The Effect of Instantaneous and Slow-Release Salt Stress Methods on Beta-Carotene Production within *Dunaliella Salina* Cells

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ABSTRACT: *The main concept of the present study is taken from the growth pattern of Dunaliella salina (green unicellular eukaryote microalgae) in Urmia Lake which is the second biggest saline lake in the world. In this study, different models of salt stress, including instantaneous (1 and 2 M) and consecutive slow-release salt stresses (0.5 M) were tested, and the amount of beta-carotene for each test was evaluated. According to the results, the highest amount of beta-carotene was obtained by the slow release method of salt injection with the amount of 9.01 µg of beta-carotene per mg of the dry weight of microalgae. The largest amount of beta-carotene production in 1 and 2 M instantaneous salt stresses method were recorded as 4.35 and 0.65 µg/mg respectively which were up to 2 and 14 times lower than the highest beta-carotene production under the slow-release salt stress method.*

KEYWORDS: *Microalgae; Dunaliella salina; Beta-carotene; Salt stress.*

INTRODUCTION

Microalgae are autotrophic organisms that produce high-value compounds such as pigments, proteins, carbohydrates, lipids, etc. by combining light energy and inorganic substances [1, 2]. These organisms categorize as the eukaryote (common microalgae) and prokaryote (cyanobacteria) that live in various environments such as lands, deserts, shores, etc., and could grow quickly due to their simple cell structures [3-5]. Moreover, due to their simple cultivation in many locations, they don't have any comparison to other agricultural products and their cultivation farms. They can even use swage streams as their feed. Although these cells don't have higher photosynthetic efficiency in comparison with higher plants, they have a higher growth rate and oil production yield [6-8]. *Dunaliella salina* is a unicellular green photosynthetic microalga that has two equal flagella with ovoid, spherical, and ellipsoid cell shapes that vary in different environmental conditions. The cell length and width vary from 5 to 25 µm

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and 3 to 13 µm in respectively [3]. This species is the basic beta-carotene natural source and could synthesize vitamins, lipids, glycerol, etc. under particular situations [9-12]. This species doesn't have any polysaccharide cell wall so, it can be easily digestive for humans and animals [13]. Beta-carotene, which can physiologically act as pro-vitamin A is a lipophilic pigment. Pharmaceutical industries, food, and cosmetics are its prominent commercial applications as an anti-cancer agent, colorant, and antioxidant [14]. Under stress conditions like nutrient deficiency, high salinity, high light intensity, temperature stress, beta-carotene is accumulated as oil droplets within the chloroplast of *D. salina* cells (inter-thylakoid spaces) [15,16]. Beta-carotene in the cell of *D. salina* has two main functions: energy transfer to chlorophyll during photosynthesis [17], cell protection against oxidation [18].

In the present study, the optimum NaCl concentration for the best *D. salina* growth pattern was determined, and two models of salt injection, including instantaneous and slow-release stresses were carried out. Also, the amount of beta-carotene which was produced in microalgae cells for each set of experiments was measured with the solvent extraction method.

EXPERIMENTAL SECTION

Organism and medium

Dunaliella salina (Urmia Lake species) was provided from the Iranian Biological Resource Center (IBRC) was grown in modified Johnson's culture medium. This medium contains 1 M salt concentration with the components below (per liter):

KH_2PO_4, 0.035 g, $MgSO_4{\cdot}7H_2O$, 0.5 g, KCl , 0.2 g, $CaCl_2{\cdot}2H_2O$, 0.2 g, $MgCl_2{\cdot}6H_2O$, 1.5 g, KNO_3, 1 g, $NaHCO_3$, 0.043 g, Fe-solution, 10 mL, Trace-element solution, 10 mL, Fe solution (for 1 L), $FeCl_3{\cdot}6H_2O$, 244 mg, Na_2 EDTA, 189 mg, Trace-element solution (for 1L) $CuSO_4{\cdot}5H_2O$, 6 mg, H_3BO_3, 61 mg, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 38 mg, $CoCl_2{\cdot}6H_2O$, 5.1 mg, $ZnCl_2$, 4.1 mg, $MnCl_2{\cdot}4H_2O$, 4.1 mg [19].

Culture conditions

The basic salt concentration for *D. salina* culture is a substantial issue for microalgae cultivation that varies with respect to the origin of each species. Considering our species that was purified from Urmia Lake, an experiment was performed to determine the optimum salt concentration for the best growth pattern. Six samples of *D. salina* culture (each sample was 1 L) were cultivated with 5% inoculation under 10000 lux white light intensity for 24 days. These samples contain six salt concentrations from 0.5 to 3 M. For growth surveillance, OD numbers were recorded every couple of days at 560 nm. To determine the effect of salt stress, two groups of quintuplet *D. salina* samples (all samples were 1 L) were cultivated in 1 M salt concentration under 10000 lux white light intensity and an average temperature of 25±2ºC. Continuous aeration was considered for each culture sample. The Optical Density (OD) numbers (at 560 nm) were recorded for each sample by spectrophotometer device every two days. The cultivation period was 21 days. To obtain better results in microalgae growth conditions and higher efficiency of beta-carotene production, another model of salt injection called slow-release salt stress (in the present experiment) was performed. A 1 L sample of *D. salina* culture was provided with the basic salinity of 1 M and the same conditions as the previous experiments. The cultivation period was 44 days. It should be noted that all the cultures replicated at least two times per treatment.

Method of dry weight measurement

The dry weight values were measured with the method of Delavari Ameri [20].

Methods of salt injections

To investigate the effect of salt stress on beta-carotene accumulation in *D. salina* cells, two models of salt injection were performed. In samples 1 to 5, 1 M salt stress was injected into each sample every three days. For example, sample 1 was subjected to 1 M salt stress on the 3rd day of cultivation (cell dry weight on this day was 2.21 mg/mL) and sample 2 encountered the stress on the 6th day (cell dry weight: 3.89 mg/mL), and this procedure was repeated for samples 3, 4 and 5. Furthermore, for samples 6 to 10, 2 M salt stress was injected into each sample every three days. A 2 M salt stress was injected to sample 6 on the 3rd day of cultivation (cell dry weight: 1.39 mg/mL), and the same amount was added to sample 7 on the 6th day (cell dry weight: 3.27 mg/mL) and it was repeated for other samples till sample 10 by adding 2 M salt stress on the 15th day. These data are shown briefly in Table 1.

Table 1: Cell dry weight of each sample at the moment of each instantaneous 1 and 2 M salt stress injection.

Salt Concentration (M)		Salt Injection Time (d)				
		3	6	9	12	15
1	Sample Number	S1	S2	S3	S4	S5
	Dry Weight (mg/mL)	2.21	3.89	3.19	6.93	6.59
2	Sample Number	S6	S7	S8	S9	S10
	Dry Weight (mg/mL)	1.39	3.27	4.77	6.01	4.92

Table 2: Cell dry weight at the moment of each 0.5 M salt stress injection (11 steps) in slow-release salt injection method).

	Salt Injection Time (d)										
	12	15	18	21	24	27	30	33	36	39	42
Salt Concentration (M)	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5
Cell dry weight (mg/mL)	4.78	5.11	5.04	5.02	4.64	4.38	4.67	4.91	5.00	4.67	4.12

For the slow-release salt stress method, 0.5 M salt was added to the medium in each step, and this comportment was repeated 11 times every three days (each dry weight related to each corresponding step is shown in Table 2).

This process was stopped as a result of reaching the death phase, which could be confirmed by changes in culture color to light green and by perusing OD numbers, which showed the death phase of growth pattern. By this method, salt concentration was raised gradually, and the whole experimental features became more similar to the real condition of natural lagoons and lakes. In the natural environment, continuous evaporation increases water salinity and with the combination of different factors, beta-carotene globes were accumulated in the microalgae cells to preserve cell structure in such a stressed situation. In Urmia Lake, a large amount of beta-carotene is accumulated within *D. salina* cells when they grow in saline water in warmer seasons with a high water evaporation rate and turns the color of the lake to reddish.

Beta-carotene extraction method

Each solvent has different biocompatibility, solubility, and extraction ability on *D. salina* cells. The highest beta-carotene extraction ability is for methanol, and ethanol and the highest solubility of beta-carotene are for hexane solvent. Also, the greatest biocompatibility is for decane and dodecane [21]. To obtain beta-carotene, extraction by the solvent method was used. For this reason, 1 mL of *D. salina* culture was centrifuged at 3000 rpm for 10 min. Then the upper supernatant part was removed, and 2 mL of ethanol, 1 mL of hexane, and 2 mL of water were added to the microalgae sediment. After the vortex, the solution was centrifuged for 10 min at 3000 rpm again. After centrifugation, the supernatant phase was divided into two distinct phases. The upper phase was hexane, including beta-carotene compounds. At the end of the process, the upper phase was separated, and beta-carotene determination was performed spectrophotometrically at 450 nm. The amount of beta-carotene was calculated in µg/mL, based on the correlation below [22]:

Beta-carotene content (µg/mL): $25.2 \times A_{450}$

RESULTS AND DISCUSSION

Growth evaluation

As described in previous sections, six samples of *D. salina* cultures with different salt concentrations (0.5, 1, 1.5, 2, 2.5, 3 M) were considered under the aforementioned conditions to find out which salt concentration had a better effect on the growth procedure of microorganisms. According to the results shown in Fig. 1, the optimum growth was obtained at 1 M salt concentration.

Cell dry weight values which are presented in Fig. 1, demonstrated salt concentration higher than 1.5 M (salts were injected on the first day of cultivation) had a negative effect on the growth of microalgae. *Tammam, Fakhry* [23] showed that at 4 M salt stress, the growth rate was reduced by 39% in *D. salina* species, and they used 1.25 M salt concentration for normal culture conditions. *Hadi, Shariati* [24] and *García, Freile-Pelegrín* [25] have reported 1 and 2 M as the salt concentration for optimum growth of

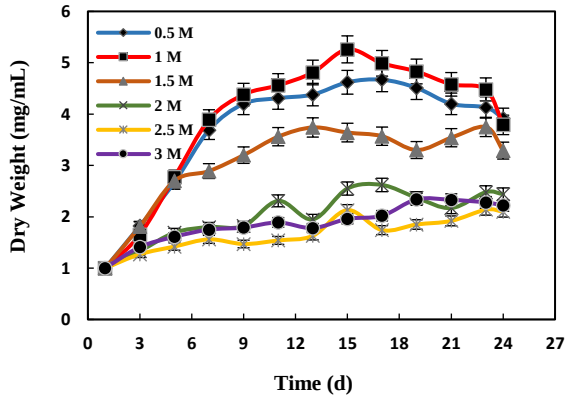

Fig. 1: Cell dry weight versus time for different salt concentrations (0.5-3 M) in D. salina culture medium.

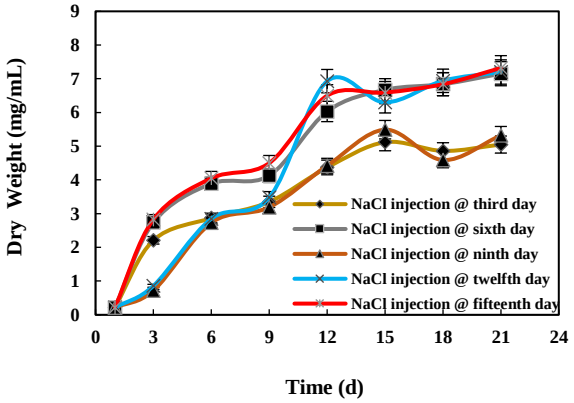

Fig. 2: Cell dry weight versus time for 1 M instantaneous salt stress every 3 days in the first five samples (initial medium salinity: 1M).

D. viridis and *D. salina*, respectively. *Fazeli, Tofighi* [26] have shown that the salt concentrations of 0.1 and 0.5 M were the optimum salinity for *D. tertiolecta* that was isolated from Urmia Lake.

Investigation of different salt stress methods

Microalgae are categorized as halophytic (needing salt for optimum growth) and halo-tolerant organisms (having physiological responses to survive the cells in saline culture) [27]. In the present paper, we used the term 'stress' as a key parameter for the experiment design. Stress is the factor that causes changes in algal metabolisms or growth patterns, and generally, it changes the homeostasis condition of the cell. Briefly, this term has the following stages to respond to a stressor or stimulus: alarm, regulation, acclimation, and adaptation. The microalgae physiological condition, the stage of the growth at the time in which the stimulus is applied, type of stressor, its magnitude, and the duration of the stress are the important factors when considering the effect of the stressor on microalgae cell and its response. Disruption of cell functions is the quick effect of a stressor. The nature and magnitude of the stressor define the level of the disruption. When a stressor impresses a microalgae cell, the homeostasis condition of the cell is interrupted, and an alarm signal initiates, which leads to a response to reinstating the homeostasis condition of the cell (regulation stage). In many cases, this situation leads to a reduction in photosynthetic activity and motility of the cell. Then, the cell tries to retrieve its steady-state condition through the acclimation process by the cellular response (acclimation stage) [28]. If the cell can fully acclimate and can restore homeostasis condition, a new steady state is obtained. If this situation lasts long enough, the cells may adapt to the new condition by genetic changes which are resulted from mutations (adaption stage) [29]. In some cases, the cell can't fully acclimate, and cell death takes place because of acute stress, which happens when the initial stimulus is so formidable [30]. Also, when the full acclimation does not accomplish due to the acclimation resources are greater than the capacity of the cell (chronic stress), cell death may happen [31, 32]. In this section, two quintuplet groups of samples were injected with 1 and 2 M salt stresses. Samples 1 to 5 were injected with 1 M, and samples 6 to 10 were injected with 2 M salt stress, respectively. Based on the results shown in Fig. 2, the best microalgae growth procedure was recorded for the sample, which was subjected to 1 M salt stress on the 15th day of cultivation (when its cell dry weight was 6.59 mg/mL), and in other groups (2 M stresses) as shown in Fig. 3, the best growth pattern was recorded for the sample that was subjected to 2 M salt stress on the 12th day of cultivation (with the cell dry weight of 6.01 mg/mL).

It could be proved that the salt stresses in the early days of cultivation could lead to destructive effects on the microalgae growth due to cell divisions and cell adaption in the early days of cultivation and any unexpected changes in culture condition could affect cells growth and when the amount of salt stress increased from 1 to 2 M, this procedure became more crucial (acute stress). A decrease in photosynthetic rate and photosynthetic pigments during the salt stress can be due to the non-adaptability of the cells to the salt stress [23]. A sudden increase in salinity caused a lag phase in the growth pattern of *D. salina*. The duration of this phase

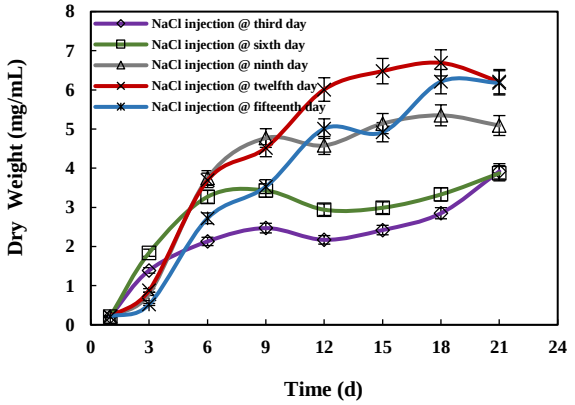

Fig. 3: Cell dry weight versus time for 2 M instantaneous salt stress every 3 days in the second five samples (initial medium salinity: 1M).

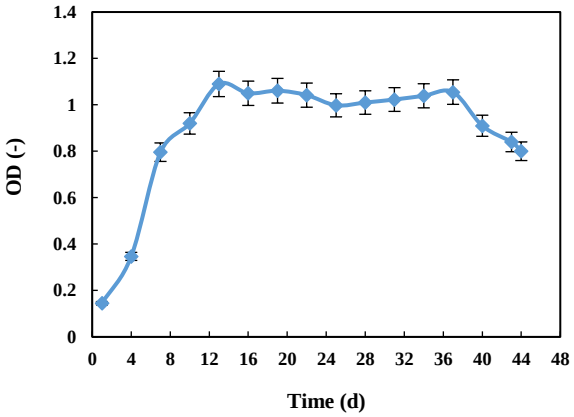

Fig. 4: Cell dry weight versus time for 0.5 M salt stress every 3 days (slow-release method in consecutive 11 steps 0.5 M salt stress).

was dependent on the amount of the change in salt concentration and its final salinity. There was no lag at 10-15% salinity increase and 4-day lag phase at a 30% increase of NaCl within the culture [33]. Unlike the previous method with instantaneous salt injection, another model of increasing salinity (slow-release salt injection) was presented. First, a 1 L culture sample with the basic salinity of 1 M was provided. Increasing culture salinity was started from the 12th day after cultivation (when the dry weight of cells was 4.78 mg/mL). Just as indicated in the previous section, salt injection in the early days of cultivation could cause serious damage to cells due to cell divisions and cell adaption in the early days of cultivation, and any changes in culture conditions could affect cell growth. 0.5 M of salt was added to the medium every three days, and this procedure was repeated 11 times. Unlike the method of instantaneous salt injection, it was evident that the growth pattern was enhanced by this method of increasing salinity (as shown in Fig. 4).

From another perspective, through this procedure of salt injection, the total salinity of the culture of *D. salina* was increased gradually and reached a considerable amount of 6.5 M NaCl concentration, which was higher than many possible upper thresholds which were reported in the literature. *Ginzburg* and *Ginzburg* [34] claimed that *D. salina* could tolerate 0.1– 6.0 M NaCl concentration. Also, *Hosseini Tafreshi* and *Shariati* [9] stated that *D. salina* is the most halotolerant eukaryotic microorganism known which could withstand wide salt concentration from 0.5 to as high as 35% v/w NaCl. Acclimation to salt stress in *Dunaliella* includes the large accumulation of glycerol, osmotic adjustment, synthesis of carbohydrate and amino acid, particular proteins, and enhanced evacuation of Na+ ions without needing new enzymes synthesis [35, 36]. Although, at very high salinity (3.5 M), regulation of some genes and accumulation of particular proteins within the plasma membrane is reported [35]. Some microalgae cells show a stress-resistant reaction to counter chronic stress [28]. For example, some *Dunaliella* species in high salinity change from palmellae (non-motile cells in polysaccharide matrix) [37]. Also, at low salt concentrations and low temperatures, *Dunaliella* cells can make a thick-walled stagnant period known as aplanospore [38]. Briefly, microalgae cells may use sexual reproduction [39] and make resistant spores [38] or cysts [40], to prevail in a stress situation.

Evaluation of beta-carotene production

Raising the culture salinity is one of the common and economical ways (in comparison with high artificial light intensity) to enhance the beta-carotene accumulation within *D. salina* cells. Beta-carotene is mainly synthesized in the chloroplast (thylakoid membrane) and performs as an accessory photosynthetic pigment, and in *D. salina* cells, it accumulates forming lipophilic globules and has the protective role, suppressing free-radicals and photo-inhibitory processes [41]. High salt concentration in microalgae culture can cause metabolic toxicity, membrane disorganization, and an increase in ROS (reactive oxygen species) by hyper-ionic and hyper-osmotic phenomena [23]. Reactive oxygen species molecules include hydrogen peroxide (H_2O_2), superoxide

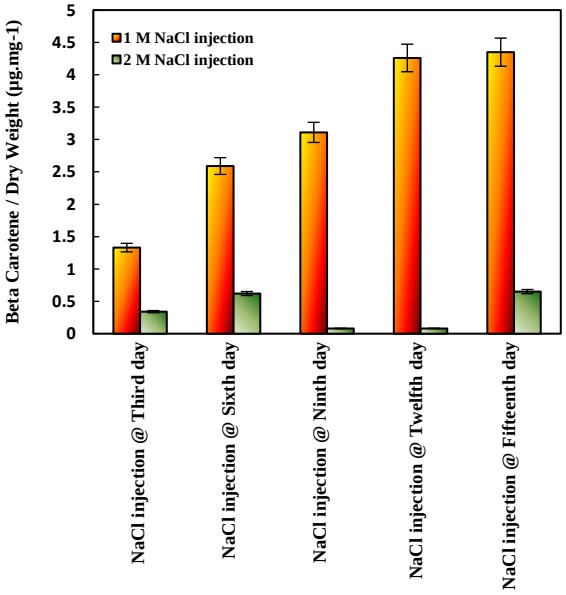

Fig. 5: Accumulated beta-carotene within D. salina cells per dry weight of microalgae (µg mg-1) in 1 and 2 M instantaneous salt stresses every 3 days.

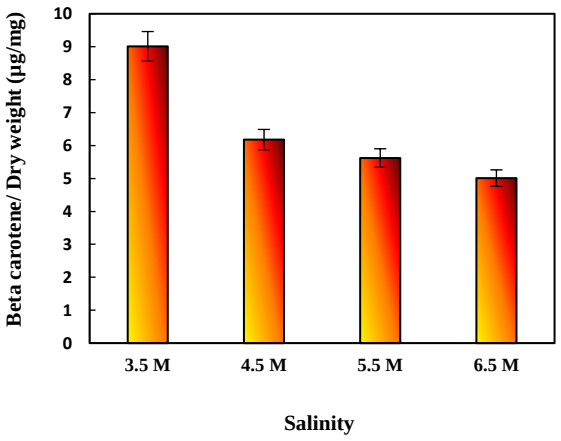

Fig. 6: Accumulated of beta-carotene in D. salina cells per dry weight of microalgae (µg mg-1) in slow-release 0.5 M salt stress method every 3 days.

radical (O_2^-), singlet oxygen ($^1O_2$), and hydroxyl radical ($OH^·$) [42]. *Dunaliella* species has various special defiance systems under salinity stress with the accumulation of enzymatic and non-enzymatic antioxidants for eliminating or quenching ROS. Under stress conditions, the protective system of the cells can't control the ROS molecules. These molecules can cause devastation of the lipids of membrane or lipid peroxidation, proteins denaturation, chlorophyll bleaching, photo-system impairing due to DNA mutation, within the microalgae cells. So, the induction of antioxidants defiance is an important defensive mechanism for salinity tolerance of *Dunaliella* species [23, 43]. Beta-carotene and other carotenoids (astaxanthin and lutein) protect the cell from the reactive oxygen species [44]. Thus, the beta-carotene accumulation within *D. salina* cells raises significantly under salt stress conditions. Also, under high light intensity, the photosynthetic system of the cells can't exploit sufficient light energy, so, free radicals of oxygen molecules which can lead to peroxidation reaction and cell damages, form by this additional energy [45]. Therefore, beta-carotene production in *Dunaliella* cells increases considerably. As shown in Fig. 5 the highest amount of beta-carotene production was recorded as 4.35 µg of beta-carotene per mg of the dry weight of microalgae for the sample which had received 1 M salt stress on the 15th day of cultivation (the total salinity of the culture included 1 M as the basic culture salinity and 1 M salt stress which performed 2 M total salinity).

According to Fig. 5, for 2 M salt stress samples, the best result was achieved for the sample that was injected with 2 M salt stress on the 15th day of cultivation (total salinity: 3 M) by the amount of 0.65 µg of beta-carotene per mg of the dry weight of microalgae. These results revealed that greater instantaneous salt stress could cause lower beta-carotene production in microalgae cells. This could be attributed to a lack of adaption between cells and rough new environmental conditions. Based on Fig. 6, the highest amount of beta-carotene was achieved in the slow-release model of adding salt to the culture by 9.01 µg of beta-carotene per mg of the dry weight of microalgae after the 5th stage of salt stresses with total salinity of 3.5 M.

By investigating the beta-carotene production pattern in different ways, the results revealed that greater instantaneous salt stress could cause lower beta-carotene production in microalgae cells. This could be attributed to a lack of adaption between cells and rough new environmental conditions. According to the results, the highest beta-carotene value which was obtained from the slow-release salt injection method (9.01µg of beta-carotene per mg of the dry weight of microalgae) is more than two times higher than the biggest reported amount of beta-carotene in 1 M instantaneous salt injection method and up to 14 times higher than the biggest reported beta- carotene value by 2 M salt stress method. This is

Table 3: Different studies on Dunaliella species and carotenoid accumulation within the cells by high NaCl concentration.

Reference	Species	Objective	Carotenoid content	NaCl concentration
[51]	two Chilean *Dunaliella salina*	Effect of salinity on the carotenogenesis in two mediums (J/1 and PES).	7.4 and 14.6 (mg/L) 18.3 (mg/L) 23.9 (mg/L)	20% salinity 10% salinity 25% salinity
[26]	*D. tertiolecta*	carotenoid content as a result of salinity	11.73 (mg/L)	0.5 M
[52]	*D. salina*	highest carotenoid content in 1, 2 and 3 M salinity	29.5 ± 0.7 (mg/L)	3 M
[53]	*D. salina*	Beta-carotene content	50-150 pg/cell	20% salinity
[54]	eight strains of *D. salina* Teodoresco of Chile	total carotenoid content	35.6 (mg/L) (42.4 pg/cell)	25% salinity
[24]	*D. salina.*	carotenoid content under five different salinities	4.57 (µg/mL)	2 M

a considerable amount of pigments could be due to the special method of salt injection to the microalgae culture where cells could preserve themselves from unfavorable conditions by producing secondary metabolites such as beta-carotene, but in an instant injection of salt, many cells could not adapt to the new environmental conditions such as sudden reverse osmosis phenomena which could burst the cells and evacuate their content to the culture medium. A sudden increase in salt concentration in the *Dunaliella salina* culture medium causes a great osmotic differential between the cytoplasm of the cells and the external environment, which leads to quick water efflux from the cells and cells shrinkage. This process takes place just in several seconds [46]. In some cases, by increasing the NaCl concentration, the cells stop swimming, and they may also miss their flagella if the instant salinity change is large enough. After the salt stress, the cell growth stops, and just after the revival of osmotic and ionic equilibrium, it starts again. It is still ill-defined that how the cell feels the environmental osmotic change. But it is obvious that this phenomenon is adjusted at the level of the plasma membrane by the sensors and the channels which is located within the cell membrane [28]. Changes in pH of cytoplasm and ions concentration are the consequences of several minutes of cell shrinkage. Actually, the Na^+ concentration within the cells increases very fast as a result of pH fluctuation [46, 47]. Na^+, K^+, and Cl^- are the major ions to maintain osmotic balance in *D. salina* cells [46]. Intracellular concentrations of Na^+ and Cl^-, as a result of hyper-osmotic stress, were calculated 10-30 mM and 20-40 mM, respectively for *D. salina* under 0.5-4 M salt concentration [48]. In *Dunaliella* cells, Ca^{2+} has an important function in osmotic stress response [49]. Intracellular Ca^{2+} concentration raises gradually via Ca^{2+} channel during the first 200 s, under the hyper-osmotic salt stress (2-4.5 M salt shock), and it increases quickly for the first 110 s under the hypo-osmotic salt stress (2 to 0.5 M salt shock), and this ion has the essential role in the response to osmotic stress [50]. Some of the appropriate studies are shown in Table 3.

Tammam, Fakhry [23] raised the salt concentration to 4 M and recorded a 41% increase in cells beta-carotene production (3.98 mg/L). *Cifuentes, Gonzalez* [51] assessed the salinity effect on the carotenogenesis in two strains of Chilean *Dunaliella salina* in two different mediums (J/1 and PES). PES medium stands for Provasoli enriched seawater culture medium. The results revealed that the maximum levels of total carotenoid were 7.4 and 14.6 mg/L for both strains in 20% NaCl concentration within the J/1 medium. Furthermore, the highest levels of carotenoid for two strains in PES medium were 18.3 and 23.9 (mg/L) in 10% and 25% salt concentration, respectively. *Fazeli, Tofighi* [26] observed the highest carotenoid content (11.73 mg/L) in 0.5 M NaCl during the stationary growth phase for *D. tertiolecta* DCCBC26, which was isolated from the Urmia hypersaline Lake. In another study, *Gomez, Barriga* [52] cultivated *D. salina* samples in 1, 2, and 3 M salinity in two different media, and the highest carotenoid content was 29.5 ± 0.7 (mg/L) in 3 M salt concentration. In each salinity, almost 80%

of total carotenoid was beta-carotene, and about 20% was alfa carotene. *Moulton* and *Burford* [53] reported 50-150 pigment per cell of beta-carotene at 20% salinity for *D. salina*. *Cifuentes*, *González* [54] have studied eight strains of *D. salina* Teodoresco collected from two salt ponds of Chile. The highest total carotenoid was reported as 35.6 mg/L (42.4 pigment cell) at 25% salt concentration. *Hadi*, *Shariati* [24] investigated the carotenoid content under five various NaCl concentrations in *D. salina*. The results showed the highest amount of carotenoid in 2 M salt concentration with 4.57 (µg/mL).

CONCLUSIONS

As reported in the literature for obtaining more beta-carotene in the cells, the salt concentration had to increase as a basic factor of increasing secondary metabolites production. When the salinity of the culture increases, the ROS molecules accumulate within the cell, and one of the defensive solutions of the cell is to produce more carotenoid (beta-carotene) to quench these molecules. The results of the present research demonstrated that a large amount of instantaneous salt stress could damage the cells and denature their structures (in this study, 2 M NaCl stresses were the best evidence for this claim as the acute stressors). Therefore, we should try another method for raising salt concentration, such as adding salt step by step consecutively to allow the cells to adapt to the new environmental situation. The best result for beta-carotene production was obtained with this method. Also, the best result for biomass production occurs when the stresses are injected a few days after cultivation almost at the end of the logarithmic phase of growth. Because in the early days of cultivation (lag and logarithmic phase), cells try to reproduce immediately and can't tolerate the rough changes in culture medium conditions. Given that *D. salina* is a halophile microalga, its growth pattern was improved by the slow increase in salinity of the culture medium. However, it was obvious that cell growth deteriorated at very high salinity because cells were trying to save their viability.

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