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EFFECTS OF INDOLE, 3-ACETIC ACID ON TWO MARINE DIATOMS *SKELETONEMA COSTATUM* (GREVILLE) CLEVE AND *THALASSIOSIRA FLUVIATILIS* HUST

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ABSTRACT

In the present study effect of the exogenously applied phytohormone, Indole, 3-acetic acid, on two marine diatoms *viz. Skeletonema costatum* and *Thalassiosira fluviatilis* was investigated. The phytohormonal impact on these two diatoms was measured in terms of growth rate and morphological characteristics over a period of 12 days. The effect of IAA was both promotory and inhibitory to growth and cell elongation, the extent being dependent on the concentrations. Maximum growth promoting concentrations were 0.1 and 1.0 ppm for *S. costatum* and *T. fluviatilis* respectively, while the highest concentration (5.0 ppm) used in the present investigation inhibited the growth and cell division of both the test organisms. *S. costatum* was comparatively more sensitive to higher concentrations of IAA than *T. fluviatilis*, indicating their differential auxin requirement for growth.

INTRODUCTION

EFFECT of phytohormones on the growth responses of algae has been studied and reviewed by different authors (Conrad and Saltman, 1962 ; Mowat, 1965 ; Provasoli and Garlucei, 1974 ; Augier, 1974). Existence of auxin like substances was reported in *Fucus* (Du Buy and Olson, 1937), *Bryopsis* and *Macrocystis* (Van Overbeek, 1940 a, b) and in *Ulva pertusa, Undaria pinnatifida* and *Gelidium amansi* (Abe Uchiyama and Sato, 1972). Exogenous application of hormones like indole-acetic acid (IAA) was also made in *Fucus, Ascophyllum* and *Laminaria.*

Several attempts have been made to study the effect of IAA on fresh water algae like *Chlorella vulgaris* (Brannon and Bartsh, 1939), *Rhizoclonium hieroglyphicum* (Davidson, 1952), *Oedogonium cardiacum* (Kim and Greulach, 1961), *Cosmarium subtriordinatum* (Sundaralingam and Govindaraj, 1977), *Oedogonium nanum* (Premila, 1983), *Spirogyra paraguayensis* (Thulasi, 1983) and *S. columbiana* (Raman, 1984). Similar studies documenting the effect of IAA on blue green algae were also made (Adhikary and Patnaik, 1979 ; Anand, 1982). Such studies pertaining to marine macro-algae are much more limited (Williams, 1952 ; Provasoli, 1958 ; Davidson, 1950). Similarly, reports on the effect of growth hormones on marine phytoplankton which form the first link in the marine food chain, are also scanty (Chifang K'uo and Ying Fang Chang, 1960 ; Ramamoorthy and Seshadri, 1966, Bentley and Reid, 1969). Hence the present attempt was made to study the influence of Indole, 3-acetic acid (IAA) on to marine diatoms *viz. Skeletonema costatum* (Greville) Cleve and *Thalassiosira fluviatilis* Hust.

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Material and Methods

Bacterial free cultures of *S. costatum* and *T. fluviatilis* were obtained from the culture collection center, CAS in Botany, Madras and were maintained in Guillard f/2 medium under the following laboratory conditions :

light intensity, 3000 lux from overhead cool white fluorescent tubes,
lighting cycle, 14L : 10D,
temperature, 28 ± 1°C at room temperature,
salinity of the filtered sea water used, 29‰ and
pH of the medium after autoclaving 8 ± 0.1.

IAA (Indole, 3-acetic acid, no. 1-1250 ; Sigma) was dissolved in 2 ml of ethyl alcohol and to avoid the presence of alcohol, it was freeze dried. Subsequent dilution was made using distilled water (Bentley and Reid, 1969). Different concentrations (Table 1) were prepared from this stock solution and added to the medium. Conical flasks (250 ml), each with 100 ml culture medium were inoculated with the exponentially growing algal cells. The initial concentration in each test flask was adjusted to give approximately 1×10^4 cells/ml. Duplicates were run in all experiments and the cultures were incubated over a period of 12 days under conditions mentioned earlier.

At four days interval, samples from each test flask were withdrawn for the determination of cell counts using 0.1 mm deep haemocytometer and mean values were taken. Growth rate (μ) was calculated using the formula provided by Eppley and Strickland (1968). Microscopical examinations of the test algae were also carefully made to record the morphological characteristics.

Results

Table 1 and Figure 1 highlight the effect of IAA on the growth of *S. costatum* and *T. fluviatilis* over a period of 12 days. IAA promoted the growth rate of *S. costatum* at the lower concentrations (0.01 and 0.1 ppm) with the maximum enhancement of growth (0.587 divisions/24 h) at 0.1 ppm. Inhibition of growth was noticed at 1.0 ppm which reduced the growth rate to 0.261 divisions/24 h (Table 1). Striking inhibitory effect on growth rate was evident at 5.0 ppm, in which concentration the number of cells declined to the lowest level.

Table 1. *Effect of IAA on the growth rate (μ) of* S. costatum *and* T. fluviatilis *after 12 days of incubation*

IAA Concentration (ppm)	μ (Divisions/24 h)	
	S. costatum	*T. fluviatilis*
0	0.516	0.541
0.01	0.521	0.546
0.1	0.587	0.571
1.0	0.261	0.628
5.0	..	0.084

In *T. fluviatilis*, growth was promoted at 0.01, 0.1 and 1.0 ppm of IAA. Maximum enhancement of growth was noticed at 1.0 ppm where a greater growth rate of 0.628 divisions/24 h was registered. Growth at the next higher concentration (5.0 ppm) remained hampered throughout the study period (Fig.1b).

Microscopical observations revealed the fact that the different IAA concentrations employed in the present study exerted no appre-

ciable change in the cell dimensions particularly the diameter of the cells of the test algae. But the effect on cell elongation was evident in all the concentrations. *S. costatum* exposed to 0.01 and 0.1 ppm of IAA exhibited healthy elongated cells while the test organism exposed to the next higher concentration (1.0 ppm) showed many dumbel shaped cells and cells with 2-fold elongation. Further, a number of cells in the chain at the intercalary positions were found to be totally bleached. At the highest concentration of IAA (5.0 pp m) used in the present study, chains of *S. costatum* were found either broken into bits or devoid of cell contents.

There were no morphologial abnormalities in the cells of *T. fluviatilis* at 0.01, 0.1 and 1.0 ppm of IAA. At 1.0 ppm, the maximum growth promoting concentration, cells were healthy and the dividing ones had 2-fold increase in length. At the highest concentration (5.0 ppm) employed, cells with 2-5 fold increase in length and non-separation of divided cells were noticed. Even those few apparently normal cells found in this concentration were either invaginated or partially bleached.

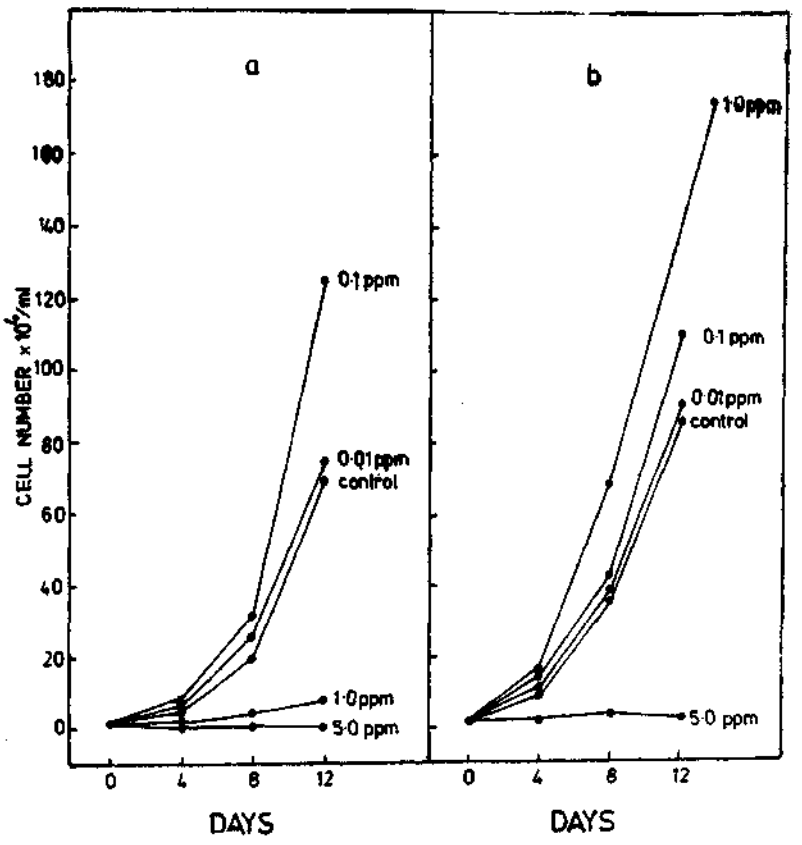

FIG. 1. Effect of IAA on the growth (cell number) of : a. *S. costatum* and b. *T. fluviatilis* over a period of 12 days.

DISCUSSION

Effect of phytohormones on various marine micro-organisms is important from the view point of ecophysiology as the marine environment contains a considerable amount of excreted organic compounds and growth regu-

lators (Fogg, 1966 ; Laren and Peterson, 1967). Previous studies on the effects of such growth regulators predominantly relate to green algae (Brannon and Bartsch, 1939 ; Davidson, 1952 ; Kim and Greulach, 1961 ; Sundaralingam and Govindaraj, 1977 ; Devi-Prasad, 1982 ; Premila, 1983 ; Thulasi, 1983 ; Raman, 1984) and marine macroalgae (Davidson, 1950 ; Williams, 1952; Provasoli, 1958),whereas the present investigation concerns with the effect of IAA on two marine micro-algae, commonly occurring in tropical estuaries. Results of the present study depicted in Table 1 and Fig. 1 showed that the growth rate is promoted at the lower concentrations of IAA and inhibited at the higher concentrations, in *S. costatum* and *T. fluviatilis*. Maximum growth promoting concentrations (0.1 and 1.0 pmm) in the present study showed approximately 2-fold increase in cell census as compared to their respective controls in *S. costatum* (Fig. 1 a) and *T. fluviatilis* (Fig. 1 b). Similarly stimulation of growth at the lower concentrations of IAA was noticed in *Rhizoclonium hieroglyphicum* (Davidson, 1952) and *Westiellopsis prolifica* (Adhikary and Pattnaik, 1978).

In *T. fluviatilis*, while 1.0 ppm of IAA induced the maximum growth, 5.0 ppm reduced it. In *S. costatum*, there was total inhibition of growth at these concentrations. In *Chlorella pyrenoidosa* higher concentration of IAA (10 mg/l) produced a 4-fold increase in dry weight (Brannon and Sell, 1945) but in *C. vulgaris* the same concentration produced no increase in the rate of cell division but only an increase in cell size (Yin, 1937). A still lower concentration of IAA (0.1-1.0 mg/l) showed a moderate increase in cell numbers of *Euglena gracilis* (Elliot, 1939). In *Scenedesmus obliqus*, 0.1 mg/l of IAA exhibited a 3-fold increase in cell number whereas *S. dimorphus*, *S.accumi natus* and *S. quadricauda* were either unaffected or inhibited at this concentration (Algeus, 1946). Such a high degree of variability among the different test organisms in response to IAA could be attributed to the interspecific differences of the test organisms (Conrad and Saltman, 1962 ; Devi-prasad, 1982), difference in the period of incubation and size of the inoculum used. The stimulatory effect of growth at lower concentrations could be ascribed to the hormonal effect of IAA on the process of cell elongation and high frequency of cell division. The inhibitory effect might have resulted due to the effect of higher concentrations of the exogenously applied hormone, on algae with high concentration of (naturally occurring) endogenous auxins (Williams, 1949).

Morphological observations in *S. costatum* and *T. fluviatilis* revealed that growth promoting concentrations in the present study caused signiffcant increase in cell length but not any appreciable change in cell diameter. This is in conformity with the earlier observations made in *Chlorella vulgaris* (Yin, 1937), *Rhizoclonium hieroglyphicum* (Davidson, 1952) *Oedogonium cardiacum* (Kim and Grelach 1961) and *Cosmarium substriordinatum* (Sundaralingam and Govindaraj, 1977). It was also interesting to note in the present investigation that the anomalous cell elongation (observed at the higher concentration of IAA) was always associated with the suppression of cell division. The occurrence of elongated cells with 2-5 fold increase in length over the control cells in *T. fluviatilis* signifies the inhibitory effect of higher concentrations of IAA on cell division, more markedly on cytokinesis. Eventhough very little is known about the mechanism of action of IAA on lower plants, the auxin is known to modify cell wall properties (Evans, 1983), membrane premeability (Sen and Das, 1982) and influence the enzyme protein of plant metabolism (Barendse, 1983) in higher plants. Evidently, totality of such effects might determine the overall physiological responses of all the test organisms including the present ones.

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