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EFFECTS OF OXALIC ACID ON CHLOROPHYLL CONTENT, VEGETATIVE SURVIVAL AND REPRODUCTION OF THE FRESHWATER GREEN ALGAE

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ABSTRACT

The present study investigate the effects of oxalic acid on photosynthetic pigments, vegetative survival and reproduction of freshwater green algae *Pithophora oedogonia and Rhizoclonium hieroglyphicum* growing in Bolds Basal medium under control culture conditions at temperature of 25 ± 1°C and 16 hrs: 8 hours (light : dark) time period. The total chlorophyll content of the algae varied on the addition of oxalic acid at different concentrations of 0.1, 1, 10 and 100 ppm level. At 10 and 100 ppm concentrations of oxalic acid decreased the chlorophyll a and chlorophyll b as well as total chlorophyll content of the algae with the time while at 0.1 and 1 ppm of oxalic acid algal chlorophyll content was higher than control culture. Oxalic acid decreased the vegetative survival in and zoosporangia formation in *R. hieroglyphicum* while akinete formation increased in *P. oedogonia* as the concentrations of oxalic acid increased from 0.1 to 100 ppm.

KEYWORDS: Bolds Basal Medium, Chlorophyll Content, Concentrations, Oxalic Acid, *Pithophora oedogonia, Rhizoclonium hieroglyphicum*

Algae are the principal primary producers of aquatic ecosystems. Growth of algal as well as other populations of the aquatic biota is restricted even by micro molar concentrations of different chemicals such as pesticides, petrochemical compounds, xenobiotics etc. Various works has been done on production of oxalic acid and its role in higher plants but very little is known about effects of Oxalic acid on the total chlorophyll content of algal filaments. Calcium oxalate crystals are common cellular constituents of vascular plants (Franceschi and Horner; 1980) but are rarely recorded in algae (Pueschel, 1995; Friedmann *et al*. 1972, Turner and Friedmann; 1974). Blue green alga *Nostochopsis lobatus, Westiellopsis prolifica* and green alga *Chaetophora attenuata* have been reported to produce various organic acids, (oxaloacetic acid and oxalic acid), amino acids and sugars in the culture medium and producing stressful conditions affecting the growth and reproduction of other algae in culture medium (Agrawal; 1994). The decrease in dry weight and carotene accumulation were observed by algal cultures that grown with oxalic acid and other carbon chemicals (Battah *et al*. 2013). It has been reported that measurement of chlorophyll can be used to monitor physiological state and growth in algae (Li *et al*.1980, Morries *et al*. 1981).

Fresh water green alga *P. oedogonia* reproduced through the akinete formation, the only asexual method of reproduction. During akinete formation, it was observed that the major part of the protoplasm of vegetative cell which was going to differentiated into an akinetes was contracted towards one end of the cell and separated by the septum and developed the thick cell wall around it. Akinetes became dark greenish to black in color. The alga *R. hieroglyphicum* reproduced through the zoospore formation in zoosporangia. During differentiations of zoosporangia, vegetative cells became broader than usual cells with thick cell wall. Fully matured zoosporangia were dark green in color and larger than vegetative cells.

The objective of the present study was to investigate the effects of oxalic acid on chlorophyll content as well as vegetative survival and reproduction of the green algae *P. oedogonia* and *R. hieroglyphicum* under different concentrations.

MATERIALS AND METHODS

Test Organisms

For the present study, green algae *Pithophora oedogonia* and *Rhizoclonium hieroglyphicum* were collected as growing and forming tangled mats in a fresh water pond at the Botany Department, University of Allahabad. The source of water of the pond is ground water.

Chemical Used For Investigations

Oxalic acid is an organic compound with IUPAC name Ethanedioic acid and formula $H_2C_2O_4$. It is colorless, crystalline solid (QUALIGENS FINE CHEMICALS, India).

Method Of Measuring Chlorophyll Content

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Algae *P. oedogonia* and *R. hieroglyphicum* were collected from their natural habitats and its equal amount (10 mg) measured with electronic balance (CONTECH, India) was inoculated separately into equal volumes (10 ml) of Bold Basal Medium as well as in desired concentrations of oxalic acid solution of 0.1, 1, 10 and 100 ppm. All inoculated culture tubes were placed in the culture chamber at control culture conditions with illumination of 2 K lux white Light intensity for 16 hrs a day at temperature of 25 ± 1°C.

Cultures were examined periodically for observing the effects of oxalic acid on total chlorophyll content of the alga by Mckinney method (1941).

Estimation Of Percentage Of Vegetative Survival Of Algal Filaments

Percentage vegetative survival of each of both algae was determined by observing the percentage of live vegetative cells versus dead vegetative cells. About 1000-2000 vegetative cells of inoculated algal mass of each of two algae were counted under microscope from each of 3 replicates to determine the percentage vegetative survival of the alga. Values were mean of 3 replicates. The vegetative cells of live algal filaments of algae were light green to dark green in color, full of chlorophyll, turgid, while dead cells of the filaments were hyaline, had shrinkage of cytoplasm, low turgidity and no greenery in the cell sap.

Estimation Of Percentage Of Akinete Formation In *Pithophora oedogonia*

Percentage of akinete formation in *P. oedogonia* was determined by counting the number of akinetes formed with respect to total number of vegetative cells in algal filaments under microscope. Values were mean of 3 replicates of algal inoculants.

Estimation Of Zoosporangia Formation In *Rhizoclonium hieroglyphicum*

Zoosporangia formation in *R. hieroglyphicum* was determined by the counting the number of zoosporangia formed with respect to total number of the live vegetative cells in algal filaments under microscope. Values were mean of 3 replicates of algal inoculants.

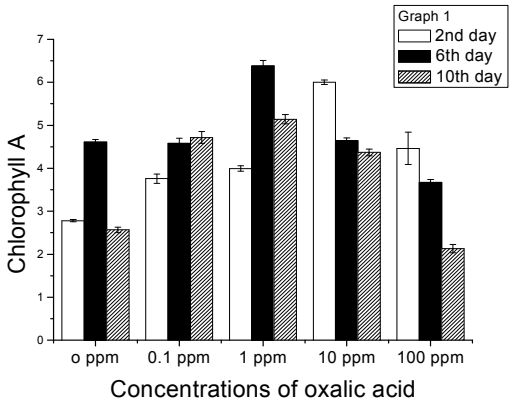
Statistical Analysis

The results presented are the means ± standard deviation data obtained from each of three replicates. All data are subjected to one way analysis of variance (ANOVA) with SPSS 16.0 software. Duncan's multiple range test was performed at the 0.05 level of significance.

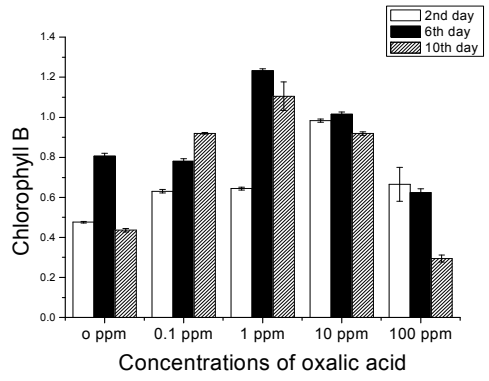
RESULTS AND DISCUSSION

Effects Of Oxalic Acid On Chlorophyll Content Of Algal Filaments

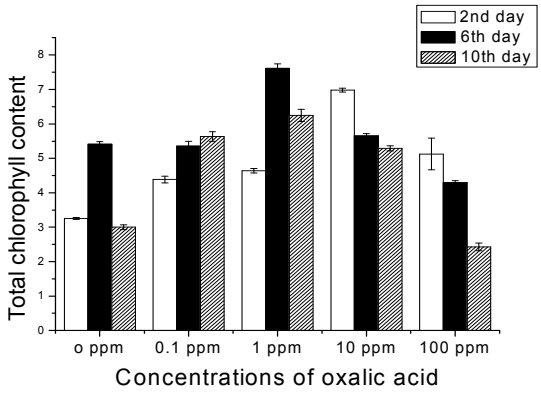
The total chlorophyll content estimation is well known method used for measuring the growth and survival of the alga. It was estimated by determining the amount of chlorophyll a and chlorophyll b of the algal mass with the help of spectrophotometer (Mckinney, 1941). As the oxalic acid concentration increased from 0.1, 1, 10 and 100 ppm in comparison to control culture (0 ppm) chlorophyll content varied. It might be due to changes in acidity or pH of the growth medium. The chlorophyll content of the algal filaments increased from second day to sixth day in 0.1 and 1 ppm of oxalic acid, while its value decreased in 100 ppm of oxalic acid continuously (Graph 3 and Graph 6). pH 7 - 8 is optimum for good survival of vegetative cells (Agrawal and Mishra 2002) as well as in other stages of life cycle like akinete initiation from vegetative cells, akinete maturation, akinete germination etc. Reduction and complete suppression in the various stages of life cycle of algae occurred below pH 7 and above pH 8. At 0.1, 1 and 10 ppm concentrations of oxalic acid there was increased in total chlorophyll content in comparison to control culture (0 ppm). The amount of chlorophyll a was always higher than chlorophyll b (Graph 1, 2, 4 and 5).



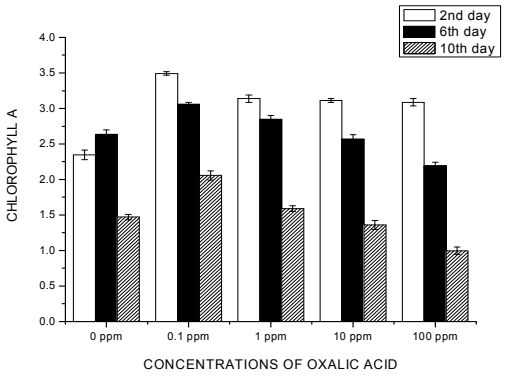
Graph 1: Effects of oxalic acid on chlorophyll a of alga *P. oedogonia*



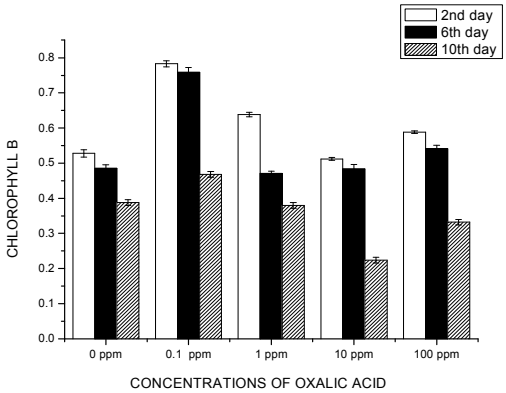
Graph 2: Effects of oxalic acid on chlorophyll b of alga *P. oedogonia*



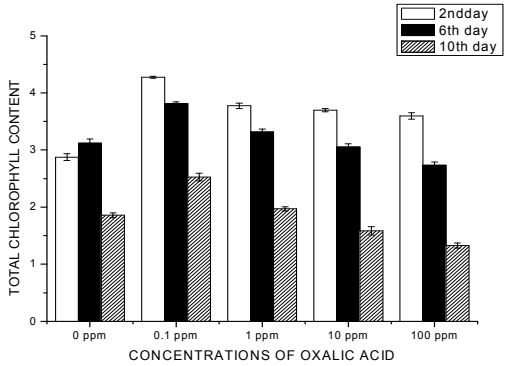
Graph 3: **Effects of oxalic acid on total chlorophyll content of alga *Pithophora oedogonia***



Graph 4 - Effects of oxalic acid on the chlorophyll A of alga *Rhizoclonium hieroglyphicum*



Graph 5: Effects of oxalic acid on the chlorophyll B of alga *Rhizoclonium hieroglyphicum*



Graph 6: Effects of oxalic acid on the total chlorophyll content of alga *Rhizoclonium hieroglyphicum*

Effects Of Oxalic Acid On Vegetative Survival And Reproduction Of Alga *P. oedogonia*-

The percentage vegetative survival of filaments of *P. oedogonia* decreased with an increase in concentration of oxalic acid (Table 1). The decrease in vegetative survival of alga as in case of oxalic acid might due to acidity of the solution or acid reaction of growth media (Battah *et al*. 2013) which negatively affects the growth of green alga P*ithophora oedogonia*.

In control culture 23% and 52% akinetes over total live vegetative cell were formed at 10 and 15 days of inoculations respectively while as oxalic acid concentrations increased from 0.1 to 100 ppm, percentage of akinetes formation in alga *P. oedogonia* also increased (Table 1). The formation of viable akinete or other reproductive bodies is shown to be directly linked with vegetative cell survival and growth (Agrawal and Misra; 2002). As the concentration of oxalic acid increased from

0.1 to 100 ppm, it imposed a stress on alga resulting in formation of akinete rapidly to escape the acidity for survival of the alga.

At 0.1, 1, 10 and 100 ppm of oxalic acid, akinete persists but could not germinate. While in control culture algal filaments were started akinete germination within 12– 14 days of inoculation.

Table 1: Percentage of akinetes formation and vegetative survival in *P. oedogonia* in presence of different concentrations of oxalic acid*

Concentrations of oxalic acid (in ppm)	10th days of inoculations		15th days of inoculations	
	V. S. (%)	Akinetes (%)	V. S. (%)	Akinetes (%)
0	75 ± 2.08	23 ± 2.0	69 ±2.64	52 ± 2.08
0.1	73 ± 2.0	9 ± 2.08	67 ± 2.64	12 ± 2.51
1	72 ± 2.0	7 ± 2.51	66 ± 2	15 ± 2.51
10	71 ± 2.64	10 ± 3.05	65 ± 2	17 ± 1.52
100	70 ± 1.52	23 ± 1.0	58 ± 2	25 ± 1.52

*all readings are mean of three replicates with standard deviation; V. S. (vegetative survival)

Effects Of Oxalic Acid On Vegetative Survival And Reproduction Of Alga *R. hieroglyphicum*-

In case of *R. hieroglyphicum*, 28% zoosporangia were formed at 15th days of inoculations in control culture. The rate of formation of zoosporangia increased with the increase in number of days after inoculations. In 0.1 and 1ppm algal filaments were 35 % and 37 % zoosporangiated respectively, higher than control culture. As the oxalic acid concentration increased from 0.1 to 100 ppm, the percentage of zoosporangia formation as well as percentage of vegetative survival both decreased (Table 2). The decrease in vegetative survival and zoosporangia formations in algal filaments might be due to change in acidity of the solution which negatively affects alga *R. hieroglyphicum*.

Table 2: Percentage of zoosporangia formation in *R. hieroglyphicum* in presence of different concentrations of oxalic acid*

Concentrations of oxalic acid (in ppm)	10th days of inoculations		15th days of inoculations	
	V. S. (%)	Zoosporangia (%)	V. S. (%)	Zoosporangia (%)
0	76 ± 2.0	13 ± 2.0	71 ± 2.0	28 ± 1.0
0.1	75 ± 1.5	14 ± 2.0	69 ± 2.0	35 ± 2.0
1	75.5 ± 1.5	15 ± 2.5	70 ± 3.2	37 ± 2.0
10	74 ± 2.0	10 ± 1.5	66 ± 2.0	15 ± 2.6
100	72.6 ± 2.5	11 ± 2.0	65 ± 3.0	17 ± 2.0

*all readings are mean of three replicates with standard deviation; V. S. (vegetative survival

CONCLUSIONS

Oxalic acid as an organic acid influenced the growth of both algae by affecting the chlorophyll content of the algal filaments under different concentrations. The increasing concentrations of oxalic acid decreased the vegetative survival of both algae and zoosporangia formations in *R. hieroglyphicum* but increased the akinete formation in *P. oedogonia.*

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