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A Comparative Study on Growth of *Dunaliella Salina* Treated with Ethyl Methane Sulfonate (EMS)

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Original Research Article

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Abstract: Micro algae *Dunaliella salina* is isolated from Sambhar Salt Lake, which has very high amount of carotenoids. We studied growth of *D. salina* in various concentration of EMS through rapid addition and having 1 hour treatment in dark to find out effective and efficient dose of EMS for *D. salina*. Studies revealed the effectiveness and efficiency decreased with increase in EMS concentration. Lower concentration of EMS was more effective and efficient for promoting growth. Maximum growth was found in control as compare to EMS treated cultures. The concentration of EMS chosen reduced the rate of growth, cell movement and cell survival as compare to control. This reduction increased with increasing concentration of EMS. Thus the mobility and survival rate of *D. salina* was negatively related to the concentration of EMS. Rapid addition of EMS exhibited high growth in comparison to 1hour treatment of EMS in dark.
Keywords: Algae, *D. salina*, *chlorophyceae*, cell movement, Growth, effectiveness, efficiency and Ethyl Methane Sulfonate (EMS).

INTRODUCTION

Algae are useful to man in a variety of ways [1]. *D. salina* is rich in essential minerals, vitamins, proteins, amino acids, and abundant unsaturated fatty acid, especially linolenic acid, carbohydrates, chlorophyll and other important nutrients which are easily absorbed by the body [1]. This halophilic algae has potential commercial value as a food or nutritional supplement [2].

D. salina is richest source of carotenoids [3] (which are pigments) can mask the green color of chlorophyll, and giving distinct red and pink or orange color [4] often characteristic of saltern ponds [5]. *D. salina* is the best commercial source of natural β-carotene which used as a natural food coloring agent in in processed foods and cosmetics [6].

In a rat study, cancer in the upper digestive tract was inhibited about 55% by *Dunaliella* extract [7]. Its extract (contains β-carotene converts into active vitamin A) protects skin cancer and help to protect premature ageing and giving a youthful skin [8, 9]. It protects cornea from UVB induced damage and cataracts [10]. Extract also decrease the human lung cancer cell proliferation by 48% by inducing cell death [11]. In another rodent study, researchers demonstrated that *Dunaliella Salina* protects against liver damage or toxicity [12]. Its valuable antioxidant property may help to protect atherosclerosis or cardiovascular disease and other chronic diseases. Therefore it maintains the normal rhythmic activity of the heart and maintains the blood pressure [13, 14].

Living things easily take in various chemical substances from the environment (atmosphere, water, or food). *Dunaliella's* saline requirements also make it easier to grow and cultivate with very few of the nutrients, because it has few natural competitors in the high-salt environment (0.2% to 35 %) [15]. It can also be exposed to different type of mutagens for quality and quantity improvement through genetic variability. Such variability comes from spontaneous or artificially induced chemical or physical mutations.

In addition to, Monofunctional alkylating agents such as ethyl methanesulfonate (EMS) is a chemical mutagen which has been found to be mutagenic from viruses to mammals [16, 17]. There are many studies which proves [18-20] EMS is more effective than many another mutagens. EMS induces modification of nucleotides, which results in mispairing and base changes. EMS cannot couple during DNA replication [21]. EMS has also been shown to be carcinogenic in mammals and able to produce significant levels of alkylation at oxygen such as the O^6 of guanine and in the DNA phosphate groups. Genetic data obtained using microorganisms suggest that EMS may produce both GC to AT and AT to GC transition. Some evidence shows that EMS can cause base-pair insertions or deletions more extensively intragenic deletions [21]. In higher organisms, there is clear-cut

evidence that EMS is able to break chromosomes. There are some reports which revealed that EMS ethylated DNA bases gradually hydrolyze from the deoxyribose leaving behind an apurinic or apyrimidinic site that is unstable. It has been reported that ethylation by EMS on some chromosomal proteins of mouse spermatids may be an important factor in causing chromosome breakage [21].

There are many recent studies reported by scientists which suggest that effective and efficient concentration of EMS affects survival and growth characteristics of cell [19, 20, 22]. There are many evidences that EMS affects algal growth [24, 25]. At present no conclusive information on relative effectiveness and efficiency of EMS is available for *D. salina*.

The present study was aimed to compare the growth of *D. salina* under 1hour dark treatment of EMS and Rapid addition of EMS and to find out effective and efficient dose of EMS affects survival and growth of *D. salina*.

MATERIALS AND METHODS

To find out effective and efficient concentration of EMS**,** *Dunaliella salina* was isolated from Sambhar Lake. Isolation and purification was made by dilution, plating technique. Culture were grown and maintained under ASWM [23] at 26±2^{0}C temperature under cyclic fluorescent illumination (12 hrs dark: 12hrs light) of 2500 lux. Uni-algal culture of *D. salina* was added to 250ml conical flasks containing 100ml artificial sea water medium (ASWM) with concentration of EMS i.e. 0.005%, 0.01%, 0.02%, 0.04% and 0.08% for 2 days duration in subjected to controlled culture conditions. Treated cultures of *D. salina* were observed under microscope for cell count and cell movement on 2th day of experiment. The mutagenic effectiveness and efficiency were computed using formula by Konzak *et al.*, [26] after doing significant modification in formula-

$$\textbf{Mutagenic effectiveness} = \frac{M \times 100}{T \times C}$$

$$\textbf{Mutagenic efficiency} = \frac{M \times 100}{X}$$

Where,
M = Cell count of viable and nonviable algal cells under mutagenic treatment (in Table-1),
C = Concentration of mutagen (in Table-1),
T= Duration of EMS treatment that is 2days or 48hrs (in Table-1),
X = Percentage of biological damage, i.e., percentage of reduction in movement on 2nd day (in Table-1)

We construct two type of arrangements to compare the growth of *D. salina* under various concentration of EMS for 1 hour in dark and rapid addition of EMS.

Rapid addition of EMS

Uni-algal culture was added to 1000ml conical flask containing 500ml artificial sea water medium (ASWM) with different concentration of EMS i.e. 0.005%, 0.01%, 0.02%, 0.04% and 0.08% for 12 days duration in subjected to controlled culture conditions. The cultures were grown in EMS treated media at 26±2^{0}C temperature under cyclic fluorescent illumination (12 hrs dark: 12 hrs light) of 2500 lux. Non-treated *D. salina* cultures in artificial sea water media were used as control. In order to find out growth of *D. salina* under rapid addition of EMS culture were observed on 2nd, 4th, 6th, 8th, 10th, and 12th days for growth parameters such as optical density and dry weight.

1 hour treatment of EMS in dark

Uni-algal culture were centrifuged at 5000 rpm for 5 min. centrifuged cells were treated with varied concentrations of EMS i.e. 0.005%, 0.01%, 0.02%, 0.04% and 0.08%. Volume of EMS solution was used 5 ml for each concentration for soaking. The cells were treated with EMS of specified concentrations for 1 hour in dark under controlled conditions with intermittent shaking. After 1 hour, the EMS solution was removed through centrifuge and the cells were thoroughly washed in ASWM for 4 times to remove the EMS residues using centrifuge. After 4 time washing treated algal cells were added to 1000ml conical flask containing 500ml artificial sea water media (ASWM) for 12 days duration in subjected to controlled culture conditions. The cultures were grown at 26±2^{0}C temperature under cyclic fluorescent illumination (12 hrs dark: 12 hrs light) of 2500 lux. Non-treated *D. salina* cultures in artificial sea water media were used as control. In order to find out growth of *D. salina* under 1 hour treatment of EMS in dark, culture were observed on 2nd, 4th, 6th, 8th, 10th, and 12th days for growth parameters such as optical density, and dry weight.

Growth Measurement

Growth was followed through optical density (figure 1a, 1b) and dry weight (figure 2a, 2b). 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. 0.50-100 ml sample of culture were filtered on whatman GF/C filters, rinsed with distilled water and weighed after drying for 24h at 80°c.

RESULTS AND OBSERVATIONS

In our present study mutagenic effectiveness and efficiency was computed. Resultant low concentration of EMS was found to be an effective and efficient dose for growth induction.

Mutagenic effectiveness

The mutagenic effectiveness was found to be the highest at lower concentration of EMS. EMS dose 0.005% was found to be more effective. EMS dose 0.005% followed by 0.01% and 0.02% (Table-1, Figure-1a, 1b, 2a and 2b).

Mutagenic efficiency

Mutagenic efficiency of EMS with respect to viable mutations was based on biological damage such as injury/lethality in mobility of algal cells. Maximum efficiency was achieved by 0.005% of EMS followed by 0.1% and 0.02%. At high EMS dose mutagenic efficiency reduces due to decrease in mutagenic induced movable mutants (Table-1, Figure-1a, 1b, 2a and 2b).

Table-1: Cell Count and Movement of *D. salina* on 2nd day based on Microscopic Observation

	Control	.005%	.01%	.02%	.04%	.08%
Cell count ($x10^4$cells/m)	95±4.9	95±3.5	63±2.3	42±2.1	36±1.5	21±1.2
cells showing difference in movement	Active Movement	Active Movement*	Slow Movement in 2/100 cells	Slow Movement in 4/100 cells	Movement retard in 10/100 cells	Inactive 16/100 cells

*All cells showing actively movement except 2/1000cells.

Effect of different concentration of EMS on growth of *D. salina*:

In comparison to different concentration of EMS, overall maximum growth was observed in control culture at 12th day (Figure-1a, 1b, 2a and 2b) Highest OD was 1.002 increased 4 times than to initial optical density value (Figure-1a, 1b).

Rapid addition of EMS was next highest growth measured at 0.005% dose level at 9th day. EMS dose level 0.08% was found least effective in promoting the growth of *D. salina*. Minimum growth was observed in for 1 hour EMS treatment in dark at 0.08% dose level on 12th day. Growth reduced in both treatments as compare to control but in rapid addition of EMS growth was higher than 1 hour treatment of EMS in dark. Optical density record in both type of treatment (Figure-1a, & 1b) also supported by dry weight record (Figure-2a, & 2b).

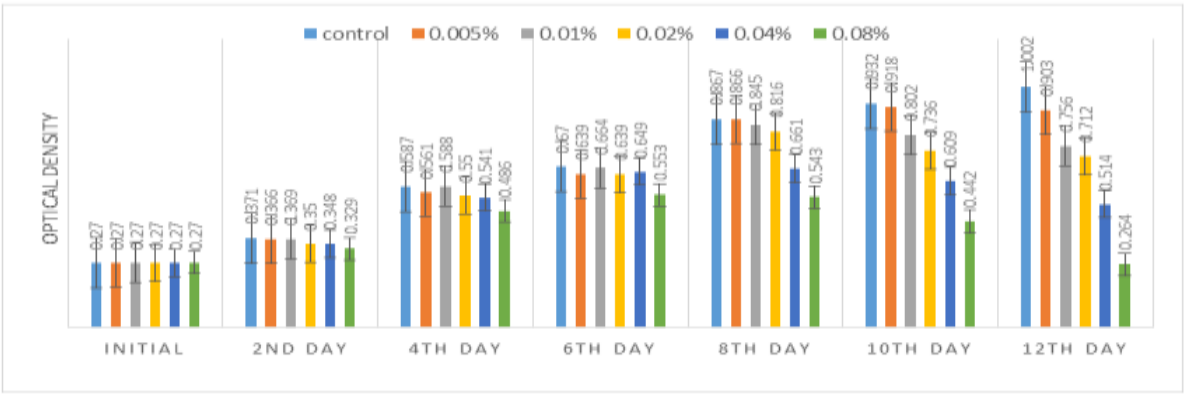
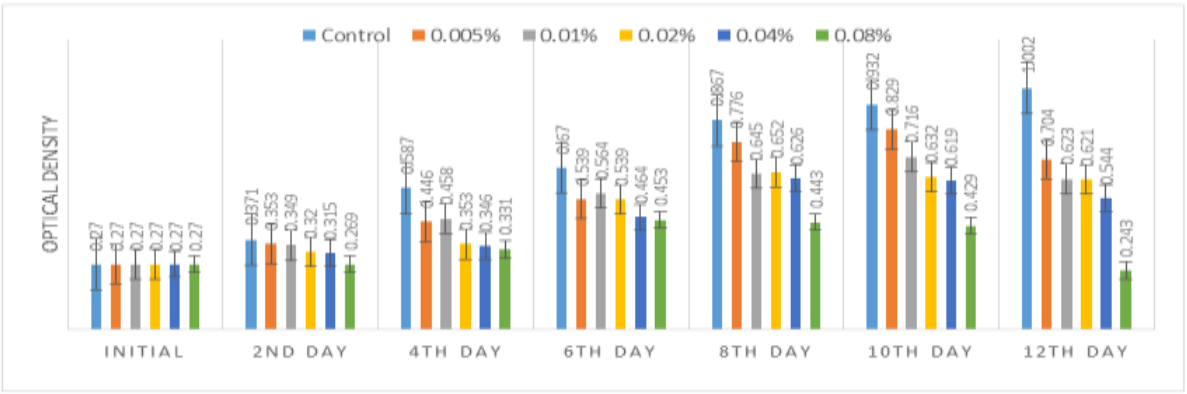


Fig-1a: Growth of *D. salina* measured through optical density in Rapid addition of EMS



Figu-1b: Growth of *D. salina* measured through optical density in 1 hour treatment of EMS in dark

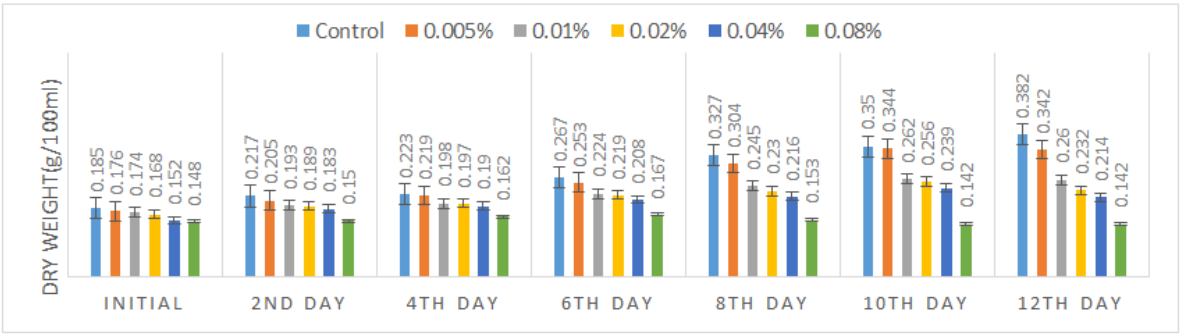


Fig-2a: Growth measured of *D. salina* through Dry Weight in Rapid addition of EMS

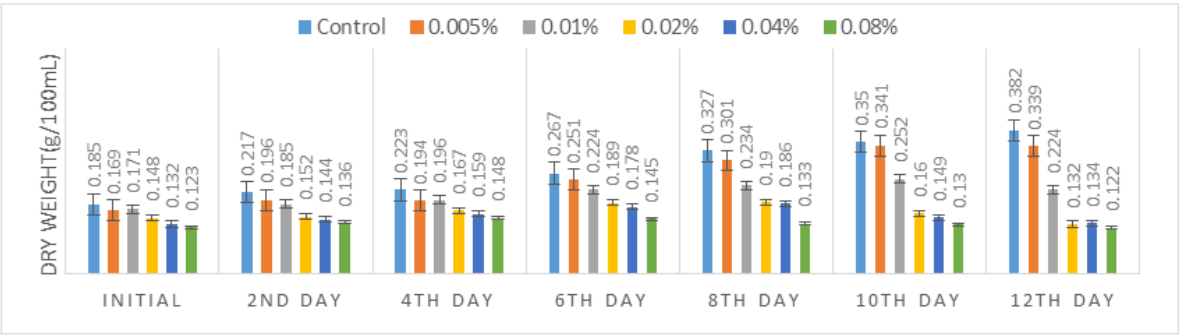


Fig-2b: Growth measured of *D. salina* through Dry Weight in 1 hour treatment of EMS in dark

DISCUSSION

Dunaliella Salina as a wonderful algae with its tremendous nutraceutical and pharmaceutical applications which are very useful to human welfare. In vitro culture of *D. salina* was treated with EMS, resultant survival rate decreased with increase in concentration of EMS as compared to control. These results also supported by findings [25] which showed the growth rate of *S. platensis* had significantly negative relation to EMS concentration. However, in control showed maximum growth and hence it can be said that control contains optimum constituent require for the growth of cells. In control increment in growth was observed till 12th days of the experimental period, which may indicate that under these environmental conditions the amount of nutrients added at the beginning of the experiment was able to support the culture till 12th days.

Here, we discussed the potential role of the efficient and effective dose of EMS in terms of their comparative advantage over their wild type control for growth with respect to their potential utilization by the algal biotechnology industry. EMS caused significant effect in a dose- and time-dependent manner. During rapid addition of EMS, *D. salina* resist and adapt to low doses of EMS (0.005% to 0.04%). But as concentration increases EMS *D. salina* could not resist high dose of EMS and *D. salina* was showing high sensitivity at 0.08% EMS dose and above. EMS dose 0.08% and above were about as inhibitory and shows inhibitory effect. The doses of EMS chosen reduced the rate of cell growth, cell movement and cell survival as compare to control. But the mutants with high growth showing active movement indicate high efficiency of that dose of mutagen. At high EMS dose mutagenic

effectiveness and efficiency reduces due to increase in biological injury /lethality and decrease in mutagenic induced movable mutants. There are many studies reported by scientists [18] which suggested that Mutagenic effectiveness and efficiency increased with the decreased in dose or concentration.

Together with available evidence on overproduction of carotenoid many studies supporting that when carotenoid increases, growth and survival rate decreases [27-29]. In our study the doses of EMS chosen reduced the rate of cell growth which give a positive feedback or a way for overproduction of carotenoids and other products of *D. salina. and According to these studies* [27-29] *our* 1 hour EMS Treatment in dark protocol may become an effective method to induce carotenoid and other products in future application because by using this protocol we get more negative growth then to control and to growth found in rapid additon of EMS. This will facilitate innovative idea for production of specific carotenoids and other products of *D. salina*

The potential role of the efficient and effective dose of EMS in terms of their comparative advantage over their wild type control for growth with respect to its commercial value and their potential utilization by the algal biotechnology industry for the production of carotenoid (such as carotene and other products). Moreover, future directions that might further our knowledge in this area will be given.

CONCLUSION

It was concluded lower doses of mutagens were effective and efficient in promoting growth, survival and active movement in cells .Mutagenic effectiveness and efficiency increased with the decreased in concentration of mutagen. Therefore we find a negative relationship between mobility, survival, growth of *D. salina* with EMS concentration

Rapid addition of EMS protocol is more effective in promoting growth then to 1 hour EMS Treatment in dark protocol. But our 1 hour EMS Treatment in dark protocol may became effective in carotenoid overproduction in future.

Improvement in growth by lowering the concentration of EMS could be a good basis for the exploitation of *Dunaliella Salina* for its nutraceutical and pharmaceutical application for human welfare.

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