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Mutagenic studies in a filamentous alga, employing a chemical mutagen–ethyl methane sulphonate

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Abstract
The biological activity of certain chemical substances is referred to as radiomimetic because they produce all the biological effects observed after treatment with ionizing radiations (E.M.S) Ethyl Methane Sulphonate is a chemical mutagen which falls under the category of radiomimetic compound as it is a electrophilic alkylating agent which reacts with structures which are rich in electrons. An attempt has been made to study the effect of Ethyl Methane Sulphonate on the filaments of green alga, *Cladophora crispata* with varying percentage of concentrations ranging from 0.0001, 0.1 for duration of 1, 2 and 4 hours for a period of three weeks. Control samples were also maintained. Observations were recorded with reference to survival percentage, dry-weight, chlorophyll content, morphological and cytological variations.

Keywords: Chemical mutagen, Ethyl Methane Sulphonate, *Cladophora crispata,* morphological and cytological variations.

INTRODUCTION

Mutations in Drosophila were produced in 1914 by treatment with alcohol and ether but could not succeed [1]. In the twenties and thirties another technique for the determination of mutation rates in Drosophila were applied to variety of chemical [2]. The first chemical mutagen discovered during Second World War was Ethyl Urethane [3]. Mutagenicity of mustard gas was discovered in 1946 [4, 5]. Mustard gas was treated because of its pharmacological properties which are very similar to those of X-rays.

Ethyl Methane Sulphonate donating its ethyl group is a mono-functional agent. Ethyl Methane Sulphonate (EMS) has been prepared by reaction of methane sulphonic acid and ethylene in the presence of boron trifloride. Ethyl Methane Sulphonate is an alkylating agent. Alkylating agents are chemicals that transfer alkyl groups to biological macromolecules under physiological conditions. General chemistry and reactions of nucleic acids were reviewed [6, 7]. More attention to alkylating mutagens showed the induction of permanent change in the character of various organisms. A general view is chromosomal breakage. The mutation exposed may be as gene mutation or visible chromosomal aberrations [8].

Earlier studies on the effects of chemicals on algae dealt mostly with physiological and mutational aspects. Previously, various chemical mutagens were tested on many algal members. The effect of Ethyl Methane Sulphonate was studied on *Rhizoclonium* [9]. The effect of Ethyl Methane Sulphonate on *Spirogyra paradoxa* was also studied which showed various chromosomal aberrations such as vacuolization of nuclei and nucleoli, chromosome and chromated breaks, bridges and laggards [10]. High toxicity of E.M.S was demonstrated [11].

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MATERIALS AND METHODS

The experimental material *Cladophora crispata* was collected from a fresh water cistern. It's unialgal clonal cultures mere raised and maintained in the laboratory conditions at 18-22^0 C. Of nine varied cultured media tried Chu-10 media was found to be must suitable for optimum growth of the alga under study. Various concentrations of the chemical mutagen E.M.S (0.0001, 0.001, 0.01 and 0.1 in percentage) was treated on the alga for duration of 1, 2 and 4 hours for a period of three weeks.

Algal cells were isolated by using sterilized needles. With the help of these needles healthy growing filaments were carefully inoculated for 10-15 days. The cleaned small fragments of the filaments were transferred to test tubes and saline bottles containing biphasic medium and these were used as stock cultures. From these stock cultures, healthy and profusely growing cultures were further inoculated in 250 C.C. conical flasks containing sterilized culture medium. The filaments of the alga were treated at hourly intervals of 1, 2 and 4 hours respectively. After the treatment the cultures were washed, transferred to fresh culture medium and were allowed to grow in culture cabinet with alternate light and dark periods (16 hours light and 8 hours darkness).

For survival percentage 100 cells were observed at random and these cells were differentiated into healthy and affected cells. Cells with normal structure and green chloroplasts were considered as healthy cells and those with shrunken or deformed chloroplasts or colourless cells due to loss of pigmentation were counted as unhealthy cells. Survival data was observed weekly for three weeks.

Regarding dry-weight the filaments were picked up with needles, washed thoroughly twice with distilled water and all the filaments were picked up and were allowed to drip off excess of water. Filter paper was weighted in single pan balance and the readings were noted. The algal material was mounted after the moisture on a filter paper has dried completely as it was folded twice and put in an oven at 100^0 C for three hours. Further, these filter

papers with material were removed carefully and placed in a desiccators for the complete absorbance of moisture. After the paper has cooled it was weighed again and finally the dry weight was determined.

For extracting chlorophylls, cultures were centrifuged with acetone at 7500 rpm for 10 minutes and the supernatant green solvent was taken as pigment extract. The extraction from various culture samples were scanned for their optical density with the help of spectrophotometer.

Regarding cytological work, the filaments were fixed in Carnoy's fluid containing glacial acetic acid and absolute alcohol in the proportion of 1:3 and stained with acetocarmine.

Morphological and cytological observations were made weekly and microphotographs were taken. These observations were continued for a period of three weeks. Control samples were also examined in these determinations.

Data regarding survival percentage, dry-weights, chlorophylls, morphological and cytological variations is illustrated in the form of figures, tables, histogram and microphotographs.

RESULTS

Effect of Ethyl Methane Sulphonate (EMS) was studied on *Cladophora crispata*. The values of survival percentage for control samples slightly increased from first week and were maximum for third week. These values increased in lower concentration treated cultures upto second week. When cultures were treated for duration of 1,2 and 4 hours in concentrations 0.01% and 0.1% a gradual decline in survival values was observed from first week onwards. Thus, the fall in survival percentage was found to be concentration and duration dependent (Fig.1).

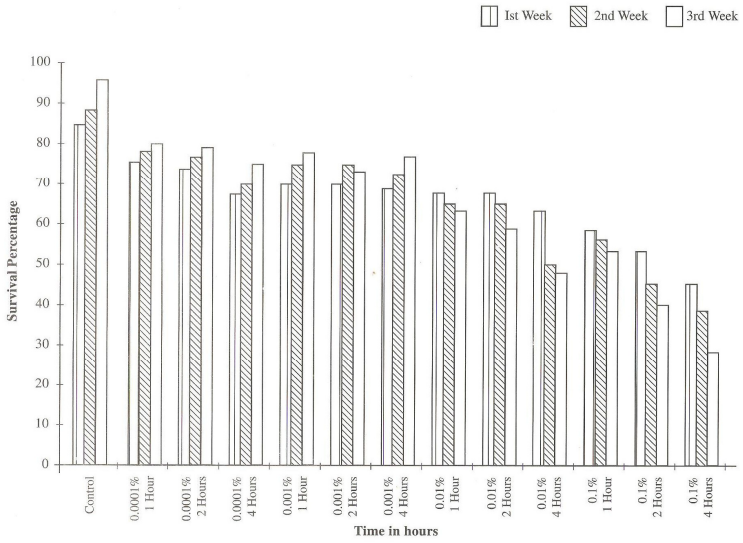

Fig 1. Showing survival percentage of *Cladophora crispate* treated with EMS (Ethyl Methyl Sulphonate)

There was a linear increase in dry-weight of control samples whereas in cultures treated in lower concentrations 0.0001% and 0.001% for 1,2 and 4 hours duration, slight increase in values was observed upto third week, cultures treated with higher concentration (0.1%) for 1,2 and 4 hours duration, the dry-weight values decreased abruptly (Fig.2).

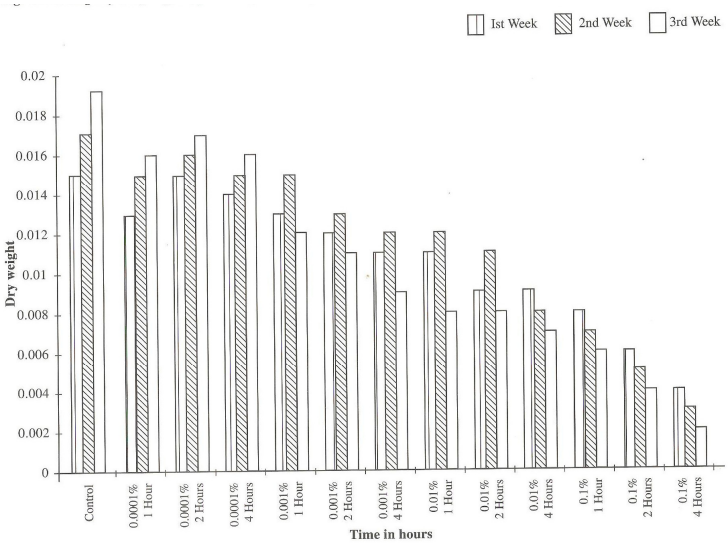

Fig 2. Showing dry-weight (gms) percentage of *Cladophora crispate* treated with EMS (Ethyl Methyl Sulphonate)

In control samples, there was a linear increase in the chlorophyll content whereas, treated cultures showed lower contents. Higher the concentration, lower the chlorophylls. The values were decreased by the third week for all the durations and concentrations of the treatment. (Table -1).

Table 1. Statement showing chlorophyll content (mg/gm) of *Cladophora crispate* treated with EMS (Ethyl Methyl Sulphonate)

Duration in hours	Concentration in Percentage	OBSERVATION IN WEEKS								
		1st			2nd			3rd		
		Chlorophyll			Chlorophyll			Chlorophyll		
		a	b	Total	a	b	Total	a	b	Total
Control	–	0.66	0.45	1.10	0.69	0.58	1.34	0.85	0.62	1 .45
1	0.0001	0.68	0.46	1.15	0.64	0.49	1 .17	0.63	0.41	1.05
	0.001	0.63	0.41	1.05	0.61	0.40	1 .01	0.56	0.40	0.96
	0.01	0.55	0.37	0.93	0.50	0.37	0.87	0.46	0.37	0.84
	0.1	0.51	0.30	0.81	0.43	0.30	0.73	0.37	0.31	0.70
2	0.0001	0.67	0.43	1.11	0.63	0.48	1.12	0.61	0.41	1 .03
	0.001	0.60	0.40	1.00	0.52	0.41	0.94	0.53	0.40	0.93
	0.01	0.53	0.35	0.90	0.47	0.35	0.83	0.45	0.34	0.80
	0.1	0.45	0.30	0.75	0.40	0.30	0.70	0.34	0.30	0.64
4	0.0001	0.65	0.41	1.07	0.61	0.48	1 .09	0.60	0.40	1 .00
	0.001	0.58	0.36	0.95	0.51	0.38	0.91	0.50	0.40	0.90
	0.01	0.50	0.35	0.85	0.45	0.31	0.77	0.41	0.31	0.73
	0.1	0.37	0.31	0.70	0.33	0.25	0.60	0.31	0.23	0.55

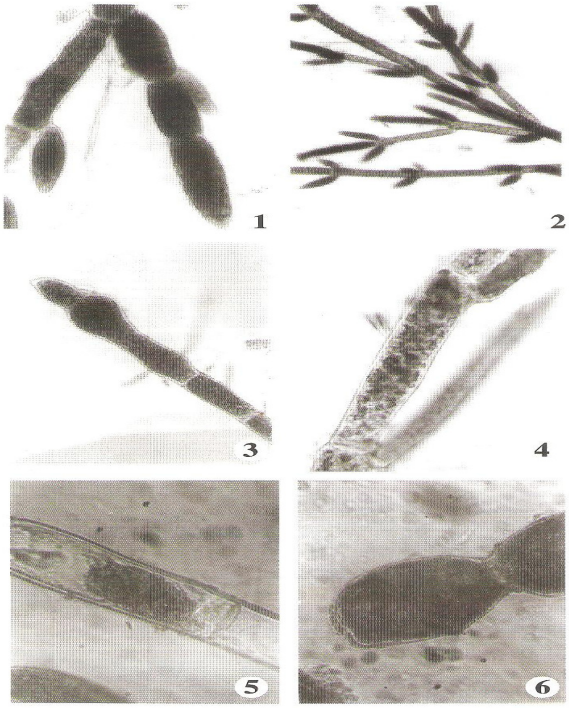

Fig 1-6. Showing morphological variation in *Cladophora crispate* after treated with EMS (Ethyl Methyl Sulphonate)

1. Filament showing branches with abundant chloroplast x 200
2. Branched filament with multiple branching x 100
3. Filament showing terminal akinete with thick chloroplast x 200
4. Contraction of chloroplast x 400
5. Cell showing damaged chloroplast x 400
6. Swollen giant cell with granulated chloroplast x 800

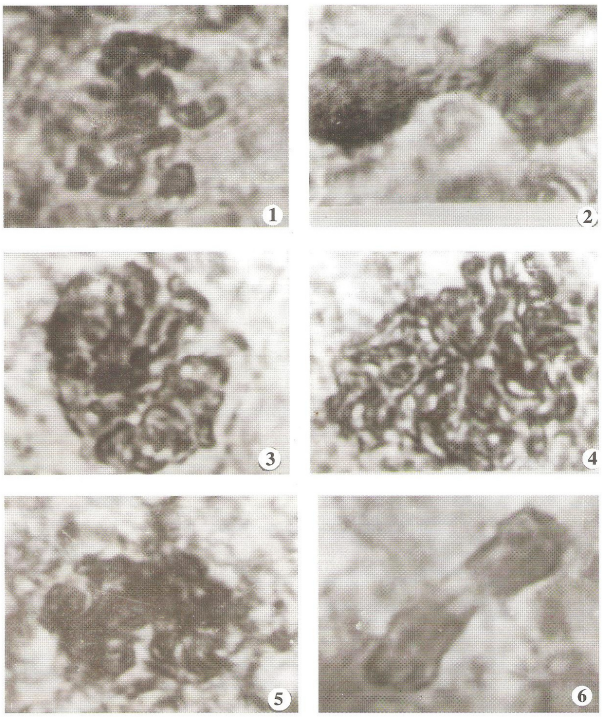

Fig 1-6. Showing various cytological abnormalities in *Cladophora crispate* after treated with EMS (Ethyl Methyl Sulphonate)

1. Condensation of chromosomes at late metaphase x 4500
2. Unequal mitotic division x 300
3. Bulging and erosion of chromosomes x 3000
4. Doubling of nuclear material x 3000
5. Breakage and pycnosis and unequal separation of chromosomes x 3000
6. Sticky multiple bridge at telophase x 4500

Morphological abnormalities were found be dose and duration dependent. Filaments treated with 0.0001% and 1 hour duration, were quite healthy and colour of the chloroplast was dark green comparable to the control (Plate-I, Fig.1). In 0.001% concentration treated for 2 and 4 hours duration majority of the cells showed multiple branching (Plate-I, Fig.2). Branching of the filaments was observed to be less in lower concentrations and showed terminal akinetes with thick chloroplast (Plate-I, Fig.3). Contractions of chloroplasts were observed in 0.01% and 0.1% concentrations (Plate-I, Fig.4). The cell showing damaged chloroplasts resulted in 0.001% and 0.01% concentration treated for the duration of 4 hours (Plate-I, Fig.5). In cultures treated with 0.1% concentration the swollen cells with granulated chloroplast were recorded (Plate-I, Fig.6).

Among cytological observations, chromosomal aberration showed a linear increase with increasing concentrations and durations. The frequency of chromosomal aberration was observed the following the analysis of cells at mitosis. Cytological abnormalities like condensation of chromosomes at late metaphase (Plate-II fig.1) and unequal mitotic division (Plate-II fig.2) were recorded in 0.0001 and 0.001 % concentrations. Bulging and erosion of chromosomes was observed (Plate-II, Fig. 3). Polyploid nuclei with enlarged size and doubling of the chromosome number (Plate-II fig.4) were noticed in 0.1% treatment of Ethyl Methane Sulphonate (EMS). Breakage and Pycnosis and unequal separation of chromosomes were also observed (Plate-II fig.5).

Multiple telophase bridges were recorded at 0.1% concentration (Plate-II fig.6). Recovery of cells was not observed in any of the concentration employed during the present studies. This may be due to heavy damage and total imbalance of nuclear material.

DISCUSSION

Based on the results that have been obtained in course of the present investigations, it may be assumed that Ethyl Methane Sulphonate (EMS) is most effective chemical mutagen and is found to be mito-depressive in action. All the four concentrations tried during the present study showed varied degrees of damage. 0.1% concentration was more effective and least effective was 0.0001% concentration. The degree of growth inhibition increased with increase in the concentration of chemical mutagen. The phenomenon of growth inhibition at higher concentration coincides with those of earlier studies with chemicals on various algal members [12-15].

The values of chlorophyll contents decreased in higher concentrations. Decreased chlorophyll content with increasing concentrations of chemical mutagen confirmed the results obtained by earlier workers [16-20]. Regarding morphological variation shrinkage of protoplast, deformation and disintegration of chloroplasts were seen. Cytological aberrations were prominent. The frequency of chromosomal aberration was found to be higher. These results coincide with the earlier work done by various workers [21-22].

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