

RESEARCH PAPER

Optimization of biomass and some metabolites productivity of *Merismopedia tenuissima* and *Spirulina (Arthrospira) platensis* grown under stress conditions

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ABSTRACT:

The attention to using cyanobacteria as a nutrient supplement has increased due to their nutritional value and high bioactive metabolites contents. The reason behind designing such a study is to illustrate and clarify stress conditions effects like nitrogen and phosphorus supplementation and deficiency, salinity stress, and different pH values on the biomass, lipid, protein, amino acid, and carbohydrate productivities of *Merismopedia tenuissima* and *Spirulina (Arthrospira) platensis*. The obtained results revealed that an increase in sodium nitrate by 100% caused an improvement in biomass, protein, and amino acid's productivity of *S. platensis* and *M. tenuissima* by 7.02 % and 7.05, 9.2% and 47.5%, 11.8% and 19.5, respectively while 100% nitrogen deficiency enhanced lipid productivity of *S. platensis* and *M. tenuissima* to 41% and 94%. Moreover, phosphorus limitation led to a reduction in biomass, protein, amino acid, and carbohydrate *S. platensis* to 24.3%, 21.1%, 43.3%, and 28.1%, respectively. However, phosphorus-free medium showed an increase in lipid productivity of *S. platensis* and *M. tenuissima* by 46.8% and 81.8%, respectively. The addition of 0.05 M NaCl concentration to *S. platensis* medium stimulates the biomass, protein, and carbohydrate productivity by 6%, 7.75%, and 18.1%, respectively, whilst, among all concentration, zero M NaCl (control) resulted in increasing biomass, protein, and amino acids, whilst, high concentration (0.3M) of NaCl enhanced lipid productivity to 125.9% and 153.5% at *S. platensis* and *M. tenuissima*, respectively. Applications of high alkalinity (pH 9) increased the productivities of all studied metabolites in *S. platensis* and reduction of all mention metabolites in *M. tenuissima*.

KEY WORDS: *Spirulina platensis*, *Merismopedia tenuissima*, Nutrients stress, pH, Metabolites, Productivity.

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1. INTRODUCTION:

Cyanobacteria, the blue-green algae, are gram-negative eubacteria widely distributed throughout the world. Besides the valuable metabolites produced by cyanobacteria, it represents a wide range of phytoplankton that helps in assessing the quality of the water system (Toma, 2019, Aziz and Yasin, 2019). Cyanobacteria are a treasure of valuable bioactive compounds where comprise high protein content, soluble, and non-soluble carbohydrates, lipids, mineral, antioxidant substances, and essentials vitamins essential and

non-essential amino acids, the later have a feature of providing humans and animals with the essential one (Jung *et al.*, 2019). Because of its importance as a nutritional supplement and a fundamental source of food, cyanobacterial protein has gained global interest and consideration. Therefore, some *Spirulina* species (nowadays are named *Arthrospira*) for the high level of protein in addition to their fiber content, are being consumed as food supplements (Seghiri *et al.*, 2019). In general, cyanobacteria are rich of structurally novel and biologically active metabolites (De Morais *et al.*, 2015).

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Nowadays, the experimental trends are over-concerned with increasing the highly valuable compounds, and biomass contents using cultural stress strategies, manipulation the favorable conditions for the algae to grow and develop. Wherefore, as a kind of resistance, the algae either increase biomass or increase valuable metabolites. Among the stress strategies applied to microalgae cultures can be divided into two main groups: physical and nutrimental factors. The physical strategies are considered as variation in surrounding environmental parameter and operating conditions which overlap with the microalgae growth (pH, salinity, light intensities, an electromagnet), whilst nutrimental strategies are described as a change in the essential ingredients of culture growth media (nitrogen, phosphorus, carbon source, and iron deficiency). A recently conducted study has indicated that the phosphorus deficiency led to a remarkable improvement in the contents of carbohydrate and lipid of *Spirulina platensis* (Markou et al., 2012). However, the productivity of carbohydrate and lipid contents in *Synechococcus* sp. PCC7942 has recorded an enhancement through increasing salinity concentration (Verma et al., 2019). Setta et al. (2014) reported that the carbohydrate content was enhanced by more than 42% of dry weight under a nitrogen-free medium in *Synechococcus subsalsus*. The current study targeted isolating some cyanobacteria species from lakes and freshwater bodies of Assiut Governorate in Upper Egypt, and estimating the effects of manipulating the concentrations of phosphorus, nitrogen, and sodium chloride as well as different pH values on the biomass productivity and productivities of some metabolites of *S. platensis* and *M. tenuissima*.

2. MATERIALS AND METHODS

2.1. Isolation and Purification:

Cyanobacteria strains were isolated from aquatic habitats (freshwater bodies and lakes) in Assiut Governorate, Egypt (Figure 1). Isolation and purification of cyanobacterial species were done by common microbiological isolation methods through standard plating methods described by (Rippka, 1988), and identified as well as authenticated based on a standard manual (Prescott, 1959). Individual colonies were picked

up under sterilized conditions and streaking at Petri dishes (9 cm diameter) containing a solidified medium. This was repeated until uni-algal colonies were established. Isolated and purified species of algae were cultured in Rippka modified medium (Rippka, 1992); (Table 1). *Merismopedia tenuissima* and *Spirulina platensis* were selected for this study based on their potential for biological activities (unpublished data). The purified *M. tenuissima* was inoculated into liquid Rippka, modified medium, and incubated under continuous illumination fluorescent light of $48.4 \mu\text{mol.photon.m}^{-2}.\text{s}^{-1}$ and $27 \pm 2^\circ\text{C}$. The culture flasks were aerated with sterile air by using aquarium air pumps (3 W, Venus Aqua Air Pump Model AP-208 A, China). On the other hand, *S. platensis* was inoculated into liquid Zarrouk's medium (Zarrouk, 1966), and inoculated under the same mentioned conditions (Table 2).



Figure 1: Maps shows A: Map of Egypt, B: Map of Assiut Governorate and C: Sample collection sites (Googal Earth).



Figure 2: A photographic view of the cultivation cultures.

Table (1) Composition of liquid Rippka modified growth medium (Rippka, 1992).

Component	g/L
NaNO ₃	1.5
K ₂ HPO ₄	0.04
MgSO ₄ ·7H ₂ O	0.075
CaCl ₂ ·2H ₂ O	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (disodium salt)	0.001
Na ₂ CO ₃	0.02
Micronutrient solution (for 1 liter)	1 ml
H ₃ BO ₃	2.86
MnCl ₂ ·4H ₂ O	1.81
ZnSO ₄ ·7H ₂ O	0.222
NaMoO ₄ ·2H ₂ O	0.39
CuSO ₄ ·5H ₂ O	0.079
Co(NO ₃) ₂ ·6H ₂ O	0.049
Distilled water	1.0 L
PH to 7.1	

2.2 Algae strain and growth conditions

Cyanobacterial algae (*S. platensis* and *M. tenuissima*) were cultivated axenically as batch cultures in 500 ml Erlenmeyer flasks with Zarrouk's medium (Zarrouk, 1966) and Rippka modified medium (Rippka, 1992), respectively. Different nutrients effects, namely nitrogen [100% (2.5 and 1.5 g L⁻¹), - 50 % (1.25 and 0.75 gL⁻¹), - 75%, (0.625 and 0.375 gL⁻¹), -100% (0 and 0 gL⁻¹) and +100% (5 and 3 gL⁻¹], phosphorus [100% (0.5 and 0.04 gL⁻¹), -50% (0.25 and 0.02 gL⁻¹), -75% (0.125 and 0.01 gL⁻¹), -100% (0 and 0 gL⁻¹) and +100% (1 and 0.08 gL⁻¹], sodium chloride [(control (0 g L⁻¹), 0.05M (2.92 gL⁻¹), 0.1M (5.84 gL⁻¹), 0.2M (11.68 gL⁻¹), 0.3M (17.52 gL⁻¹)] and pH value [control (7), 5, 6, 8 and 9] on the growth, lipid, protein, amino acid and carbohydrate productivities of *S. platensis* and *M. tenuissima*, respectively, were studied (Figure 2).

2.3 Biomass assay

Two approaches were followed to monitor cyanobacterial growth, initially; a daily monitoring approach was done by using chlorophyll (A) according to Metzner *et al.*

(1965), and optical density, according to Fatma *et al.* (1994), whilst the final approach was achieved through determining the cellular dry weight (CDW) after harvesting phase. The following equation illustrates the calculation of the productivity of biomass:

$$\text{Biomass productivity (mg CDWL}^{-1}\text{d}^{-1}\text{)} =$$

$$(\text{CDW}_L - \text{CDW}_E) / (t_L - t_E)$$

Where; CDW_E and CDW_L are representing the CDW (mgL⁻¹) at the start of the culture (t_E) and late exponential phase (t_L), respectively.

Table (2) Composition of Zarrouk's growth medium (Zarrouk, 1966).

Component	g/L
NaHCO ₃	16.8
K ₂ HPO ₄	0.5
NaNO ₃	2.5
K ₂ SO ₄	1.0
NaCl	1.0
MgSO ₄ ·7H ₂ O	0.2
CaCl ₂ ·2H ₂ O	0.04
FeSO ₄ ·7H ₂ O	0.01
Na ₂ EDTA·2H ₂ O	0.08
Trace mineral mix A5	1.0 ml
H ₃ BO ₃	2.86
MnCl ₂ ·4H ₂ O	1.81
ZnSO ₄ ·7H ₂ O	0.222
NaMoO ₄ ·2H ₂ O	0.39
CuSO ₄ ·5H ₂ O	0.079
Co(NO ₃) ₂ ·6H ₂ O	0.0494
Distilled water	1.0 L
Trace mineral mix B6	1.0 ml
Modified	
NH ₄ VO ₃	0.23
K ₂ Cr ₂ (SO ₄) ₄ ·24H ₂ O	0.096
NiSO ₄ ·7H ₂ O	0.0478
Na ₂ WO ₄ ·2H ₂ O	0.0179
Ti ₂ (SO ₄) ₃	0.04
Distilled water	1.0 L
pH to 9	

2.4. Estimation of total lipid:

The sulfophosphovanillin (SPV) technique was used to estimate the total lipid content (Drevon and Schmit, 1964).

2.5. Estimation of total protein:

The Lowry protein assay was used to measure the total protein, which is measured

spectrophotometrically at 750 nm (Lowry *et al.*, 1951).

2.6 Estimation of total carbohydrate:

The anthrone-sulfuric acid technique described by Hedge and Hofreiter (1962) was used to determine the total carbohydrate content. Briefly, the processes of this method involve breaking down the polysaccharides using diluted HCL and turn them into simple sugar, which chemically interacts with anthrone-sulfuric acid as a result of this interaction a green color appears, which is measured spectrophotometrically at 630 nm. To specify the exact numeral values of the total carbohydrate, the calibration standard curve was prepared using glucose.

2.7. Estimation of free amino acids:

The ninhydrin method described by Lee and Takahashi (1966) was used to estimate free amino acids.

2.8. Productivities calculation

Productivities of lipid, protein, carbohydrate, and amino acid were calculated by:

$$Productivity (mgL^{-1}d^{-1}) = Biomass productivity \times C_f$$

The abbreviation, C_f is used to refer to amino acids, carbohydrates, lipids, or proteins' final contents.

2.9. Statistical Analysis

The data were obtained from four independent experiments and measured as a means \pm SE using Excel 2010 program. The statistical software SPSS (Version 16, Released 2007, SPSS Inc., Chicago, IL, USA) was applied to analyze the effect of various factors in this study. In the current study, the ANOVA table was consulted to determine significantly the various concentrations of different factors' effects on studied cyanobacterial metabolites. For comparison of the means, Duncan's multiple range tests ($p < 0.05$) were used.

3. RESULTS

In the initial work stage, from the three different freshwater bodies, samples were collected and eight genera (15 species) of cyanobacteria were identified (Table 3). Of cyanobacterial identified strains, two isolates viz., *S. platensis*, and *M. tenuissima* selected for this study based on their biological activity (unpublished data). The changes in growth patterns caused by applying

different concentrations of nitrogen on the growth of *S. platensis* and *M. tenuissima* were recorded as Chlorophyll a (Chll a) and optical density for 10 days of incubation (Figure 3a, b, c, and d). The obtained results revealed that a decrease in sodium nitrate concentration led to a reduction in Chll a, optical density, and biomass productivity.

Table (3) List of cyanobacteria recorded (+) in water samples

Algal taxa	Water samples		
	S.1	S.2	S.3
Cyanophyta			
<i>Chroococcus limneticus</i>	+		
<i>Chroococcus pallidus</i>	+	+	+
<i>Chroococcus</i> sp.	+	+	
<i>Chroococcus turgidus</i>	+	+	+
<i>Coelosphaerium</i> sp.	+		+
<i>Gomphosphaeria</i> sp.		+	
<i>Merismopedia convoluta</i>	+	+	+
<i>Merismopedia tenuissima</i>	+	+	+
<i>Microcystis aeruginosa</i>	+	+	+
<i>Microcystis</i> sp.	+	+	+
<i>Microcystis wesenbergii</i>			+
<i>Nostoc</i> sp.	+		
<i>Oscillatoria limosa</i>		+	+
<i>Oscillatoria</i> sp.			+
<i>Spirulina platensis</i>			+

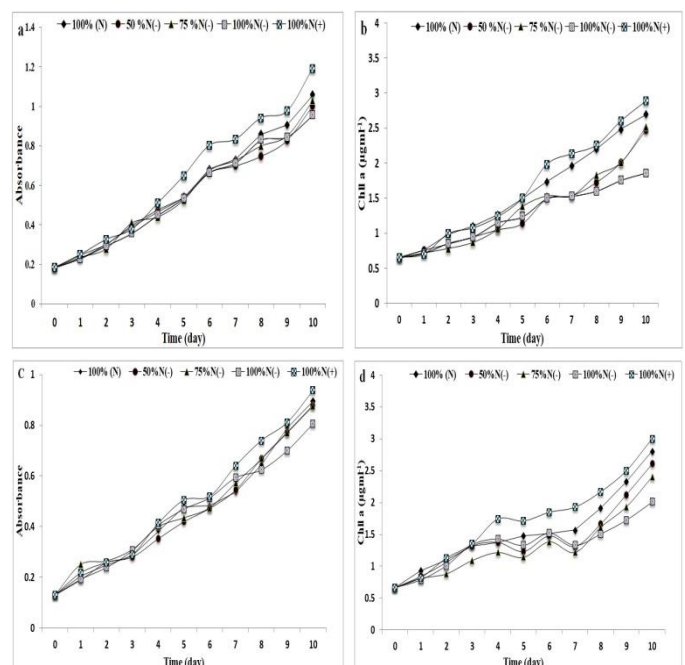


Figure 3: Effect of different concentrations of $NaNO_3$ on a) optical density of *S. platensis* b) chlorophyll a content of *S. platensis* c) optical density of *M. tenuissima* d) chlorophyll a content of *M. tenuissima*

Table (4) Effect of different nitrogen concentrations on biomass, lipids, proteins, amino acids, and carbohydrates productivities of *M. tenuissima* and *S. platensis*

	Productivities (mg/L/day)					
	Biomass	Lipids	Proteins	Amino acids	Carbohydrates	
<i>Merismopedia tenuissima</i>	100% N(C)	61.53±3.34 ^b	5.35±0.43 ^a	4.25±0.3 ^b	1.79±0.12 ^{bc}	1.49±0.09 ^a
	50% N (-)	46.76±2.66 ^a	8.92±0.60 ^b	2.79±0.30 ^a	1.36±0.09 ^b	2.19±0.32 ^a
	75% N (-)	44.76±7.72 ^a	10.11±1.9 ^b	3.42±0.6 ^{ab}	1.48±0.24 ^b	1.82±0.33 ^a
	100% N (-)	38.76±3.62 ^a	10.38±1 ^b	3.18±0.2 ^{ab}	0.68±0.09 ^a	1.52±0.10 ^a
	100% N(+)	65.87±3.86 ^b	5.28±0.50 ^a	6.27±0.43 ^c	2.14±0.20 ^c	1.44±0.12 ^a
<i>Spirulina platensis</i>	100% N(C)	68.17±0.6 ^{bc}	8.39±0.10 ^a	10.9±0.2 ^{cd}	5.15±0.07 ^c	2.99±0.09 ^b
	50% N (-)	62.75±1.4 ^{ab}	9.35±0.12 ^b	7.77±0.38 ^a	3.84±0.47 ^b	1.41±0.07 ^a
	75% N (-)	59.79±2.87 ^a	9.71±0.24 ^b	8.65±0.6 ^{ab}	4.84±0.50 ^{bc}	1.75±0.22 ^a
	100% N (-)	56.13±1.56 ^a	11.83±0.4 ^c	9.8±0.23 ^{bc}	2.74±0.03 ^a	1.42±0.05 ^a
	100% N(+)	72.96±4.12 ^c	8.27±0.13 ^a	11.9±0.3 ^d	5.76±0.34 ^c	3.87±0.24 ^c

The data are given as averages of three replicates ± standard error. Values in each row with different letters are significantly different at P≤0.05. Values in rows with same letters are not significantly different.

productivity. The most distinct increase in lipid productivity of *S. platensis* and *M. tenuissima*

Table (5) Effect of different phosphorus concentrations on biomass, lipids, proteins, amino acids, and carbohydrates productivities of *Merismopedia tenuissima* and *Spirulina platensis*

	Productivities (mg/L/day)					
	Biomass	Lipids	Proteins	Amino acids	Carbohydrates	
<i>Merismopedia tenuissima</i>	100% P(C)	66.42±6.8 ^{ab}	6.54±0.79 ^a	7.87±0.89 ^b	2.40±0.26 ^b	1.92±0.26 ^a
	50% P (-)	55.53±8.6 ^{ab}	9.67±1.87 ^{ab}	5.34±0.12 ^a	1.83±0.28 ^{ab}	2.50±0.11 ^a
	75% P (-)	52.20±8 ^{ab}	11.04±1.69 ^b	6.02±0.92 ^{ab}	1.79±0.29 ^{ab}	2.02±0.29 ^a
	100% P (-)	50.31±1.3 ^a	11.89±0.94 ^b	6.40±0.72 ^{ab}	1.13±0.02 ^a	2.10±0.38 ^a
	100% P (+)	70.53±1 ^b	5.97±0.44 ^a	9.85±0.15 ^c	3.22±0.23 ^c	1.90±0.07 ^a
<i>Spirulina platensis</i>	100% P(C)	73.3±4.38 ^{bc}	8.36±0.36 ^a	11.20±0.5 ^b	6.23±0.35 ^c	2.99±0.22 ^c
	50% P (-)	69.04±1.1 ^b	9.86±0.21 ^b	8.48±0.13 ^a	4.34±0.38 ^{ab}	1.23±0.09 ^a
	75% P (-)	61.42±1.6 ^a	10.34±0.31 ^b	8.72±0.40 ^a	4.97±0.33 ^{bc}	2.30±0.26 ^b
	100% P (-)	55.50±1.5 ^a	12.27±0.08 ^c	8.84±0.27 ^a	3.53±0.55 ^a	2.15±0.19 ^b
	100% P (+)	78.17±1.5 ^c	8.60±0.13 ^a	12.35±0.5 ^b	8.17±0.39 ^d	3.77±0.11 ^d

The data are given as averages of three replicates ± standard error. Values in each row with different letters are significantly different at P≤0.05. Values in rows with same letters are not significantly different.

The most pronounced reduction in biomass productivity of *S. platensis* and *M. tenuissima* amounted to 17.7% and 37%, respectively, at a 100% decrease in NaNO₃ (Table 4). On the other hand, the biomass productivity was increased when cultured under testing has grown in a 100% increase of nitrogen. The present study has proven that nitrogen starvation causes an increase in lipid

were observed at a 100% decrease in NaNO₃, which amounted to 41%, 94%, respectively, compared to control (Table 4). However, the increase in nitrogen concentration by 100% decreased the lipid productivity of *S. platensis* and *M. tenuissima* by 1.4%, 1.3%, respectively. Increasing nitrogen concentration by 100% resulted in an increase of protein and amino acid productivities by 9.17%, 47.5%, and 11.8%,

19.5% in *S. platensis*, and *M. tenuissima*, respectively, whilst, the decrease in sodium nitrate led to a reduction in protein and amino acid productivities (Table 4). The increase or decrease of nitrogen concentration was not dependent on the carbohydrate productivity of *M. tenuissima*. In contrast, the carbohydrate productivity of *S. platensis* significantly increased to 29.4% under nitrogen-rich conditions (Table 4).

The changes in growth patterns caused by applying different concentrations of phosphorus on the growth of *S. platensis* and *M. tenuissima* that recorded as Chl a and optical density were studied for 10 days of incubation (Figure 4a, b, c, and d). The results in this study cleared that a decrease in phosphorus concentration led to a reduction in Chl a and optical density as well as biomass productivity.

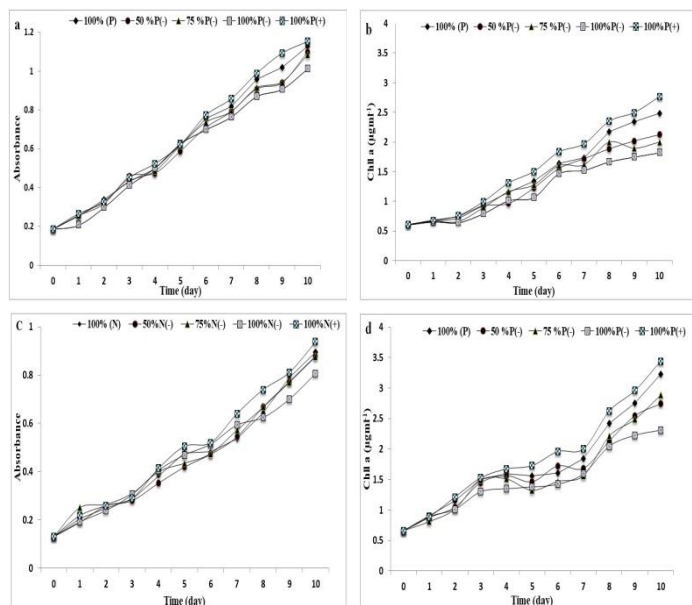


Figure 4: Effect of different concentrations of KH_2PO_4 on a) optical density of *S. platensis* b) chlorophyll a content of *S. platensis* c) optical density of *M. tenuissima* d) chlorophyll a content of *M. tenuissima*

The reduction in biomass productivity amounted to 24.28 and 24.25% in *S. platensis*, and *M. tenuissima*, respectively at a 100% decrease in phosphorus (Table 5). However, no significant change in biomass productivity when grown in a 100% increase in phosphorus concentration. Phosphorus free medium led to the enhancement of the productivity of lipid in *S. platensis*, and *M. tenuissima* by 46.8%, 81.8% respectively. On the other side, no significant effect appeared when an

application of a high concentration of K_2HPO_4 (+100 %). Increasing phosphorus concentration by 100% resulted in an increase of protein and amino acid productivities by 10.2%, 25.1%, and 31.1, 34.1% in *S. platensis*, and *M. tenuissima*, respectively.

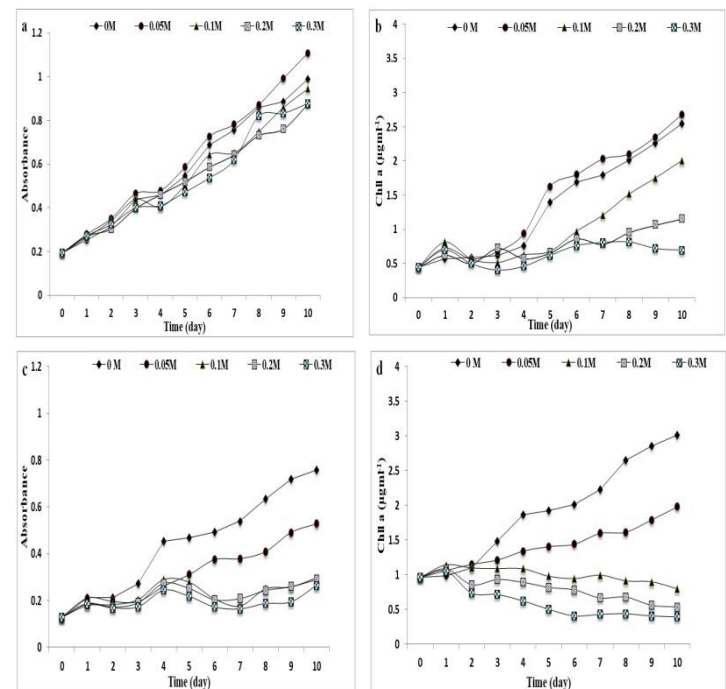


Figure 5: Effect of different concentrations of NaCl on a) optical density of *S. platensis* b) chlorophyll a content of *S. platensis* c) optical density of *M. tenuissima* d) chlorophyll a content of *M. tenuissima*

Whereas, when the *S. platensis* cultures were experienced to free phosphorus concentrations, the productivity of protein and the amino acid was put down by 21% and 43.3%, respectively, while phosphorus depletion resulted in shifting down amino acid productivity by 52.9% comparing to control.

The salinity effects on the growth pattern of *S. platensis* and *M. tenuissima* for 10 days of incubation are illustrated in Figure (5a, b, c, and d). Results indicated that a remarkable increase in Chl a and optical density as well as biomass productivity of *S. platensis* and *M. tenuissima* at 0.05 M NaCl and zero M NaCl, respectively. High salinity concentrations (0.1, 0.2, and 0.3 M) significantly decreased biomass productivity (Table 6). The obtained results clarified that the increase in NaCl concentration increases the productivity of lipids (Table 6). At 0.3 M NaCl, *S. platensis* and *M. tenuissima* showed the most

pronounced increase in lipid productivity amounted to 125.9% and 153.5%, respectively.

productivity of *S. platensis* by 18.1%, whilst, application of 0.3 M NaCl concentration

Table (6) Effect of different sodium chloride concentrations on biomass, lipids, proteins, amino acids, and carbohydrates productivities of *M. tenuissima* and *S. platensis*

	Productivities (mg/L/day)					
	NaCl (M)	Biomass	Lipids	Proteins	Amino acids	Carbohydrates
<i>Merismopedia tenuissima</i>	C. (0)	75.09±2.39 ^c	4.26±0.28 ^a	6.51±1.5 ^b	2.07±0.13 ^c	0.62±0.09 ^a
	0.05	73.09±2.35 ^c	5.16±0.14 ^a	3.0±0.28 ^a	1.74±0.03 ^{bc}	0.82±0.09 ^{ab}
	0.1	62.53±0.77 ^b	6.74±0.41 ^b	2.87±0.7 ^a	1.38±0.001 ^{bc}	0.85±0.18 ^{ab}
	0.2	52.79±0.97 ^a	9.06±0.29 ^c	1.35±0.2 ^a	0.89±0.15 ^a	0.96±0.03 ^{ab}
<i>Spirulina platensis</i>	0.3	48.09±0.56 ^a	10.8±0.38 ^d	1.8±0.04 ^a	0.88±0.21 ^a	1.03±0.09 ^b
	C. (0)	86.8 ±0.7 ^c	5.18±0.34 ^a	12.9±0.5 ^c	7.26±0.05 ^c	3.14±0.05 ^c
	0.05	92±1.2 ^c	6.56±0.20 ^{ab}	13.9±0.3 ^c	7.52±0.06 ^c	3.71±0.31 ^d
	0.1	71.33±8.98 ^b	8.01±1.13 ^{bc}	9.02±0.5 ^b	5.50±0.74 ^b	2.53±0.15 ^b
	0.2	51.58±3.10 ^a	9.36±0.48 ^c	5.1±0.58 ^a	2.64±0.25 ^a	1.99±0.12 ^{ab}
	0.3	46.50±0.38 ^a	11.7±0.05 ^d	4.56±0.2 ^a	2.76±0.03 ^a	1.89±0.13 ^a

The data are given as averages of three replicates ± standard error. Values in each rows with different letters are significantly different at P≤0.05. Values in rows with same letters are not significantly different.

The protein and amino acid productivities were decreased with increasing salt concentration; the high concentration (0.3M) of NaCl resulted in 64.6%, 72.3%, and 62%, 57.4% reduction in protein, and amino acid productivity of *S. platensis* and *M. tenuissima*, respectively (Table 6). Moreover, promoting medium with 0.05 M NaCl concentration stimulates the carbohydrate

Table (7) Effect of different pH values on biomass, lipids, proteins, amino acids, and carbohydrates productivities of *Merismopedia tenuissima* and *Spirulina platensis*

	Productivities (mg/L/day)					
	Biomass	Lipids	Proteins	Amino acids	Carbohydrates	
<i>Merismopedia tenuissima</i>	C. (7)	94.76±1.93 ^c	10.9±0.27 ^a	5.9±0.30 ^b	2.20±0.24 ^d	1.83±0.21 ^a
	5	73.20±6.74 ^a	9.65±0.95 ^a	7.96±0.31 ^c	1.15±0.10 ^a	1.89±0.11 ^a
	6	93.87±0.84 ^{bc}	9.65±0.38 ^a	7.81±0.16 ^c	1.86±0.03 ^{cd}	2.42±0.42 ^a
	8	82.09±3.95 ^{ab}	8.22±0.61 ^a	6.75±0.38 ^b	1.62±0.07 ^{bc}	2.08±0.13 ^a
<i>Spirulina platensis</i>	9	72.64±2.70 ^a	8.14±1.40 ^a	4.71±0.34 ^a	1.35±0.10 ^{ab}	1.83±0.52 ^a
	C. (7)	66±9.89 ^a	10±0.24 ^{bc}	8.92±0.07 ^a	5.95±0.03 ^{ab}	1.60±0.06 ^a
	5	62.17±0.44 ^a	5.71±0.26 ^a	10.1±0.43 ^{ab}	3.68±0.21 ^a	1.60±0.13 ^a
	6	69.58±2.72 ^{ab}	7.4±0.56 ^{ab}	12.1±0.4 ^{abc}	4.47±0.67 ^a	2.24±0.04 ^{ab}
	8	75.88±1.09 ^{ab}	11.1±0.19 ^c	13.7±0.34 ^{bc}	8.19±0.10 ^{bc}	2.92±0.28 ^{bc}
	9	84±0.89 ^b	13.4±2.27 ^c	15.1±2.37 ^c	8.59±1.50 ^c	3.03±0.40 ^c

The data are given as averages of three replicates ± standard error. Values in each rows with different letters significantly different at P≤0.05. Values in rows with same letters are not significantly different.

stimulates the carbohydrate productivity of *M. tenuissima* by 66.1%.

The data in table 7 and Figure (6 a, b, c, and d) shows that the best-suited pH value for the growth of *S. platensis* and *M. tenuissima* was 9 and 7, respectively, with a noticeable enhancement in the productivity of biomass. The alkaline pH value (pH 9) significantly increases the lipid, protein, amino acids and carbohydrate productivities in *S.*

platensis by 34%, 69.3%, 44.4% and 89.4% respectively and decrease protein and amino acids productivities in *M. tenuissima* by 20.2% and 38.6% (Table 7). However, the lipid and carbohydrate productivities in *M. tenuissima* are pH-independent, which means increasing or decreasing the value of the pH does not affect the increased lipid and carbohydrate.

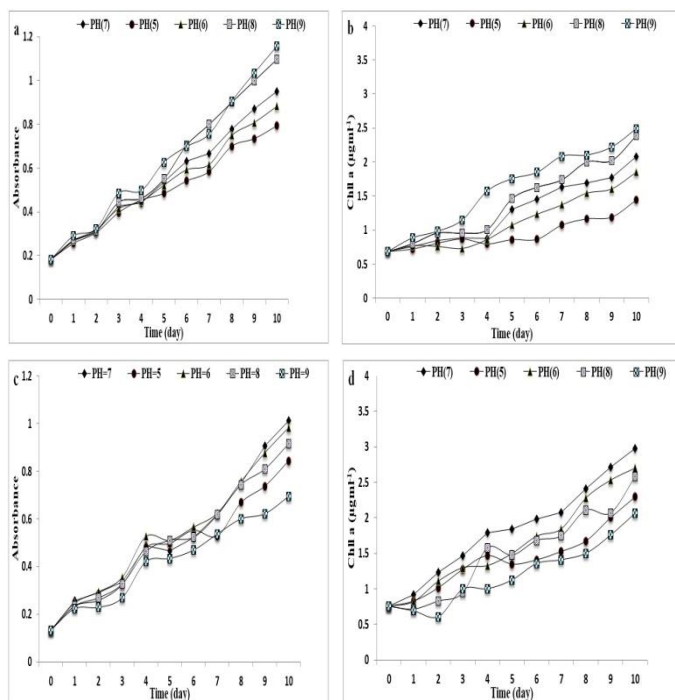


Figure 6: Effect of different pH values on a) optical density of *S. platensis* b) chlorophyll a content of *S. platensis* c) optical density of *M. tenuissima* d) chlorophyll a content of *M. tenuissima*

The increase and decrease of phosphorus concentration showed consistency in carbohydrate productivity of *M. tenuissima*, whilst, the culture of *S. platensis* grown in phosphorus limitation of K_2HPO_4 resulted in a decrease in carbohydrate productivity, furthermore, the 50% decrease in phosphorus concentration was followed by a reduction in productivity of carbohydrate by 58.9%, compared to the control. The most distinct increase in carbohydrate productivity was observed at a 100% increase in K_2HPO_4 amounted to 26.1% (Table5).

4. DISCUSSION

Cyanobacteria have gained importance as human food and pharmaceutical agent because it is rich with vitamins, protein, essential fatty acids carotenoids, lipids, and carbohydrates (De Morais *et al.*, 2015); so many studies were established to optimize these critical compounds production. The previously mentioned experiment resulted that the concentrations of sodium nitrate inversely proportionate to the productivity of biomass and the growth parameters of the investigated strains. The same findings are recorded by Hifney *et al.* (2013), who observed completely removed nitrogen sources from medium caused a severe drop in the biomass of *Spirulina* sp. Yang *et al.* (2018) also observed that nitrogen-deficient conditions decreased the growth pattern for *Chlamydomonas reinhardtii*. Moreover, Menegol *et al.* (2017) noticed a reduction in algal biomass of *Heterochlorella luteoviridis* under low nitrate concentration. However, the biomass productivity of studied strains was improved when cultured under nitrogen enrichment conditions. It has been authorized that higher levels of nitrogen affect positively on the growth of cyanobacteria (Jonte *et al.*, 2013). Nitrogen has an influential role in the metabolism of fatty acids and lipids existing in different kinds of microalgae. Furthermore, as nitrogen is easily manipulated and cheap compared to other factors, it is intensively used for enhancing the lipid accumulation, which is used as a feedstock for biofuel production.

In the current study, nitrogen starvation caused an increase in lipid productivity of the studied cyanobacteria. This finding is in accordance with the observation of Fawzy, (2017) who noticed that the deficiency of nitrogen enriched the lipid content and productivity of *Asteromonas gracilis* by (29.26 % and 10.21 $mgL^{-1}d^{-1}$, respectively. Also, Yeesang and Cheirsilp (2011) reported that under nitrogen-starvation conditions, the lipid accumulation of microalgae increases as a result of their ability to store energy in form lipids, which serves as an essential carbon source, helping them to resist such conditions. On the other hand, De Bhowmick *et al.* (2015) reported that under nitrogen depletion conditions, the increase in the lipid content occurs as a result of the activation of acyl hydrolase, a decrease in the cellular content of the thylakoid membrane and stimulation of phospholipids hydrolysis. In the current study, the increase in nitrogen

concentration resulted in decreased lipid productivity of the studied cyanobacteria. The same findings are recorded by sassano *et al.* (2010), who recorded the negative effect of nitrogen-rich medium on the accumulation of lipids in the case of *Arthrospira platensis*. The supplementation of medium with 100% NaNO₃ followed by a remarkable enhancement in the productivity of amino acid and protein in the studied strains. An increase in biomass and protein production by increasing nitrogen concentration has been widely supported by various reports from *S. platensis* (Colla *et al.*, 2007), *Dunaliella viridis* (Loaiza *et al.*, 2010), and *Chlorella vulgaris* (Neha and Khan, 2016).

On the other hand, the decrease in sodium nitrate led to a reduction in protein and amino acid productivities. These findings were in correspondence to the results of Uslu *et al.* (2011) who studied the effects of nitrogen deficiency on the protein content of *Spirulina* cultivated on Zarrouk medium and recorded 67, 54, 6% of cellular dry weight protein for groups of control, 50% and 100% deficient nitrogen, respectively. The possible reason behind a decrease in protein contents in these conditions is that the cells might have neutralized intracellular nitrogen quota for their normal metabolic function through degrading the nitrogenous compounds (Imran *et al.*, 2014). Nitrogen and phosphorus play a critical role in most processes of cell metabolism, as the nitrogen contributes to structural, functional, and cellular processes of living cells while phosphorus plays a crucial role in energy transfer, signal transduction, respiration, photosynthesis, and macromolecular biosynthesis (Minhas *et al.*, 2016). Therefore, the manipulating in the concentration of nitrogen or phosphorus directly affects the biomass, the lipid content, and other metabolites.

The results in this study cleared that, a decrease in phosphorus concentrations led to a reduction in Chll a and biomass productivity of cyanobacterial species. The same results were also recorded in *Arthrospira platensis* cultured at very low phosphorus concentration in batch culture (Markou *et al.*, 2012). From a different perspective, no significant change in biomass productivity was noticed when grown in a 100 % increase in phosphorus concentration. Similar results were reported by El-Shouny *et al.* (2015) who examined the effects of phosphorus concentration on biomass production content of

Arthrospira platensis and recorded that, the increase of phosphorus concentration up to 50% and 100% didn't show any significant change in biomass production. The obtained data cleared that, in general, the phosphorus depletion led to an improvement in the lipid content and productivity of all studied cyanobacteria. However, the increase of phosphorus concentrations caused a non-significant reduction in lipid content and productivity of all studied cyanobacteria. Xin *et al.* (2010) noticed that by the time phosphorus concentration reduces from 2.0 to 0.1 mg l⁻¹, the lipid content of *Scenedesmus* sp. improves from 23 % to 53 %. In general terms, under nutrient limitation, microalgae accumulate lipids when photosynthesis process conditions such as carbon source (CO₂), energy source (light), and cellular mechanisms for this process are available (Courchesne *et al.*, 2009). The obtained results revealed that a decrease in phosphorus concentrations led to a decrease in protein productivity by all studied cyanobacteria. In this respect, Wang *et al.* (2018) observed that the limitation of phosphorus accessibility caused the soluble protein to decrease. They attributed the previously mentioned decrease to the repression of nonessential proteins' synthesis due to the affectation of the enzymes responsible for protein synthesis under these circumstances.

The current study revealed that high salinity concentrations significantly decreased biomass productivity. This observation is parallels the results of Hu *et al.* (2014), who illustrated that the biomass and Chll a production of *Scytonema javanicum* was reduced when exposed to high NaCl concentration. In an excessive salinity condition, the toxic ionic stress and salt osmotic cause the reduction of photosynthetic rate, which in turn will cause a decrease in chlorophyll contents (Moradi and Ismail, 2007). Wang *et al.* (2010, 2011) suggested that salinity stress may inhibit electron transport at the PSII donor site. In general, the high concentration of NaCl (0.3 M) caused a remarkable reduction in protein content and the productivity of all studied strains. Kirroliia *et al.* (2011) clarified that the shortage in protein and chlorophyll contents is a direct outcome of cultivating the *Scenedesmus quadricauda* under intensive salinity concentration. Under the same conditions, a similar result in *Anabaena cylindrica* was observed (Sheikh *et al.*, 2006). The most

pronounced increase in lipid productivity for *S. platensis* and *M. tenuissima* was recorded at 0.3 M NaCl. This finding is in accordance with the findings of Church *et al.* (2017), who reported that the increase in total lipid content of *Chlorella vulgaris* from 11.5% to 16.1% when the culture under salinity stress. The carbohydrate content was enhanced by the treatment with different NaCl concentrations. The distinguished enhancement of carbohydrate synthesis was achieved in *Scenedesmus quadricauda* under stress conditions (Kirrolia *et al.*, 2011). Gill *et al.* (2002) explained that during stress conditions and reproduction, the soluble carbohydrate is the key to the osmotic regulation of cells. The increase in the sugar content may be an adaptive measure under salinity conditions.

For maintaining the internal pH necessary for cell function, the cell consumes energy at high or low pH (Rai and Rajashekhar, 2016). The flourishing of cyanobacteria species is pH dependence; it has been suggested that pH can affect Chl a and biomass productivity of cyanobacteria. In acidic as well as alkaline conditions, the cultures of tested algae were able to grow, but the most appropriate pH value, which is needed for *M. tenuissima*, and *S. platensis* to grow were 7 and 9, respectively. According to former studies, the most suitable pH for cyanobacteria growth fluctuates from 7.4 to 8.0 (Thingujam *et al.*, 2016). Besides the ability of cyanobacteria to grow in a neutral medium, it can survive in a wide range of uncertain pH conditions (Burja *et al.*, 2002). Thornton (2009) indicated that the acidic environment caused the reduction in the efficiency rate of photosynthesis of *Chaetoceros muelleri*, while when the pH value is between 7.4 to 8.2 could not hold any reduction in the growth rate of diatom; rather at pH 6.8, the growth rate was remarkably decreased. The enhancement of triacylglycerol (TAG) accumulation and the reduction in membrane lipids of *Chlorella* CHLOR1 were achieved under alkaline pH stress without significant effect by carbon and nitrogen limitations (Sharma *et al.*, 2012). Based on morphological observations, alkaline pH inhibited the growth of microalgae, thus diverting the energy to form TAG. Change in pH facilitates changes in the net charge of the protein and also affects the partitioning behavior of the protein (Waghmare *et al.*, 2016).

5. CONCLUSION

In the current study, the protein, biomass, lipid, amino acid, and carbohydrate productivity of *S. platensis* and *M. tenuissima* grown under stress conditions was analyzed. In general, biomass productivity was passively affected by stress conditions. The best-suited pH value for the maximum biomass, protein, amino acids, and carbohydrate productivity of *S. platensis* was 9 with an increase of 27.3%, 69.3%, 44.4%, and 89.4, respectively compared to the control and other treatments. Whereas the treatment of *S. platensis* and *M. tenuissima* with high salinity concentration (0.3M NaCl) resulted in the highest lipid productivity amounted to 125.9% and 153.5%, respectively.

Conflict of interest:

The authors declare no conflict of interest.

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