53 and 3.54 µg/mL in 6% *G. acerosa* SLF grown biomass which were found to be significantly higher than control (4.15 and 2.89 µg/mL). In *T. ornata* SLF grown biomass the maximum value of Chl-*a* and *b* was found as 2.96 and 2.01 µg/mL in 2% concentration which was significantly lower than the control (Figures 11a and 11b). Maximum carotenoid production of 4.01 and 3.99 µg/mL by *D. salina* biomass was achieved in 10% *K. alvarezii* and 6% *G. acerosa* SLF treatments and these values were significantly higher in comparison to commercial media grown culture (2.84 µg/mL). In *T. ornata* SLF treated groups, the maximum carotenoid value of 2.31 µg/mL was achieved in 2% SLF concentration (Figure 12). In red SLF treated groups the maximum carotenoid production was nearly 1.41 times higher than the value of commercially produced biomass. Addition of *K. alvarezii* and *T. conoides* extract as supplement with F/2 and Walne's media, respectively, resulted in increase of pigment synthesis in *C. muelleri* and *D. salina*. Chlorophylls *a* and *b* of *C. muelleri* increased by 32.5%, 30.2%, respectively, whereas Chlorophyll *a* and *c* of *D. salina* by 31.4% and 28.3%, respectively, when compared with control that has no supplement (Raja et al., 2015). Similar results of increase in growth and photosynthetic

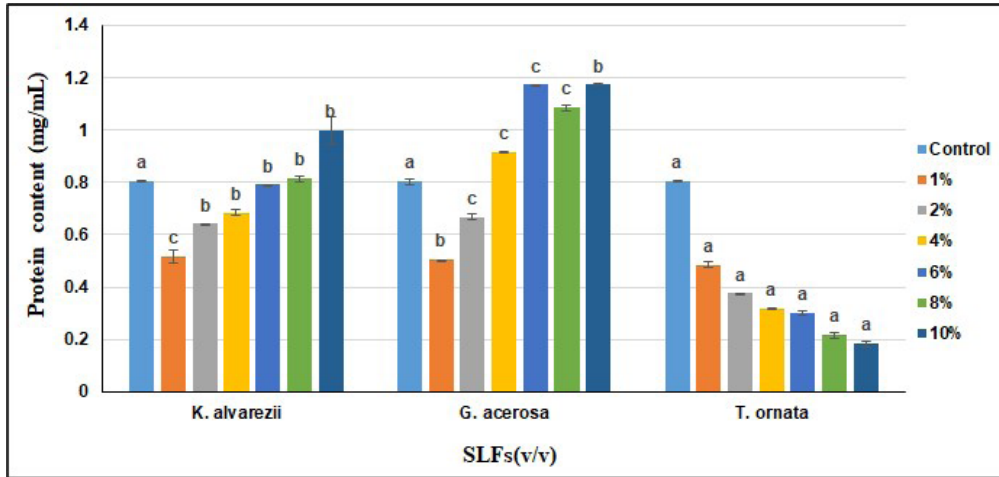


Figure 7. Protein content of *N. oculata* grown in three different SLFs at various concentrations. The Bars=SD.

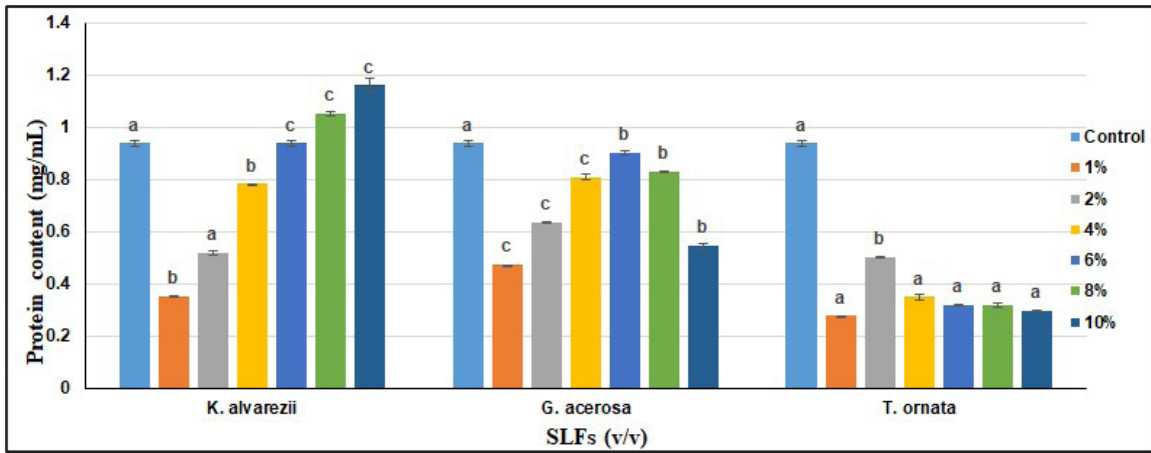


Figure 8. Protein content of *D. salina* grown in three different SLFs at various concentrations. The Bars=SD.

performance of *Chlorella variabilis* were observed when cultured in Zarrouk's medium supplemented with liquid extract from *K. alvarezii* (Sati et al., 2021). The reason for increase in pigment synthesis of microalgae when treated with SWE may be due to the phytohormones (mainly cytokinins) which enhances the nutrient uptake capacity and thereby stimulating the pigment synthesis (Zhang, 1997). Another main key factor determining the pigment synthesis in microalgae is the iron availability in culture media which is very essential for pigment biosynthesis (Kong et al., 2014; Chereskin and Castelfranco, 1982). This reason clearly correlates with the present study results that both the red SLFs had high Fe concentration than the brown SLF which resulted in more chlorophyll synthesis in red SLF grown microalgae than the brown SLF.

### 3.5.3. Carbohydrate analysis

Compared with other biochemical composition, the carbohydrate content was significantly higher in SLF

grown biomass than the commercial media grown culture. The maximum carbohydrate content of 0.058 and 0.049 mg/mL was found in 6% and 8% *G. acerosa* SLF followed by 0.042 and 0.053 mg/mL in 8% and 10% *K. alvarezii* SLF grown microalgae. The carbohydrate content was found to be increasing with an increase in SLF dose for *K. alvarezii* but in *G. acerosa* 6% showed the maximum carbohydrate content. These results also correlate with the cell density of the microalgae. *N. oculata* grown in brown SLF had the lowest carbohydrate value of 0.025 mg/mL in 1% concentration. In *K. alvarezii* and *G. acerosa* SLF treated group, carbohydrate content of green algae was 1.82 and 2 times higher than the F/2 media grown microalgae (Figure 13). The carbohydrate content of *D. salina* was found to be highly increasing when compared to control. The maximum of 0.05 and 0.044 mg/mL was found in 8% and 10% *K. alvarezii* SLF followed by 0.048 and 0.046 mg/mL in 6% and 8% *G. acerosa* SLF,

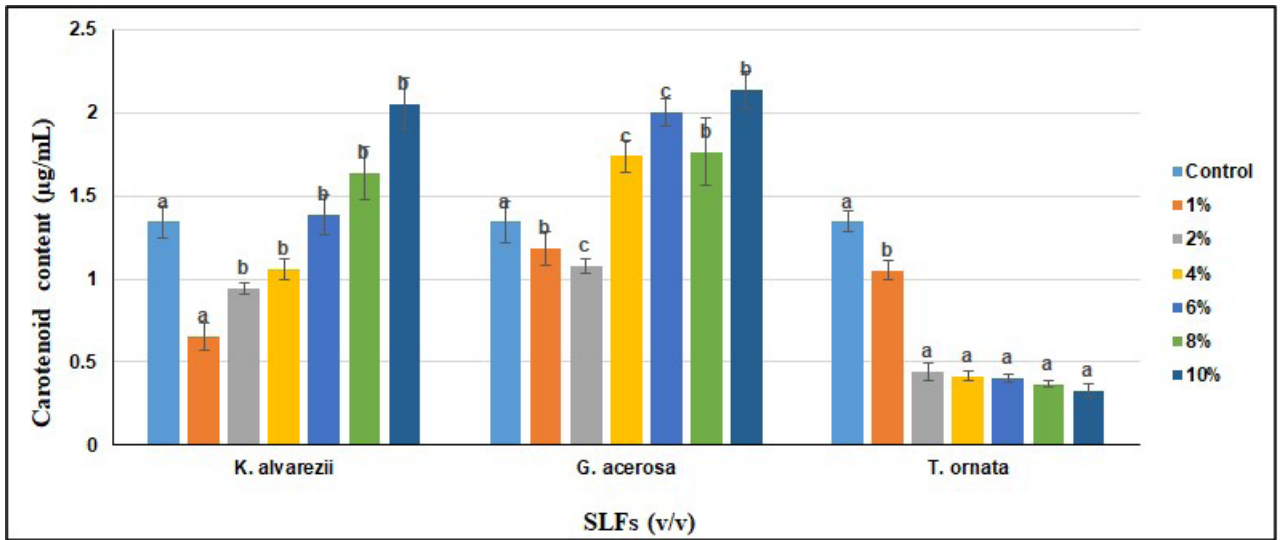


Figure 9. Chlorophyll- *a* content of *N. oculata* grown in three different SLFs at various concentrations. The Bars=SD.

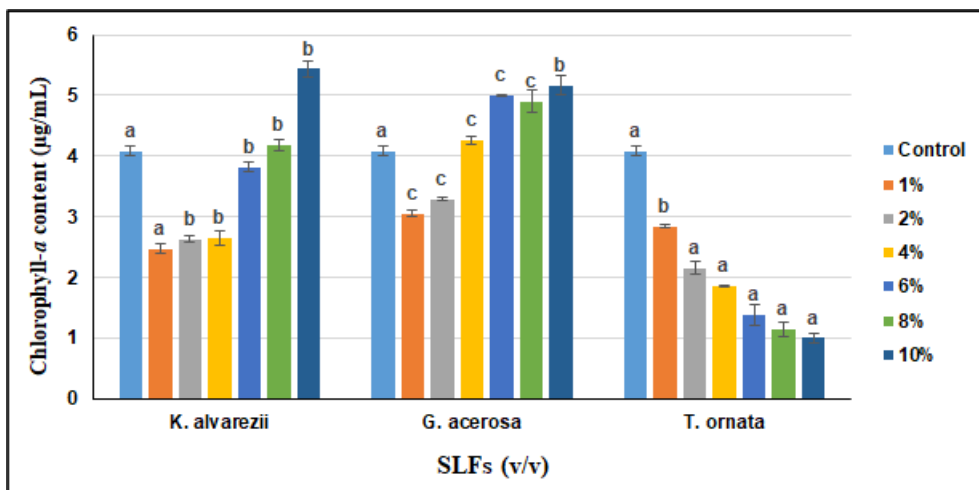


Figure 10. Carotenoid content of *N. oculata* grown in three different SLFs at various concentrations. The Bars=SD.

respectively, whereas in control it was 0.03 mg/mL. The lowest value of 0.022 mg/mL was found in 2% *T. ornata* SLF treated groups which was significantly lower than the control. In *K. alvarezii* and *G. acerosa* SLF treated group, carbohydrate content of dinoflagellate was 1.6 times higher than the F/2 media grown microalgae (Figure 14). The carbohydrate content of both algae was significantly higher in both red SLF treatments than the commercial F/2 media. Similar results of significantly higher carbohydrate value was documented by Rohani-Ghadikolaei et al. (2012) that when *I. galbana* was grown in the F/2 media with supplement of SWE of *U. lactuca*, *E. intestinalis* and *G. corticata* there was an increase in carbohydrate content than the control. Another study by Lakshmi and Sheeja (2021) also suggests the same results of drastic increase

in carbohydrate compared to protein and pigments when grown in BBM media supplemented with SWE i.e. *G. corticata* and *G. lithophila* SLF grown *C. vulgaris* showed the maximum protein value of 115 and 113 µg/mL which was significantly higher than the control. The biochemical composition of the microalgae *Picochlorum maculatum* cultured using extracts of *Sargassum wightii*, *Sargassum muticum* and *Turbinaria ornata* as additive along with the commercial culture medium resulted in higher protein, carbohydrate and lipid levels in comparison with the control (Bharathi et al., 2021). According to the result study in comparison with the literature, we can conclude that the SLF has high influence on the carbohydrate production of microalgae in comparison with other biochemical parameters.

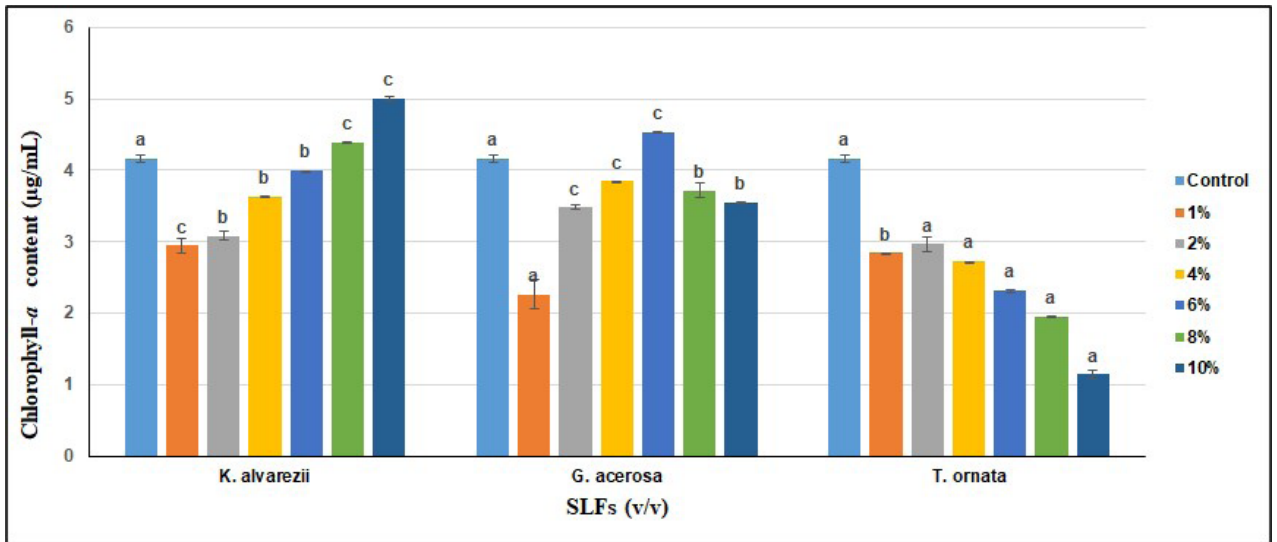


Figure 11a. Chlorophyll- a content of *D. salina* grown in three different SLFs at various concentrations. The Bars=SD.

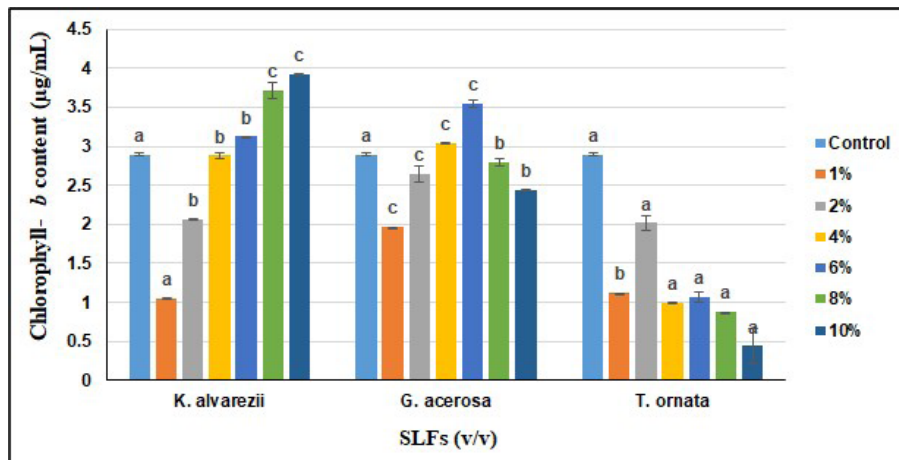


Figure 11b. Chlorophyll- b content of *D. salina* grown in three different SLFs at various concentrations. The Bars=SD.

### 3.5.4. Lipid analysis

Maximum lipid production was obtained in the high *K. alvarezii* SLF dose grown *N. oculata* i.e. 10% (0.19 mg/mL) and 8% (0.177 mg/mL) followed by 0.18 mg/mL in 6% *G. acerosa* SLF biomass. The microalga cultured in *T. ornata* SLF had the lipid content of 0.09 mg/mL which was lower than control. The lipid production of microalgae cultivated in both red SLFs were significantly higher than the control (0.15 mg/mL) stating the efficiency of SLF to increase the lipid yield of algae (Figure 15). The study conducted by Alvarado et al. (2008) for evaluating the effect of two different concentrations of brown seaweed compost amended with disodium silicate on the biochemical composition of the microalgae *C. muelleri* states that the seaweed compost resulted in increasing the lipid accumulation rate of the microalgae. In control

(Walne medium), the lipid production rate was 28% whereas in seaweed compost media grown microalgae it was estimated as 42%. Similar results of an increase in lipid content than the control were stated by Gireesh (2009) that *D. salina* grown in SLF showed higher lipid content than the control (Conveyor Walne’s media).

Lipid yield of *D. salina* was found to be high in 8% and 10% *K. alvarezii* SLF as 0.25 and 0.3 mg/mL whereas in *G. acerosa* SLF grown microalgae it was 0.27 and 0.2 mg/mL in 6% and 8% concentrations. These values were significantly higher than control (0.21 mg/mL). Similar results of an increase in lipid content than the control were stated by Gireesh (2009) that *D. salina* grown in SLF showed higher lipid content than the control (Walne’s or Conway medium). In *T. ornata* SLF treated groups the maximum lipid value was 0.08 in 2% concentration. In *K.*

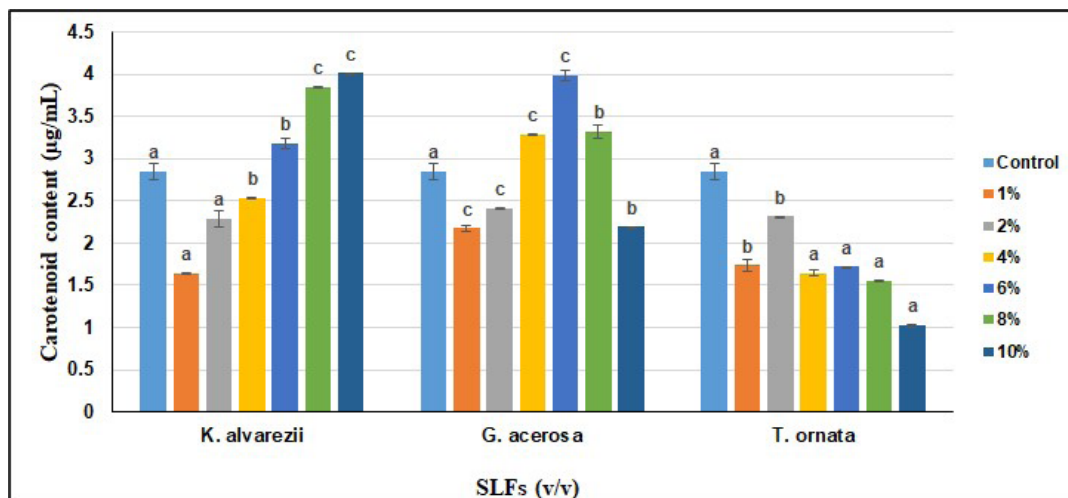


Figure 12. Carotenoid content of *D. salina* grown in three different SLFs at various concentrations. The Bars=SD.

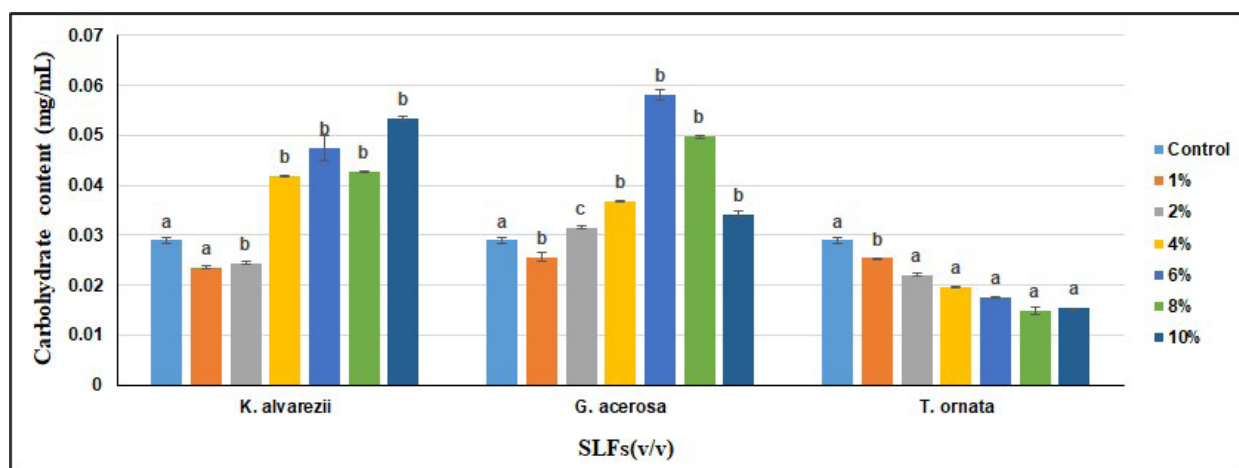


Figure 13. Carbohydrate content of *N. oculata* grown in three different SLFs at various concentrations. The Bars=SD.

*alvarezii* and *G. acerosa* SLF treated group, lipid content was 1.42 and 1.28 times higher than the commercial media grown biomass (Figure 16). Research results of Rohani-Ghadikolaei et al. (2012) also stated the same results that lipid production of the microalgae *I. galbana* cultivated in SWE as an alternative or supplement resulted in similar value with the control (F/2 medium). The reason for the increased lipid production was provided by Liang et al. (2009) that the seaweed extracts contain abundant carbon sources in the convertible form that can stimulate the accumulation of lipid in microalgae.

### 3.6. Production cost of SLFs

Lam and Lee (2012) stated that the production of organic fertilizer for microalgal growth stimulation cost around 1.20 USD/400 g. Another study by Zheng et al. (2016) documented the cost of production of one litre kelp waste extract (culture media for microalgae) cost around

0.28 USD. However, in our present study, the production cost of one litre of SLF was 0.1 USD (10 g cellulase cost) which is much cheaper than the reported fertilizers for microalgal cultivation. Hence, the present study provides a cost effective, eco-friendly and effective algal culture medium which will be very useful for aquaculture sectors and industries by reducing the production cost.

### 4. Conclusion

The study provides a substantial data and information for the feasibility of the application of seaweed liquid fertilizers (SLFs) to reduce the dependence on costly synthetic chemicals for algae cultivation. The study report presents the inexpensive and standardized method for preparing organic media that can completely replace the expensive commercial media without compromising the nutritional properties. The results of the present study suggest that the

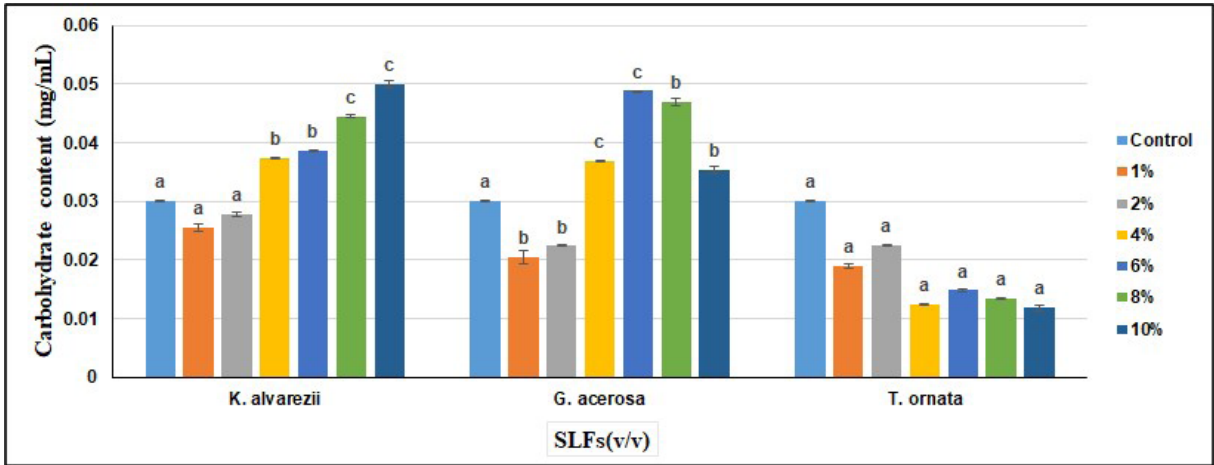


Figure 14. Carbohydrate content of *D. salina* grown in three different SLFs at various concentrations. The Bars=SD.

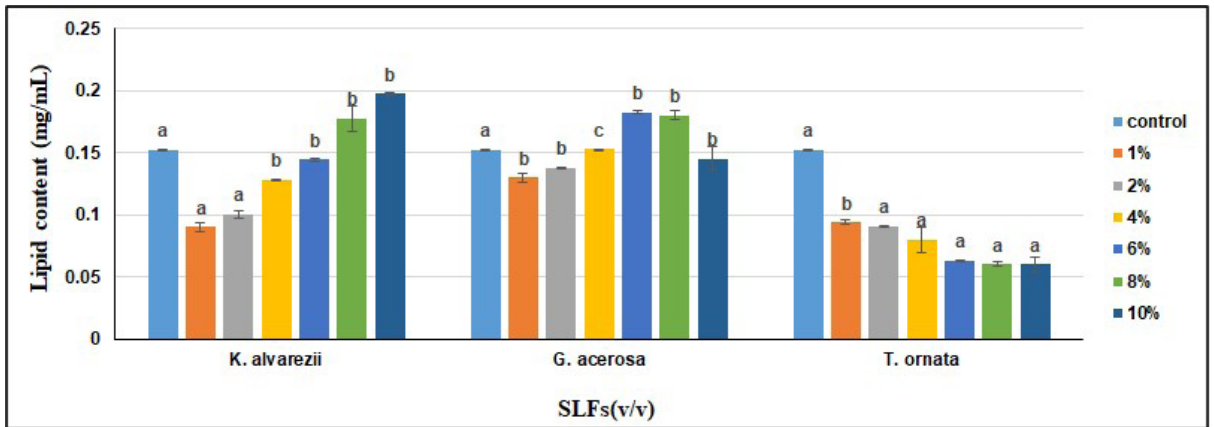


Figure 15. Lipid content of *N. oculata* grown in three different SLFs at various concentrations. The Bars=SD.

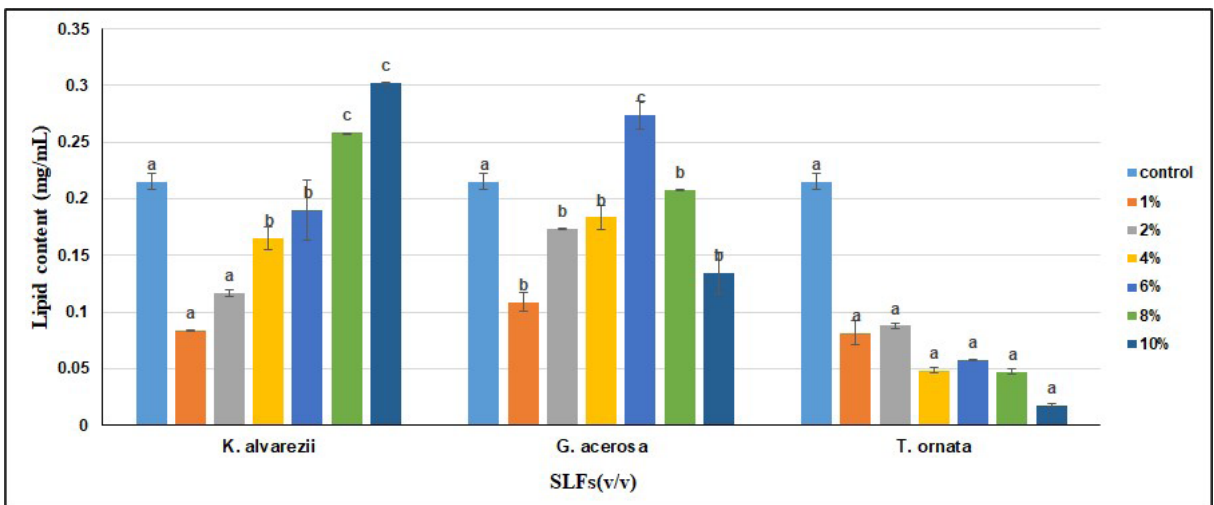


Figure 16. Lipid content of *D. salina* grown in three different SLFs at various concentrations. The Bars=SD.

biochemical properties of the microalgae were significantly influenced by red seaweed SLFs. Both the red seaweed SLFs enhanced the protein, pigment, carbohydrate and lipid synthesizing potential of both the marine microalgae. This study also documents the biochemical response of the microalgae grown in three different SLFs and provides evidence about the lack of adverse effects of SLFs on algal growth. From the present study, it can be concluded that *K. alvarezii* SLF as the best culture media. Since this seaweed species can easily be cultivated there is no need to depend on the natural resource, suggesting its practical suitability for industrial scale production. In addition, this low-cost organic media is suitable for practical application in industrial sectors and farmers due to the simplicity of preparation and use of SLFs.

### Contribution of authors

Krishna Moorthy Abarna: Investigation, Conceptualization, Writing - original draft, Data analysis and interpretation; Rani V: Supervision, Project

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### Conflict of interest

The authors have no conflict of interest to declare.

### Ethical approval

In this research no human or animal were harmed.

### Authorship consent

All authors agree and approve the submission of the research article.



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