

## EFFECT OF DES ON *PITHOPHORA OEDOGENIA* (MONT) WITTROCK (CLADOPHORALES, CHLOROPHYCEAE)

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*Pithophora oedogonia* was treated with various concentrations of DES (0.1, 0.01, 0.001 and 0.0001) in Chu-10 inorganic culture medium. The 0.1 concentration of DES proved lethal for the alga and the growth rate was inhibited in various concentrations of treated samples compared to control. The control samples showed only akinete formation. No sporulation could be observed in 0.1 concentration of DES which proved lethal. The percentage of spore formation improved in lower concentrations of DES. These values were low when compared to control. Morphological variations include irregular swellings of the cells, bulging nature of branching tips and fragmentation into individual cells in DES treated samples.

**Key Words :** *Pithophora oedogonia*, DES.

A wide range of chemical mutagens were employed to study their effects in algae since a long time with particular reference to their morphology, cytology and the stimulation or inhibition of growth. However, few studies seem to have reported on the effect of mutagens on the *Pithophora oedogonia*, by using various chemicals like, colchicine, antibiotics IAA & GA, vitamins, heavy metals and atropin (Patel, 1971; Chaudhary & Singh, 1989; Agrawal, 1984, 1988; Venumadhav *et al.*, 1991; Purnachander *et al.*, 1991). The chemical mutagens have been employed as effective analytical tools for the study of organisms and causes deleterious effects to the algal members. The alkylating agent, diethyl sulphate was chosen for this work. It has been known to induce mutations, which constitute a class of potent mutagens in certain microorganisms and higher plants. Some of the earlier reports of DES effect on algal members are (Gupta and Kumar, 1970) on *Anacystis* sp., (Sathaiyah and Vidyavati, 1983) on *Cosmarium* sp., (Bharati and Giriappanvar, 1988) on two nitrogen fixing BGA *Anabaena* and *Hapalosiphon* sp. The present investigation aims to determine the effect of various concentrations of DES on the growth rate and morphology of *Pithophora oedogonia*.

### MATERIAL AND METHODS

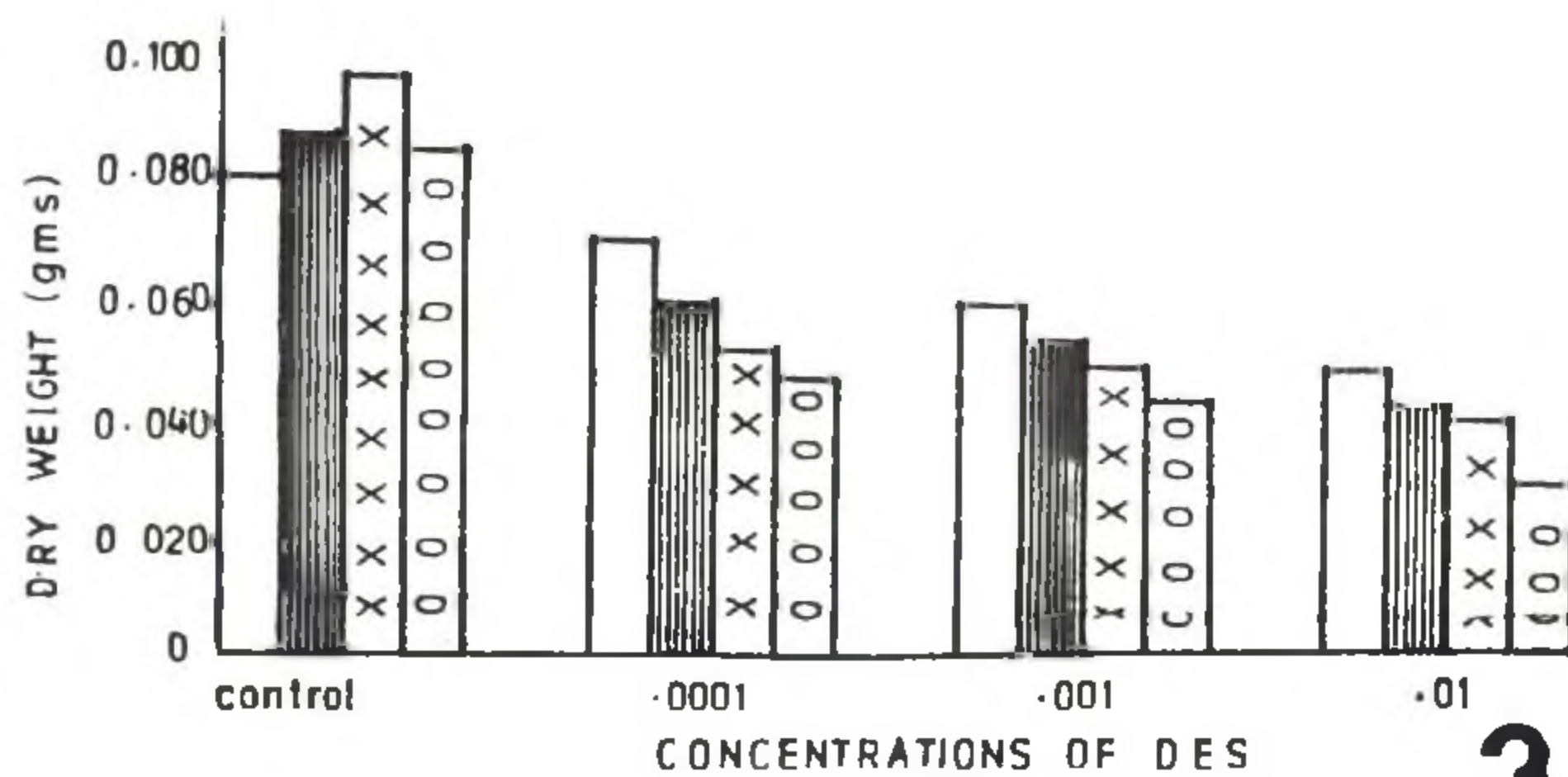
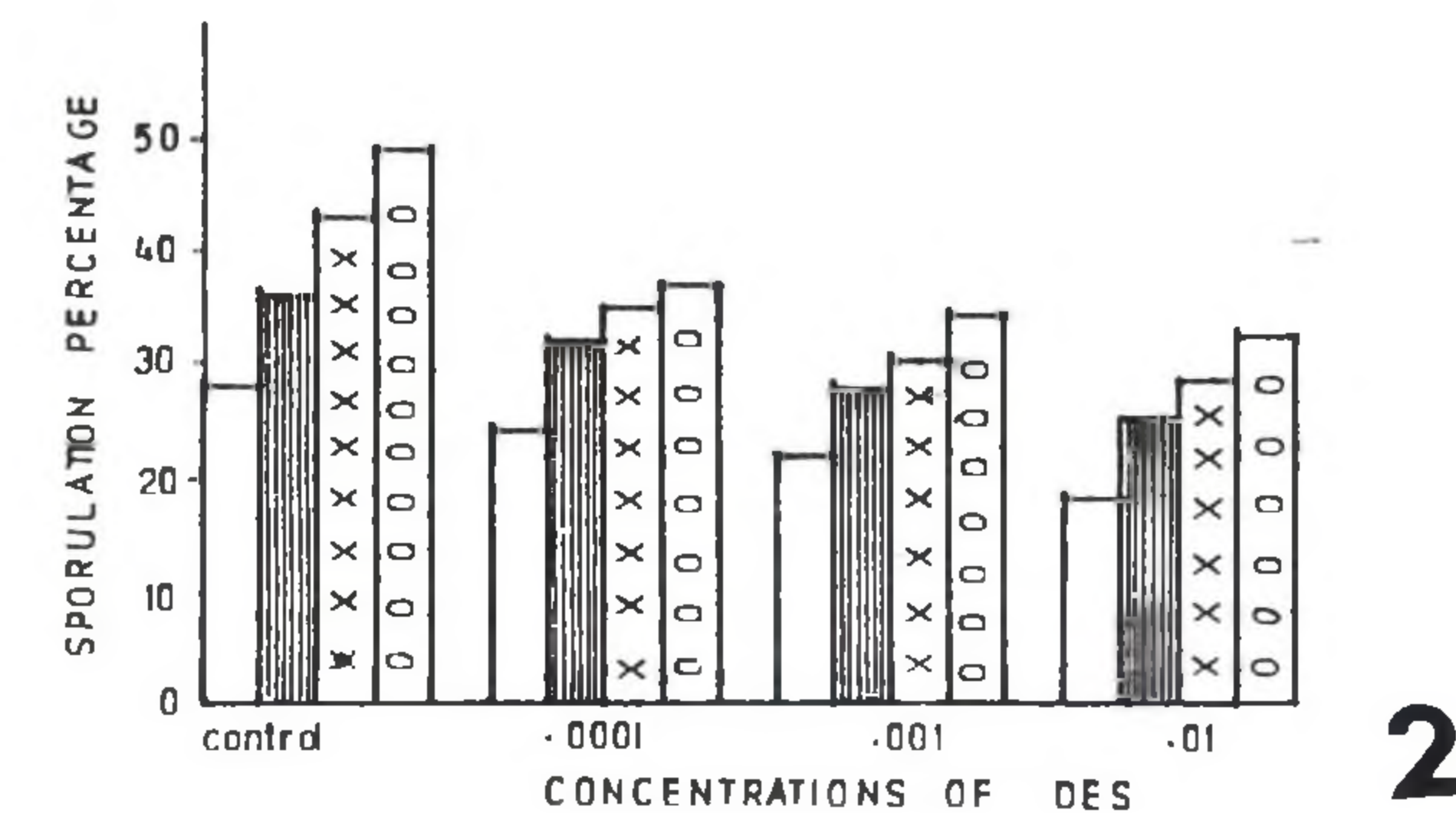
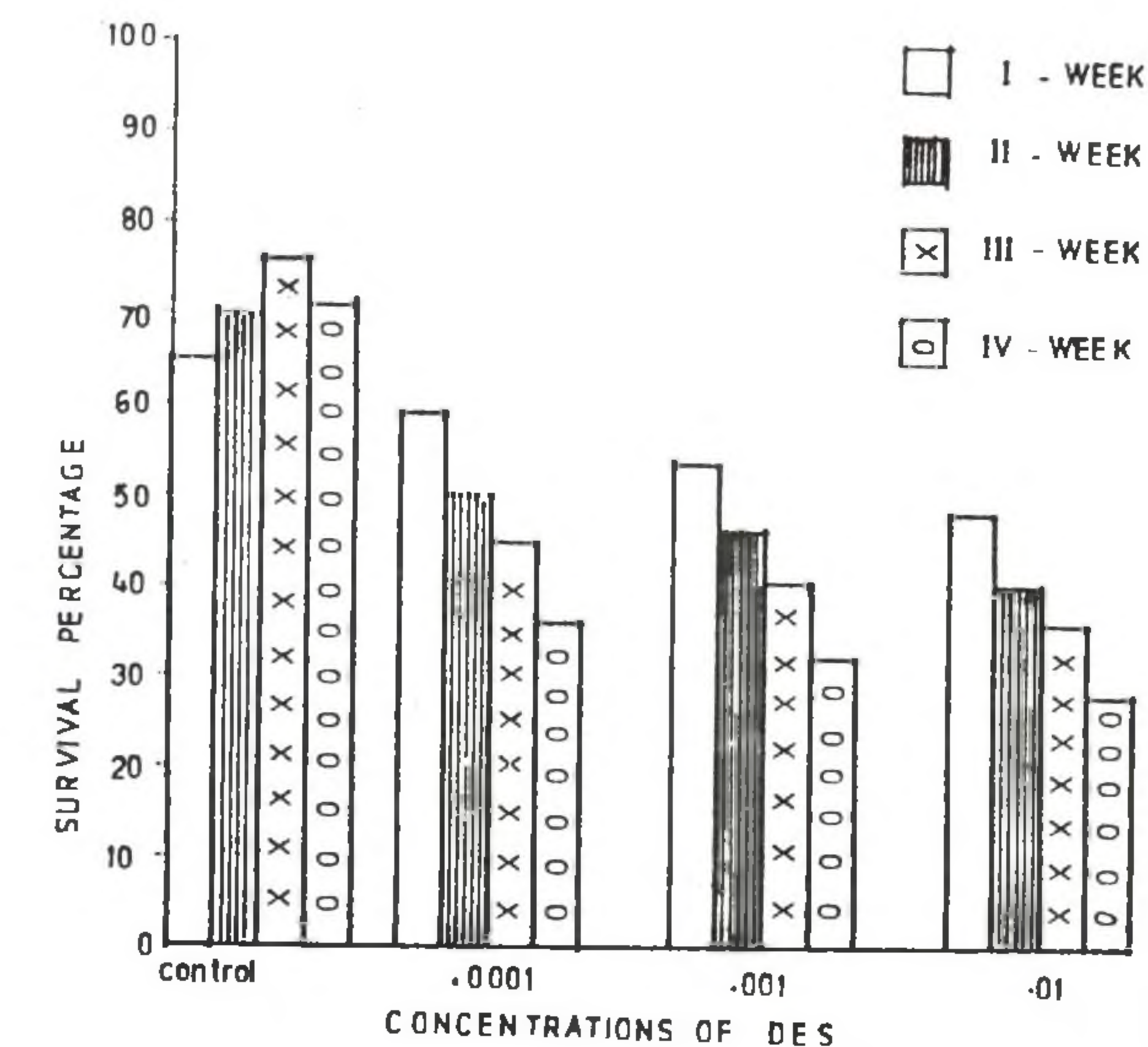
*Pithophora oedogonia* was collected from a fresh water pond of Bhadrakali temple in Warangal district of Andhra Pradesh. The clonal cultures were raised under suitable laboratory conditions. The mutagenic chemical, diethyl sulphate was obtained

from Sisco Research Laboratories Pvt. Ltd., Bombay. The alga was treated with various concentrations of DES (0.1, 0.01, 0.001 and 0.0001) in culture medium (Chu-10) and maintained by receiving 16 hrs light, 8 hrs dark at a temperature of 18-22°C along with control. The observations were made every week and continued for over a period of four weeks including the parameters survival percentage, sporulation, chlorophyll content, dry weight. For survival percentage from each culture sample 20 slides were prepared and from each slide 20 randomly selected fields of view were scored. The akinete sporulation 200 cells of different filaments were counted from each sample. The pigments were extracted using 80% cold acetone and estimated by using the formulae of Maclachlan and Zalik (1963). Spectrophotometer analysis of pigment extract revealed two peaks at 645 and 663 in all the samples to measure the chlorophyll-a, b and total chlorophyll contents. From each sample 20 mg of alga weighed and put on filter paper folded twice, dried completely in an oven at 100°C for 3 hrs. After the filter papers were removed and carefully placed in a desicator. It was weighed again and dry weights were determined.

### OBSERVATIONS

In control survival percentage (Fig. 1), chlorophyll contents (Table 1) and growth (Fig. 3) of the alga were improved upto three weeks. The sporulation started on 7th day of the culture and only akinetes were developed, which were terminal and intercalary and may be formed singly or in small





Figures 1-3. Fig. 1 Showing survival percentage of *Pithophora oedogonia* after DES treatment. Fig. 2 Showing sporulation in same. Fig. 3 Showing dry weight in the same.

series. Healthy growing normal cells of the filaments developed akinetes with higher values (Fig. 2).

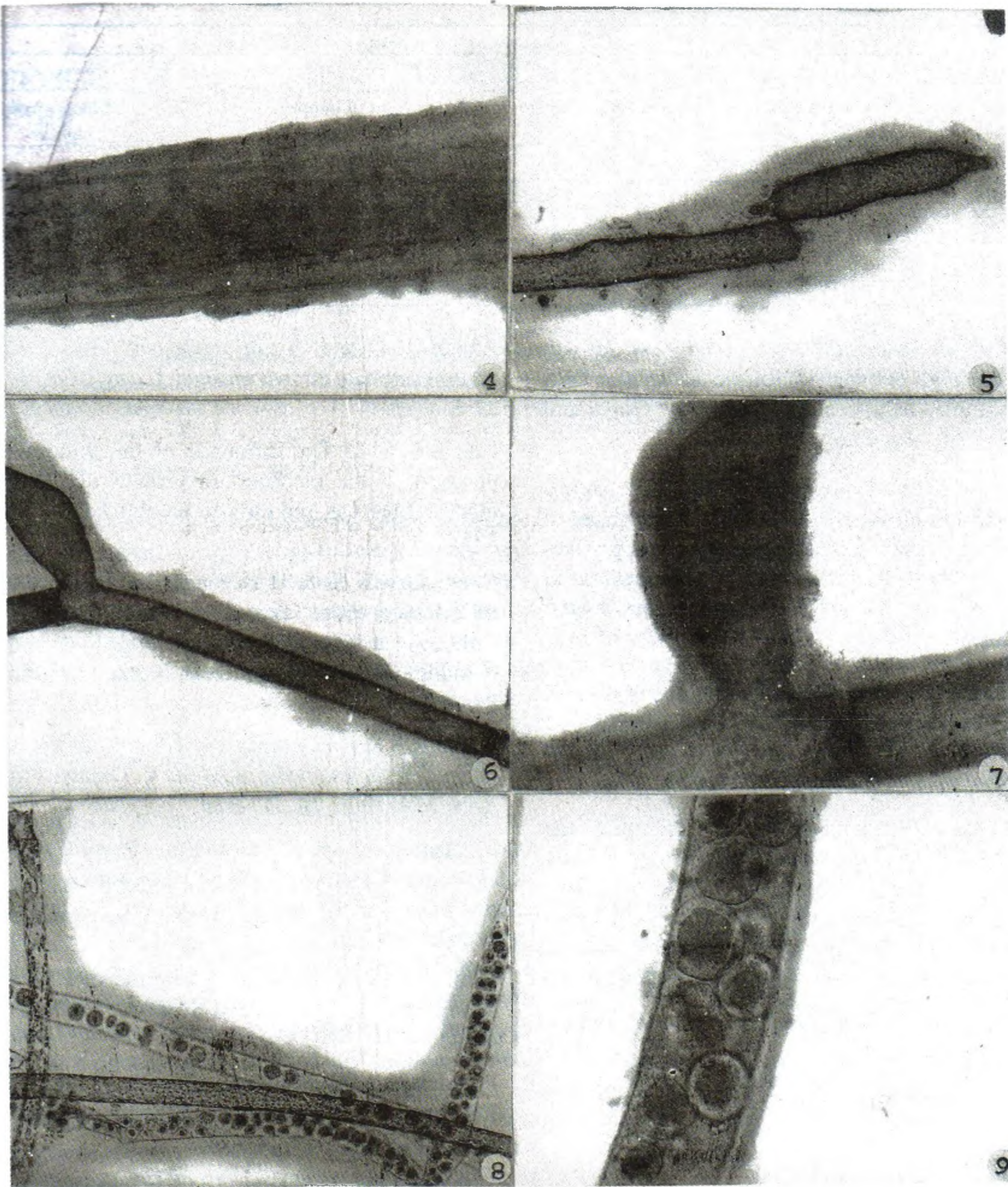
In the 0.0001 percentage of DES concentration the cells were healthy and quite normal under this treatment. The sporulation started from the 1st week

onwards and slowly improved till the end of the experiment. These values were found to be lower than the control (Fig. 2). Two types of spore formation were developed. Akinete formation appeared with thick wall and contained dark-green healthy pigmentation, whereas, some of the cells developed more number of spores in a single cell showing yellowish pigmentation which later turned into brown pigmentation. Each of the spore developed separate spore wall, smaller in size with many spores within the cell resulting in the formation of aplanospores. Sometimes, the spores also possessed only yellow contents. The survival percentage (Fig. 1), chlorophylls (Table 1) and growth (Fig. 3) of the alga gradually decreased with increasing durations but there values showed higher than the 0.01 and 0.001 concentrations of DES. No abnormalities of the cells however, could be scored in the cultures.

In the 0.001 percentage of DES concentration, most of the yellowish-green pigmented cells started developing from 2nd week onwards and showed aplanospore formation. The akinetes were formed only from healthy cells. The akinete sporulation values decreased with lower to higher concentration of DES (Fig. 2). It is quite interesting to observe that the aplanospore formations, under the concentration showed two types. Some of the cells developed large size spores and formed less in number in a cell. Each of the spore contained separate spore wall and may be called macrospores whereas, other cells developed into smaller size and formed many spores within a cell which may be called microspores. The spore content was brown coloured but sometimes, the content was completely disintegrated in which case the spores could not survive. The survival percentage (Fig. 1), chlorophyll contents (Table 1) and growth (Fig. 3) of the alga decreased with the increasing period of treatment. Morphological variations more irregular swellings and fragmentation into individual cells were noted.

In the 0.01 concentration, the cells were more effected and showed yellowish-green pigmentation. Sporulation started and the values were improved with the period. But the rapid decrease in the survival percentage was observed (Fig. 1). Chlorophyll contents and growth (Fig. 3) of the alga in this concentration of DES showed more inhibition (Table 1). Dark-green pigmented akinetes and brown pig-





Figures 4-9. Showing various morphological variations in *Pithophora oedogonia* with DES treatment, fig. 4 Normal cell in the control X 100. fig. 5 Fragmentation into individual cell under the 0.001 concentration of DES x 100. fig. 6 Cell showing more bulging of the growing branch under the 0.01 concentration of DES x 40. fig. 7 Enlarged part of more bulging branch x 100. fig. 8. Aplanospore formation in the cells under the 0.0001 concentration of DES x 40. fig. 9 Enlarged cell with aplanospores x 100.

mented aplanospores developed with varied shapes in the cells. Bulging nature of growing tips of branches were noted.

In the 0.1 percentage of DES concentration proved as a lethal dose for the alga and no sporu-

lation could be observed.

#### DISCUSSION

During the study, it was observed that the present organism, *Pithophora oedogonia* was markedly ef-



Table 1. Showing chlorophyll content (mg/gm) of *Pithophora oedogonia* after DES treatment. (Observations in weeks)

| Concentration of DES (%) | (Observations in weeks) |      |       |                     |      |       |                     |      |       |                     |      |       |
|--------------------------|-------------------------|------|-------|---------------------|------|-------|---------------------|------|-------|---------------------|------|-------|
|                          | I                       |      |       | II                  |      |       | III                 |      |       | IV                  |      |       |
|                          | Chlorophyll content     |      |       | Chlorophyll content |      |       | Chlorophyll content |      |       | Chlorophyll content |      |       |
|                          | a                       | b    | Total | a                   | b    | Total | a                   | b    | Total | a                   | b    | Total |
| Control                  | 0.72                    | 0.68 | 1.40  | 0.81                | 0.75 | 1.56  | 0.90                | 0.80 | 1.70  | 0.82                | 0.66 | 1.48  |
| 0.0001                   | 0.70                    | 0.61 | 1.31  | 0.62                | 0.58 | 1.20  | 0.51                | 0.46 | 0.97  | 0.45                | 0.37 | 0.82  |
| 0.001                    | 0.62                    | 0.57 | 1.19  | 0.50                | 0.46 | 0.96  | 0.45                | 0.40 | 0.85  | 0.36                | 0.25 | 0.61  |
| 0.01                     | 0.51                    | 0.49 | 1.00  | 0.38                | 0.36 | 0.74  | 0.29                | 0.27 | 0.56  | 0.20                | 0.18 | 0.38  |

ected with chemical mutagen, DES. The growth rate of the alga showed inhibition in all the DES treated samples as also shown earlier by Gupta and Kumar (1970) in *Anacystis nidulans*.

Bharati and Giriappanvar (1988) have shown the growth inhibition by the chemical employed at different time doses 5, 10, 20 minutes. The production of heterocysts increased in *Anabaena fertilissima* at 5 and 10 minutes and *Haplosiphon stuhlmannii* for 10 minutes.

Sathaiah and Vidyavati (1983) observed the growth inhibition due to DES in all the concentrations tried on *Cosmarium botrytis* along with the maximum number of binucleate cells.

In the present investigation, the growth of the organism was inhibited with simultaneous stimulation for the spore formations due to chemical mutagen. Various types of spore formations with varied pigments were observed. Earlier such spore variations were not reported employing various chemicals on the alga, *Pithophora oedogonia*.

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