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EFFECT OF SALINITY ON GROWTH AND BIOPIGMENT COMPOSITION OF *DUNALIELLA SALINA* ISOLATED FROM SAMBHAR SALT LAKE, RAJASTHAN, (INDIA)

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ABSTRACT

A study was conducted to investigate the impact of salinity (NaCl) on the growth and biopigment of a green alga *Dunaliella salina* isolates from Sambhar salt lake, Rajasthan (India). In order to determine the impact of NaCl, an algal species *D. salina* was exposed to different concentrations of NaCl ranging from 1M to 5M along with control (1.5M) over a period of 30 days. It was found that the algal biomass yield was highest at 2M NaCl concentration and then it subsequently decreases with increase in NaCl concentration. Total chlorophyll content of the algal species decreases as the salinity was increased. While total carotenoids content of the algal species increases as the increases salt concentration and it was found that the green alga species changed its appearance from green to deep orange. The results indicated that algal species showed diverse response to salinity stress.

KEYWORDS: **NaCl, Caroteniods, Chlorophyll, Biomass, Growth, *Dunaliella salina.***

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INTRODUCTION

Algae are among the fastest growing autotrophs on the earth. Around 70% of earth's surface is covered with water and the phytoplankton especially algae believed to contribute around 85-90% in photosynthesis. They are able to survive in diverse habitats and produce a vast array of natural products including proteins, enzymes, bioactive compounds and carotenoids. Humans use algae as food, for production of useful compounds, as biofilters to remove nutrients and other pollutants from wastewaters, to improve water quality, as indicators of environmental change, in space technology,and as laboratory research system. An alga is commercially cultivated for Pharmaceuticals, Cosmetics and Aquaculture purpose. In the present era of biotechnology, scientists are investigating microalgae as a solution for the problems world is facing today. Microalgae are very diverse[1].They can create a range of useful products, and there are a variety of ways in which they can be cultivated, manipulated, harvested and utilized[2]. *Spirulina* is now being commercial produced as a food supplement which is very rich in protein content. Many microalgae are being investigated for biofuel production. In particular, some species have been identified as promising producers of useful lipids for biofuels production. *Dunaliella salina* is one such species, which shows lipid accumulation. *D.salina* is a unicellular species with no cell wall[3]. *Dunaliella* have some advantages such as disruption of cells is much easier than that in other algae because of its cell wall less nature, continuous culture in the laboratory is easy and the growth rate is relatively high and resistance to various environmental conditions is higher than in other algae. *D.salina* is occurring naturally in a number of locations worldwide. It lives in areas of fluctuating salinity and can tolerate extreme salinities of between 0.5 and 5 M NaCl. It does this by maintaining a steadily low intracellular ion concentration[4], and by forming compatible solutes such as glycerol that maintain the structure and volume of the cell[5]. This ability to adapt, and its high metabolic and physiological versatility, has led to its identification as a high potential for large-scale cultivation of beta carotene[6]. A number of studies have revealed that growth[7] and pigment compositions[8] of this alga are affected by halostress conditions. It was found that the β-carotene to chlorophyll a ratio gradually increased with an increase in NaCl concentration, and as a result, the algae changed its appearance from green to deep orange[9]. *Dunaliella* species provides the best examples of microalgae that can tolerate high salt concentractions. The ability of *Dunaliella* species to proliferate over practically the saturation range of salinities makes them one of the favorite candidates to study salinity effects on microalgae. The present study was an attempt to observed the effect of different salt concentration on the growth, biomass and biopigment composition of *D. salina*. In a study cells of *D.salina* were transferred from 1 M to 5 M salinity concentration along with the control(1.5M) in the ASWM[10] (ARTIFICIAL SEA WATER MEDIUM).

MATERIALS AND METHODS

Microalgae source

The test alga *D. Salina* was isolated from the Sambhar salt lake, Rajasthan, which is located about 35 km.from Jaipur, (Rajasthan).

Experimental set up

To evaluate salt concentration for the growth and biopigment of *D.salina*, five different salt concentration viz.1M, 2M, 3M, 4M, 5M along the control (1.5M) were experimented upon. The inorganic media for the above experiment set up remain the same and it was ARTIFICIAL SEA WATER MEDIA (ASWM).The inorganic media was sterilized in autoclave at 121°c for 20 min before inoculation. Conical flasks of 500 ml capacity were prepared containing 250ml media and 50 ml sample culture (inoculum). The cultures were incubated at 25°c in a thermo-statically controlled room, with a 12:12 h light: dark period and at a light intensity of 2,500 lux[11-12].Observations were carried out every week over a period of weeks. Cultures were shaken manually thrice a day to avoid clumping and accelerate the growth process. Experiment for each salt concentration was carried out in triplicates.

Growth Measurement

Growth was followed through optical density (OD), dry weight, and growth rate .Biomass was determined by optical density of cultures at 670 nm using Shimadzu UV/VIS spectrophotometer. The dry weight against standard absorbance unit was followed throughout the experiment period.50-100 ml sample of culture were filtered on whatman GF/C filters, rinsed with distilled water and weighed after drying for 24h at 80°c.

Growth rate was calculated on dry weight basis according to the equation given below

$$\mu(divisions/day) = \frac{3.322(\log DW2 - \log DW1)}{t2 - t1}$$

Where t =time and DW=dry weight. Subscripts denote values at different times.[13]

Biopigments Analysis

The Chlorophyll contents of samples were estimated by Parson and Strickland method[14] and carotenoids by Jensen method[15].

Statistical Analysis

Effect of different salt concentration on growth and biopigment of *D.salina* were compared by one way analysis of variance (ANOVA).[16] Statistical analysis of data was carried out using MS Office Excel analysis Tool Pak. The significance between pairs of variable means was analysed using least significant difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

The results yielded from this research show that a great variability in different salinities (NaCl) of *D.salina*. Because salt (NaCl) is a necessary ingredient in the growth medium (ASWM) of this alga. This study suggest *D.salina* was adapted well to the wide salinity range 1 to 5 M salt concentration .

Effect on Growth

Growth analysis of *D.salina* in different salt concentrations shows different growth patterns[17]. As compared to the other salt concentrations, the best growth of *D.salina* was calculated in 2M salinity condition. The optical density increased exponentially up to the end of the experiment. It was increased 3.6 times more than the initial value and the dry weight also supported optical density, it increased expontially4.99times than initial values. Highest growth rate(i.e.0.091divisions/day) was recorded in 2M (Graph1-A,B). 1.5M (control) was the next to 2M, in terms of growth of the alga. The optical density and dry weight recorded of algal sample were increases, optical density was about 3.3 times and dry weight found 4.81 times than their initial values and growth rate of culture at this salt concentration was just next to 2M (0.083divisions/day). 1M Salinity was the next to 1.5M (control), in terms of growth of *D.salina*. The optical density and dry weight calculated of algal sample were increases, O.D. was about 3.2 times and dry weight calculated 4.72 times than their initial value and growth rate of culture at this salinity was just next to 1.5M (i.e.0.073divisions/day). The salt concentration of 3M was next to 1M salinity in supporting and maintaining the growth of the alga. At this salinity, optical density increased 2.9 times and dry weight 4.56 times than the initial values and growth rate of cultures was found next to 1M salinity (i.e.0.066divisions/day). 4M salinity was the next to 3M salt concentration, in terms of growth of the alga. The optical density was found increased 2.6 times and dry weight 4.21 times than the initial values and growth rate of alga in this salt concentration was calculated (0.030divisions/day). In comparison to all the salinities tested, 5 M salt concentration was found least effective in promoting the growth of *D. salina*. The optical density and dry weight results were calculated to increases 1.8 times and 3.64 times respectively than the initial values, at the end of experiment. The least growth rate of alga (i.e.0.021 divisions/day) was recorded at this salt concentration. (Graph1-A,B)

Effect on Biopigment content

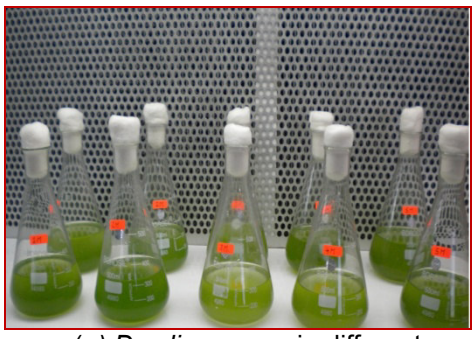
The Biopigment composition of algae correlates with the growth of *D.salina*. The maximum biopigment (chlorophyll & carotenoids) content were found at 2 M salinity (NaCl). Total chlorophyll content was recorded in culture grown in 2M salt concentration(5.246µg/ml) followed by control culture at 1.5M (4.822µg/ml), 1M (4.513µg/ml), 3M (2.708µg/ml), 4M(1.147µg/ml) and least chlorophyll content was recorded at 5M salt concentration(i.e.0.591µg/ml).Total carotenoids content was observed highest value at 2M salinity (4.732µg/ml), followed by 3M (3.529µg/ml), 4M(2.860µg/ml), 1.5M(2.682µg/ml), 1M(2.614µg/ml)and least carotenoids was found at 5M NaCl (1.537µg/ml). (Table1) and (Graph 1-C). The results demonstrate that at higher salinity condition, photosynthesis activity adversely affected, hence the chlorophyll contain was decreased with increase in salinity and carotenoids are secondary metabolite, produced by the cell in stress condition as cell protecting process[18]. When the salt concentration is increased it acts as stress (halostress) that enhances carotenoids production. It was found that the carotenoids to chlorophyll ratio gradually increased with increases in NaCl concentration and as a result the alga changed its appearance from green to deep orange. However cells of *D.salina* adapt to the higher salt concentration due to the presence of glycerol as it has an ability to balance the extracellular osmotic stress[19].

Table 1

Effect of various salt concentrations on biopigment composition of D.salina. Values are means±SD(n=3).

Salinity NaCl(M)	Chlorophyll-a(µg/ml)	Chlorophyll-b (µg/ml)	Total Chloro-phyll(µg/ml)	Total Carotenoids(µg/ml)	Alga color
1M	2.926	1.587	4.513	2.614	Green
1.5M	3.221	1.601	4.822	2.682	Green
2M	3.614	1.632	5.246	4.732	Green
3M	1.875	0.833	2.708	3.529	Orange
4M	0.795	0.352	1.147	2.860	Orange
5M	0.569	0.022	0.591	1.537	Orange-red

Figure 1
(a,b,c) D.salina grown different salinity showing various response

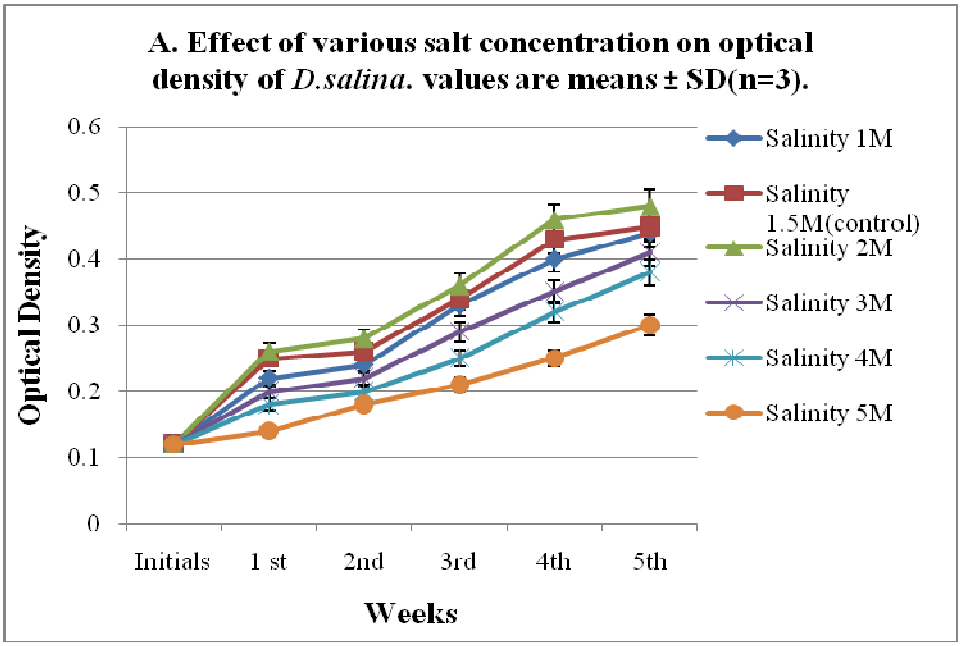


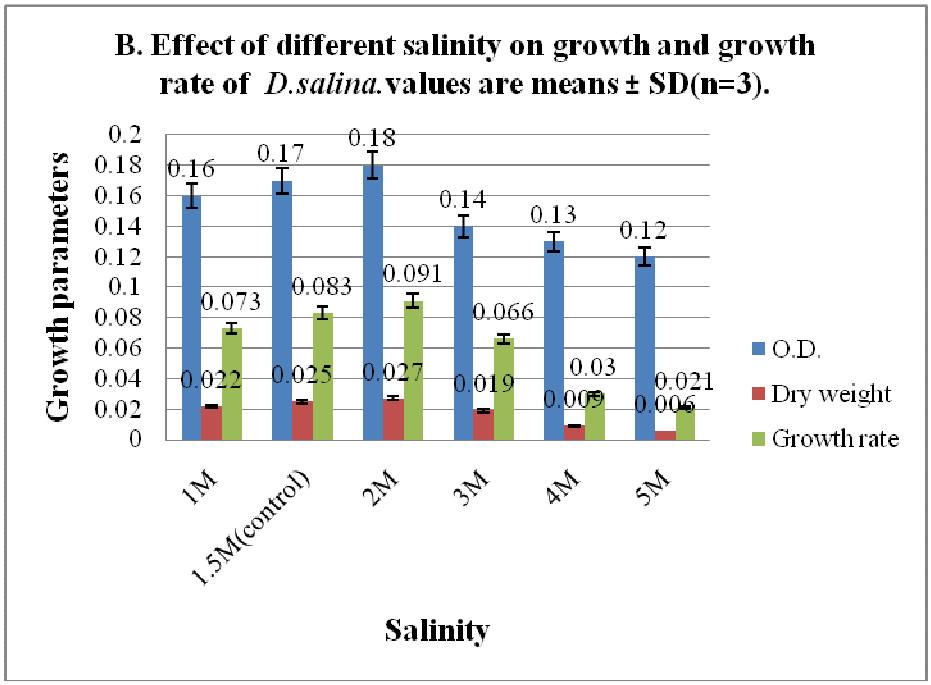
(a) *D.salina* grown in different (1-5M) initial green colour of alga

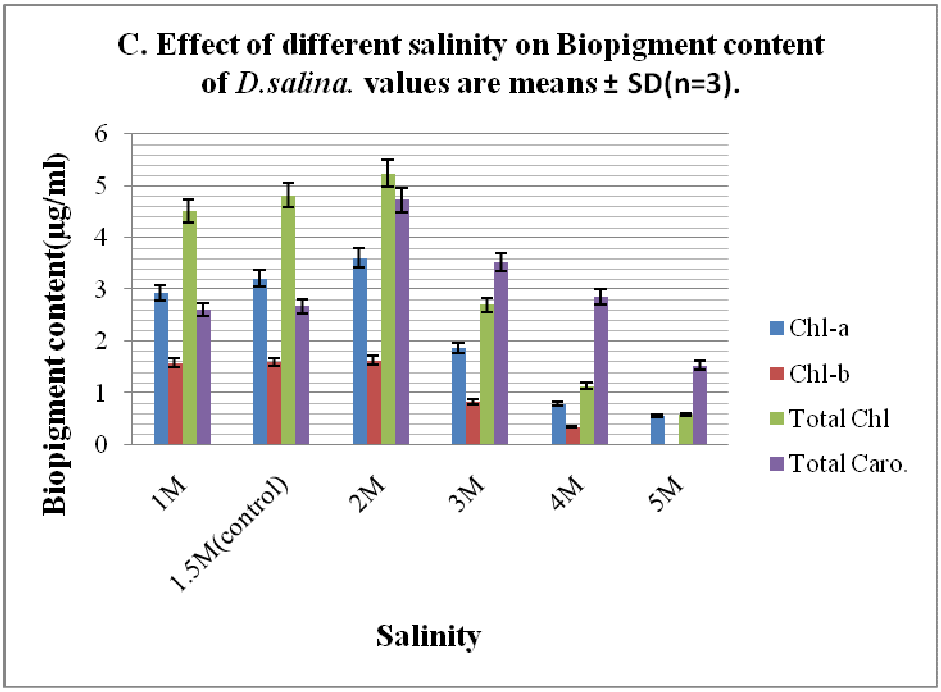
(b) *D.salina* showing slight orange colour changes at IVth week of exp

(c)Control culture (1.5 M)

Graph 1
Effect of different salinity on the growth, growth rate and biopigment content of D.salina. (A,B,C)







CONCLUSION

The alga *Dunaliella* includes many important scientific aspects and application on the biotechnology (β-carotene and glycerol production), physiology (osmoregulation), bioenergy (bioreactor and biofuel production) and pharmaceutical industries. The Sambhar Salt Lake is a natural habitat for *D.salina* and therefore it has a very promising potential as the site of biofuel and β-carotene production in the state of Rajasthan (India). The high salinity, hot climate, abundant sun light and the high temperature present at the lake provide nearly the ideal set of condition for biofuel production from *Dunaliella salina*. This natural bioreactorof *D.salina* holds great promise for the future of India. The salt production process at the lake would be coupled with biofuel and β-carotene production. Finally it seems that the tremendous potentialities of different species of *Dunaliella* for exploitation in various biotechnological areas such as production of new antibiotic substance, production of biofuel and wastewater management will make *Dunaliella* a main topic for many future microalgal researches.

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