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Research Article

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Thiamin as a Factor Affecting the Growth Parameters of Salinized *Chlamydomonas reinhardtii*

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Abstract A study was conducted to investigate the impact of thiamin ($V.B_1$) on the growth parameters of salinized algal species. In order to determine the impact of thiamin, an algal species *Chlamydomonas reinhardtii* was exposed to different concentrations of NaCl ranging from 25-100mM and 200 ppm of thiamin ($V. B_1$) along with control over a period of 7 days. It was found that the Dry weight, optical density, total photosynthetic pigments, total carbohydrates and total proteins were significantly increased up to the level 75 mM NaCl. Under higher concentration 100mM NaCl all these parameters were significantly decreased. On the other hand the total lipid contents were significantly increased up to the level 25 mM NaCl. Under higher concentration 50, 75, and 100mM NaCl the total lipid contents were significantly decreased.

When the salinized *Chlamydomonas reinhardtii* cultures treated with 200 ppm thiamine (B_1), Dry weight, optical density and total photosynthetic pigments), total carbohydrates, total proteins and total lipid contents were significantly increased, when the algal cultures subjected to lower and higher concentrations of NaCl.

Keywords Dry weight, total lipid contents, total photosynthetic pigments, optical density, total proteins, total carbohydrates

1. Introduction

A number of factors are known to influence the lipid content of microalgae, such as nitrogen [14] and silicon [19] deficiency, phosphate limitation [24] high salinity [23]. Light intensity [15] and iron content of the medium also affect algal growth [18] Plant cells are generally able to live within a certain range of enhanced salt concentrations or changing salinities, since most probably all life originated in the oceans, i.e. a highly saline environment. However, during evolution, the degree of salt resistance and salt tolerance became very divergent among the present day aquatic organisms. Algae (and cyanobacteria) have attracted considerable attention in this respect, since they are inhabitants of biotopes characterized by changing salinities and can serve as model organisms or a better understanding of salt acclimation in the more complex physiological processes of higher plants [3], [4] and [11] Salinity is a serious agro-economical problem which leads to metabolic alterations and graded reduction in the plant growth in terms of all the growth parameters leading to severe crop losses. It is also considered an important ecological variable in the fresh water and marine environment. Salinity has been suggested as being a controlling factor for blooms of cyanobacteria in estuaries and is considered as one of the major constraints on species diversity and productivity of natural population of algae [5] and [7]. Particularly in estuarine water planktonic algae are often subjected to widely fluctuating salt concentrations [13] and [21]. Such changes in the salinity of water often affect the growth, metabolism and photosynthesis of phytoplanktons [21] and [16]. Salinity stress and unfavorable light conditions are main limiting factors of plant productivity both in aquatic and terrestrial, natural and anthropically modified environments [10]. The biomass of algal species mainly comprises of protein, carbohydrate, and lipids [28]. Mainly due to the high protein content some algal

species like *Chlorella* sp. are widely used as health food for human beings and as animal nutritional supplements [28] ,[20] and [12] finds its potential applications in food, cosmetic, and pharmaceutical industries [27]. The total lipid contents of microalgae varied from 1 to 70% of the dry cell weight [20]. The lipid present in microalgae is mainly in the form of esters of glycerol and fatty acid, which are suitable for producing biodiesel [8] and [9].

2. Materials and Methods

The microalgae species used in this study (*Clamydomonas reinhardtii*), were collected from culture collection of Algal and Plant Physiology Laboratory, Faculty of Science, Al-Azhar University, Assiut, Egypt. Culture medium BG11 nutritive culture was used as a medium for enrichment and growth of the tested algae, [29].

Treatments: *Clamydomonas reinhardtii* was subjected to 00 (control) and various concentrations (25, 50, 75 and 100mM) of NaCl and the same concentrations with 200 ppm of $V.B_1$ for 7 days were followed.

2.1. Analytical methods

2.1.1. Determination of dry weight

A definite volume (100 mls.) of alga suspension was filtered through weighed glass fiber filter. The cells after being precipitated on the filter were washed twice with distilled water and dried overnight in an oven at 105 ^{o}C. The data were expressed as µg ml^{-1} algal suspension.

2.1.2. Determination of Optical Density (OD)

The cell concentration (Optical density) was determined by the method of measuring OD at 680 nm [25]. The data were calculated (g L^{-1} algal suspension).

2.1.3. Determination of total photosynthetic pigments

The pigment fractions (µg $m1^{-1}$ algal suspension) chlorophyll a, chlorophyll b and carotenoids extracted by 100 % actone were calculated using the equations [17]: -

Chlorophyll a = 11.75 A_{662} - 2.350 A_{645}

Chlorophyll b = 18.61 A_{645} - 3.960 A_{662}

Carotenoids = 1000 A_{470} - 2.270 Chloro.a - 81.4 Chloro.b / 227

2.1.4. Determination of total carbohydrates

Using of anthrone-sulphoric acid reagent according to the method by [1] the data measured as µg mg^{-1} dry weight.

2.1.5. Determination of Proteins

Using Bradford reagent according the method adapted by [6] and [33] .The data were measured as µg mg^{-1} dry weight.

2.1.6. Determination of lipid contents

The lipid consents were determined according method by [2]. The data were measured as µg mg^{-1} dry weight.

2.1.7. Statistical Analysis

Four replicates were used in this study and the data were statistically analyzed to calculate the Least Significant Difference (L.S.D) according to [26].

3. Results and Discussions

In this study, the growth criteria (dry weight, optical density and total photosynthetic pigments) of *Chlamydomonas reinhardtii* cultures were markedly increased up to level 75mMNaCl. However, under higher relatively concentration 100mM NaCl, all these parameters was markedly decreased, when compared with that of the control cultures. But when the algal cultures treated with 200ppm of $V.B_1$ all these parameters was significantly increased.

Thus, the maximum value of dry weight, of *Chlamydomonas reinhardtii* cultures was 140.7%, when the algal cultures subjected to 75 mM NaCl only, as compared with that the control cultures. When the salinized-*Chlamydomonas reinhardtii* cultures treated with 200 (ppm) of thiamin the maximum value of dry weight reached to 164.3%, as compared with that the control cultures. The minimum value of dry weight, of *Chlamydomonas reinhardtii* cultures amounted to 75.7 %, when the algal cultures subjected 100 mM NaCl only, when compared with that the control cultures. But, the minimum value of dry weight, of salinized -

Chlamydomonas reinhardtii cultures was 97.1 % of that the control cultures, when the algal cultures subjedted to 100 mM of NaCl and treated with 200 (ppm) of thiamin, as compare with the control cultures (Table 1-a).

The maximum value of optical density of *Chlamydomonas reinhardtii* was 120.2%, when the algal cultures subjected to the moderate concentration 75 mM NaCl only, as compared with that the control cultures. When the salinized- *Chlamydomonas reinhardtii* cultures treated with 200 (ppm) of thiamin the maximum value of optical density reached to 141.2%, when compared with that the control cultures. The minimum value of optical density, of *Chlamydomonas reinhardtii* cultures amounted to 62.9 %, when the algal cultures subjected to higher concentration of 100 mM NaCl only, when compared with that the control cultures. But, the minimum value of optical density of *Chlamydomonas reinhardtii* cultures was 68.4% of that the control cultures, when the algal cultures subjected to 100 mM and treated with 200 (ppm) of thiamin as compare with the control cultures (Table 1-b).

The maximum value of the total pigments of *Chlamydomonas reinhardtii* reached to 197%, when the algal cultures subject to the moderate level of 75 mM NaCl, only as compare with the control cultures. On the other hand, the maximum value of the total pigments of salinized *Chlamydomonas reinhardtii* reached to 220.4%, when the algal cultures subject to the moderate level of 75 mM NaCl and treated with 200 (ppm) of thiamin, as compare with the control cultures. The minimum values of total pigments amounted to 19.9% of that the control cultures, when the algal cultures subject to 100 mM NaCl only. Also, the minimum value of the total pigments of salinized *Chlamydomonas reinhardtii* was 28.2 % of the that control cultures, when the algal cultures subject to the higher concentration of 100 mM NaCl and treated with 200 (ppm) of thiamin, as compare with the control cultures (Table 1-c).

The maximum values of total carbohydrate contents of *Chlamydomonas reinhardtii* amounted to 152.6%, of that the control cultures, when the algal cultures subjected to moderate concentration of 75 mM of NaCl only. On the other hand, maximum values of the total carbohydrate contents of *Chlamydomonas reinhardtii* treated with (200 ppm) thiamin were 158.4%, of that the control cultures, when the algal cultures subjected moderate concentration (75 mM) of NaCl and treated with 200 (ppm) of thiamin ($V.B_1$).

The minimum values of total carbohydrate contents of *Chlamydomonas reinhardtii* were 77.6%, of that the control cultures, when the algal cultures subject to 100 mM NaCl, only, as compared with that the control cultures. Also, The minimum values of total carbohydrate contents of *Chlamydomonas reinhardtii* were 93.2% of that the control cultures, when cultures, when the algal cultures subject to 100 mM NaCl, and treated with 200 (ppm) of thiamin. (Fig. 1-a).

The total lipid contents of *Chlamydomonas reinhardtii* were significantly increased up to the lower concentration of 25 mM NaCl. But, under moderate and higher relatively concentration 50, 75, and 100 mM NaCl were significantly decreased. On the other side, the total lipid contents of salinized- *Chlamydomonas reinhardtii* were significantly increased, when the algal cultures subjected to different concentrations (25, 50, 75, and 100 mM) of NaCl and treated with 200 (ppm) of thiamin.

The maximum values of the total lipid contents of *Chlamydomonas reinhardtii* reached to 185.5%, of that the control cultures, when the algal cultures subjected to moderate concentration of 25 mM of NaCl only. On other hand, maximum values of the total lipid contents of salinized *Chlamydomonas reinhardtii* were 197%, of that the control cultures, when the algal cultures subjected lower concentration (25 mM) of NaCl and treated with 200 (ppm) of thiamin ($V.B_1$). The minimum values of total lipid contents of *Chlamydomonas reinhardtii* were 86.7%, of that the control cultures, when the algal cultures subject to 100 mM NaCl, only, as compared with that the control cultures. Also, The minimum values of total lipids contents of salinized *Chlamydomonas reinhardtii* were 95.1 % of that the control cultures, when cultures, when the algal cultures subject to 100 mM NaCl, and treated with 200 (ppm) of thiamin ($V.B_1$) (Fig. 1-b).

The maximum values of the total protein contents of *Chlamydomonas reinhardtii* reached to 143.9%, of that the control cultures, when the algal cultures subjected to moderate concentration of 75 mM of NaCl only. On other hand, maximum values of the total lipid contents of salinized *Chlamydomonas reinhardtii* were 157.2%, of that the control cultures, when the algal cultures subjected moderate concentration (75 mM) of NaCl and treated with 200 (ppm) of thiamin ($V.B_1$). The minimum values of total protein contents of *Chlamydomonas reinhardtii* were 63.1%, of that the control cultures, when the algal cultures subject to 100 mM NaCl only, as

compared with that the control cultures. Also, The minimum values of total protein contents of salinized *Chlamydomonas reinhardtii* were 79.3% of that the control cultures, when cultures, when the algal cultures subject to 100 mM NaCl, and treated with 200 (ppm) of thiamin ($V.B_1$) (Fig. 1-c).

	Growth parameters of *Chlamydomonas reinhardtii*					
Treatments	**Dry weight ($\mu g\ ml^{-1}$)**	**%**	**Optical density ($g\ l^{-}$)**	**%**	**Total pigment (μgmg^{-1})**	**%**
Control	295 ± 4.41	100.0	0.779± 0.001	100.0	11.29± 0.78	100.0
25 mM NaCl	376 ± 7.87	127.7	0.846± 0.004	108.5	14.80±0.53	131.1
50 mM NaCl	395± 1.66	133.8	0.878± 0.003	112.6	17.22± 0.03	152.5
75 mM NaCl	415± 3.33	140.7	0.937± 0.01	120.2	22.25± 0.02	197.0
100 mM NaCl	223 ± 7.87	75.71	0.491± 0.007	62.98	2.249± 0.05	19.92
Control + *thiamin*	350 ± 5.00	118.6	0.841± 0.023	107.9	14.14± 0.15	125.3
25 mM NaCl + *thiamin*	388± 5.09	131.6	0.998± 0.019	128.0	15.88± 0.09	140.7
50 mM NaCl+ *thiamin*	455± 6.00	154.2	1.046± 0.020	134.2	20.21± 0.05	179.0
75 mM NaCl + *thiamin*	484± 1.50	164.3	1.102± 0.001	141.2	24.88± 0.01	220.4
100 mM NaCl + thiamin	286± 4.19	97.18	0.533± 0.001	68.44	3.184± 0.09	28.20
LSD=0.05	26.9		0.029		1.10	

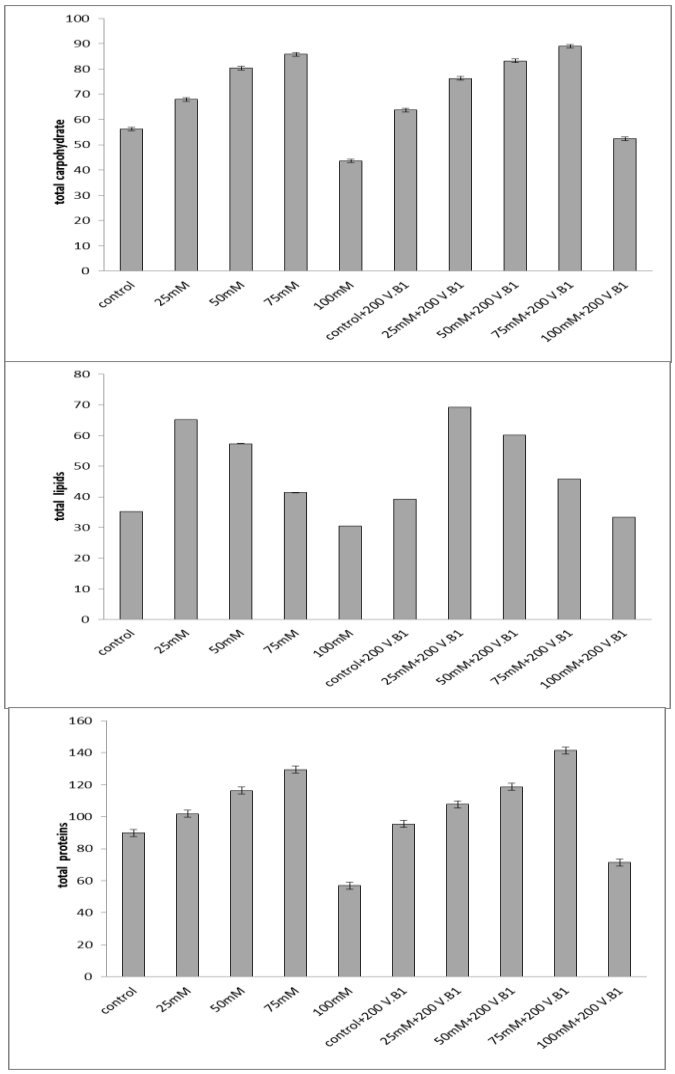

Figure 1: (a) Total carbohydrates ($\mu g\ mg^{-1}$ dry weight), (b) Total lipids ($\mu g\ mg^{-1}$ dry weight), (c) Total proteins ($\mu g\ mg^{-1}$ dry weight), of Chlamydomonas reinhardtii cultures were subjected to various concentrations of NaCl (mM) and treated with (200) ppm of thiamin (vitamin B_1) for 7 days.

4. Conclusions

This study detected the effect of NaCl on Dry weight, optical density, total pigments, total carbohydrate, total protein and total lipid contents of *C. reinhardtii* and *C.reinhardtii* treated with thiamin (vitamin B_1) cultured for 7 days was as following. The growth parameters of *C. reinhardtii* and *C.reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to (25, 50 and 75 mM NaCl). There above all these parameters were significantly decreased. According to [22], reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress. Many previous studies reported that the cultivation with higher saline concentrations had lower chlorophyll and protein contents [31].

Many previous studies reported that carbohydrates synthesis was stimulated by stress conditions [32] and [30] The total carbohydrate contents of *C. reinhardtii* and *C. reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to lower and moderate concentrations (25, 50 and 75mM NaCl), but under relatively higher concentration (100 mM NaCl) these parameters were significantly decreased. The total protein contents of *C.reinhardtii* and *C. reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to lower and moderate concentrations (25, 50and 75mM NaCl), but under relatively higher concentration (100mM NaCl) these parameters were significantly decreased.

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