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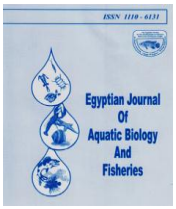

Effect of Different Nitrogen Concentrations on Primary and Secondary Metabolites of *Scenedesmus obliquus* (Turpin) Kutzing

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ABSTRACT
The work aimed to enhance growth and biochemical substances in *Scenedesmus obliquus by* creating a growing suitable condition of enrichment culture with varied concentrations of KNO_3 as a nitrogen source. Kuhl medium was used for the cultivation of *Scenedesmus obliquus* with optimized nitrogen source concentration. Growth was determined by optical density (1.94 OD_{680}) on the 10^{th} day of the culture; and protein (0.18 mg/ml) concentration was assessed with +50% conc. of KNO_3. The diminishment of KNO_3 (-50% conc. of KNO_3) showed the highest lipid output (31.08µg/ml) and the maximum carbohydrate content (0.2 mg /mL) on day 10 of culture. Also, carotenoids increased by 20.51% over control. Total phenolic, flavonoid, and ascorbic acid contents were measured, recording 127.53 ±0.79 mg gallic acid equivalents/ g crude extract, 81.59 ±1.05 mg gallic acid equivalents/ g crude extract 149.27 ±1.04 $mg.g^{-1}$, respectively. In conclusion, this work highlighted those polyunsaturated fatty acids (PUFAs) of *S. obliquus* cultivated at (-50%conc. of KNO_3) that increased by 80%, compared to the control culture; linolelaidic and α-linolenic acids were the main dominant of PUFAs and constituted 17.84 % and 22%, respectively.

INTRODUCTION

Freshwater microalgae are widely distributed in rivers, lakes and polar seas. They have a vast variety of cellular, morphological, structural, and biochemical components **(Chu *et al*., 2004)**. Microalgae have the capacity to produce a variety of physiologically active compounds. Consequently, researchers consider their rapid biomass development and capacity to modify their biochemical composition in response to cultivation circumstances as interesting organisms. Microalgae are not only single-celled, they are minute in size, having diversity in their morphological structure from the single-celled, colony, filaments, etc. These microalgal flora representatives have enormous potential for use in many fields of research and technology **(Olasehinde *et al*., 2017)**.

Proteins, polysaccharides, lipids, polyunsaturated fatty acids (PUFAs), vitamins, pigments, phycobiliproteins, enzymes, and other physiologically active components are abundant in microalgae. Antioxidant, antibacterial, antiviral, anticancer, regenerative,

antihypertensive, neuroprotective, and immune-stimulating properties are described in biologically active compounds from microalgae **(El-Sheekh *et al*., 2019; Gürleket *et al*., 2019)**. The active compounds of microalgae are considered valuable auxiliary biomolecules for manufacturing feed and functional foods in the fields of pharmacology, medicine, cosmetics, the chemical industry, fish farming, energy and agriculture **(Bhattacharjee, 2021)**.

Due to the propensity of microalgae to grow rapidly, their ability to adapt to extreme environmental conditions, and their ease of cultivation and handling, numerous species of *Scenedesmus* are used in a variety of applications throughout the world. **(Lürling, 2003; Pultz & Gross, 2004)**. The high nutritional content and bioactivities of *Scenedesmus* spp. qualified them to be utilized in numerous biotechnological applications **(Chacón-Lee & Gonzalez-Marino, 2010; Guedes *et al*., 2012)**. Regarding the chemical composition of the enrichment medium, nitrogen is a crucial nutrient influencing the biomass growth and lipid production of different microalgae **(Griffiths & Harrison, 2009)**.

Nitrogen is ranked among the most important nutrients for proliferation since nitrogen is a component of peptides, energy transfer molecules, genetic materials, chlorophylls, enzymes, and genetic materials in algal cells, as well as structural and functional proteins **(Cai *et al*., 2013)**. The rate of cell development and microalgae's biochemical makeup are greatly influenced by the nitrogen concentration in the culture medium **(Wang *et al*., 2013)**. Microalgae can modify lipid metabolism naturally through the stress response resulting from nitrogen deprivation. Despite that the lack of nitrogen appears to prevent the cell cycle and the creation of almost all cellular components, the rate of lipid synthesis is still higher, causing starved cells to accumulate oil **(El-Sheekh & Abomohra, 2012)**.

Although constant nitrogen deprivation causes microalgae to have higher lipid and carbohydrate contents, it slows down their growth rate, which lowers their overall output **(Pancha *et al*., 2014)**. Numerous studies have shown that nitrogen scarcity increases fat formation and decreases biomass productivity **(El-Kassas, 2013)**. Numerous studies have demonstrated that nitrogen deficiency alters the biochemical composition of organisms, including the amount of protein and carbohydrates, pigments, lipids, and fatty acids, adding to the photosynthetic activity of microalgae **(Fan *et al*., 2014)**.

The present work was proposed to examine the use of different concentrations of potassium nitrate (KNO_3) as a nitrogen source for inducing the biomolecules synthesis from freshwater microalga *Scenedesmus obliquus* through its effects on the level of carbohydrates, lipids, protein, and carotene contents of the investigated microalgae.

MATERIALS AND METHODS

Microalgal strain growth condition

The green microalga *S cenedesmus obliquus* was acquired from the Phycology Research Unit Culture Collection, Faculty of Science, Tanta University in Egypt. In 1L Erlenmeyer flasks stopped with cotton plugs, 700ml of Khul medium **(Khul & Lorenzen, 1964)** was used. The flasks were sterilized in an autoclave for 20 minutes at

121ºC and 1.5 atmospheric pressure. After cooling, a specific volume of *Scenedesmus obliquus* pre-cultures was added to the Erlenmeyer flasks. Aeration was sustained through silicon tubes, with one end connected to the culture flask and the other to the aerator supplied with a bacterial filter. Algal culture flasks were subjected to incubation conditions of a temperature of 25 ±2ºC and a continuous fluorescent light at 45 mole $m^{-2}s^{-1}$.

Table 1. The chemical composition of Kuhl medium **(Kuhl & Lorenzen, 1964)**.

Nutrients	**Stock solution Quantities (g/L)**
Macronutrient	
KNO_3	101.11
$Na_2HPO4.2H_2O$	8.9
$NaH_2PO_4.H_2O$	62.12
$MgSO_4.7H_2O$	24.65
$CaCl_2.2H_2O$	1.47
Micronutrients added to 1000 ml of distilled water	
H_3Bo_3	0.06100
$MnSO_4.H_2O$	0.16900
ZnSo4.7H2O	0.28700
$CuSO_4.5H2O$	0.00249
$(NH4)_6MO_7O_{24}.4H2O$	0.01235

$FeSO_4.7H_2O$ Fe-EDTA complex

The Fe-EDTA complex was made by briefly heating 0.69 g of $FeSO_4.7H_2O$ and 0.93 disodium salts of ethylene diamine tetra acetic acid (EDTA) in 80ml of deionized distilled water. The solution was diluted to a volume of 100ml after reaching room temperature.

Optimization of media composition

Nitrogen source concentrations

Potassium nitrate (KNO_3) is the source of nitrogen in the Kuhl medium and its original concentration in standard Kuhl is 101.11 $g.L^{-1}$, with original nitrate concentration in the media containing different nitrate concentrations (-50%, +50%, -100%(zero)).

Table 2. Experiment scheme showing Kuhl medium with different nitrogen concentrations

KNO_3 concentrations (g L^{-1})	
Control(100%)	101.11
-50%	50.55
+50%	151.66
-100%(zero)	0

Measurement of cell growth

Optical density

Growth curves were evaluated by measuring the optical density at 680nm every two days using spectrophotometer (SHIMADZU UV- 2401PC, Japan) **(El-Sheekh *et al.*, 2022)**.

Determination of the biochemical constituents in the tested algae

To estimate the total soluble protein and carbohydrate, the microalgal cells (3ml of culture was centrifuged at 3000rpm for 5min) were extracted with 1 N NaOH in a boiling water bath for 2 hours according to **Payne and Stewart (1988)**. The amount of protein content was determined following the method of **Bradford (1976)**. While, carbohydrate content was calculated following the study of **Kochert's (1973)**. In addition, the Park method was modified to directly measure lipid content using the Sulfophosph vanillin (SPV) reaction **(Jaeyeon Park, 2016)**.

Secondary metabolite estimation

Preparation of algal extract

The extraction was performed with methanol solvent by steeping the material in the corresponding solvent (0.3g of algae with 30ml of methanol) in a conical flask, which was subsequently closed with cotton wool. Next, the samples were retained on a rotatory shaker at 120rpm at 20- 300◦C for 2 days. Then, the extract was filtered and ready to use. Estimation of phenolic content was done according to **Taga *et al.* (1984)**, while the aluminium chloride colorimetric assay was carried out for flavonoid quantification **(Chang *et al.*, 2002)**; the estimation of the ascorbic acid was conducted according to **Oser (1979)**.

Extraction of total lipids and analysis of fatty acid

The modified Folch technique **(Folch *et al.*, 1957)** was used to extract the total lipid content. Whereas, the modified procedure of **Zahran and Tawfeuk (2019)** was used to estimate the fatty acid profile.

Statistical analysis

Three replicates' means and standard deviations (SD) were used to express the data. Using one-way analysis of variance (ANOVA) and Duncan's multiple range testing for data with a significant difference at $P< 0.05$, the collected data were statistically evaluated using the SPSS 23.0 programme.

RESULTS

Growth by optical density

Fig. (1) shows the different concentrations of KNO_3 (Control, -100% (zero),+50% and -50%) using optical density (OD). KNO_3 with concentration + 50% showed the highest growth (1.94 ±0.01), compared to control culture (1.56 ±0.01) on day 10 of cultivation, followed by - 50% conc. with a culture growth rate of 1.46 ±0.01. Culture with no nitrogen source recorded the lowest growth of *S. obliquus* (1.09 ±0.01).

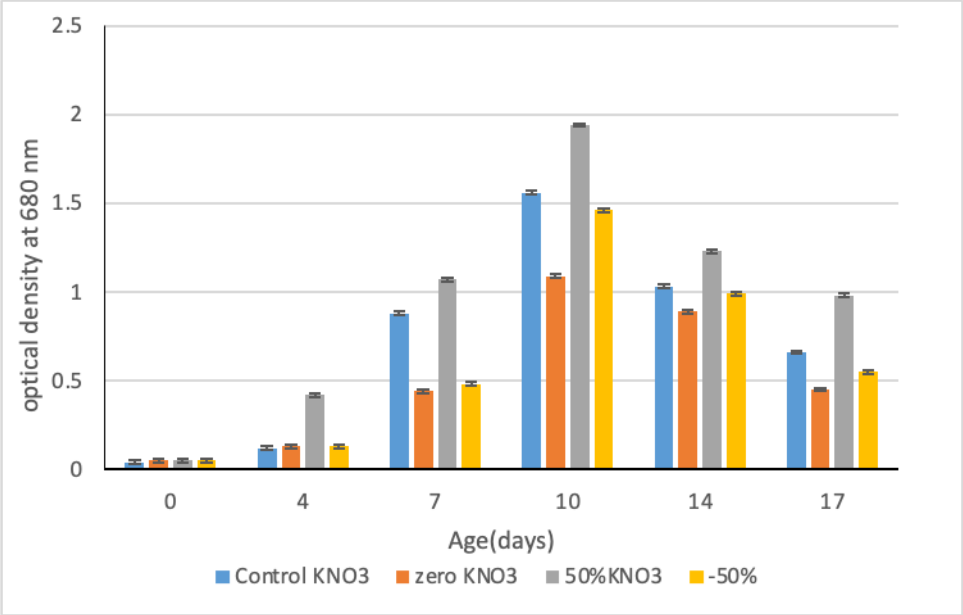

Fig. 1. Effect of different KNO_3 concentrations on the growth of *Scenedesmus obliquus* measured as optical density in different incubation periods

Each column represents the mean value of three replicates; Error bars show the SD for measurements; SD: Standard deviation. When employing one-way ANOVA ($P< 0.05$), different letters represent the statistical comparisons between groups.

Protein content (mg/ml)

Table (1) reveals that, the highest protein content (0.18 ±0.01) was observed in *S. obliquus* culture treated with +50%conc. of KNO_3, compared to the control culture (0.14 ±0.01) on the 10^{th} day of the growth, while culture treated with both -50 % conc. of KNO_3 and zero% conc. of KNO_3 caused a significant reduction in protein content (0.10 ±0.001, 0.11 ±0.01, respectively).

Table 1. Effect of different KNO_3 concentrations on protein content(mg/ml) of *Scenedesmus obliquus* at different incubation periods

Day	Control (100% KNO_3)	Zero% KNO_3	+ 50 % KNO_3	- 50 % KNO_3	F. value
0	0.01±0.001^a	0.01±0.001^a	0.01±0.01^a	0.01±0.001^a	0.354 NS
4	0.03±0.01^b	0.02±0.001^b	0.05±0.01^a	0.02±0.001^b	36.60*
7	0.07±0.01^b	0.06±0.01^c	0.08±0.01^a	0.04±0.001^d	339.70*
10	0.14±0.01^b	0.11±0.01^c	0.18±0.01^a	0.10±0.001^d	445.70*
14	0.09±0.01^b	0.07±0.01^c	0.1±0.01^a	0.05±0.001^d	336.60*
17	0.05±0.01^b	0.04±0.01^c	0.07±0.01^a	0.03±0.001^d	338.60*

The mean and standard deviation (SD) of three replicates are used to express data. When employing one-way ANOVA (P< 0.05), different letters represent the statistical comparisons between groups.

Carbohydrate content (mg/ ml)

Data recorded in Table (2) clarify that culture of *Scenedesmus obliquus* treated with -50% conc. of KNO_3 and zero% conc. of KNO_3 recorded the highest carbohydrate content (0.37 ±0.00and 0.29 ±0.00, respectively), compared to the control culture on the 10th day of the cultivation, while +50% conc. of KNO_3 caused a significant reduction in carbohydrate content (0.20 ±0.00).

Table 2. Effect of different KNO_3 concentrations on carbohydrate (mg/ ml) content of *Scenedesmus obliquus* in different incubation periods

Day	Control (100% KNO_3)	Zero% KNO_3	+ 50 % KNO_3	- 50% KNO_3	F. value
0	0.01±0.00^a	0.01±0.00^a	0.01±0.00^a	0.01±0.00^a	0.357 NS
4	0.03±0.00^c	0.04±0.00^b	0.02±0.00^d	0.06±0.00^a	37.60*
7	0.07±0.00^c	0.09±0.00^b	0.05±0.00^d	0.11±0.00^a	339.70*
10	0.22±0.00^c	0.29±0.00^b	0.20±0.00^d	0.37±0.00^a	445.71*
14	0.14±0.00^c	0.17±0.00^b	0.11±0.00^d	0.2±0.00^a	336.70*
17	0.05±0.00^c	0.08±0.00^b	0.04±0.00^d	0.09±0.00^a	335.80*

The mean and standard deviation (SD) of three replicates are used to express data. When employing one-way ANOVA (P< 0.05), different letters represent the statistical comparisons between groups.

Lipid content (µg/ ml)

For the results of carbohydrate content, *S. obliquus* showed the highest lipid content at -50% conc. of KNO_3 (31.08 ±0.03) on day 10 of cultivation. Culture with no nitrogen source showed the second highest lipid content (27.02 ±0.00) compared to control culture (26.44 ±0.00), while, KNO_3 (+50% conc.) recorded the lowest lipid content (25.74 ±0.00) on the 10^{th}day of cultivation

Table 3. Effect of different KNO_3 concentrations on lipid content (µg/ ml) of *Scenedesmus obliquus* during different incubation periods

Day	Control (100% KNO_3)	Zero% KNO_3	+ 50 % KNO_3	- 50 % KNO_3	F. value
0	0.26 ±0.03^a	0.26±0.03^a	0.26±0.03^a	0.26±0.03^a	0.351 NS
4	3.44 ±0.01^c	3.78±0.02^b	2.88±0.03^d	3.91±0.00^a	38.60*
7	11.34±0.01^c	12.75±0.00^b	9.69±0.03^d	13.61±0.00^a	339.70*
10	26.44±0.00^c	27.02±0.00^b	25.74±0.00^d	31.08±0.03^a	443.70*
14	16.66±0.00^c	18.92±0.00^b	14.29±0.00^d	22.73±0.00^a	336.60*
17	9.65±0.01^c	10.26±0.00^b	7.30±0.03^d	12.65±0.01^a	338.60*

The mean and standard deviation (SD) of three replicates are used to express data. When employing one-way ANOVA (P<0.05), different letters represent the statistical comparisons between groups.

Carotenoids content (µg/ ml)

The results illustrated in Table (4) show that the culture of S. *obliquus* under -50% of KNO_3 recorded the highest carotenoids content (15.16 ±0.01) after 10 days of growth, compared to the control culture (12.58 ±0.45); while, the lowest carotenoids content (10.33 ±0.15 and 6.68 ±0.01) was recorded in KNO_3 (+50% and zero %, respectively).

Table 4. Effect of different KNO_3 concentrations on carotenoids content of *Scenedesmus obliquus* during different incubation periods

Day	Control (100% KNO_3)	Zero% KNO_3	+ 50 % KNO_3	- 50 % KNO_3	F. value
0	0.23±0.00^a	0.23±0.00^a	0.23±0.00^a	0.23±0.00^a	0.354 NS
4	0.57±0.01^d	1.11±0.01^b	0.97±0.01^c	2.84±0.00^a	37.60*
7	5.67±0.01^b	4.01±0.00^c	4.82±0.01^c	7.84±0.01^a	338.70*
10	12.58±0.45^b	6.68±0.01^d	10.33±0.15^c	15.16±0.01^a	443.70*
14	10.48±0.08^b	5.14±0.02^d	8.24±0.05^c	13.47±0.01^a	336.60*
17	9.91±0.05^b	4.80±0.02^d	6.16±0.01^c	11.16±0.00^a	354.60*

The mean and standard deviation (SD) of three replicates are used to express data. When employing one-way ANOVA (P< 0.05), different letters represent the statistical comparisons between groups.

Estimation of secondary metabolites

At different concentrations of nitrogen (KNO_3)

Concerning phenolics, the maximum value was recorded for KNO_3 (-50% conc.) (127.53 ±0.79) mg gallic acid equivalents/ g crude extract), followed by +50%conc. of KNO_3 (110.81±1.55 mg gallic acid equivalents/ g crude extract), while the minimum value was showed with zero % conc. of KNO_3 (68.09 ±1.76mg gallic acid equivalents/ g crude extract) (Fig. 2).

Moreover, the total flavonoid content of *S. obliquus* algal extracts was at its highest at a concentration of -50% conc. of KNO_3 (81.591.05 mg quercetin equivalents/g crude extract), while the lowest value was detected at a concentration of zero% conc. of KNO_3 (67.091.70 mg quercetin equivalents/g crude extract), as shown in (Fig. 2).

Ascorbic acid content was investigated (Fig. 2); the -50% conc. of KNO_3 recorded the highest value (149.27 ±1.40 mg/g). While, the lowest value was detected at the zero % conc. of KNO_3 (37.69 ±0.87 mg/ g.

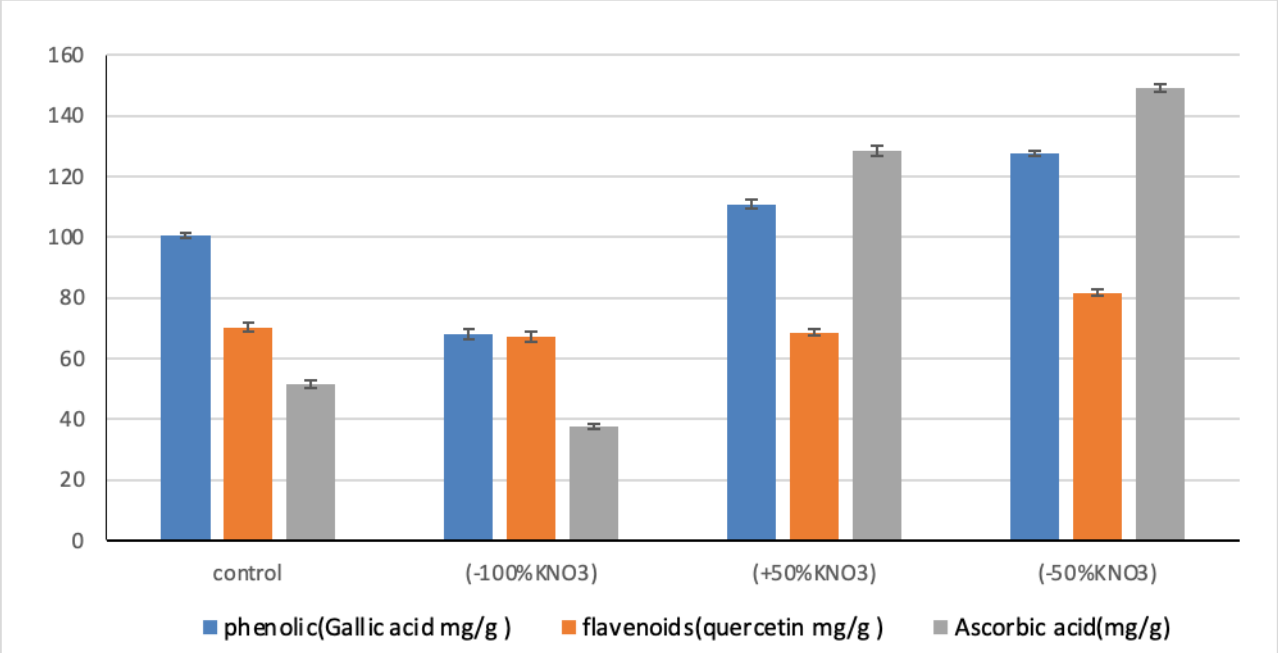

Fig. 2. Phenolics, flavonoids, and ascorbic acid at different KNO_3 concentrations in *Scenedesmus obliquus*

Each column represents the mean value of three replicates; Error bars show the SD for measurements; SD: Standard Deviation. When employing one-way ANOVA, different letters are used to denote statistical comparisons between groups (P<0.05).

Fatty acids content

The results in Table (5) emphasize that polyunsaturated fatty acids (PUFAs) of *S. obliquus* cultivated at the -50%conc. of KNO_3 increased by 80%, compared to the control culture, which contained 27.7 % of the total fatty acids; linolelaidic and α-linolenic acids were the main dominant of PUFAs, with 17.84 and 22%, respectively, while they

represented only 10.82% and 5.57%, respectively, in the control culture. On the contrary, saturated fatty acids (SFA) of the control culture (66%) were higher than the culture of the -50% conc. of KNO_3 (43.14%). Palmitic acid was the main saturated fatty acid in both the control and the -50%conc. of KNO_3 cultures. A notable reduction of mono-unsaturated fatty acid content (MUFAs) was recorded in both the control and -50%conc. of KNO_3 cultures.

Table 5. Fatty acid profiles of *Scenedesmus obliquus* grown in control and -50% KNO_3 culture

Fatty acid	C-number	Control	-50%KNO_3
SFAs			
Capric acid	C10:0	3.45	3.87
Tridecylic acid	C12:0	9.57	2.01
Myristic acid	C14:0	6.68	0.77
Palmitic acid	C16:0	29.5	27.81
Stearic acid	C18:0	16.8	8.68
MUSFAs			
Oleic acid	C19:1(ω9)	6.3	7.0
PUFAs			
Linolelaidic acid	C18:2(ω6)	10.82	17.84
-11,14Eicosadienoic acid	C21:2	4.61	ND
α-Linolenic acid	C18:3(ω3)	5.57	22.02
-7,10Hexadecadienoic acid	C17:2	6.7	10.0
Total SFAs		66.0	43.14
Total MUFAs		6.3	7.0
Total PUFAs		27.7	49.86

(SFA): Saturated fatty acids, PUSFA: Polyunsaturated fatty acid.
USFA: Unsaturated fatty acid, MUSFA: Monounsaturated fatty acid.

DISCUSSION

The mixture of macro- and micronutrients in the media and their availability in cultures significantly impact the growth of algal cultures and their nutritional structure **(Chandra *et al.*, 2020)**. Numerous media lack the ideal ratio of macro- and micronutrients. Since algal cells are not hazardous to nutrients, including nitrogen, potassium, magnesium, sulphur, and sodium; they can be added at large concentrations **(Chandra *et al.*, 2020)**.

Numerous biomolecules, including proteins, chlorophylls, and nucleic acids, which are crucial for maintaining algal development, include a significant amount of nitrogen **(Li *et al.*, 2016)**. Increases in lipid and carbohydrate contents frequently occurred as a result of nitrogen restriction or famine; however, it was at the expense of biomass production **(Sun *et al.*, 2018 ; Chandra *et al.*, 2020)**. Given this data, the most logical place to start to increase the biomass and lipid productivity of possible strains of microalgae is by choosing an appropriate growing medium and optimizing the nitrogen source **(Mutlu *et al.*, 2011; Nath *et al.*, 2012)**. Additionally, it has been noted that nutrition level regulation affects the content and accumulation of lipids **(Zhang *et al.*, 2019)**.

Obtained results showed that increasing nitrogen of concentration improves the growth of *S. obliquus* measured as optical density, so that the +50% conc. of KNO_3 showed a higher optical density compared to the control by 26.28%. Similar results were obtained by **El-Sayed (2007)**, who concluded that the optimal nitrate concentration for the growth of *Chlorella salina* was attained two times that recorded for the control at 1.5 times the basal medium's concentration. On the other hand, **Feng *et al*. (2011)** demonstrated that one of the elements encouraging *Isochrysis zhangjniangensis* cell division is the addition of nitrate.

Since nitrogen is essential for protein synthesis, its deficiency slows development, alters metabolic pathways, and, as a last resort, changes the biomass's biochemical makeup. For large-scale production purposes, it is simpler and less expensive to manipulate nitrogen concentration than other parameters **(Go *et al*., 2012)**.

Compared to the control, the protein content of *S. obliquus* is often improved by 28.57% when nitrogen levels rise in the medium to +50% conc. of KNO_3; these results are in contrast with those of **El-Sayed (2007)** who reported that, the optimization of protein synthesis in *Nannochloropsis salina* took place when nitrogen decreased to 0.5 times that of the basal medium and are similar to **Vergara and Niell (1993)** who found that, total soluble protein increased when the algal cells were incubated in nitrogen-sufficient circumstances. On the other hand, a lack of nitrogen affects the production of proteins that are necessary for cell division and photosynthesis, which slows down the pace of cell growth **(Ordög *et al*., 2012)**.

Concerning the carbohydrate content of *S. oblique*, results indicated that the decreasing level of nitrogen (-50% conc. of KNO_3) concentration improved the carbohydrate content by 68.18% above the control. Our findings agree with those of **Jia *et al*. (2015)** who found that, *N. oceanica's* cellular carbohydrate content significantly increased from 5.8 to 17.9% over a 4- day nitrogen-starved culture, which disagrees with the finding of **Markou *et al*. (2012)** who claimed that, raising nitrogen levels in the media used for growing algae enhanced the content of carbohydrates in the algae.

Limiting elements such as N_2 is necessary for algae growth and can increase lipid content **(El-Sheekh *et al*., 2013)**. This coincides with our result, regarding the effect of the decreasing level of nitrogen (-50% conc. of KNO_3) on the increase in the lipid content of *S. obliquus* after 10 days of growth, when compared to the control culture. These findings were in contrast with (Li *et al*., 2010), who found that raising the nitrogen content of the media used to cultivate microalgae increased the lipid content of the algae. On the other hand (Yeesang and Cheirsilp's, 2011) found that algal cells store carbon metabolites as lipids when nitrogen levels are low.The breakdown of nitrogenous substances like protein, which may provide the cell with carbon or energy for the synthesis of lipids, may be the cause of a trigger in lipid accumulation under nitrogen deprivation. (Adams *et al*.,2013; Fan*et al*. 2011, Zhu *et al*. 2014; Huang *et al*., 2019).

In microalgae, carotenoids have reportedly been shown to protect against oxidative stress (Zhang *et al*.,2013). The production of free radical species in the cell as a result of nutrient stress may modify the antioxidant content of the cell. Nutrient stress is well documented to cause the accumulation of carotenoids in various species of microalgae, including *Dunaliella* (Ben-amotz *et al*.,1983) and this demonstrated our results that carotenoids content of *S. obliquus* is increased by (17.55%) under (-50% conc. of KNO_3) compared to control culture. According to numerous reports, the absence or low concentration of specific nutrients, such as nitrogen, functions as a metabolic constraint for microalgae to cause an immediate physiological reaction that initiates

secondary biosynthetic pathways (Touchette and Burkholder, 2000). Additionally, it has been shown that when cultivated in nitrogen-depleting circumstances, *Dunaliella* sp. and *Haematococcus pluvialis* accumulate significant levels of carotenoids, astaxanthin, and its acyl esters (up to 13% w/w) (Harker*et al*., 1996). Also, Hejazi *et al*. (2003) discovered that employing a finite amount of nitrogen that is then consumed during growth can result in an increase in carotene intracellular accumulation. Carotenoids exhibit extremely high antioxidant qualities (Campo *et al*., 2007; Yuan *et al*., 2011; Christaki *et al*., 2013).

Algal biomass's vitamin content can differ greatly between species. According to (Brownand Miller,1992), ascorbic acid (vitamin C) exhibits the highest degree of variability, albeit this could be attributed to variations in how algae are prepared, dried, and stored because ascorbic acid is extremely heat-sensitive. As shown in (Figure 2), ascorbic acid recorded the highest value content in S. *obliquus* when grown under (-50% conc. of KNO_3). These findings are consistent with those of (El-Baz *et al*.,2002), who demonstrated that *Dunaliella salina* accumulated a lot of ascorbic acid when cultivated in conditions with a limiting nitrogen concentration. Also, our results concur with those of (Durmaz *et al*. (2007) and Abd El-baky *et al*., 2004), they cleared that nitrogen stress causes a rise in both alpha-tocopherol and ascorbic acid content in *Dunaliella salina*. Our findings show that the phenolic and flavonoid content of *S. obliquus* increases by 26.81% and 16.13%, respectively, when nitrogen is limited to (-50% conc. of KNO_3) compared to the control culture.

Recent investigations have demonstrated that phenolic chemicals have a crucial role in the antioxidant activity of microalgae (Goiris *et al*., 2012).

By altering peroxidation kinetics and lowering cell membrane fluidity, polyphenols serve as a substrate for the hydrogen peroxide-scavenging enzyme peroxidase and prevent the spread of ROS (Chokshi *et al*., 2017). The amount of phenolics often rises in response to nutritional stress in microalgae (Arnold *et al*.,1995). Nitrogen-limiting treatments(Goiris *et al*., 2015) showed that the phenolic content of three microalgal strains, *Chlorella, Phaeodactylum*, and *Tetraselmis*, decreased.S. *quadricauda's* phenolic content was shown to remain unchanged after UV exposure by (Kovacik *et al*.,2010) however *Chlamydomonas nivalis*' phenolic content increased by (Duval *et al*.,1999).

Nitrogen plays a crucial role in the synthesis of ω3 fatty acids in microalgal species (Bapjai *et al*., 1991; Yongmanitchai and Ward, 1991). Microalgae store PUFAs in their mitochondrial membranes to shield their cell membranes from oxidative damage (Matsui *et al*., 2021). Results obtained by (Abd El-Baky *et al*., 2004) showed that *Dunaliella salina* has a high total lipid content that is rich in ω3 polyunsaturated fatty acids and antioxidant chemicals when nitrogen is limited, are in accordance with our results where *S. obliquus* generates significant amounts of important long-chain PUFAs, (49.86%) of total FA content compared to control culture when nitrogen concentration limited to (-50% conc. of KNO_3) in the medium. Also (Ben-Amotz *et al*., 1985) illustrated that *Botryococcus braunii*, *Dunaliella bardawili*, and *Dunaliella salina* produced more of the fatty acid under low N levels EPA (C20:5), which is a type of omega-3 fatty acid. Fatty acids and carotenoids are two examples of non-enzymatic types of compounds that can guard the body against oxidative harm (Sies and Stahl,1995). Microalgae growth in nitrogen-deficient medium might change their lipid metabolic pathway and accumulate mainly triacylglycerides (TAGs), which are stored in the cytoplasm of microalgae as a source of carbon and energy (Chu *et al*., 2013**)**. Nitrogen-limiting conditions can decrease the thylakoid

membrane cellular content, activates acyl hydrolase, and stimulates phospholipid hydrolysis, resulting in increased intracellular content of fatty acyl-CoA (Chu *et al*., 2013**)**.

CONCLUSION

Our research concluded that an increase in nitrogen levels (+50% conc. of KNO_3) promotes an increase in *S. obliquus* growth and enhances the protein level, while a decrease in nitrogen levels (-50% conc. of KNO_3) raises lipid levels and polyunsaturated fatty acids, carbohydrate levels, and carotenoids content. *S. obliquus* is employed in aquaculture because of an increase in secondary metabolites such as phenolic, flavonoid content, and ascorbic acid. On the other hand, the high content of carotenoids and polyunsaturated fatty acids, which have antioxidant activity, makes our strain *S. obliquus* promising and applicable microalga in the field of algal biotechnology.

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