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Study of different chemical stresses on the vegetative survival of *Cladophora glomerata*

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ABSTRACT

Cladophora glomerata (L.) kütz under treatment of urea, petrol and sulphuric acid showed reduction or absolute inhibition in percentage vegetative survival at different concentrations. The survivability of vegetative filaments declined rapidly with the treatment of sulphuric acid, which reduces the whole chlorophyll content of vegetative filaments whereas urea and petrol declined the vegetative survival which is calculated as live cells versus dead cells. Petrol is found to be more effective in reducing vegetative survival of *Cladophora* than urea; for instance, observation on 21st day at 50 ppm shows '0' vegetative cells in petrol, whereas '7' live cells were still present in urea. This trend was observed at other concentrations also, showing that petrol is more efficient than urea.

Keywords: *Cladophora glomerata,* vegetative survival, akinetes, chemicals, chlorophyll content, green algae.

INTRODUCTION

Cladophora Kützing is macroscopic alga found attached to substratum by holdfast and/or rhizoids. Filaments are erect and branched dichotomously or sub-dichotomously. Cells are cylindrical in shape and consist of parietal chloroplasts. *Cladophora glomerata* (L.) kütz are mainly marine microalgae but they are also distributed abundantly in fresh water (David M. John 2007). Very little laboratory work has been done on the effects of different chemicals on the vegetative survival of different green algae. A few work has been done on green algae such as *Trentepohlia aurea* (Gupta and Agrawal 2004), *Pithophora oedogonia* (Agrawal and Misra 2002), *Scenedesmus quadricauda, Cosmarium granatum, Hormidium flaccidum, Rhizoclonium crassipelitum, Oedogonia sp.* (Gupta and Agrawal 2012). Effect of nutrients, heavy metals (Co, Cu, Zn, Hg, Pb) and pesticides (Carbofuran, 2, 4-D, Dithane, Phorate, Bavistin) have been studied on survival of vegetative cells of *Cladophora glomerata* (L.) kütz. earlier (Agrawal and Misra 2002). This research paper deals with the effect of Urea, Petrol and Sulphuric acid on the vegetative survival of green algae *Cladophora glomerata* (L.) kütz.

MATERIALS AND METHODS

Material: The material *Cladophora glomerata* (L.) kütz. was collected from the small freshwater cemented pond found growing attached to its wall, present in Roxburgh Botanical Garden, Department of Botany, University of Allahabad. The alga was identified with the help of Prescott (1962).

Source of chemicals used in the present study: Source of pesticide Urea (Nitrogen 46%) is Vijaypur Fertilizer Co., India; Petrol is Government Supply of India and source company of Sulphuric acid (98%) is EMPLURA.

Methods: Bold's basal medium (Nichols and Bold 1965) was used for culturing *Cladophora glomerata*. The pH of the culture medium was maintained at 8.0 with the help of 2% NaOH and 1% HCL (measured by using digital pH meter). Medium was prepared in well-washed glass wares with the help of distilled water and the nutrients and components present in liquid Bold's Basal Medium was too prepared in double distilled water followed by autoclaving (at 121˚C for 30 minutes under 15 psi of pressure). *Cladophora* collected was then inoculated into the medium. Temperature was

maintained at 22ºC and light intensity of 40µmol m^{-2} s^{-1} was given for 16 hours a day from fluorescent tube (Agrawal and Misra 2002). Culture was kept for 15-20 days for proper growth.

Urea which was used to study the effect on the vegetative survival of *Cladophora* at different concentrations (25 ppm, 50 ppm, 75 ppm and 100 ppm) was maintained in Bold's Basal Medium (pH was adjusted to 7.5). Effect of petrol at different concentrations (25 ppm, 50 ppm, 75 ppm and 100 ppm) was studied on the filaments of algae (pH was maintained 7.5). Due to lethal effect of sulphuric acid, low concentrations were made to study its effect i.e. 1-5 ppm (pH was adjusted to 3.0). Filaments of *Cladophora glomerata* were inoculated in different chemicals at different concentration. Biological triplicates were made of each chemical at every concentration. The effect was studied at regular interval of 7 days in Urea and Petrol, and on daily basis in sulphuric acid. Filaments of *Cladophora glomerata* were observed and vegetative cells were counted in definite number of cells. 100 cells were observed and dead cells versus live cells were studied.

Percentage vegetative survival= number of vegetative cells ÷ 100

[Number of vegetative cells = total number of live cells – dead cell formation]

RESULT AND DISCUSSION

Cladophora glomerata survived for time period of 30-115 days in controlled culture medium, but with Urea and petrol, it survived from 7-21 days depending on the concentration. However, with the sulphuric acid, the filaments started degenerating from the day of inoculation and completely degraded within 5 days. Different green algae survived from 2-7 days when treated with high concentration of urea (2000 ppm) (Gupta and Agrawal 2012). Vegetative cells of *Chlorella variegata* and *C. vulgaris* also did not show any growth in the presence of petrol (Agrawal and Manisha 2007) With the treatment of urea, vegetative survival of *Cladophora* was 88% at 25 ppm and 20% at 100 ppm concentration on 7th day. Algal growth is reduced by petroleum products (Vandermeulen and Ahorn 1976); the vegetative survival decreased from 78% on 7th day to 20% on 21st day at 25 ppm concentration of petrol whereas at 100 ppm concentration vegetative survival declined to 16% on 7th day only. %age vegetative survival was found to be nil on 28th day with the treatment of Urea and Petrol. However, *Cladophora* under treatment with sulphuric acid started showing response from the day of treatment, making the whole filament hyaline by degenerating the chlorophyll content and no reproductive bodies formation took place.

Table 1: Vegetative Survival (in %) of cells when treated with Urea.

	25 ppm	50 ppm	75 ppm	100 ppm
DAY 7	88	65	40	20
DAY 14	55	42	28	05
DAY 21	22	07	00	00
DAY 28	00	00	00	00

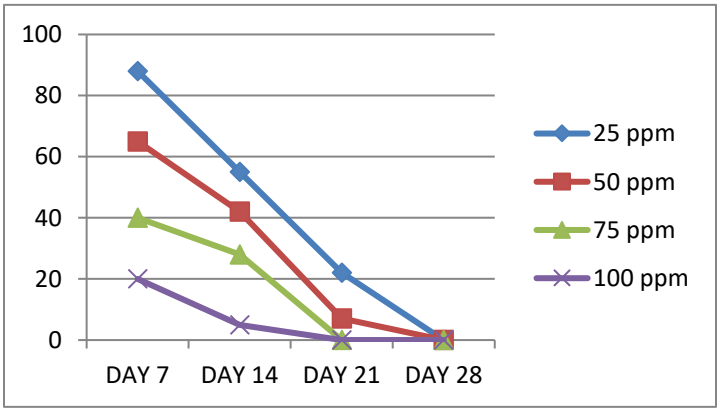

Fig. 1 Graphical representation of effect of different concentration of urea on vegetative survival of *Cladophora glomerata*. (X-axis represents time period of observation and Y-axis represents percentage vegetative survival)

Table 2: Vegetative Survival (in %) of cells when treated with Petrol.

	25 ppm	50 ppm	75 ppm	100 ppm
DAY 7	78	55	34	16
DAY 14	47	28	15	00
DAY 21	20	00	00	00
DAY 28	00	00	00	00

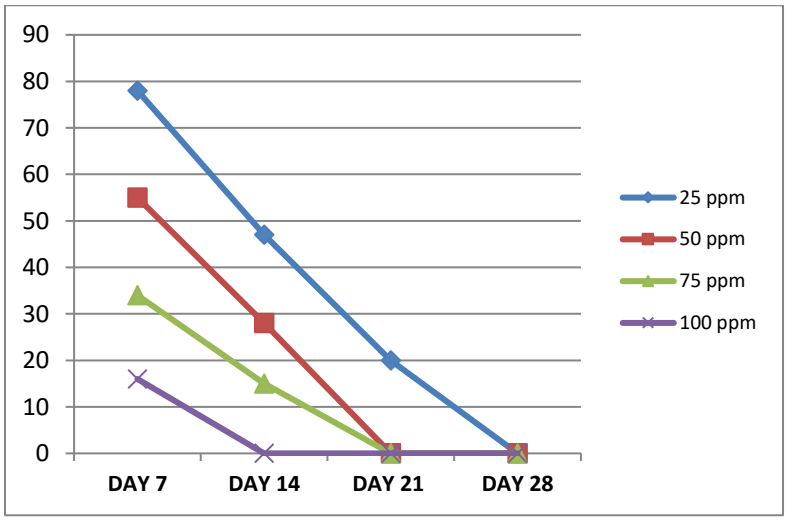

Fig. 2 Graphical representation of effect of different concentration of petrol on vegetative survival of *Cladophora glomerate*. (X-axis represents time period of observation and Y-axis represents percentage vegetative survival)

Table 3: Chlorophyll content in filaments of *Cladophora glomerata* after treatment of H_2SO_4.

	1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
DAY 1	+++	+++	++	+	+
DAY 2	++	++	+	+	_
DAY 3	++	+	+	_	_
DAY 4	+	_	_	_	_
DAY 5	_	_	_	_	_

NOTE: +++ denotes high chlorophyll content, ++ denotes progressively less chlorophyll content, + denotes very less amount of chlorophyll content in filament and – denotes hyaline filament or no chlorophyll content.

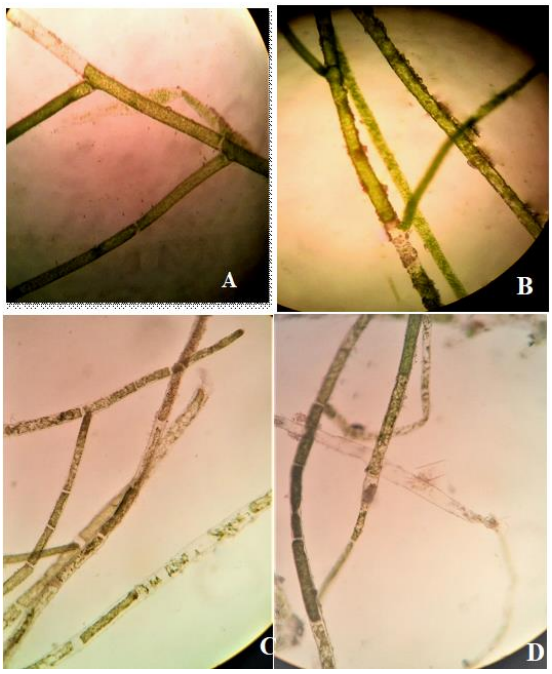

Fig. 3 A-B showing dead cell (hyaline) in the filament C. degenerating chlorophyll content D. showing whole hyaline filament.

After few days of treatment with urea and petrol, the cells started turning dark green in color, giving indication that they might be akinetes, which could have been germinated if the filaments were again cultured into nutrient media. In *Rhizoclonium hieroglyphicum*, urea showed formation of zoospores at 200 ppm (Gupta and Agrawal 2004), which indicates that formation of akinetes might have taken place.

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