Interference Effects of Salinity on Growth and Some Metabolic Activities of Two Chlorella Species

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Abstract: *The unicellular green algae Chlorella minutissima and Chlorella oocystoides were isolated from different regions in Upper Egypt. The effect of different concentrations of salinity (100, 250 and 500 mM NaCl) on growth parameters (optical density, dry weight, and total photosynthetic pigments) and primary products (total carbohydrates, total proteins, and lipids contents) were measured after 7 days. The growth parameters, and the primary products of Chlorella minutissima and Chlorella oocystoides were significantly increased at lower and moderate concentrations (100 and 250 mM NaCl). Under higher concentration 500 mM of NaCl, the growth parameters, and the primary products (total carbohydrates, and total proteins) were significantly decreased. However, the lipid contents were markedly increased.*

Keywords: *Chlorella minutissima, Chlorella oocystoides, cell growth, total carbohydrates, total proteins and lipid contents.*

Introduction

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. Microalgae differ in their adaptability to salinity and other stress conditions. The ability of cells to survive and flourish in saline environment under the influence of osmotic stress has received considerable attention. Cells develop many adaptive strategies in response to different abiotic stresses such as salinity, dehydration, cold and excessive osmotic pressure. Against these stresses, cells adapt themselves by undergoing different mechanisms including changes in morphological and developmental pattern as well as physiological and bio-chemical processes (Bohnert *et al*. 1995). Microalgae are simple photosynthetic organisms, which are widespread in nature, playing fundamental roles as primary producers in marine, freshwater and sub aerial terrestrial systems (Faria *et al* 2012). *Chlorella* is a kind of unicellular green algae living in freshwater and belongs to the phylum Chlorophyta (Barghbani *et al*., 2012). Microalgae are rich sources of proteins, carbohydrates and fatty acids. Unicellular eukaryotic microalgae have special importance due to the simplicity of their structures showing all metabolic activities and having similarities with higher plants. The growth of microalgae is retarded during salinity stress due to the accumulation of compatible solutes like proline and glycine to balance the external salt concentrations (Ahmed *et al.*, 1989). Takagi *et al*., (2006) found that the cell growth (optical density) of *Dunaliella*. *Tertiolecta* was decreased, when the algal cultures subjected to 1 M or 2 M of NaCl. But under 0.1 M NaCl the optical density was no decreased.The changes in the lipid contents of some plants types have vigorously been elevated with the rise of salinization level. In this respect some other investigations working with some plant types have suggested that the lipid contents were raised with the rise of salinization level (Ferguson, 1966 and Desouky, 1990). This increase in lipid contents could be regarded as means of adaptation to salt effect (Gale and Poljakoff-Mayber ,1970 , Ben – Amozt and

Tornabene, 1985 and Desouky 1990, 1995).In this context, Desouky (1990 and 1995) working with *Chlorella vulgaris* Bijer found that the growth parameters (Cell number, dry weight and total pigments) , total carbohydrates total proteins were significantly increased upto the level 200 mM NaCl. But the lipid contents were significantly decreased. The same author found that, all these parameters were significantly decreased under higher concentration 400 mM NaCl; however the lipid contents were significantly increased. In this context, Takagi *et.al*. (2006) found that the lipid contents of Dunaliella. *Tertiolecta* markedly increased, when the algal cultures subjected to 1.5 M of NaCl. The same investigators found that the various levels (0.5 or 1 M) NaCl markedly increased the lipid contents.

Aim of the work

At the end the study showed the effect of salinity on growth parameters (Optical density, dry weight and total pigments) , and primary products (total carbohydrates and total protein) were significantly increased under lower and moderate levels. However under higher concentration all these parameters were significantly decreased, but the lipid contents were significantly increased.

Material and Methods

Tested algae: The two microalgae species used in this study, *Chlorella minutissima* and *Chlorella oocystoides*, were collected from culture collection of Algae, 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, (Stanier.*et al*., 1971). The compontents of this medium is as the following:

	Stock solutions [g / 100ml]	Nutrient solution [ml]
NaNO3	15	10
K2HPO4 . 3H2O	0.4	10
MgSO4 . 7H2O	0.75	10
CaCl2 . 2H2O	0.36	10
citric acid	0.06	10
Ferric ammonium citrate	0.06	10
EDTA (dinatrium-salt)	0.01	10
Na2CO3	0.2	10
*Micronutrient solution		1
De-ionized or distilled water		919

*Composition of the micronutrient solution (from Kuhl and Lorenzen 1964)

Add to 1000 ml of de-ionized or distilled water:

H3BO3	61.0 mg
MnSO4 . H2O	169.0 mg
ZnSO4 . 7H2O	287.0 mg
CuSO4 . 5 H2O	2.5 mg
(NH4)6Mo7O24 . 4H2O	12.5 mg

Treatments

Chlorella minutissima *and* Chlorella oocystoides *were subjected to 00 (control) and various levels (100, 250 and 500 mM) of NaCl for 7 days were followed.*

Analytical methods:

1-Determination of Optical Density (OD): The cell concentration was determined by measuring OD at 680 nm. The data were calculated according the method by Robert (1979)

2-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 over night in an oven at 105 ^{o}C. The data were expressed as µg ml^{-1} algal suspension.

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 Lichtentaler and Wellburn (1985) : -

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

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

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

4- Determination of carbohydrates

Using of anthrone-sulphoric acid reagent according to the method by Badour (1959). The data measured as µg mg^{-1} dry weight.

5- Determination of Protein Contents:

Using Bradford reagent according the method adapted by Bradford, (1976), and Zor and Selinger, (1995) .The data were measured as µg mg^{-1} dry weight.

Proteins content was determined in the algal suspension extract using Bradford reagent (Bradford, 1976 and Zor and Selinger, (1995). A calibration curve was constructed using bovine serum albumin (BSA) and the data were expressed as µg mg^{-1} dry weight.

Reagent: *100 mg Coomassie brilliant blue G-250 was dissolved in 50 ml of 95% (v/v) ethyl alcohol. To this volume 100 ml of 85% (w/v) phosphoric acid was added. The resultant solution was diluted to a final volume of 1 liter with dist. water and stored in dark bottle at room temperature.*

Sample preparation

Total protein

A definite volume (25 mls.) of alga suspension was boiled in water bath for 1.5 hour, cool under tap water. Working centrifuged for 10 minutes at 5000 r.p.m.Take 2 mls. from water extract were mixed with 5 ml 1 N NaOH and boiled in water bath for 30 min., then completed to a definite volume by distilled water

Procedure

One ml of the reagent was added to 100 µl of sample. The blue colour developed after 5 min was detected at 595 nm. Bovine serum albumin was used as standard curve

Determination of lipid contents

A culture sample containing 30 mg cell was centrifuged participated was washed with 1 % NaCl. After extraction of lipid from the participated with methanol - chloroform (2: 1) , chloroform and 1 % NaCl solution was added to adjust the ratio of methanol , chloroform and water to 2:2:1.At the chloroform layer collected three time were evaporated dried in desiccators , and weighed as total lipid (Ben –Amozt and Tornabene 1985).

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 Snedecor and Cochray (1980).

Results

The date present in this investigation showed the interface effects of salinity on growth parameters (Optical density and dry weight, total photosynthetic pigments), primary products (total carbohydrate, total protein, and total lipid contents) of *C. minutissima* and *C. Oocystoides* cultures for 7 days. In this study, the growth criteria (Optical dessity and dry weight, and total photosynthetic pigments) of *C.minutissima* and *C. oocystoides* cultures were significantly increased up to level 250 mM of NaCl. However, under higher level (500) of NaCl, all these parameters were significantly decreased, as compared with that of the control cultures. Thus, the maximum values of growth parameters of *C.minutissima* reached to 111 %, 153 %, and 120 % of that the control cultures, respectively, when the algal cultures subject to 250 mM NaCl (Table, 1 and 2).On the other side, the maximum values of growth parameters of *C. oocystoide* reached to 126%, 126 %, and 153 % of that the control cultures, respectively, when the algal cultures subject to 250 mM NaCl (Table, 3 and 4).

On the other hand, the total carbohydrates and total proteins of *C. minutissima* and *C. oocystoides* cultures were significantly increased upto the 250 mM Nacl, when compared with that the control cultures (Fig1 and Fig.2).The maximum values of total carbohydrates and total proteins *of C. minutissima* and *C. oocystoides* amounted to 149 %, 157 %, 153, and 155 %, of that the control cultures, when the algal cultures subjrcted to 250 mM NaCl, respectively (Fig.1 and Fig.2).

Lipid contents of *C. minutissima* and *C. oocystoides* cultures were significantly increased when the algal cultures subjected to 500 mM NaCl. The maximum values of lipid contents reached to 150 % and 162 %, respectively, when the algae cultures subjected to 500 mM NaCl (Fig.1 and 2).

Table (1): Effect of NaCl on Optical Density and dry weight (µg mg^{-1} dry weight) *of Chlorella minutissima* cultures for 7 days.

Treatment NaCl mM	Optical Density	% Control	Dry weight	% Control
00	0.795	100.00	357	100.00
100	0.879**	110.560	427**	113.86
250	0.887**	111.570	577**	153.86
500	0.519**	64.900	285**	79.83
L.S.D at 0.05 %	0.001		4.214	
L.S.D. at 0.01 %	0.005		6.245	

*Significantly difference ……. as compared with absolute control
**High significantly difference…….. as compared with absolute control

Table (2) : Interference effects of NaCl on total pigments (µg ml^{-1} algal suspension) of *Chlorella minutissima* cultures for 7 days.

Treatments NaCl mM	Chloro. a	Chloro.b	Carot.	Total pigments	% Control
00	5.926	2.908	3.602	12.436	100.00
100	7.200**	2.800**	3.082**	13.082**	105.190
250	9.517**	2.111**	3.396**	15.024**	120.810
500	2.951**	1.412**	1.936**	6.029**	48.480
L.S.D at 0.05 %	0.854	0.214	0.012	0.298	
L.S.D. at 0.01 %	1.255	0.411	0.084	0.464	

*Significantly difference …… as compared with absolute control
**High significantly difference…….. as compared with absolute control

Fig (1): Interference effects of NaCl on total carbohydrates, total proteins and total lipid contents (µg mg^{-1} dry weight) of *Chlorella minutissima* cultures for 7 days.

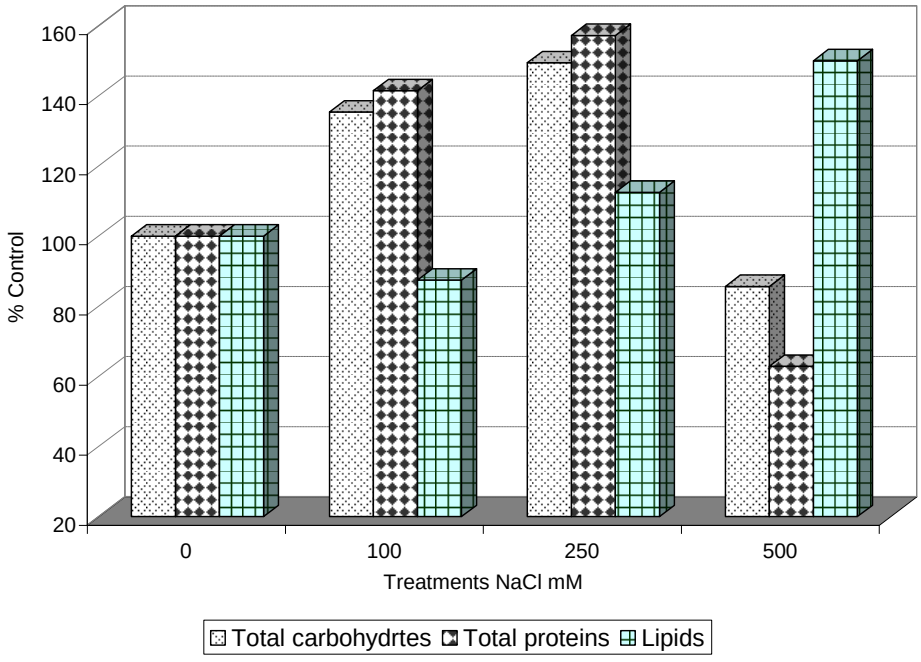


Table (3) : Effect of NaCl on Optical Density (g L^{-1}) and dry weight (µg mg^{-1} dry weight) of *Chlorella oocystoides* cultures for 7 days.

Treatment NaCl mM	Optical Density	% Control	Dry weight	% Control
00	0.856	100.00	320	100.000
100	0.991**	115.771	360**	112.500
250	1.082**	126.401	405**	126.562
500	0.240**	28.037	215**	67.187
L.S.D at 0. 05 %	0.003		7.123	
L.S.D. at 0.01 %	0.004		9.241	

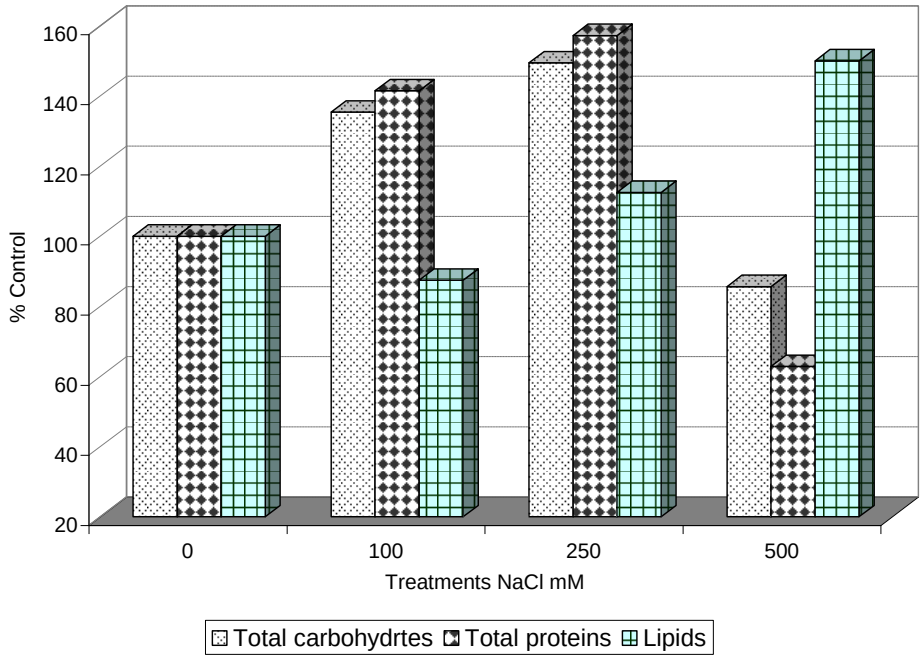
*Significantly difference …….. as compared with absolute control
**High significantly difference…….. as compared with absolute control

Table (4): Effect of NaCl on total pigments (µg ml^{-1} algal suspension) *of Chlorella oocystoides* cultures for 7 days.

Treatments NaCl mM	Chloro. a	Chloro.b	Carot.	Total pigments	% Control
00	7.414	2.133	2.209	11.756	100.000
100	8.281**	2.572**	3.184**	14.037**	119.402
250	10.727**	4.848**	2.511**	18.086**	153.727
500	3.049**	1.521**	1.581**	6.151**	43.815
L.S.D at 0.05 %	0.282	0.001	0.003	2.225	
L.S.D. at0.01 %	0.452	0.005	0.004	4.283	

*Significantly difference …….. as compared with absolute control
**High significantly difference…….. as compared with absolute control

Fig. (2): Effect of NaCl on total carbohydrates, total proteins and total lipid contents (µg mg^{-1} dry weight) of *Chlorella oocystoide*s cultures for 7 days.



Discussion

This study elucidated effect of salinity on growth criteria (optical density and total pigments), total carbohydrates, total proteins and lipid contents of *Chlorella minutissima* and *Chlorella oocystoides* cultured for 7 days.

The growth parameters of *Chlorella minutissima* and *Chlorella oocystoides* were significantly increased, when the algal cultures subjected to (100 and 250 mM NaCl). Thereabove all these parameters were significantly decreased. These results in accordance with (Desouky, 1990, 1995 and Taking *et al*., 2006).

The total carbohydrates and total protein contents of *Chlorella minutissima* and *Chlorella oocystoides* were significantly increased, when the algal cultures subjected to lower and moderate concentrations (100 and 250 of NaCl,but under relatively higher concentration (250 mM NaCl) the all parameters were significantly decreased. These results were according with some investigators working with some plant types recoded that the total carbohydrates and total protein contents were significantly increased at lower and moderate levels of salinity, but under higher level all the parameters were significantly decreased (Erdman, 1983, Gilmour *el. a*l., 1985, Kandapal and Appajirao, 1985, Ahmed, 1988, Karsten and Kirst, 1989, Desouky 1990, and Desouky, 1995).

The changes in the lipid contents of some plants types have vigorously been elevated with the rise of salinization level. In this respect some other investigations working with some plant types have suggested that the lipid contents were raised with the rise of salinization level (Ferguson, 1966 and Desouky, 1990, and Taking *et al*., 2006). This increase in lipid contents could be regarded as means of adaptation to salt effect (Gale and Poljakoff-Mayber, 1970. Haffman and Phene, 1971, Ahmed et al., 1979 and Desouky, 1990).

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