**RESEARCH ARTICLE**

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# EFFECTS OF SALINITY AND LIGHT INTENSITY ON PRODUCTION OF ß-CAROTENE AND GLYCEROL FROM THE HALOTOLERANT ALGA *DUNALIELLA SALINA* (CHLOROPHYTA) ISOLATED FROM ZARANIK NATURE RESERVE, NORTH SINAI, (EGYPT)

**ABSTRACT:**

The halotolerant green alga *Dunaliella salina* (isolate SEA003) recently isolated from salt march at Zaranik nature reserve, North Sinai, (Egypt) was cultivated in the artificial seawater medium (ASW) with different salinity levels (0.5, 1, 2, 3, and 4 M), under continuous illumination of two different light intensities: 50 and 200 µmol $m^{-2}s^{-1}$, during 30 day of incubation. The maximum growth density as number of cell was recorded in media contained 2M NaCl under the high light intensity, while Chlorophyll a giving its maximum values at 1 M salinity under high light intensity. Anent values of β carotene produced by *Dunaliella salina* revealed that, 2 M salinity had the superiority in enhancing β carotene production during the 30 days of this study, especially under high light intensity. In contrast with β carotene productivity, *Dunaliella salina* gave its maximum glycerol values at the high salinity (4M). This result revealed that, the algal productivity can be controlled, as well as directed to produce a particular substance (such as carotene and glycerol) on a wide scale by controlling the growth conditions. Adjusting salinity and light intensity is likely the best strategies to achieve optimal β-carotene or glycerol production in mass cultures.

**KEY WORDS:**

*Dunaliella,* ß-carotene, glycerol, salinity, light intensity

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## INTRODUCTION:

The halotolerant, unicellular, biflagellate alga *Dunaliella* is distinguished morphologically by absence of rigid cell wall (Ben-Amotz, 2009; Tran *et al.*, 2013; Rad *et al.*, 2015), however it shows a remarkable degree of adaptation to a variety of salt concentrations (from 0. 1 to 5.0 M NaCl) in the aquatic environments (Alizadeh *et al.*, 2015). Thus, this organism is unique in its ability to adapt in harsh environmental conditions. *Dunaliella* species has the ability to accumulate significant amounts of carotenoids (Hadi *et al.*, 2008; Mingazzini *et al.*, 2015), proteins, vitamins (Ghoshal *et al.*, 2002) and it was also recommended as a good source for glycerol production (Ben-Amotz, 2003) under stress conditions. Currently, the best commercial source of natural β -carotene (with economic value) among all organisms in the world is *Dunaliella salina* (Ben-Amotz, 1995; Rad *et al.*, 2015), which contains β-carotene up to 14% of its dry weight (Aasen *et al.*, 1969). Therefore, production of β -carotene by mass cultivation of *D. salina* has being masterful in many countries including China, USA and Australia (Rad *et al.*, 2015).

The mechanism by which *Dunaliella* cell adapted to broad salinity range based on the ability of the algal cell to change its internal concentration of carotenoids especially β-carotene and glycerol (Raja *et al.*, 2007). Where, significant amounts of β -carotene accumulated in the plastid as droplets to prevent the photo-damage of chlorophyll under stress conditions "include high temperature, light intensities, salinity and deficiency of nutrient" (Ben-Amotz and Shaish, 1992; Ben-Amotz, 2003). Beta-carotene accumulates within the lipid globules in the spaces between thylakoids located in chloroplast (Ben-Amotz, 2003). Thus, it has been proposed that the increase in fatty acid content under stress conditions can be partially attributed to the increase in β-carotene (Mendoza *et al.*, 1999).

It is of interest to mention that, liver enzymes oxidized β-carotene compound producing vitamin A, which plays a vital role

in increasing the efficiency of the immunity system and act as antioxidants agents beside of that it is necessary for many important physiological functions (Mishra and Jha, 2011). Hence, *Dunaliella*'s β-carotene can be used as health dietary supplements for both human and animals (Pulz and Gross, 2004), cosmetic and also in several promising pharmaceutical industries (Borowitzka, 1999; Chidambara-Murthy *et al.*, 2005), reducing the risk of cardiovascular diseases (Törnwall *et al.*, 2004) and controlling cholesterol. In addition β-carotene can prevent or even inhibit various types of tumors (Hallmann, 2007; Chattopadhyay *et al.*, 2008). It is well known that, all the β-carotene's therapeutic effects related to its protective ability against potentially harmful free radicals and stimulatory affects to the immunity system (Gotz *et al.*, 1999). Although, natural β -carotene can be obtained from many other sources like fruits, green leafy vegetables and fungi (Pulz and Gross, 2004) or even synthetically produced (Mayer and Isler, 1971), the *Dunaliella*'s β-carotene has more therapeutic effects, where concerning isomers composition, the synthetic β -carotene only contains all-trans isomers. While, natural β -carotene present in fruits and vegetables composed mainly of a mixture from 9-cis and trans β-carotene (Ben-Amotz and Shaish, 1992) and it is highly difficult in purification and obtained by relatively low concentration. It is well known that, the 9-cis isomer is more efficient as antioxidant than all-trans, where it is significantly more soluble in lipid and thus it may be more effectively accumulated in human tissue (Ben-Amotz *et al.*, 1989). Hence, the higher the 9-cis concentration, the higher is the anticancer and antioxidant efficiency (Hu *et al.*, 2008). In comparison, among all natural sources, *Dunaliella salina* produce the greatest amount of 9-cis isomer (more than 50% of all its isomers). In addition, a *Dunaliella* cell can accumulate β -carotene, thousands of times more than a carrot cell (Klausner, 1986). In summary, *Dunaliella salina* is considered as the best-known β -carotene natural source (Ben-Amotz, 2003; Rad *et al.*, 2015), because it has high commercial value where the cultivation of microalgae and purification with organic solvents is economically cheap. Indeed, the natural β -carotene has a market price approximately double or triple more than that of the synthetic products (Pulz and Gross, 2004).

On the other hand, glycerol is a vital commercial organic chemical act in osmoregulation beside using in food, cosmetic and pharmaceutical industries, as well as it is also used as anti-drying agent (Shariati, 2003). Under stress circumstances of high salinity, glycerol accumulated (up to 40% of the cell dry weight) by *Dunaliella* (Ben-Amotz, 1990). Nowadays, the price of glycerol is depended on the price of crude oil, where it is mostly produced from petrochemical sources. Therefore, recently advancing algal biotechnology and mass culture cultivation of *Dunaliella* sp. for glycerol and carotenoid production and other biotechnological purposes is of great interest. The ability to induce, modify and scale up *Dunaliella* sp. to produce a series of uncommon carotenoids of high nutritional and medical value and glycerol, also opens a new field in the area of *Dunaliella* biotechnology. It is necessary to mention that, among different strains of *D. salina*, there is no predictable unique condition for reaching the maximum β-carotene or glycerol contents per unit time and per unit volume for all strains. Where, there are great physiological variations in response to different inductive growth factors.

Therefore, it was very important to determine the optimal conditions of salinity and irradiance enhancing the production of both β- carotene and glycerol from this strain of *D. salina*, recently isolated from salt march at Zaranik nature reserve, North Sinai, (Egypt), as well as determine its productivity at different stress conditions for mass cultivation.

## MATERIAL AND METHODS:

The marine microalga *Dunaliella salina* (Eukaryota, Viridiplantae, Chlorophyta, Chlorophyceae, Chlamydomonadales, Dunaliellaceae, *Dunaliella*, Species: *D. salina*) isolated from salt march at Zaranik nature reserve, North Sinai, (Egypt), was generously donated by doctor: Eltanahy E. G., from the Culture Collection of Botany Department**,** Faculty of Science, Mansoura University, Egypt. The test isolate was identified by morphological and genetic analyses. *Dunaliella salina* isolate SEA003 was deposited in the GenBank under the accession number JX220893.1 (Eltanahy *et al.*, 2015).

### Growth Conditions:

*Dunaliella salina* was cultivated in the artificial seawater medium (ASW) which contained: 4.5 mM $MgCl_2.6\ H_2O$, 3 mM $CaCl_2.\ 2H_2O$, 1.5 mM $NaVO_3$, 5 mM $KNO_3$, 0.13 mM $K_2HPO_4$, 0.5 mM $MgSO_4.\ 7\ H_2O$, 0.02 mM EDTA, 0.02mM $FeCl_3$, 1 mg $l^{-1}$ trace element stock with 10 mM $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, 25 mM $NaHCO_3$, 50 mM $H_3BO_3$, 2mM $NaMoO_4.2H_2O$, 0.8 mM $ZnSO_4.7H_2O$, 0.2 mM $CoCl_2.6H_2O$. Different NaCl concentrations (0.5, 1, 2, 3 and 4 M) were added to one litter of medium. The pH value was adjusted at 7.5 by addition of 40 mM of Tris -buffer. All stock solutions were sterilized separately for 30 minutes and pooled aseptically, in order to avoid

precipitation of certain compounds (Hejazi *et al.*, 2003). After autoclaving, the culture flasks were left to cool at room temperature for one day to allow for pH stabilization at 7.5 (Olmos *et al.*, 2000). To achieve an initial cell density of 4000 cell $ml^{-1}$, 10 ml of actively growing vegetative inoculums of *Dunaliella salina* cells were inoculated into sterilized flasks containing 250 ml of ASW medium. Flasks were incubated at 28 ± 1°C under continuous illumination (24 h light) of two different light intensities: 50 µmol $m^{-2}s^{-1}$ (low light intensity) and 200 µmol $m^{-2}s^{-1}$ (high light intensity) by cool-white fluorescent lamps (Philips, 50 w). To ensure a uniform illumination of the cells, the media were shaked manually twice a day. The experiment was performed in triplicate for 30 days, and all experiments were repeated at least twice.

**Growth Estimation:**

Cell number was determined by direct counting under a light microscope (magnification × 40) by using a 0.1 mm deep counting chamber "haemocytometer" (James and Al-Khars, 1990). Once in every 5 days the samples were collected and cell counting was performed. Since the alga is motile, one drop of Lugol's iodine solution was added to arrest the motility.

**Estimation of Chlorophyll a:**

Chlorophyll extraction was carried out according to Pistal and Lele (2005). Four ml aliquots sample from each culture medium of *Dunaliella salina* cultures were centrifuged at 5,000 rpm for15 min. The precipitated pellets were washed with distilled water, then suspended in an acetone/water solution (80:20 v/v) for 30 min. after that it was thoroughly vortexed for 5 min. to ensure complete extraction and then centrifuged again for 5 min at 5000 rpm to extract pigments until the pellets turned clear/white. The amount of extracted pigments in the solvent phase was spectrophotometric quantified using method described by Lichtenthaler and Wellburn (1985):

$$\text{Chl a } (\mu g\ ml^{-1}) = 11.75\ (A_{662}) - 2.35\ (A_{645})$$

**Determination of β-Carotene Content:**

Contents of β -carotene in each sample were determined spectrophotometrically. Four ml from the aqueous phase of each *Dunaliella* culture medium was centrifuged at 4000 rpm for 15 min, discarding supernatant and then, each precipitated pellet was added to 4 mL (75%) acetone solution, shacked until separation. The above processes repeated till the extract turned to white, then 1/10 volume of 60% KOH was added at 49°C, to 10 ml volumetric flask, the supernatant without chlorophyll was transferred and 2ml acetone was added to the volume. After determining the $A_{453}$ value, the concentration ($A_{453}$) of the β -carotene solution could be found from the standard curve. Standard curve was prepared by dissolving different concentrations of standard β -carotene in pure acetone and measured at 453 nm which is the characteristic absorption for β -carotene. Graph was constructed by plotting the -carotene concentrations on X axis and OD values on Y-axis (Semenenko and Abdullaev, 1980).

**Determination of Glycerol Content:**

Four ml of the growth culture of *D. salina* was centrifugated at 1500 rpm for 10 min at room temperature, and then the precipitated pellets were washed twice in a solution of 1.5 M NaCl and 5 mm phosphate buffer at pH 7.5. One ml of periodate reagent and 2.5 ml of acetylacetone reagent were added to 200 µl of precipitated pellets,. The mixture was incubated at 45°C for 20 min. After that, optical density was determined at 410 nm. Results were compared with the standard curve prepared by using known amount of glycerol (Chitlaru and Pick, 1991).

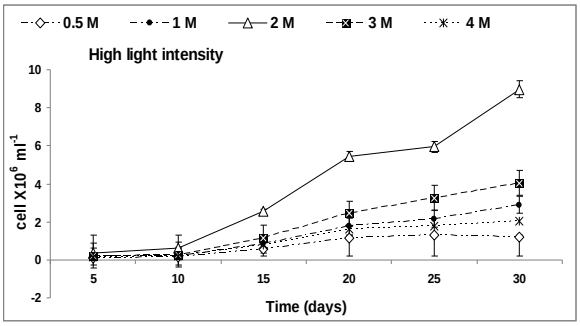
**Statistical Analysis:**

The data obtained are represented as Mean ± Standard deviation (mean ± SD). The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) using the SPSS-PC computer software package version 10.

## RESULTS:

The effect of different salt concentrations on cell number of *Dunaliella salina* under both high (200 µmol $m^{-2}s^{-1}$) and low (50 µmol $m^{-2}s^{-1}$) light intensities were studied. Inspection of Fig.1 revealed that, growth density as number of cell, reached its maximum (8.97 cell $X10^6$ $ml^{-1}$) after 30 days of incubation in media contained 2M NaCl under the high light intensity. Meanwhile, the same sample under low light intensity gave lower cell number (2.68 cell $X10^6$ $ml^{-1}$), however the maximum density gained under the low light intensity was considered during entire period of investigation. In contrast to the above, low cell density were recorded in 0.5 M salinity, followed by that values recorded within 1M and 4M respectively. Concerning the incubation period, it is noticeable that, the first ten days had convergent the lowest cell number (Fig. 1) in all parameters. Generally, compared with the other salt concentrations, the 2 M salinity supported the *Dunaliella* growth during the entire period of investigation under the two light intensities after the first ten days. Also it is of interest to mention that, 2M salinity significantly+ enhanced growth of *D. salina* over the other NaCl concentrations under high light intensity during the incubation

period, however in case of low light intensity; the growth was higher but with values closely to that recorded in 3 M salinity.



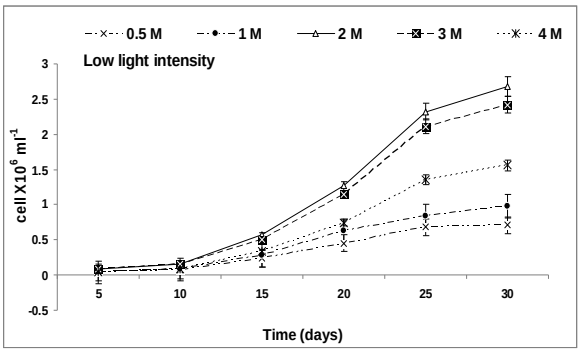
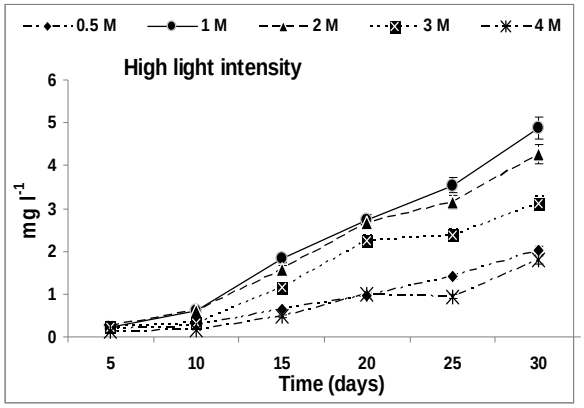




Fig. 1. Effect of different salinity concentrations on *Dunaliella salina* cell number (cell X10$^6$ ml$^{-1}$) under high and low light intensity during 30 days

A glance on figure 2 reveal that Chlorophyll "a" giving its maximum values in 1 M salinity under both high and low light intensity (0.26 - 4.88 mg l$^{-1}$ - 0.07 -2.2 mg l$^{-1}$, respectively), followed by that values recorded at 2 and 3 M of salinity respectively during the entire period of investigation. In this context, the high intensity of light enhanced chlorophyll "a" production more than the low light intensity. However, both 0.5 and 4 M NaCl suppressed the chlorophyll a production in tested *Dunaliella salina*. It is noticeable that, generally the chlorophyll "a" values under the high light intensity were higher than that recorded under low light intensity.



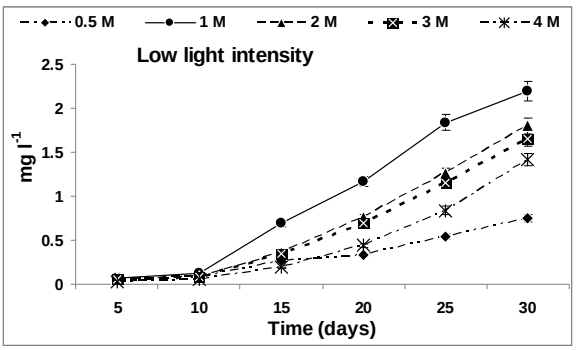




Fig. 2. Effect of different salinity concentrations on chlorophyll "a" (mg l$^{-1}$) in *Dunaliella salina* under high and low light intensity during 30 days

Anent values of β carotene produced by *Dunaliella salina* the results showed that, 2 M salinity had the superiority in enhancing β carotene production during the 30 days of this study, giving significant high values especially under high light intensity (2.41 - 34.62 mg l$^{-1}$) followed by that values recorded with 3M salinity (1.41- 18.91 mg l$^{-1}$) and then 1M (0.77- 17.02 mg l$^{-1}$) under the same conditions (Fig. 3). Nevertheless, generally low light intensity enhanced β carotene productivity at all NaCl concentrations but with lower rate from that recorded at high light intensity, however at low intensity the difference between the recorded values with all NaCl concentrations were convergent, where it produced 14.2, 12.48, 10.03, and 9.92 mg l$^{-1}$ after 30 days with 2M, 3M, 1M and 4M, respectively.

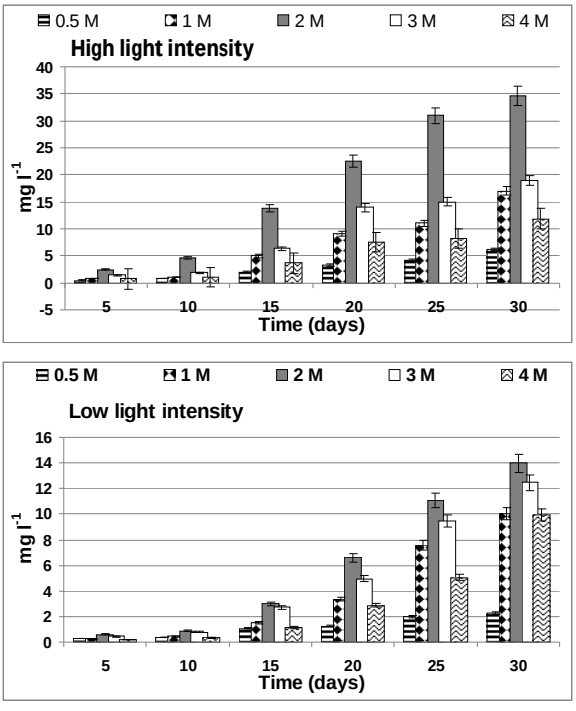



Fig. 3. Effect of different salinity concentrations on β-carotene (mg l$^{-1}$) yield of *Dunaliella salina* under high and low light intensity during 30 days

In contrast with β carotene productivity, *Dunaliella salina* gave its maximum glycerol values (48.49 - 31.7 mg $l^{-1}$ under both, high and low light intensity, respectively) within the high salinity (4M). Compared with this high values (4.06- 48.49 mg $l^{-1}$) of glycerol related to high salinity concentration, the recorded values in 0.5M (0.12 - 2.91 mg $l^{-1}$) are very low. Again, comparable to β carotene, the maximum values of glycerol were recorded under high light intensity especially within the highest NaCl concentrations (Fig. 4).

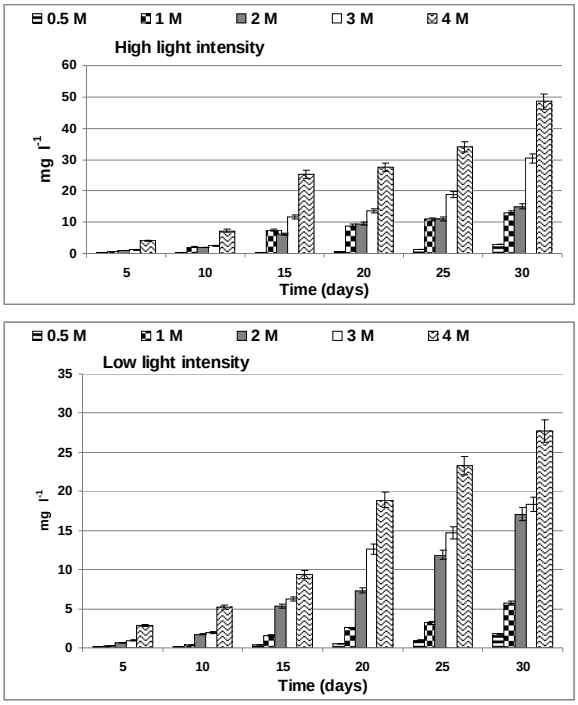



Fig. 4. Effect of different salinity concentrations on glycerol (mg $l^{-1}$) yield of *Dunaliella salina* under high and low light intensity during 30 days

The considerable variation between β carotene, Chlorophyll a and glycerol production within the different tested salinity concentrations during the period of investigation, reflect the importance of focusing on the ratios between β carotene and both of Chlorophyll a and glycerol. As regard in figure 5, the β carotene/Chlorophyll ratio under both high and low light intensities following the same approach of high values, where it fluctuated from 9.82 to 5.49 under high light intensity and from 9.66 to 6 under low light intensity with high salinity (2, 3, and 4 M). However, within lower salinity concentrations (0.5 and 1 M) it tended to give lower values. Nevertheless, the situation was reversed in case of β carotene/Glycerol ratio, where the cited results revealed that low NaCl concentrations gained the maximum β carotene/Glycerol ratio (Fig. 6). Meanwhile, minimum values were related to the high NaCl concentrations, under both, high and low light intensity, during the investigation period.

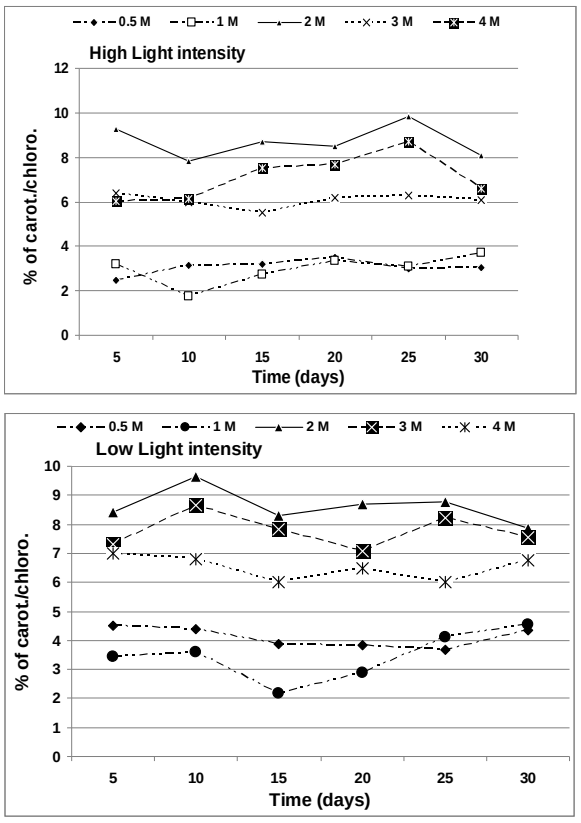



Fig. 5. Ratio of β-carotene : chlorophyll "a" in *Dunaliella salina* under high and low light intensity during 30 days

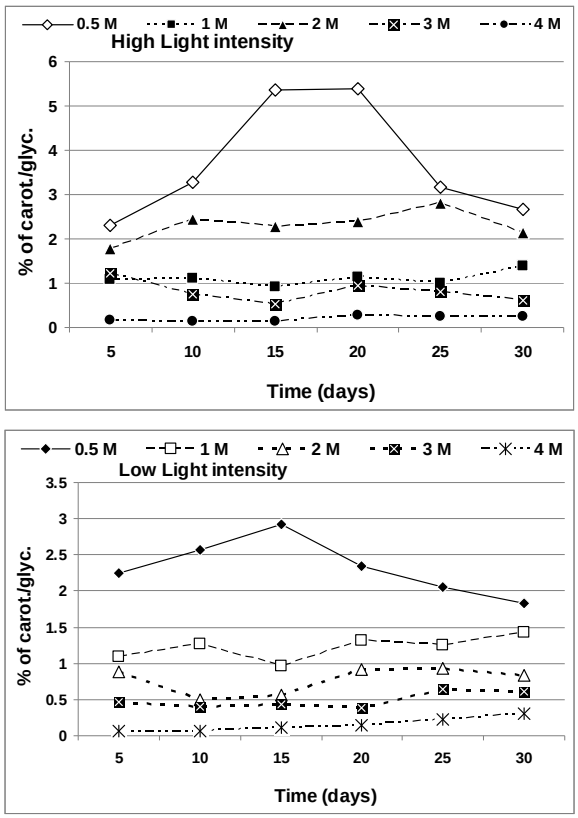



Fig. 6. Ratio of β-carotene : glycerol in *Dunaliella salina* under high and low light intensity during 30 days

**DISCUSSION:**

On the contrary to other chlorophyta, *Dunaliella*'s cells have no rigid cell wall, but only enclosed by an elastic plasma membrane covered by a mucus surface layer, which facilitates rapid swelling or shrinking when subjected to hypotonic or hypertonic condition, (Ben-Amotz, 2003), then it rapidly responds to any change in osmotic pressure by changing the volume of the cell (Rad *et al.*, 2015). Hence, *Dunaliella* species can physically resist 3 to 4 fold decreases or increases in osmotic pressure (which know as osmoregulation). Nevertheless, more hypoosmotic stress will lead to bursting of the cell, while the hyperosmotic stress causes irreversible shrinkage (Ben-Amotz and Avron, 1992; Prieto *et al.*, 2011; Suong *et al.*, 2014; Mingazzini *et al.*, 2015). In the present study, the maximum cell number (8.97 - 4.05 - 2.68 - 2.42 cell X $10^6$ $ml^{-1}$) of the recently isolated strain of *Dunaliella salina* recorded within media contains 2M NaCl under both high (200 µmol $m^{-2}s^{-1}$) and low (50 µmol $m^{-2}s^{-1}$) light intensity followed by that recorded in 3M NaCl concentration during entire period of investigation. Meanwhile, the lower salinity (0.5 and 1 M) responsible for the minimum growth, which reflected that low salinity, was inappropriate for the growth of this strain of *D. salina*. This agreed with Hadi *et al.* (2008) results, who reported that the optimal growth of *Dunaliella salina* was obtained at 2 M NaCl.

Chlorophyll is the pigment of life in the photosynthetic organisms, therefore this study interested with the influence of light intensity and salinity on chlorophyll "a" production. The cited results revealed that, Chlorophyll a giving its maximum values within 1 M salinity under both, high and low light intensity. However 0.5 M NaCl suppressed the chlorophyll a production in *Dunaliella salina*. Previous studies have described that, salinity exerted inhibitory effects on the activities of plastid's thylakoid membrane and some soluble enzymes of *Dunaliella* (Finel *et al.*, 1984). Rad *et al.* (2015) also reported that, hypo-osmotic shocks stimulate a temporary inhibition of photosynthesis and then the main photosynthetic pigment (chlorophyll), while hyper-osmotic shock induce a substantial inhibition of photosynthesis, but stimulate the glycerol synthesis. Since light intensity is a major contributing factor for the pigment production, the present study demonstrated that, high light intensity enhance chlorophyll a production than the low light intensity, the result which agreed with the results obtained by Liu and Shen (2004) in their study on *D. salina*.

β-carotene considered as extra-photosynthetic product, stored and accumulated to protect cells against chlorophyll catalyzed single reactive oxygen and possibly other excited chlorophyll damaging agents, and also to protect the cell against the deleterious effects of high radiation intensity by acting as a light filter or a screen to absorb excessive irradiation (Ben-Amotz and Avron, 1992; Ben-Amotz, 2003 & 2009), where the possible reasons for increasing the synthesis of β-carotene in *Dunaliella* seems to be photo-protection through its absorption properties. The oily nature of the cup shaped chloroplast is most efficient for this purpose. Prieto *et al.* (2011) also indicated that, β-carotene has various roles include acting as pro-vitamin "A", absorbing light energy, antioxidation activity, oxygen transport and general colorizing agent for many organisms.

Maximizing the production of β-carotene per unit time in mass culture of the halo-tolerant green algae *Dunaliella*, have been experimented by several strategies. These strategies based on the hypothesis that, severe physiological growth parameters, such as high light intensity (Coesel *et al.*, 2008), high salinity (Hadi *et al.*, 2008), temperature (Ben-Amotz, 2009; Mingazzini *et al.*, 2015), and nutrient deficiency (Shariati, 2003; Aghaii and Shariati, 2007) induce the rate of synthesis and the accumulation of significant amounts of β-carotene (up to 14% of dry weight). Anent, values of β-carotene produced by the tested strain of *Dunaliella salina* in this study reveal that, 2 M salinity had the superiority in enhancing β carotene production during the 30 days of this study, giving significant high values especially under high light intensity. Pisal and Lele (2005) also indicated that β-carotene can be greatly enhanced at higher irradiation. Hence, it was thought desirable to explore the possibility of using higher light stress for carotenogenesis. On the other hand, salinity affected the β carotene production as shown in cited results. Ben-Amotz (2009) indicated that, the highest accumulation of anti-oxidant vitamin A (β-carotene) in *Dunaliella salina* was observed when it subjected to high intensity of light in media containing high salinity (30%).

Beside, β-carotene and under stress conditions of salinity, *Dunaliella* species possess the ability to accumulate significant amounts of glycerol (Hadi *et al.*, 2008; Hosseini Tafreshi and Shariati, 2014). The mechanism by which *Dunaliella* cells can balance the extracellular osmotic stress by wide range of NaCl (from 50 mM up to 5 M NaCl) based on the ability of the microalga to modify its intracellular glycerol concentration (Shariati, 2003, Raja *et al.*, 2007; Hadi *et al.*, 2008). In fact, when *Dunaliella* cells grown at high salinity, the intracellular concentration of glycerol increased up to 50% and is sufficient

to account for essentially all of the osmotic pressure required to balance the extracellular osmolarity, while the internal NaCl concentrations remaining very low (Hosseini Tafreshi and Shariati, 2014). Some studies have confirmed that, measurements of freezing point depression in cell sap of *D. salina* indicated that, the internal osmotic concentration is higher than that expected due to the presence of the intracellular glycerol (Liu and Shen, 2004; Chen *et al.*, 2011), the phenomena known as osmoregulation. In this condition, glycerol acts as a 'compatible solute' that protects enzymes against both inhibition and inactivation (Hosseini Tafreshi and Shariati, 2014). The maximum values of glycerol in the present study related to high salinity (4M) under both, high and low light intensity. Generally, the concentration of glycerol in *Dunaliella salina* increase with increasing salinity, which are consistent with the previous hypothesis.

Glycerol produced in *Dunaliella* either by photosynthetic carbon dioxide fixation or by degradation of starch. The contribution of these two metabolic pathways to glycerol synthesis mainly depends on the degree of salinity stress, the availability of light and the reserved starch. Hosseini Tafreshi and Shariati (2014) reported that, both the glycerol synthesis under hypertonic conditions and its elimination under hypotonic condition occur in the dark or light (Rad *et al.*, 2015). In the dark, *Dunaliella* produces glycerol by degradation of starch, and the capacity of the cells to recover from hyperosmotic shock depends on the amount of reserved starch. However, in the light the hyperosmotic shock greatly encourage the production rate of glycerol and also stimulate starch degradation, which indicate that, in the light both the two metabolic pathways have significant contributions in the production of glycerol (Hosseini Tafreshi and Shariati, 2014; Tran *et al.*, 2014). This hypothesis greatly supported the results of this study where, the maximum values of glycerol were recorded under high light intensity especially within high salinity concentrations. Meanwhile the minimum concentrations were obtained under low Light intensity.

The β carotene/Chlorophyll ratio under both, high and low light intensity following the same approach of high values, which reflect that β carotene production increase at that concentration. However, within lower salinity concentrations (0.5 and 1 M) it tended to give low values proving that the production of β carotene was related to salinity stress.

On the other side, in case of β carotene /Glycerol ratio, where the cited results reveal that, low NaCl concentrations gained the maximum β carotene/Glycerol ratio. Meanwhile, minimum values were related to the high NaCl concentrations under both, high and low light intensity during the investigation period, which reflect that the cell has shifted its activity to produce glycerol by increase in salinity level. Therefore logically, from a commercial point of view, the best strains of *D. salina* for use in mass cultivation should have the maximum specific growth rate and the highest β-carotene and/or glycerol content per unit time and culture volume under optimized conditions (Prieto *et al.*, 2011).

## CONCLUSION:

In this context, the algal productivity can be controlled as well as directed to produce a particular substance (such as carotene and glycerol) on a wide scale by controlling the growth conditions. Adjusting salinity and light intensity is likely the ideal strategies to achieve optimal β-carotene or glycerol production in mass cultures of *Dunaliella salina*.

## REFERENCES:


Aasen AJ, Eimhjellen KE, Liaaen-Jensen S. 1969. An extreme source of Beta-carotene. Acta Chem. Scand., 223(7): 2544-2545.

Aghaii P, Shariati M. 2007. The effect of sulfate deficiency on cell division, β-carotene, chlorophyll accumulation, and photosynthesis in green alga *Dunalliella salina* (isolated from salt marsh of Gavkhoni, Isfahan). Iran. J. Biol., 2(20): 1-11.

Alizadeh GI, Aliev II, Magerramova Kh, Galandarli ZI, Dibirova GH, Jalilova AR. 2015. The response reaction of Dunaliella cells against the influence of Methylene blue and Norflurazon under the low temperature stress conditions. Eur. J. Biotechnol. Bioscie., 3(3): 7-10.

Ben-Amotz A. 1990. The Beta-carotene accumulating halo-tolerant alga *Dunaliella*. Biotechnology, 7(1): 28–32.

Ben-Amotz A. 1995. New mode of *Dunaliella* biotechnology two phase growth for β-carotene production J. Appli. phycol., 7(1): 65-68.

Ben-Amotz A. 2003. Industrial production of microalgal cell-mass and secondary products – major industrial species. In: "Handbook of Microalgal Cultures, Biotechnology and Applied Phycology. (Richmond A. Ed.)". Blackwell, UK. pp. 273-280.

Ben-Amotz A. 2009. Bioactive compounds: glycerol production, carotenoid production, fatty acids production. In: "The Alga *Dunaliella,* Biodiversity, Physiology, Genomics and Biotechnology. (Polle JEW, Subba Rao DV. Eds.)". Science Publishers, Enfield, USA, pp. 189–208.

Ben-Amotz A, Mokady S, Edelstein S, Avron M. 1989. Bioavailability of natural isomer mixture as compared with synthetic all-trans b-

carotene in rats and chicks. J. Nutr., 19(7): 1013- 1019.

Ben-Amotz A, Avron M. 1992. *Dunaliella*: Physiology, Biochemistry, and Biotechnology. CRC Press, Boca Raton, pp. 256.

Ben-Amotz A, Shaish A. 1992. β-carotene biosynthesis. In: "*Dunaliella*: Physiology, Biochemistry, and Biotechnology. (Avron M, Ben-Amotz A. ed.)". CRC Press, Boca Raton, FL, USA, pp. 205-216.

Borowitzka MA. 1999. Commercial production of microalgae: pond, tanks, tubes and fermenters. J. Biotechnol., 70(1-3): 313–321.

Chattopadhyay P, Chatterjee S, Sen SK. 2008. Biotechnological potential of natural food grade biocolorants. Afr. J. Biotechnol., 7(17): 2972-2985.

Chen H, Lao YM, Jiang JG. 2011. Effects of salinities on the gene expression of a (NAD+)-dependent glycerol-3-phosphate dehydrogenase in *Dunaliella salina*. Sci. Total Environ., 409(7):1291–1297.

Chidambara-Murthy KN, Vanitha A, Rajesha J, Mahadeva Swamy M, Sowmya PR, Ravishankar GA. 2005. In vivo antioxidant activity of carotenoids from *Dunaliella salina* – a green microalga. Life Sci., 76(12): 1381–1390.

Chitlaru E, Pick U. 1991. Regulation of glycerol synthesis in res ponse to osmotic changes in *Dunaliella*. Plant Physiol., 96(1): 50-60.

Coesel SN, Baumgartner AC, Teles LM, Ramos AA, Henriques NM, Cancela L, Varela JC. 2008. Nutrient limitation is the main regulatory factor for carotenoid accumulation and for Psy and Pds steadystate transcript levels in *Dunaliella salina* (Chlorophyta) exposed to high light and salt stress. Mar. Biotechnol. (NY), 10(5): 602–611.

Eltanahy EG, Soliman AI, Shaaban SA. 2015. Salinity effect on growth and antibacterial activity of Egyptian *Dunaliella* isolates. Mansoura J. Environ. Sci., 44(1): 1-16.

Finel M, Pick U, Selman-Reimer S, Selman BR. 1984. Purification and Characterization of a Glycerol-Resistant CF0-CF1 and CF1-ATPase from the Halotolerant Alga *Dunaliella bardawil*. Plant Physiol., 74(4): 766–772.

Ghoshal D, Mach D, Agarwal M, Goyal A, Goyal A. 2002. Osmoregulatory isoform of dihydroxyacetone phosphate reductase from *Dunaliella* tertiolecta: Purification and characterization. Protein Expr. Purif., 24(3): 404-411.

Gotz T, Windhovel U, Boger P, Sandmann G. 1999. Protection of photosynthesis against ultraviolet-B radiation by carotenoids in transformants of the *cyanobacterium Synechococcus* PCC7942. Plant Physiol., 120(2): 599-604.

Hadi MR, Shariati M, Afsharzadeh S. 2008. Microalgal biotechnology: Carotenoid and glycerol production by the green algae *Dunaliella* isolated from the Gave-Khooni salt marsh, Iran. Biotechnol. Bioprocess Eng., 13(5): 540-544.

Hallmann A. 2007. Algal transgenics and biotechnology. Transgenic Plant J., 1(1): 81-98.

Hejazi MA, Andrysiewicz E, Tramper J, Wijffels RH. 2003. Effect of mixing rate on b-carotene production and extraction by *Dunaliella salina* in two-Phase bioreactors. Biotechnol. Bioeng., 84(5): 591–596.

Hosseini Tafreshi A, Shariati M. 2014. *Dunaliella* biotechnology: methods and applications. J. Appl. Microbiol., 107(1): 14-35.

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and ad-vances. Plant J., 54(4): 621-639.

James CM, Al-Khars AM. 1990. Intensive continuous culture system using tubular photo-bioreactor for producing microalgae. Aquaculture, 87(3-4): 381-393.

Klausner A. 1986. Algaculture: food for thought. Nature, 4: 947-953.

Lichtenthaler HK, Wellburn AR. 1985. Determination of total carotenoids and chlorophylls A and B of leaf in different solvents. Biol. Soc. Trans. 11: 591-592.

Liu XD, Shen YG. 2004. NaCl-induced phosphorylation of light harvesting chlorophyll a&b proteins in thylakoid membranes from the halotolerant green alga, *Dunaliella salina*. FEBS Lett., 569(1-3): 337–340.

Mayer H, Isler O. 1971. Total syntheses. In: "Carotenoids. (Isler O. Ed.)". Basle: Birkhauser, pp. 328–575.

Mendoza H, Martel A, Jiménez del Río M, García Reina G. 1999. Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J. Appl. Phycol., 11(1): 15-19.

Mingazzini M, Teresa M, Palumbo H. 2015. Open Mass Cultures of Marine Microalgae for Biodiesel Production: Laboratory Approach to Study Species Competition in Mixed Cultures. Nat. Resour., 6: 174-180.

Mishra A, Jha B. 2011. Antioxidant response of the microalga *Dunaliella salina* under salt stress. Bot. Mar., 54(2011): 195–199.

Olmos J, Paniagua J, Contreras R. 2000. Molecular identification of *Dunaliella* sp. utilizing the 18S rDNA gene. Lett. Appl. Microbiol., 30(1): 80-84.

Pisal DS, Lele SS. 2005. Carotenoid production from microalgae, *Dunaliella salina*. Indian J. Botechnol., 4: 476-483.

Pistal DS, Lele SS. 2005. Carotenoid production from microalga, *Dunaliella salina*. Indian J. Biotechnol., 4: 476-483.

Prieto A, Pedro Cañavate J, García-González M. 2011. Assessment of carotenoid production by *Dunaliella salina* in different culture systems and operation regimes. J. Biotechnol., 151(2): 180–185.

Pulz O, Gross W. 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol., 65(6): 635-648.

Rad FA, Aksoz N, Hejazi MA. 2015. Effect of saltiness on the growth and production of β-carotene in isolated Dunaliella sp. microalga from qom salt Lake of Iran. Int. J. Biosci., 6(2): 164-171.

Raja R, Hemaiswarya S, Rengasamy R. 2007. Exploitation of *Dunaliella* for β-carotene

production. Appl. Microbiol. Biotechnol., 74(3): 517-523.

Semenenko VE, Abdullaev AA. 1980. Parametric control of β-carotene biosynthesis in *D. salina* cells under conditions of intensive cultivation. Fiziol. Rast., 27: 31-41.

Shariati M. 2003. Characterization of three species of *Dunaliella salina*, *Dunaliella parva* and *Dunaliella pseudosalina* isolated from salt marsh of GaveKhoni of Isfahan- IRAN. Iran. J. Sci. Technol., 27: 185-190.

Suong N, Duc T, Sixto P, Trung V. 2014. Medium improvement for higher growth and longer stationary phase of *Dunaliella*. J. Plant Sci., 2(1): 9-13.

Törnwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Taylor PR, Albanes D, Huttunen JK. 2004. Effect of a-tocopherol and b-carotene supplementation on coro-nary heart disease during the 6-year post-trial follow-up in the A TBC study. Eur. Heart J., 25(13): 1171–1178.

Tran A, Sudre B, Paz S, Rossi M, Desbrosse A, Chevalier V, Semenza JC. 2014. Environmental predictors of West Nile fever risk in Europe. Int. J. Health Geogr., 13: 26.

Tran D, Vo T, Portilla S, Louime C, Doan N, Mai T, Tran D, Ho T. 2013. Phylogenetic study of some strains of *Dunaliella*. Am. J. Environ. Sci., 9(4): 317-321.

## تأثير الملوحة وشدة الاضاءة علي انتاج كلا من β- كاروتين والجلسرول من طحلب الدونياليلا سالينا المقاوم للملوحة (السلالة المعزولة من محمية الزرانيق، شمال سيناء، مصر)

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يعتبر طحلب *Dunaliella* المقاوم للملوحة احد المصادر الطبيعية الهامة لإنتاج مركبات البيتا- كاروتين والجليسرول هذا بالإضافة الي مركبات حيوية اخري. لذا فقد عنيت هذة الدراسة بتحديد تركيزات الملوحة و كذا شدة الإضاءة التي تعزز إنتاجية كلا من البيتا- كاروتين و الجليسرول من سلالة لطحلب *Dunaliella salina* تم عزلها حديثا (isolate SEA003) من ملاحات بمحمية الزرانيق- شمال سيناء –مصر. تضمنت النتائج المتحصل عليها كلا من عدد الخلايا و نسبة كلوروفيل أ و كذالك تركيز البيتا- كاروتين و الجليسرول خلال 30 يوم من الزراعة. أبرزت النتائج ان أعلي كثافة للنمو (كعدد خلايا) قد سجلت عند درجة ملوحة 2M تحت شدة الاضاءة العالية بينما سجل أعلي معدل لانتاج الكلوروفيل عند درجة الملوحة 1M عند نفس شدة الاضاءة تليها القيم المسجلة عند 2M ثم 3M . في حين أعطت هذة السلالة أعلي إنتاجية للبيتا - كاروتين عند درجة ملوحة 2M تليها انتاجيتها عند 3Mثم 4Mعند شدة الاضاءة العالية. وعلي النقيض فقد سجلت أعلي قيم من الجلسرول لهذة السلالة عند درجة الملوحة القصوي (4M). و بهذا يمكن الجزم أنة يمكن توجية انتاجية الطحالب بصفة عامة و هذا الجنس بصفة خاصة لانتاج مواد مطلوبة أمثلة البيتا- كاروتين والجليسرول على نطاق واسع من خلال التحكم في ظروف النمو. ولا يغيب عن أذهاننا أن المناطق ذات الملوحة العالية هي مناطق فقيرة الإنتاج علي جميع الأصعدة و غير مستغلة و بذالك نكون قد تمكنا من الإستفادة منها عن طريق إنتاج مواد حيوية هامة من خلال إستغلال هذا الجنس من الطحالب المقاومة للملوحة.

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