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Influence of High Light Intensity and Nitrate Deprivation on Growth and Biochemical Composition of the Marine Microalgae *Isochrysis galbana*

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HIGHLIGHTS

- Influence of abiotic stress on the biochemical composition of microalgae has considerable physiological and biotechnological importance.
- High light conditions and nitrogen limitations enhanced lipid and carbohydrate productivity in *Isochrysis galbana*.
- Limitation of nitrogen significantly decreased all photosynthetic pigments, TPC and antioxidant activity.
- These stresses induce significant increase of palmitic, and oleic, acids and decrease polyunsaturated fatty acid except docosahexaenoic acid.

Abstract: *Isochrysis galbana* is a brown microalgae widely used as a feed for marine organism in aquaculture. The aim of present study is to investigate the growth, biochemical composition, fatty acid profile, photosynthetic parameters and antioxidant activity (radical scavenging activity) of *Isochrysis galbana* cells cultivated under different levels (sub-optimum; 50 ± 1.5 , optimum; 125 ± 2.5 and supra-optimum; 325 ± 3.5 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) of photosynthetic active radiation (PAR), and subsequently treated with different concentration of nitrate deprivation (8mM, 2mM and 0.5mM). The experiment was carried out under controlled conditions utilizing a factorial design 3x3 (light intensity and nitrate concentration). The result depicts that PAR positively influences the growth of *Isochrysis galbana* which is maximum under supra-optimum PAR. Nitrate deprivation (2mM & 0.5mM) induced decline in growth in terms of dry weight is observed as 60.1% & 61.9% in suboptimum and 26.5% and 34.9% in supra-

optimum respectively over the values recorded in their respective controls. Supra-optimum PAR decreased primary photosynthetic pigment Chl a and Chl c by 15.7% and 8.5%, whereas carotenoid content increased by 45.9% in supra-optimum PAR which displays potential interest as antioxidant agent in addition to total phenolic content and radical scavenging activity. The results suggest that combined stress of high light and nitrogen deprivation shifts the metabolic physiology from protein synthesis to energy reserve (carbohydrate and lipid) and accumulation of saturated fatty acid on expense of unsaturated fatty acid except docosahexaenoic acid. These valuable compounds exhibit potential applications in mariculture, nutraceutical and biofuel industry.

Keywords: *Isochrysis galbana*; Photosynthetically active radiation; Nitrate deprivation; Biochemical composition; Pigments, Antioxidant.

INTRODUCTION

Marine phytoplankton which includes different groups of microalgae, cyanobacteria and diatoms are primary producer of organic matter to ocean interior and to higher trophic levels via food chain. Recently, Microalgae seeks the interest for biotechnological purposes due to their valuable biomass components and ability to accumulate and store secondary metabolites that have diverse application in aquaculture, nutraceutical, pharmaceutical and biofuel industry [1,2,3]. Interesting biomass compounds that could be produced by microalgae includes carotenoids, fatty acids, amino acid, proteins, polysaccharides, vitamins, and phenolic compounds, which exhibits potential application in food and pharmaceutical industries. *Isochrysis galbana* is a golden brown flagellated microalgae belonging to haptophyta species. This genus have been used for centuries in aquaculture as animal feed due to its comparatively high growth rate, small size, non-toxic and superior nutritional value [4]. Various environmental factors as temperature, pH, salinity, nitrogen availability, and light exert an impact on growth and biochemical composition of microalgae cells. Since variation in irradiance is a typical event in cultivation system under natural conditions, indoor or outdoor photobioreactors etc. Its influence on the biochemical composition of microalgae has considerable physiological and biotechnological importance. Light is one of the key factors that determines the amount of available energy for the photosynthetic process and thereby affects growth. Previous study reported that nitrate is a significant nutrient in the media that can provoke important changes in the growth and biochemical composition of microalgal species [5,6,7]. There are meager reports on combined impact of high light intensity and nitrate deprivation induced oxidative stress on biomass production and biomass composition of *I. galbana*. Therefore, the objective of this paper is to point out how change in light intensity along with nitrate concentration in batch culture affects the growth and biochemical composition of microalgae. Beside, aqua culturist, demand for nutraceutical substance and biofuel on the world market, raised the needs of improved knowledge of its growth characteristic and biochemical composition for higher production of particular valuable metabolites with high mass productivity. Therefore, it is important to investigate the interactive effect of light and nitrate limitation on growth and biochemical composition of microalgae.

MATERIALS AND METHODS

Algal sample and growth condition

The starter culture of marine microalgae *Isochrysis galbana* was obtained from Center of Marine and Fishery Research Institute (CMFRI) Kochi, India. The experiment was carried out in 1litre conical flask containing 200-300ml of growth media. The

growth media contained autoclaved filtered natural seawater, supplemented with f/2 culture medium [8]. The f/2 culture medium was composed of 75 mg L⁻¹ NaNO₃, 5 mg L⁻¹ NaH₂PO₄H₂O, 1 ml L⁻¹ trace metal solution (3.15 mg L⁻¹ FeCl₃6H₂O, 4.36 mg L⁻¹ Na₂EDTA, 0.0098 mg L⁻¹ CuSO₄5H₂O, 0.0063 mg L⁻¹ Na₂MoO₄2H₂O, 0.022 mg L⁻¹ ZnSO₄7H₂O, 0.01 mg L⁻¹ CoCl₂6H₂O, 0.18 mg L⁻¹ MnCl₂4H₂O) and 0.5 ml/L of vitamin solution (0.001 mg L⁻¹ vitamin B12, 0.2 mg L⁻¹ vitamin B1, 0.001 mg biotin⁻¹) were added. In the laboratory, the culture of *Isochrysis galbana* were grown in a batch culture at temperature of 22-25°C, pH 8-8.2 and illumination of 125 μmol photon m⁻² s⁻¹ provided by white fluorescent tube for 18:6 h light–dark period. The cultures were shaken manually 2-3 times daily.

Experimental design

The early log phase culture of *I. galbana* were cultivated under three levels of photosynthetic active radiation (PAR) - Sub-optimum (50 ± 1.5 μmol photons m⁻² s⁻¹); Optimum (125 ± 2.5 μmol photons m⁻² s⁻¹); and Supra-optimum (325 ± 3.5 μmol photons m⁻² s⁻¹). Then within each light regime, nitrate content was reduced from 8mM to 2mM and 0.5mM in respective culture medium. A factorial 3X3 (light intensities X nitrate concentration) experimental design with 3 replicates per treatment was performed. Treatments consisted of three levels of light intensity: 50 μmol photon m⁻² s⁻¹ (sub-optimum), 125 μmol photon m⁻² s⁻¹ (optimum), and 325 μmol photon m⁻² s⁻¹ (supra optimum), and three level of nitrate concentration (8mM, 2mM and 0.5mM).

Measurement of algal growth

The growth of *Isochrysis galbana* cells were estimated in terms of optical density (O.D.) and dry weight. Optical density was measured daily at 710nm by UV visible spectrophotometer. For dry weight measurement, 50 ml of culture samples were centrifuged at 8000 rpm for 10 minutes. After rinsing twice with ammonium formate, the pellets were dried 6hr in an oven at 110°C and then cooled down in desiccators before weighing. All the experiments were carried out in triplicates.

Biochemical analysis and pigment content

The crude protein was determined by modified Lowery method [9]. The absorbance of the samples was taken at 650nm and the concentration was determined using standard curve. Total Protein Content = Wt. of protein (from curve) X 100/ dry cell mass (mg). The content of carbohydrate was estimated by phenol-sulfuric acid method with slight modification [10]. The optical density of the sample was determined against the blank at 490 nm in a UV-visible spectrophotometer. Carbohydrate Content (%) = Wt. of carbohydrate (from glucose standard curve) X 100/ dry cell mass (g) and total lipid content of samples were analyzed gravimetrically after extraction with chloroform–methanol (2:1) modified by Bligh and Dyer [11]. Extracted lipid were converted to methyl esters with methanolic HCL and 2,2-dimethoxy propane via transesterification. Then fatty acid methyl esters were determined by using gas chromatograph (GC) of series SP-2560 (100×0.25 mm; 0.20 μm), followed by flame-ionization detection (Hewlett-Packard, USA). Pigments (*Chl a*, *Chl c* and carotenoids) were extracted in methanol (90%) at 4°C overnight and measured by spectrophotometric methods [12].

Antioxidant activity

Antioxidant activity of the various treatment of *Isochrysis* cells were determined by quantifying total phenolics content (TPC) present with methanol and calculating the antioxidant activity of the extract by measuring the DPPH free radical scavenging activity. Total phenolics content (TPC) of crude extract was determined by the Folin-phenol method following the method of Waterhouse [13]. 0.1 mL of methanolic extract was mixed with 1.5 mL distilled water and then 0.1 mL of folin cioaltea phenol reagent

was added. After 3 minutes 1 mL of sodium carbonate (10%) was added and 10ml volume was makeup with distilled water. After incubation of 90 min absorbance was read at 765 nm and compared with the standard curve prepared by gallic acid. The amount of TPCs was expressed as gallic acid equivalent g^{-1} dry weight. The DPPH radical scavenging effect of the extracts was determined by the method of Sanja *et al* with slight modification [14]. 100 μl of extract was mixed with 150 μl of DPPH solution and diluted up to 3ml with pure methanol. Incubate for 15 mins at room temperature in dark and the absorbance of solution was taken at 517 nm on UV spectrophotometer using methanol as blank. The radical scavenging activity (%) was expressed as the percentage discoloration of DPPH solution by using the following equation:
 Radical scavenging activity (%) = $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$

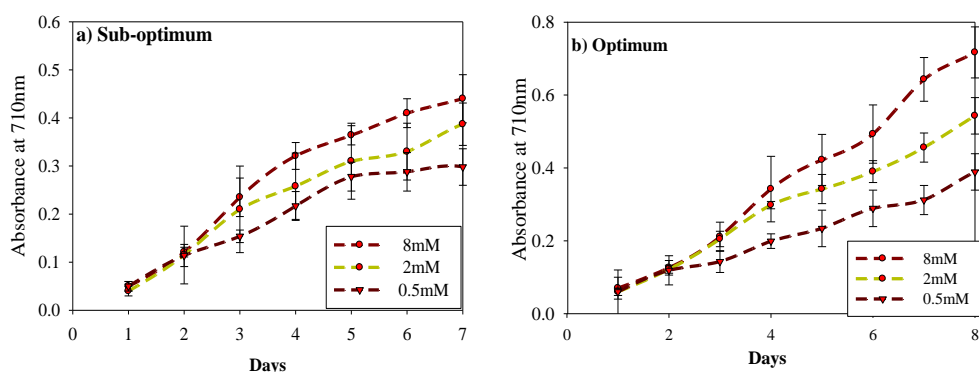
Statistical analysis

All experiments were done in triplicates and data represent the means \pm SD. The means were analyzed by two- way ANOVA and significant differences between treatments were tested using Duncan's multiple range test (DMRT). *P*-values <0.05 were considered significant. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 10.0.

RESULTS

Growth pattern and dry weight

To evaluate the effect of light and nitrate concentration on growth, absorbance at 710 nm were measured. The growth curve of *I. galbana* cells treated with different concentration of nitrate (8 mM, 2mM, 0.5mM) illustrated in figure 1. Result reveals that nitrogen depletion negatively affects the growth of cells at all light regime. It was also observed that stress of high light intensity for short period stimulates the growth of *I. galbana* more efficiently than low light intensity. The maximum growth was observed at $325 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Growth measured in term of dry weight was shown in figure 2. The biomass content significantly decreased with deprivation of light and nitrate concentration from 0.97 to 0.43 g L^{-1} . The culture grown under supra-optimum PAR with sufficient nitrate concentration attained a highest dry weight ($0.97 \pm 0.028 \text{ g L}^{-1}$) followed by culture grown in optimum PAR ($0.83 \pm 0.042 \text{ g L}^{-1}$). After deprivation of nitrate content in growth medium, growth was declined considerably in dose dependent order. Result reveals that with deprivation of nitrate (2mM, 0.5mM) growth lowers by 60.1% and 61.9% in sub-optimum PAR and 26.5% and 34.9% in supra-optimum PAR respectively, over the values recorded in their respective controls.



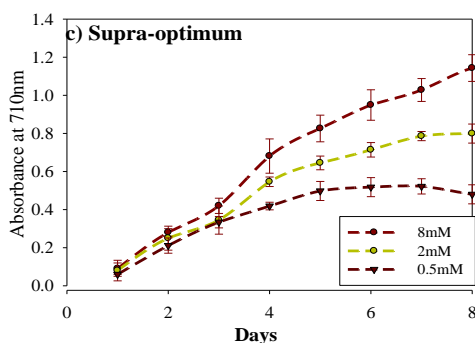


Figure 1: Growth curve at three different nitrate concentrations under a) Sub optimum b) Optimum level 1 c) Supra-Optimum of PAR.

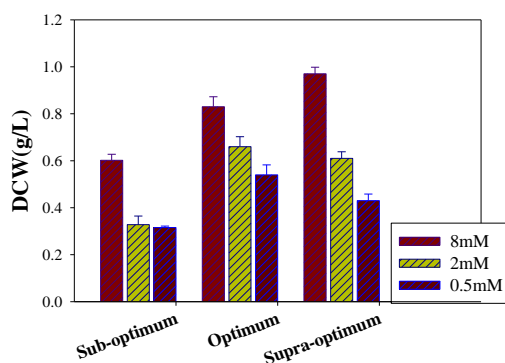


Figure 2. Impact of nitrate deprivation on dry weight of *Isochrysis galbana* cultivated under three levels (sub-optimum, optimum and supra-optimum level) of PAR. Data are the means \pm standard error of three replicates in each experiment.

Biochemical composition and pigment contents

In figure 3, the variations of the determined biochemical compositions during the cultivation of *I. galbana* under different light intensity and nitrate concentration were illustrated. It had reported that in each light regime, all biochemical parameters were significantly different ($p \leq 0.05$) at all three levels of nitrate deprivation, although post hoc test (Duncan's test) reveals some culture were insignificant in different light regime. The result of present study revealed slight increase in protein content with increase in light intensity in nitrate untreated cells whereas deprivation of nitrate content (2mM, 0.5mM) decreased protein content by 13.7% and 33.9% respectively, in sub-optimum PAR and 36.9% and 51.9% respectively, in supra-optimum PAR. In nitrate untreated cells, carbohydrate and lipid accumulates by 22.3% and 14.4% respectively, in supra-optimum PAR. Further, with deprivation of nitrate content in media carbohydrate and lipid accumulates with increasing order of doses (figure 3). The pigment contents of *I. galbana* significantly ($p \leq 0.05$) affected by deprivation of nutrient and light intensity in most of cultures (figure 4). Our result found that with untreated nitrate cells maximum content of photosynthetic pigment Chl *a* and Chl *c* (4.67 ± 0.28 mg L⁻¹ and 3.82 mg L⁻¹) attained under supra-optimum PAR while maximum carotenoid content (4.89 ± 0.256 mg L⁻¹) reported in cell grown under sub-optimum PAR. Similar trend is found in other studies *Dunaliella salina*, *Chlorella zofingiensis*, *Isochrysis galbana* [14,15].

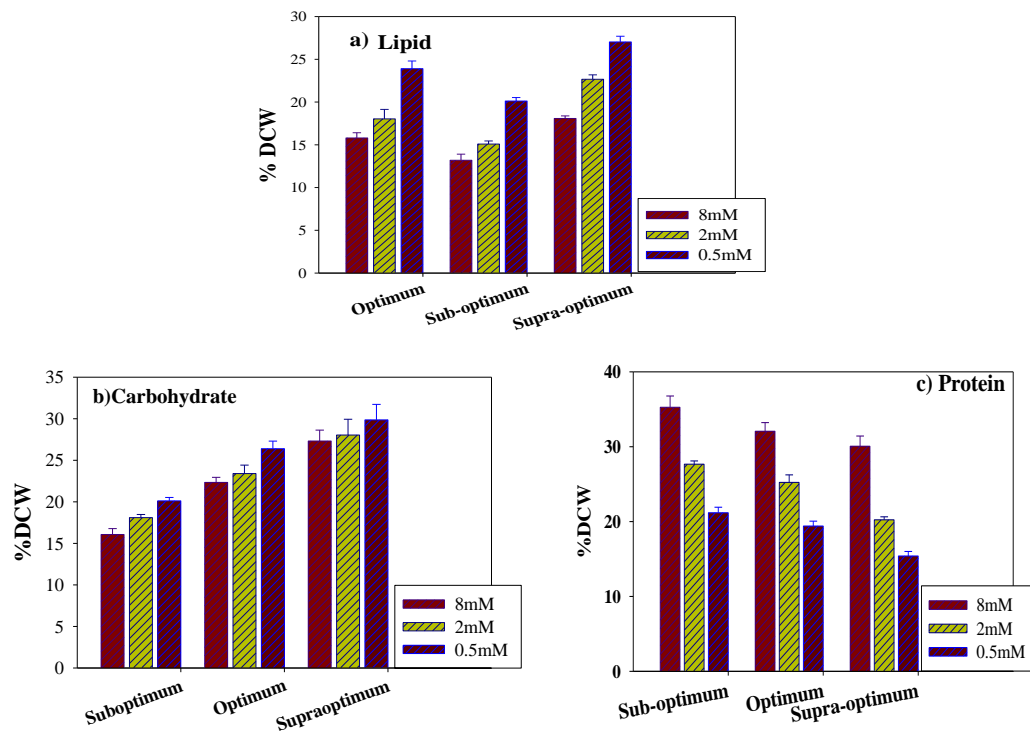


Figure 3. Effect of nitrate deprivation on a) Lipid b) Carbohydrate c) Protein content of *Isochrysis galbana* cell grown under sub-optimum, optimum and supra-optimum level of PAR. Data are the means \pm standard error of three replicates in each experiment.

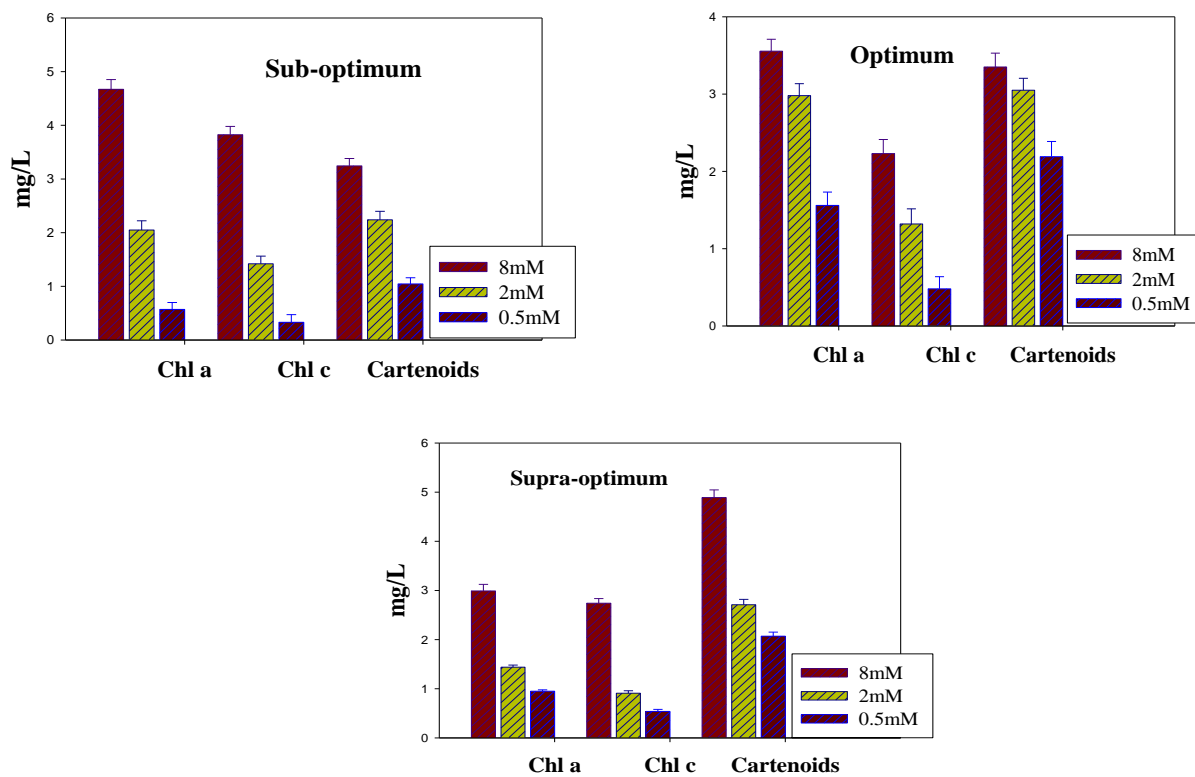


Figure 4: Effect of nitrate deprivation on pigments under a) Sub-optimum b) Optimum light, c) Supra-optimum PAR. Data are the means \pm standard error of three replicates in each experiment.

Fatty acid Profile

The previous studies on fatty composition of *Isochrysis galbana* were revealed that it contains Myristic acid (C16:0), Palmitic acid (C18:0), Oleic acid (C18:1, n-9) and DHA (C22:6, n-3) as major fatty acid. In this study, we investigated the effect of varying light intensity and nitrate deprivation on five major fatty acid compositions (FAME) which are presented in table 1. In parallel to previous studies, we observed that high light conditions or severe nitrogen limitations were associated with significant increase of palmitic, C16:0 (10.38-14.3 mg g⁻¹) and oleic, C18:1 (15.89-21.78 mg g⁻¹) acids and decrease in the percentage of the polyunsaturated fatty acid, predominately Stearidonic acid, C18:4(18.54-11.47). Although, the highly unsaturated fatty acid docosahexaenoic acid, C22:6 slightly increased from 12.38 mg g⁻¹ to 15.48 mg g⁻¹ under high PAR and low nitrogen concentration.

Table 1: Effect of nitrogen depletion on fatty acid profile (% of total) of *Isochrysis galbana* cultivated in three different light regimes. All fatty acids are expressed as mg g⁻¹.

Fatty acids	Common name	Sub-optimum			Optimum			Supra-optimum		
		8mM	2mM	0.5mM	8mM	2mM	0.5mM	8mM	2mM	0.5mM
C14:0	Myristic acid	16.96	16.93	17.09	17.06	17.1	17.21	17.11	17.2	17.34
C16:0	Palmitic acid	10.38	11.46	12.1	11	11.7	12.3	12.08	13.1	14.3
C18:1,n-9	Oleic acid	15.89	16.5	17.08	16.4	17.23	18.32	19.1	20.45	21.78
C18:2, n-6	Linoleic acid	6.29	5.99	5.2	5.05	3.78	3.55	2.94	2.58	2.1
C18:3, n-3	α -Linolenic acid	2.27	2.15	1.27	2.51	1.89	1.51	3.54	2.32	1.54
C18:4, n-3	Stearidonic acid	18.54	15.7	13.7	16.2	15.8	14.3	14.9	12.3	11.47
C20:5, n-3	Eicosa pentaenoic acid (EPA)	0.98	0.82	0.7	0.92	0.8	0.76	0.87	0.67	0.55
C22:6, n-3	Docosahexaenoic acid (DHA)	12.38	12.66	13.07	13.3	13.91	14.28	15.01	15.25	15.48

Antioxidant activity

Total phenolic compounds and free radical scavenging activities of methanol extract of different treatments were studied at 250 μ l/ml concentrations. The result of present studies found that highest quantity of TPC (3.77mg GAE DW⁻¹) and antioxidant activity (72.3%) were observed in culture grown in supra-optimum PAR with 8mM of sodium nitrate. Figure 5 illustrated that deprivation of nitrate concentration decreases both TPC and free radical scavenging activity and lowest quantity of TPC (1.85 mg GAE DW⁻¹) and DPPH activity (37.5%) were observed in culture grown in sub-optimum PAR with 0.5mM of sodium nitrate.

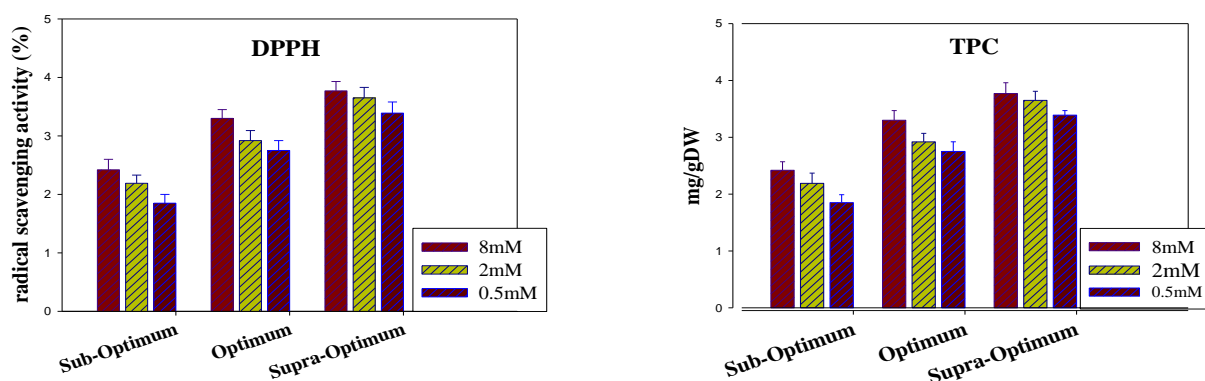


Figure 5: Impact of nitrate deprivation on TPC and DPPH free radical scavenging activity of *Isochrysis galbana* cultivated under three levels (sub-optimum, optimum and supra-optimum level) of PAR. Data are the means \pm standard error of three replicates in each experiment.

DISCUSSIONS

Growth pattern and dry weight

I. galbana is a photoautotrophic microalgae its growth is highly influence by irradiance supply. In present study, wide growth permissive range of light intensity for this alga has been identified. It had found that stress of high light for short period stimulates the growth of *Isochrysis galbana* cell more efficiently than low light intensity. This could be because under stress (such as high light intensity) microalgae might increase their size in order to survive in stressful environments. Other study advocated this result and reasoned that a under supra-optimum PAR microalgae would turn out increased amounts of light energy into usable chemical energy which would result in an increase in algal biomass. At sub-optimum PAR, minimum growth has been observed and further growth decreased linearly with nitrate deprivation. In support of this, one of previous study stated that the growth of phytoplankton in large-scale batch cultures usually slowed by light limitation or terminated by nutrient limitation (N, P, Si) especially in old culture because excess of cells produces self shading in culture [15]. A maximum growth was found in cell grown at $325 \mu\text{mol m}^{-2} \text{s}^{-1}$ this suggesting that this species is able to grow under a wide range of light conditions with suffering an extensive stress that makes necessary cell adaptation. Since nitrogen is a major component in many biological macromolecules like protein, chlorophyll, photosystem, enzymatic and genetic materials [16,17]. Nitrogen deprivation is also a major limiting factor affecting the growth and biomass production [18,19]. Previous study observed that nitrogen limitation decreases the synthesis of photosynthetic pigments thereby also hampers the processes of photosynthesis and assimilation in turn positively affects the cell density or microalgal biomass [17,20,21]. The present study observed that nitrate deprivation has more pronounced effect on growth pattern and dry weight of microalgae than light deprivation [22]. *Isochrysis galbana* attained maximum cellular density at 8mM NaNO_3 and biomass content significantly decreased with a decrease in nitrate. This indicates that nitrogen starvation slows down the metabolic activity and cell division in *I. galbana*. Similar results have also been observed in previous studies [23,24]. The lowest dry weight have been found in cell grown in low light with N deficient condition (sub-optimum-0.5mM) resulted in only 0.43 gL^{-1} (dry cell weight). The lower biomass yield in the N-limited treatment was primarily caused by lower specific growth rate and may be due to a combination of lower cell abundance and smaller cells in the N-limited treatment. This result is supported by observation of other studies in which nitrate and salinity influence the growth in *Tetraselmis sp.* and reported that limitation of nitrogen may limit the cell growth [25]. Previous research demonstrated that low levels of nitrogen in media reduced the productivity of

microalgae as they may have an adverse effect on the metabolism, cell division and thereby reducing cell population levels [26].

Biochemical compositions and pigments

Stress of high light and nitrate deprivation affects the metabolic activity of cell and divert the cell from protein synthesis to carbohydrate and lipid accumulation. Under supra-optimized PAR, synthesis of carbohydrate and lipid increased by 22.3% and 14.4% respectively. This is because under high light intensity cell fix more carbon dioxide to storage products lipids and carbohydrates because they are hydrophobic in nature and have highly reduced states. In addition they efficiently packed in small compartment of cells and can also be used during adverse conditions for cell survival and proliferation [27]. Figure 3 illustrated the proximate composition of *Isochrysis galbana* under different levels of stress and observed a similar trend widely documented in the previous literatures [28,29,30]. Result of study observed that nitrate deprivation facilitates the accumulation of carbohydrate and lipid under both sub-optimum and supra-optimum PAR. Nitrogen limitation interrupts the Krebs's cycle thus contributing to fatty acid formation [31]. This increased lipid concentration was coupled to decrease in both dry weight and total protein content. It has been observed that cultivation under nitrogen deficient conditions for short time leads to a sharp increase in the lipid or carbohydrate content because nitrogen starvation limited the rate of cell division and shifts anabolic pathways from protein synthesis to produce reserve substance like carbohydrate or lipid. This behavior is consistent with numerous studies demonstrated that under N-limited conditions, lipid and carbohydrate contents were improved in many algal species like *Neochloris oleoabundans* [32] HK-129, *Thermosynechococcus* sp., *Chlorella vulgaris* [33], *Scenedesmus obliquus* CNW-N [34], *Nannochloropsis* sp., and *Tetraselmis suecica* [35]. Under supra-optimum PAR, *I. galbana* similarly to other photosynthetic organisms showed an acclimation response by decreasing the Chl *a* content and accumulating carotenoids. Previous studies observed that under high light intensities to reduce the light harvesting ability chlorophyll *a* content decreases and carotenoids accumulates to prevent the absorption of surplus light energy by the photosynthetic machinery and thereby protect the photosynthetic apparatus from oxidative stress [36,37].

Since, chlorophyll is a nitrogenous compound; its content and composition are greatly influenced by nitrate concentration in the growth medium. Figure 4 illustrated that with limitation of nitrate content, photosynthetic pigments and carotenoids decreased significantly ($p < 0.05$) whereas ratio of Chl *a*/Chl *c* and car/total Chl increased in all three light regime. Nitrogen is a key element for the synthesis of protein and its limitation lowers protein synthesis rate. Since proteins involves in photosystem reaction center and photosynthesis electron transport system therefore nitrogen limitation impairs the rate of photosynthesis too. These are similar to previous studies [38]. In our study, under same light regime the limitation of nitrogen significantly decreased all photosynthetic pigments viz. Chl *a*, Chl *c* and total carotenoids. As shown in figure 4, reduction in nitrate in cultivation medium from 8mM to 0.5 mM has reduced the total chlorophyll and carotenoids by about 83.8% to 89.4% DW and 78.1% to 67.7% DW respectively under different light regime. However, the ratio of Chl *a*/*c* and caro/total Chl was found to increase in nitrogen limited cultures, thus indicating the decrease in light harvesting complex and PS II activity. This is further supported by previous studies [39].

Fatty acid profile

The results of present study were found in consistent to the earlier finding [40,41,42] and suggested that fatty acid composition within cells changes with changing the growth condition such as light intensity or nitrate concentration. We observed that there is shift of one group of FAs, includes myristic acid, LA, ALA

and SDA, to a second group of FAs including palmitic acid and oleic acid, with nitrogen limitation and high light intensity. Under high light intensity, an excess of energy is used by the cells for producing storage lipids, mostly SFA and MUFA which are commonly involved in the formation of thylakoid membranes and under low PAR elevation in percentages of PUFA predominantly 18:4(n-3), 20:5(n-3) and 18:5(n-3) were observed which was accompanied by an increase in chlorophyll a content. The increase in MUFA (oleic acid) can be explained with the central role of oleic acid in elongation and saturation of fatty acid [43,44,45]. The decrease in PUFA would be due to its role in synthesis of structural components and to increase light absorption and light utilization efficiency [46], [47] explained the changes in fatty acid composition of cells under different light regimes and explained the relation of functioning of photo systems and processes of production and desaturation of fatty acids. DHA is the prominent omega 3 fatty acid in *Isochrysis galbana* which increases under high light intensity and severe nitrogen depletion. There are multiple theories for contradictory increasing trend of DHA which includes its imperative role in membrane physiology of *I. galbana* [42], association of DHA with anti-photooxidation procedure [48] and some studies suggested that the light promotes its synthesis by activation of desaturase enzymes [49].

Antioxidant activity

Result showed that altering the culture conditions (light intensity and nitrate deprivation) has significant effect on antioxidant potential of microalgae. Cells grown under high PAR showed highest amount of TPC and antioxidant activity this highlights the key role of light on pigment photoprotection mechanism. Figure 5 elicited that the deprivation of nitrate in media decline the TPC and antioxidant activity of *Isochrysis galbana* cells. Similar results were observed and explained that nitrogen is required for synthesis of the aromatic amino acids (AAA), which may be playing an important role as precursors for biosynthesis of phenolic compounds [50,51].

CONCLUSION

Our study observed that combined manipulation of the nutrient deficiency and high light intensity is important to achieve algal cells with high lipid and carbohydrate productivity. The present experiment found that high light (325 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) facilitates rapid growth and accumulation of reserve like carbohydrate and lipid content. The combination of high light and nitrogen-deficiency stresses are of particular interest for establishing the photobiotechnology of this alga, as lipid especially docosahexaenoic acid (DHA) is a valuable nutraceutical component of *Isochrysis galbana*. It is possible to manipulate the biochemical composition of *Isochrysis galbana* by changing the light regime and nutrient content which could be used for aquaculture, human nutrition, pharmaceutical, biofuel and other industrial application. The stress of high light intensity increases the total carotenoids content and the antioxidant activity which displays some potential interest as antioxidant agent. Stress of high light intensity and nutrient deficiency could also be used to induce carbohydrate and lipid accumulation, which could be used in mariculture feeds, nutraceutical and biofuel industry.

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Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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