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PRODUCTIVITY OF BIOMASS AND SOME METABOLITES OF *ANABAENA ORYZAE* AND *NOSTOC* SP. GROWN UNDER STRESS CONDITIONS

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The current study aimed to investigate the effect of stress conditions such as nitrogen and phosphorous deficiency and supplementation, salinity stress and different pH values on the biomass, lipid, protein, amino acid and carbohydrate productivities of *Anabaena oryzae* and *Nostoc* sp. The obtained results revealed that an increase in sodium nitrate by 100% led to increase in the productivities of biomass, protein and amino acid of *A. oryzae* and *Nostoc* sp. While 100% nitrogen deficiency enhanced lipid productivity to 40.8% and 48.7% for *A. oryzae* and *Nostoc* sp., respectively. In addition, phosphorus limitation led to a reduction in biomass, lipid, amino acid and carbohydrate for *A. oryzae* to 36%, 48.4%, 59.3% and 20%, respectively. However, phosphorus free medium showed an increase in lipid productivity by 35% over the control in *Nostoc* sp. Application of all concentrations of NaCl decreased the productivities of all metabolites in *A. oryzae.* While high concentration (0.3M) of NaCl enhanced carbohydrate productivity to 127.2% for *Nostoc* sp. In addition, the biomass productivity of *A. oryzae* and *Nostoc* sp. was increased at pH 7 and at pH 8, respectively. The maximum production of amino acid in *A. oryzae* and *Nostoc* sp. was obtained at pH 8. On the other hand, the acidic and alkaline conditions did not cause any significant changes on the productivity of carbohydrate in case of *A. oryzae* and *Nostoc* sp.

Keywords: *Anabaena oryzae*, *Nostoc* sp., Nutrients stress, pH, Productivity.

1. INTRODUCTION

Cyanobacteria, the blue green algae are an assemblage of Gram-negative eubacteria widely distributed throughout the world. Cyanobacteria are rich of structurally novel and biologically active metabolites [1]. Cyanobacteria include high protein content; capacity to synthesize all amino acids (and provide the essential ones to humans and animals); presence of carbohydrates composed of starch, glucose, sugars and non-digestible polysaccharides (carrageenan and alginate); lipids in the form of glycerol and fatty acids of the ω-3 and ω-6 families; and a valuable content of many essentials vitamins, minerals and antioxidant substances [2]. Filamentous cyanobacteria *Nostoc*,

Anabaena, and many others are particularly attractive for the production of high quality biomass, because they represent a source of protein and a variety of chemicals and pharmaceuticals [3]. Cyanobacterial protein has received worldwide attention for either as food supplement or as an alternative source of food. Some species of *Anabaena, Nostoc* and *Spirulina* are consumed as food due to their high protein and fiber content [4]. Actually, some strategies have been studied for enhancing biomass and high value compounds yields. Stress strategies have been used as culture strategies. These conditions are defined as a significant deviation from the optimal conditions for the normal development and growth of microalgae and cause changes in all functional levels of the organism. Among the stress factors applied to microalgae cultures can be classified into two groups: nutrimental and physical. The nutrimental factors are considered as manipulation of culture media composition (carbon source, nitrogen, phosphorus and iron deficiency), while physical are described as manipulation in operation conditions and external factors that affect the microalgae growth (high light intensities, temperature, pH, salinity and electromagnet [5,6]. Recent studies have indicated that the phosphorus limitation causes a significant increase in the carbohydrate and lipid content of *Spirulina platensis* by 63.09%, 128%, respectively [7,8]. While the increasing salinity caused significant increase of lipid, carbohydrate productivities up to 93, 84%, respectively, in *Arthrospira platensis* [8]. Setta *et al.* [9] reported that the nitrogen limitation increments in carbohydrate concentrations were remarkable, achieving more than 42% of the dry weight in *Synechococcus subsalsus*. The present study focused on the isolation of cyanobacteria from lakes and freshwater ponds in Assiut Governorate and determined the effect of different concentrations of nitrogen, phosphorus and sodium chloride as well as different pH values on the biomass productivity and productivities of some metabolites of *Anabaena oryzae* and *Nostoc* sp.

2. MATERIALS AND METHODS

2.1. Isolation and purification

Cyanobacteria were collected during spring (2015) from lakes and freshwater ponds in Assiut Governorate. Isolation and purification of cyanobacterial species were done by common microbiological isolation methods through streaking and spreading on plates descriptive by Rippka [10]. Isolated and purified species of algae were cultured in Rippka and Herdman [11] modified medium. The medium contained (g/L) $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; Citric acid, 0.006; Ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02 and one ml of a microelement solution consisting of (g/L) H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $Na_2MoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.08;

$Co(NO_3)_2.6H_2O$, 0.05. The pH was 7.0, and the medium was maintained at 27±2ºC under a light intensity of 48.4 µmol.photon.m^{-2}.s^{-1}. The cultures were subjected to purification by serial dilution followed by plating. The individual species were isolated and inoculated into liquid Rippka and Herdman [11] modified medium and incubated under the same previous conditions. Identification of algae has been done by the following references [12,13].

2.2. Algae strain and growth conditions

Cyanobacteria (*Anabaena oryzae* and *Nostoc* sp.) were cultivated axenically as batch cultures in ٥٠٠ ml Erlenmeyer flasks with Rippka and Herdman [11] modified medium at 27±2ºC under a light intensity of 48.4 µmol.photon.m^{-2}.s^{-1}. The effect of different nutrients, namely nitrogen [control (1.5 g L^{-1}), - 50 % (0.75 gL^{-1}), -75%, (0.375 gL^{-1}), -100% (0 gL^{-1}) and +100% (3 gL^{-1}], phosphorus [control (0.04 gL^{-1}), -50% (0.02 gL^{-1}), -75% (0.01 gL^{-1}), -100% (0 gL^{-1}) and +100% (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 *Anabaena oryzae* and *Nostoc* sp. was studied.

2.2.1. Biomass assay

The growth of cyanobacteria was daily monitored by the determination of chlorophyll a according to Metzner *et al.* [14], and by determination of cyanobacterial cellular dry weight (CDW). Biomass productivity was calculated as follows:

Biomass productivity (mg $CDWL^{-1}d^{-1}$) = $(CDW_L-CDW_E)/(t_L-t_E)$

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

2.3. Estimation of total lipid

The total lipid content was determined by the sulfophosphovanilin method (SPV) according to Drevon and Schmit [15].

2.4. Estimation of total protein

The total protein was spectrophotometrically measured at 750 nm using the method described by Lowry *et al.* [16].

2.5. Estimation of total carbohydrate

The carbohydrate content was measured by hydrolyzing of polysaccharides into simple sugars using dilute HCL and estimating the resultant monosaccarides by the anthrone sulfuric acid method [17]. Glucose was used as a standard for the preparation of calibration curve.

2.6. Estimation of free amino acids:

Free amino acids were estimated according to the method adopted by Lee and Takahashi [18].

2.7. Productivities calculation

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

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

Where C_f is the final content of lipids, proteins, carbohydrates or amino acids and was given as percent dry weight.

2.8. Statistical Analysis

The data were obtained from four independent experiments and measured as a means ± SE using Excel 2010 Program. The statistical programmed SPSS version sixteen was used to analysis the effect of different factors in this study. ANOVA table was employed to study the effect of different concentrations of the various factors on the metabolites of studied cyanobacteria. For comparison of the means, the Duncan's multiple range tests ($p< 0.05$) were used.

3. RESULTS AND DISCUSSION

Cyanobacteria have gained importance as human food and pharmaceutical agent for its high content of protein, vitamins, carotenoids, lipids, carbohydrates and essential fatty acids [5], so many studies were established to optimize these important compounds production. The present study aimed to enhance the production of some of these compounds, which were calculated as productivity in $mgL^{-1}d^{-1}$, by modification of media composition. Different nitrogen ($NaNO_3$) and phosphorus (K_2HPO_4) as well salinity (NaCl) concentrations and different pH values were selected for this purpose.

The two studied cyanobacteria were grown in the absence and in the presence of sodium nitrate as a source of nitrogen. The effect of different nitrogen concentrations on the growth of *Anabaena oryzae* and *Nostoc* sp. was recorded as chl. a for 7 and 9 days of incubation, respectively (Fig. 1a,b). The obtained results revealed that a decrease in sodium nitrate concentration led to a reduction in chl. a and biomass productivity in both cyanobacteria species. The most pronounced reduction in biomass productivity amounted to 29.8% and 5.6% at 100% decrease in $NaNO_3$ for *A. oryzae* and *Nostoc* sp., respectively (Table 1). These results are in accordance with Rosales *et al*. [19] who observed the reduction in biomass production of *Anabaena* strains when exposed to low nitrate concentration. In this respect, Hifney *et al.* [20] reported that, completely removed nitrogen source from medium severely

drop in the biomass of *Spirulina* sp. Mandal and Mallick [21] were also observed a decrease in growth pattern for *Scenedesmus obliquus*, under nitrogen deficient conditions. Decrease in algal biomass concentration in low nitrate concentration was also seen in *Nannochloropsis* sp. [22]. On the other hand, the biomass productivity was increased when the cultures of cyanobacteria under testing grown in a 100% increase of nitrogen. Similar studies have been verified that cyanobacteria grow better with higher levels of nitrogen [23].

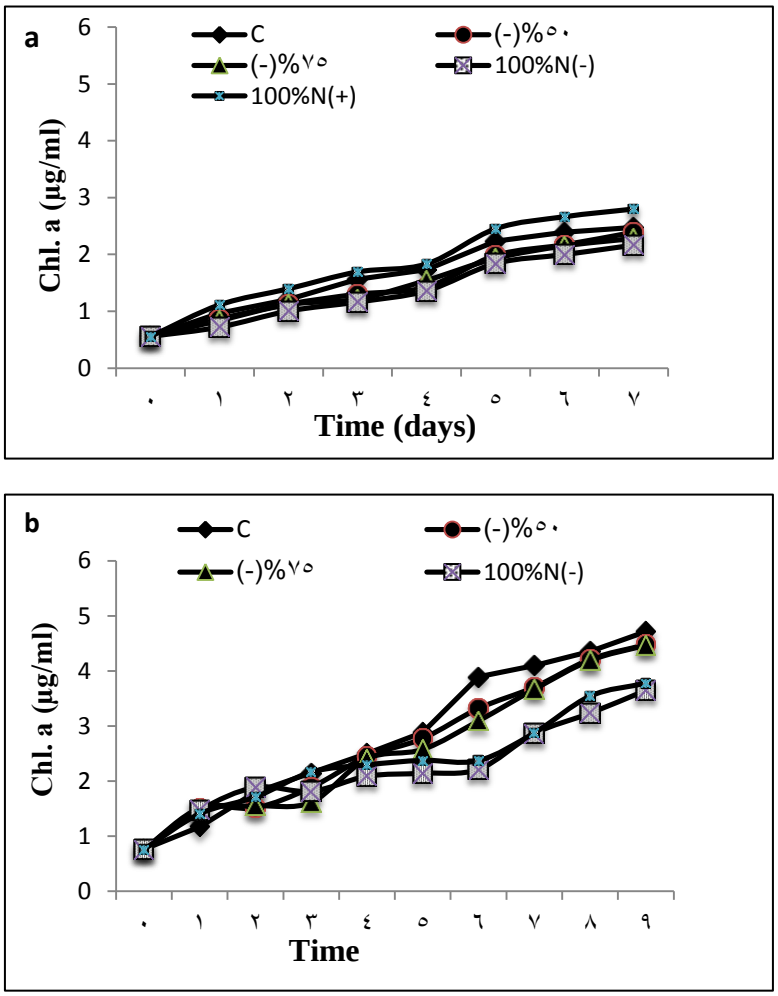

Fig. 1. Effect of different concentrations of $NaNO_3$ on the growth of a) *Anabaena oryzae* and b) *Nostoc* sp.

The present study proved that the nitrogen starvation causes increased in lipid productivity by the studied cyanobacteria. The most distinct increase in lipid productivity was observed at 100% decrease in $NaNO_{3,}$ which amounted to 40.8 % and 48.7% for *A. oryzae* and *Nostoc* sp., respectively, compared to standard medium (Table 1). These results agree with the data obtained by Yeesang and Cheirsilp [6] who reported that under nitrogen deficient conditions, algal cells accumulate carbon metabolites as lipids.

On the other hand, the increase in nitrogen concentration by 100% decreased the lipid productivity of *A. oryzae* and *Nostoc* sp. by 3.3% and 7.6%, respectively. An increase in nitrogen concentration in the medium leads to reduction in lipid yields for *Arthrospira platensis* [24].

Table 1. Effect of different nitrogen concentrations on biomass, lipid, protein, amino acid and carbohydrate productivities of *Anabaena oryzae* and *Nostoc* sp.

	Productivities (mg/L/day)					
		Biomass	Lipid	Protein	Amino acid	Carbohydrate
Anabaena oryzae	C.	114.1 ± 4^{c}	12.0 ± 0.2^{a}	11.4 ± 0.8^{b}	2.8 ± 0.08^{c}	1.9 ± 0.2^{c}
	50% N (-)	96.7 ± 4^{b}	15.6 ± 0.4^{b}	7.6 ± 0.3^{a}	1.7 ± 0.16^{a}	1.0 ± 0.1^{b}
	75% N (-)	96.7 ± 4^{b}	15.5 ± 0.3^{b}	8.3 ± 0.02^{a}	1.8 ± 0.13^{a}	0.9 ± 0.1^{a}
	100% N (-)	80.79 ± 5^{a}	16.9 ± 0.5^{c}	8.2 ± 0.1^{a}	1.0 ± 0.06^{a}	1.1 ± 0.1^{b}
	100% N(+)	١17.3±٣^{c}	11.6 ± 0.1^{a}	12.6 ± 0.05^{c}	3.9 ± 0.13^{d}	2.9 ± 0.1^{d}
Nostoc sp.	C.	57 ± 1^{ab}	7.8 ± 0.2^{a}	8.7 ± 0.6^{a}	2.0 ± 0.2^{b}	0.9 ± 0.02^{a}
	50% N (-)	57.7 ± 1^{ab}	9.2 ± 0.2^{b}	8.0 ± 0.1^{a}	1.8 ± 0.1^{b}	1.2 ± 0.1^{a}
	75% N (-)	56.5 ± 1^{ab}	11.1 ± 0.1^{c}	8.8 ± 0.6^{a}	1.9 ± 0.1^{b}	1.0 ± 0.1^{a}
	100% N (-)	53.8 ± 1^{a}	11.6 ± 0.1^{c}	7.8 ± 0.4^{a}	1.2 ± 0.03^{a}	1.2 ± 0.2^{a}
	100% N(+)	60.4 ± 1.6^{b}	7.2 ± 0.3^{a}	9.1 ± 0.4^{a}	2.5 ± 0.2^{c}	1.0 ± 0.02^{a}

The data are given as averages of three replicates of standard error. Values followed by the different letters are significantly different at $p < 0.05$.

Increasing of nitrogen concentration by 100% resulted in increase of protein and amino acid productivities of *A. oryzae* and *Nostoc* sp. by 10.5%, 39.2% and 4.6%, 25%, respectively (Table 1). Increase in biomass and protein production by increasing nitrogen concentration, as it seen in results of *A. oryzae* and *Nostoc* sp., has been widely supported by various reports from *Anabaena* [25], *Oscillatoria* [26], *Dunaliella* [27] and *Spirulina platensis* [28]. On the other hand, the decrease in sodium nitrate led to reduction in protein and amino acid productivities amounted to 28.1%, 10.3% and 64.2%, 40% at 100% decrease in $NaNO_3$ for *A. oryzae* and *Nostoc* sp., respectively (Table 1). These results were in agreement with the finding of Uslu *et al.* [29] who studied the effects of nitrogen deficiency on 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. A possible explanation towards decrease in protein content is the cells might have degraded the nitrogenous compounds to maintain intracellular nitrogen quota for their normal metabolic function [30]. The carbohydrate productivity of *A. oryzae* significantly increased to 52.6% under nitrogen-rich conditions (Table 1). The same result recorded in *Anabaena* strains treated with high nitrate concentration [19]. There was no significant effect of decreasing and increasing of sodium nitrate on the carbohydrate productivity of *Nostoc* sp.

Effect of phosphorus on the growth of *Anabaena oryzae* and *Nostoc* sp. that recorded as chl. a was studied by changing of phosphorus concentration (Fig. 2a,b). The results in this study cleared that the decrease in phosphorus concentration led to a reduction in chl. a and biomass productivity in both cyanobacteria species. The reduction in biomass productivity amounted to 36% and 4.1% at 100% decrease in phosphorus for *A. oryzae* and *Nostoc* sp., respectively (Table 2). These same results were also recorded in *Arthrospira platensis* cultured at very low phosphorus concentration in batch culture [7]. However, no significant change in biomass productivity of *A. oryzae* and *Nostoc* sp. when grown in a 100% increase in phosphorus concentration (Table 2). These results were in agreement with the finding of El-Shouny *et al.* [8] who studied the effect of phosphorus concentration on biomass production content of *Arthrospira platensis* and recorded that the increasing of phosphorus concentration up to 50% and 100% didn't show any significant change in biomass production.

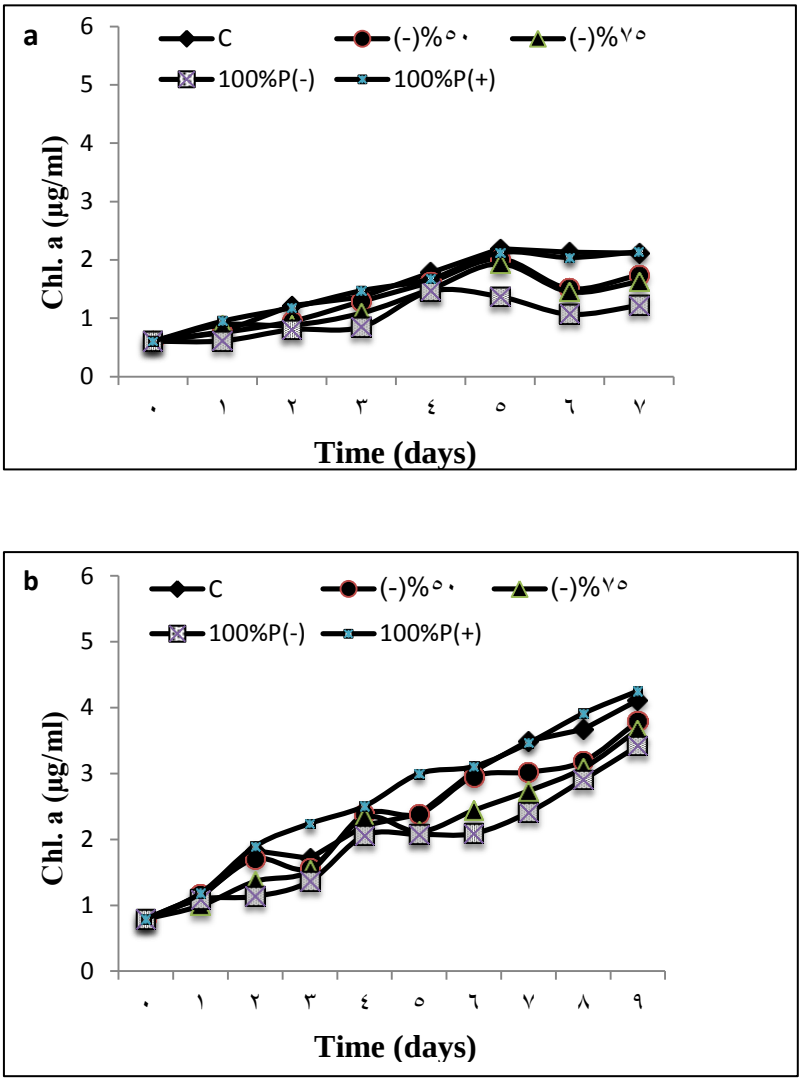

Fig. 2. Effect of different concentrations of K_2HPO_4 on the growth of a) *Anabaena oryzae* and b) *Nostoc* sp.

Compared to the control, cultures treated with 100% decrease in K_2PO_4 showed the significant decrease in lipid productivity of *A. oryzae* by 48%. While, phosphorus free medium showed 35% increase in lipid productivity more than the control in *Nostoc* sp. (Table 2). However, application of high concentration of K_2PO_4 (+100 %) to the culture of *A. oryzae* and *Nostoc* sp. led to a reduction in lipid productivity by about 7.4% and 36% than the control in case of *A. oryzae* and *Nostoc* sp., respectively. Generally, microalgae accumulate lipid under nutrient limitation when the energy source

(light) and carbon source (CO_2) are available and when the cellular mechanisms for the photosynthesis are active [31].

Table 2. Effect of different phosphorus concentrations on biomass, lipid, protein, amino acid and carbohydrate productivities of *Anabaena oryzae* and *Nostoc* sp.

		Productivities (mg/L/day)				
		Biomass	Lipid	Protein	Amino acid	Carbohydrate
Anabaena oryzae	C.	118.8 ± 4^{d}	19.0 ± 0.03^{bc}	9.2 ± 0.3^{b}	3.2 ± 0.41^{b}	2.0 ± 0.1^{b}
	50% P (-)	104.6 ± 4^{c}	20.1 ± 0.7^{c}	7.5 ± 0.3^{a}	1.7 ± 0.12^{a}	1.6 ± 0.02^{a}
	75% P (-)	90.3 ± 3^{b}	17.8 ± 0.3^{b}	8.4 ± 0.2^{ab}	1.5 ± 0.02^{a}	1.8 ± 0.03^{b}
	100% P (-)	76 ± 4^{a}	9.8 ± 0.4^{a}	9.1 ± 0.1^{b}	1.3 ± 0.13^{a}	1.6 ± 0.1^{a}
	100%P (+)	117 ± 3^{d}	17.6 ± 0.5^{b}	7.3 ± 0.3^{a}	2.0 ± 0.03^{a}	1.8 ± 0.1^{b}
Nostoc sp.	C.	65.7 ± 1^{bc}	9.4 ± 0.3^{b}	9.8 ± 0.02^{c}	2.4 ± 0.2^{b}	1.0 ± 0.1^{a}
	50% P (-)	58.9 ± 1^{a}	10.6 ± 0.9^{bc}	9.8 ± 0.9^{c}	2.5 ± 0.3^{b}	1.0 ± 0.1^{a}
	75% P (-)	64 ± 3^{abc}	12.1 ± 0.6^{cd}	7.9 ± 0.5^{ab}	1.5 ± 0.0^{a}	1.0 ± 0.1^{a}
	100% P (-)	63 ± 2^{ab}	12.7 ± 0.1^{d}	6.3 ± 0.1^{a}	2.0 ± 0.1^{ab}	0.7 ± 0.02^{a}
	100%P (+)	69 ± 1^{c}	6.0 ± 0.1^{a}	9.2 ± 0.4^{bc}	2.5 ± 0.1^{b}	0.8 ± 0.03^{a}

The data are given as averages of three replicates of standard error. Values followed by the different letters are significantly different at $p < 0.05$.

The obtained results in table 2 revealed that a decrease or increase of K_2PO_4 concentration led to decrease in protein productivity by *A. oryzae* and *Nostoc* sp. The most pronounced reduction amounted to 20.6 % and 35.7% than the control at +100 % and -100% of K_2PO_4 for *A. oryzae* and *Nostoc* sp. (Table 2). In this respect, Baiee *et al*. [32] reported that, the soluble protein content decreased under phosphorus limitation. They suggested that the decreasing because the synthesis of nonessential proteins may be repressed during phosphorus limitation because the enzymes that are responsible for protein synthesis affected by phosphorus concentration**.**

The decrease or increase in K_2PO_4 concentrations also significantly decreased the amino acid productivity of *A. oryzae*, but the low significant amino acid productivity was observed by using -75% of K_2PO_4 for *Nostoc* sp., which showed 37.5 %, decrease in amino acid productivity with respect to the control (Table 2). The same results were observed on carbohydrate productivity shown in table 2 .Whereas, the culture of *A. oryzae* grown in different concentrations of K_2PO_4 resulted in a decrease in carbohydrate productivity, whereas, the most pronounced reduction amounted to 20% below the control at -50% and -100% of K_2PO_4 for *A. oryzae*. However, the different concentrations of K_2PO_4 didn't cause any significant changes on the carbohydrate productivity of *Nostoc* sp. (Table 2). At higher N:P ratios the increased cell size allows for greater accumulation of carbohydrates in the cell, which is also indicated by the similar cellular carbohydrate content [33].

Fig. 3a,b shows the effect of different concentrations of NaCl on the growth of *A. oryzae* and *Nostoc* sp. for 7 and 9 days of incubation respectively. Application of all concentrations of NaCl decreased the chl. a in the two studied cyanobacteria, with respect to the control.

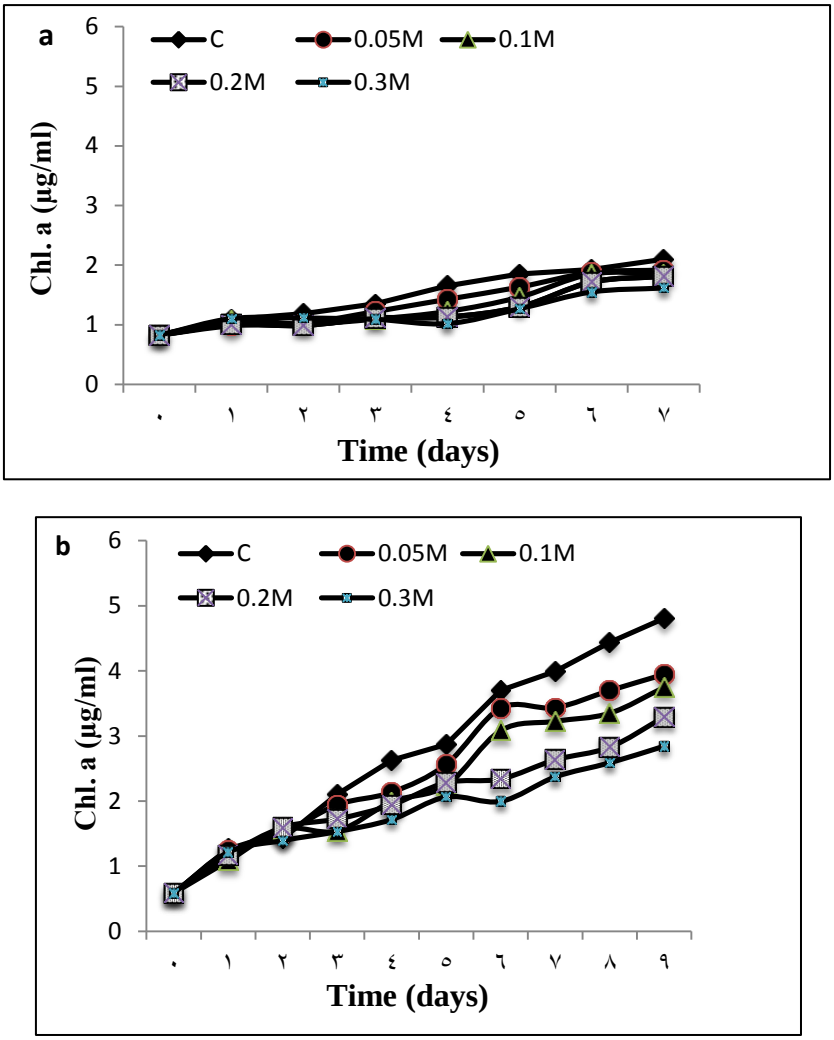

Fig. 3. Effect of different concentrations of NaCl on the growth of a) *Anabaena oryzae* and b) *Nostoc* sp.

High salinity concentrations (0.1, 0.2 and 0.3 M) significantly decreased the biomass productivity of *A. oryzae*by 24.5%, 31.4%, 29.5% and 32 %, 44.6, 54.8% for *Nostoc* sp., respectively than the control (Table 3). These observations were in accordance with the results of Hu *et al.* [34] who showed that the biomass and chl. a production of *Scytonema javanicum* were reduced when exposed to high NaCl concentration. The reduction of chlorophyll contents at high salinity is due to a decrease in photosynthetic rate because of salt osmotic and toxic ionic stress [35]. Wang *et al*. [36,37]

suggested that salinity stress may inhibit electron transport at the PSII donor site. The obtained results in this investigation showed a significant decrease in lipid productivity of *A. oryzae* at different concentrations used of NaCl. However, in case of *Nostoc* sp. a non-significant increase in lipid productivity was observed at 0.05M concentration of NaCl (Table 3). The most pronounced reduction in lipid productivity amounted to 56.3% and 22.1% at 0.3M and 0.2M of NaCl for *A. oryzae* and *Nostoc* sp., respectively. This result agrees with the data obtained by Chaffin *et al*. [38] who reported the reduction in lipid production when *Fragilaria capucina* exposed to high NaCl concentration.

Table 3. Effect of different sodium chloride concentrations on biomass, lipid, protein, amino acid and carbohydrate productivities of *Anabaena oryzae* and *Nostoc* sp.

	Productivities (mg/L/day)					
	NaCl (M)	Biomass	Lipid	Protein	Amino acid	Carbohydrate
Anabaena oryzae	C. (0)	121.5 ± 2^{b}	18.1 ± 0.4^{c}	9.4 ± 0.1^{c}	3.0 ± 0.12^{c}	2.1 ± 0.2^{b}
	0.05	106.5 ± 7^{ab}	12.1 ± 0.9^{b}	6.6 ± 0.4^{b}	2.5 ± 0.23^{bc}	1.6 ± 0.1^{ab}
	0.1	91.7 ± 6^{a}	12.5 ± 0.8^{b}	5.2 ± 0.3^{ab}	2.4 ± 0.1^{bc}	1.3 ± 0.0^{a}
	0.2	83.3 ± 9^{a}	10.9 ± 0.9^{b}	6.2 ± 0.4^{ab}	2.2 ± 0.20^{ab}	1.5 ± 0.1^{ab}
	0.3	85.56 ± 8^{a}	7.9 ± 0.7^{a}	4.5 ± 0.2^{a}	1.7 ± 0.26^{a}	1.9 ± 0.3^{ab}
Nostoc sp.	C. (0)	68.5 ± 5^{b}	9.5 ± 1.2^{ab}	10.9 ± 0.7^{b}	2.3 ± 0.4^{a}	1.1 ± 0.1^{a}
	0.05	65 ± 7^{b}	10.3 ± 1^{b}	7.8 ± 0.7^{ab}	3.7 ± 0.4^{b}	1.2 ± 0.1^{a}
	0.1	46.6 ± 6^{b}	8.6 ± 0.0^{ab}	9.3 ± 1.4^{ab}	3.2 ± 0.3^{ab}	1.4 ± 0.2^{a}
	0.2	37.9 ± 1^{a}	7.4 ± 0.2^{a}	8.3 ± 0.3^{ab}	2.6 ± 0.1^{a}	2.3 ± 0.1^{b}
	0.3	31 ± 3^{a}	7.9 ± 0.2^{a}	7.2 ± 0.3^{a}	2.7 ± 0.3^{ab}	2.5 ± 0.1^{b}

The data are given as averages of three replicates of standard error. Values followed by the different letters are significantly different at $p < 0.05$.

The protein productivity of the studied cyanobacteria was decreased with increasing salt concentration, whereas, high concentration (0.3M) of NaCl resulted in a 52% and 34% reduction in protein productivity of *A. oryzae* and *Nostoc* sp., respectively (Table 3). Kirroliaa *et al.* [39] reported that the cultivation with higher saline concentrations had lower chlorophyll and protein contents. Sheikh *et al*. [40] obtained the same results in *Anabaena cylindrica* when exposed to NaCl stress.

The same results were observed on the amino acid and carbohydrate productivities of *A. oryzae* when exposed to salinity stress. While, the addition of 0.05 and 0.1M NaCl concentration stimulates the amino acid productivity in *Nostoc* sp. by 61% and 39%, respectively (Table 3). Hifney [41] reported that the concentration of free amino acids increased by salinity stress when applied to *Anabaena circinalis*. Stewart *et al.* [42] found that salinity stimulated the conversion of saccharides to amino acids and/or inhibit amino acid incorporation into protein. The highest value of carbohydrate productivity was obtained when the culture of *Nostoc* sp. was supplemented with 0.2 and 0.3M NaCl concentration (Table 3). Many previous studies reported that carbohydrate synthesis was stimulated by stress conditions [39]. Gill *et al*. [43] observed that soluble sugars play an important role in the osmotic regulation of cells during reproduction and stress conditions. The increase in the sugar content may be an adaptive measure under salinity conditions.

The sequence of changes in growth of the cyanobacteria under different pH values (5, 6, 7, 8 and 9) is represented in (Fig. 4a,b). At high or low pH, cells may have to spend energy for maintenance of an internal pH necessary for cell function [44]. 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 *A. oryzae* were able to grow, but growth in these conditions was comparatively less than the control culture (pH 7) (Table 4). The biomass productivity of *A. oryzae* was increased at pH 7 compared to the other treatments. The best suited pH value for the growth of *Nostoc* sp. was 6 with significant increase of 13% in biomass productivity. Earlier studies related to the effect of pH on the growth of cyanobacteria has revealed that pH between 7.4 and 8.0 is favorable for the optimum growth of cyanobacteria species [45]. The fact that all cyanobacteria were able to grow in acidic (pH 6.5) medium indicates that cyanobacteria can adapt to variable pH conditions as suggested by Burja *et al.* [46]. Thornton [47] reported that photosynthetic efficiency of *Chaetoceros muelleri* decreases as the environment surrounding the cells becomes more acidic and also observed that the growth rate of diatom was not affected by pH between 7.4 and 8.2, but the growth rate at pH 6.8 was significantly low.

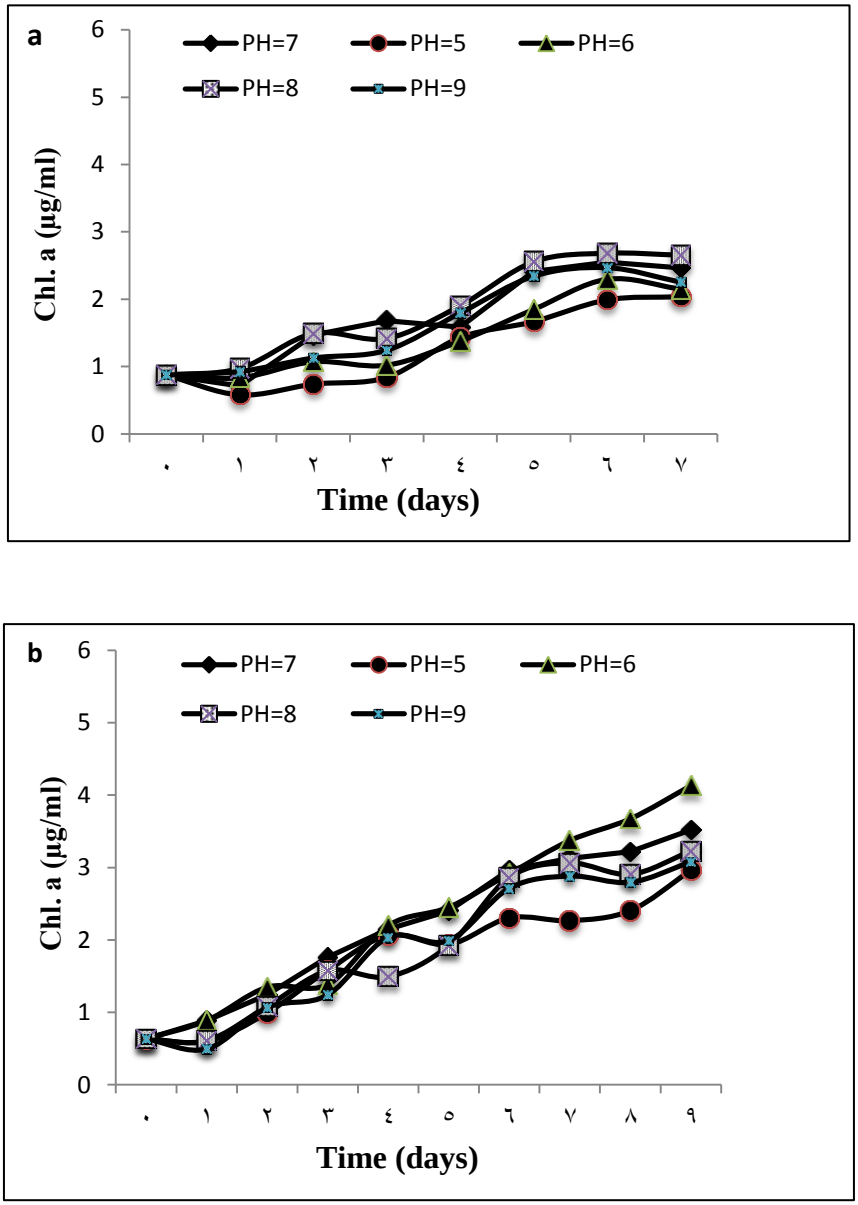

Fig. 4 Effect of different pH values on the growth of a) *Anabaena oryzae* and b) *Nostoc* sp.

The acidic pH value (pH 5) significantly reduced the lipid productivity in *A. oryzae* and *Nostoc* sp. by 28% and 27%, respectively, compared to the control culture (Table 4). The most pronounced increase in lipid at pH 7 and pH 9 for *A. oryzae* and *Nostoc* sp., respectively. Alkaline pH stress led to triacylglycerol (TAG) accumulation in *Chlorella* CHLOR1 and was not

dependent on nitrogen or carbon limitation levels, and led to a decrease in membrane lipids [48]. Based on morphological observations, alkaline pH inhibited the growth of *Chlorella* CHLOR1, thus diverting the energy to form TAG.

The protein productivity of *Nostoc* sp. was significant increased at pH 8 amounted to 20.6% with compared to the control culture. There was no significant effect of decreasing and increasing of pH on the protein productivity of *A. oryzae* (Table 4). Change in pH facilitates changes in the net charge of protein and also affects the partitioning behavior of the protein [49].

Table 4. Effect of different pH values on biomass, lipid, protein, amino acid and carbohydrate productivities of *Anabaena oryzae* and *Nostoc* sp.

	Productivities (mg/L/day)					
		Biomass	Lipid	Protein	Amino acid	Carbohydrate
Anabaena oryzae	C. (7)	116 ± 4^{b}	14.3 ± 1.7^{b}	8.4 ± 0.2^{a}	2.6 ± 0.005^{ab}	1.6 ± 0.1^{a}
	5	77.9 ± 9^{a}	10.3 ± 1.4^{a}	9.2 ± 0.3^{a}	2.7 ± 0.16^{ab}	1.6 ± 0.1^{a}
	6	77.7 ± 5^{a}	12.4 ± 0.6^{ab}	9.1 ± 0.1^{a}	3.0 ± 0.2^{bc}	1.7 ± 0.1^{a}
	8	76.8 ± 6^{a}	12.3 ± 2.4^{ab}	7.5 ± 0.3^{a}	3.4 ± 0.05^{c}	1.5 ± 0.1^{a}
	9	82.6 ± 10^{a}	13.4 ± 3^{ab}	7.3 ± 0.3^{a}	2.2 ± 0.37^{a}	1.3 ± 0.2^{a}
Nostoc sp.	C. (7)	68.7 ± 2^{b}	9.2 ± 0.4^{b}	9.7 ± 0.2^{bc}	2.3 ± 0.01^{c}	0.9 ± 0.1^{a}
	5	46.3 ± 4^{a}	6.7 ± 0.6^{a}	5.6 ± 0.3^{a}	1.1 ± 0.1^{a}	0.8 ± 0.02^{a}
	6	78.9 ± 8^{c}	10.0 ± 0.6^{b}	10.4 ± 0.9^{c}	2.2 ± 0.1^{c}	1.1 ± 0.1^{a}
	8	67 ± 3^{b}	8.7 ± 0.3^{b}	11.7 ± 0.5^{c}	2.4 ± 0.3^{c}	1.1 ± 0.1^{a}
	9	52 ± 2^{a}	10.1 ± 0.9^{b}	7.3 ± 0.7^{ab}	1.7 ± 0.2^{b}	1.1 ± 0.01^{a}

The data are given as averages of three replicates of standard error. Values followed by the different letters are significantly different at $p < 0.05$.

The maximum production of amino acid in *A. oryzae* and *Nostoc* sp. cultures obtained at pH 8 amounted to 30.7% and 4.3% respectively. Taraldsvik and Myklestad [50] observed that increase in free amino acid in *Skeletonema costatum* when cells were subject to an increase in pH from 6.5 to 8.0. While pH did not cause any significant changes on the productivity of carbohydrate in case of *A. oryzae* and *Nostoc* sp. (Table 4). This observation

agrees with the finding of Touloupakis *et al.* [51] who showed that no change in the carbohydrate production of *Synechocystis* sp. when exposed to pH values between 7.5 and 10.0.

4. CONCLUSION

In this study, the biomass, lipid, protein, amino acid and carbohydrate productivity of *A. oryzae* and *Nostoc* sp. grown under stress conditions were investigated. In general, the biomass productivity of two cyanobacteria species was passively affected by the stress conditions. Nitrogen starvation was considered as the most preferred condition for the maximum lipid productivity in *A. oryzae* and *Nostoc* sp. than the other factors. The best suited pH value was 8 for the maximum protein productivity in *Nostoc* sp. with an increase of 20.6%, compared to the control and other treatments. On the other hand, the highest amino acid productivity was recorded by the treatment of *Nostoc* sp. with a low concentration of NaCl amounted to 60.8%. However, the high concentration of NaCl enhanced carbohydrate productivity to 127.2% for *Nostoc* sp. in comparison to the control culture and other used treatments.

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"إنتاجية الكتلة الحيوية وبعض المركبات الايضية لطحلبى أنابينا أوريزا والنوستوك تحت ظروف الإجهاد"

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توضح الدراسة الحالية تاثير ظروف الإجهاد المختلفة كنقص عنصرى النيتروجين والفوسفور وكذلك الإجهاد الملحى وتغير تركيز ايون الهيدروجين على إنتاجية الكتلة الحيوية، الليبيدات، البروتينات، الأحماض الأمينية والكربوهيدرات لطحلبى الانابينا اوريزا والنوستوك.

وتشير النتائج إلى أن زيادة تركيز النتيروجين إلى ١٠٠% ينتج عنة زيادة نسبة الكتلة الحيوية والبروتين والأحماض الأمينية لطحلبى الانابينا اوريزا والنوستوك. بينما نقص النيتروجين بنسبة ١٠٠% أدى إلى زيادة ملحوظة فى إنتاجية الليبيدات بنسبة ٤٠.٨% و ٤٨.٧% لطحلبى الأنابينا اوريزا والنوستك على التوالى. كما أوضحت النتائج أن نقص الفوسفور أدى إلى نقص فى إنتاجية الكتلة الحيوية والليبيدات والاحماض الامينية والكربوهيدرات لطحلب الأنابينا اوريزا بنسبة ٣٦%، ٤٨.٤٢%، ٥٩.٣% و٢٠% على التوالى. أما نمو طحلب النوستوك فى بيئة خالية من الفوسفور أدى إلى زيادة معنوية فى إنتاجية الليبيدات بنسبة ٣٥%. وقد أدى معاملة طحلب الأنابينا اوريزا بتركيزات مختلفة من كلوريد الصوديوم إلى نقص فى إنتاجية المركبات الايضية سالفة الذكر. أما زيادة تركيز كلوريد الصوديوم إلى ٠.٣ مول أدى إلى تحفيز إنتاجية الكربوهيدرات الى ١٢٧% لطحلب النوستوك. وأوضحت النتائج أيضا أن افضل إنتاجية للكتلة الحيوية لطحلبى الأنابينا اوريزا والنوستوك عند تركيز أيون الهيدروجين ٧و ٨ على التوالى، ولوحظ ان أفضل إنتاجية للأحماض الأمينية لطحلبى النوستوك والأنابينا اويزا عند تركيز أيون الهيدروجين ٨. ومن ناحية اخرى فان الظروف الحامضية أو القاعدية لا يتبعها اى تغير فى إنتاجية الكربوهيدرات لطحلبى الأنابينا اوريزا والنوستوك.