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Effect of Aspartic acid and glucose on growth rate and pigmentation of *Spirulina platensis*: An aquatic source of food

Harish Chandra Singh, Samuel G Singh and Dhruv Kumar

Abstract
Sodium salt of 2'4 dichlorophenoxyacetic acid, growth promoter commonly used as a herbicide, has been used in the present investigation to observe its effect on growth and pigmentation of filamentous cyanobacterium *Spirulina platensis. S. platensis* was found to be very sensitive towards 2, 4-D. With increase in concentrations growth and pigmentation decreased gradually and complete growth inhibition was observed at 5 mg/ml-1. Attempt was also made to study the effect of some exogenous substances on the toxicity of 2, 4-D.

Keywords: aspartic acid, growth rate, *Spirulina platensis*, aquatic source, food

Introduction
Spirulina platensis, a paddy soil cyanobacterium having highest protein content has a special place in the algal biotechnology as it offers unlimited potential to use as food and fertilizer.
The use of blue- green algae as bio fertilzer in paddy fields for increasing the crop productivity has been suggested (Venkataraman, 1972) [14]. However the increasing use of herbicides to paddy fields particularly 2, 4-D is likely to create microbial imbalance by affecting their growth and development (Lundkvist, 1970; Das and Singh, 1977; Singh, 1974) [6, 1, 11].
In the present investigation an attempt has been made to study the influence of Glucose and aspartic acid on 2, 4-D toxicity on growth and pigment production in *Spirulina platensis.*

Materials and Methods
Bacteria free cultures of *Spirulina platensis* was maintained in CFTRI medium. Experiments were conducted by inoculation of equal amount of exponentially growing algal material (equivalent to 1 mg dry weight) in to 25 ml solution contained in 100 ml Erlenmeyer flasks. Stock solution of sodium salt of 2, 4- Dichlorophenoxyacetic acid (obtained from E. Merck, Bombay, 98% active ingredient) were added to the desired concentration in a final volume of medium after autoclaving. The pH of the medium was adjusted to 8.0 after sterilization. Experiments were done on the culture room set at 24.6 ºC temperature and 2200 lux light intensity for incubation.
Growth was estimated in dry weight basis (Padhi, 1983) [8] and the specific growth rate constants (K) were plotted from mg dry weight per kratz and Myers (1955) [5]. Chlorophyll-a and carotenoids were extracted with 80% acetone and estimated according to Talling and Drive (1963) [12] and Davies (1976) [2] respectively.

Results
The effect of different concentration of 2, 4-D (0.05-5.0 µg ml^{-1}) on growth and pigmentation of *Spirulina platensis* in presence and absence of glucose and aspartic acid were examined. Growth of *S. platensis* measured in terms of dry weight of algal cultures at different concentration of 2, 4-D showed concentration dependent decrease in total dry weight except 0.05 µg ml^{-1} 2, 4-D which showed comparable increase in dry weight over control (Table-1). Complete growth inhibition was observed at 5.0 µg ml^{-1}. However aspartic acid (25 µg ml^{-1}) and glucose (500 µg ml^{-1}) reversed the toxic effect of 2, 4-D when treated with lethal doese, Aspartic acid exhibited of significant growth enhancement than glucose throughout the period

of investigation. A gradual decrease in the specific growth rate (K) was noticed at higher concentrations. Table 2 shows the effect of aspartic acid (25 µg ml^{-1}) and glucose (500 µgml^{-1}) at stimulatory and lethal concentration of 2, 4-D on chlorophyll-a and carotenoid production of the test organism which indicated that pigment production was in consequence of the growth of the organism.

Discussion

The results indicated that aspartic acid and glucose play a protective role against the toxicity of 2, 4-D on growth and chlorophyll content of *Spirulina platensis.* Differential protective action of glucose and aspartic acid was observed throughout the period of investigation.

Table 1: Effect of Aspartic acid and Glucose on growth rate of *Spirulina platensis* at different concentrations of 2, 4-D. Each value represents the means of three closely concordant determinations.

Treatment	Growth in mg 4^{th} 16^{th}		Specific growth rate x 100 16^{th} 4^{th} 16^{th}	
Control	4.50	38.50	16.3	7.92
2.4-D 0.05 mg ml^{-1}	4.72	40.38	16.84	8.03
2,4-D 0.1 mg ml^{-1}	3.97	34.25	14.96	7.67
2,4-D 0.5 mg ml^{-1}	3.16	28.11	12.49	7.24
2,4-D 1.0 mg ml^{-1}	2.05	17.28	7.79	6.18
2,4-D 0.05 mg ml^{-1}	1.62	-	5.23	-
+Aspartic acid (25 mg ml^{-1})	6.25	46.38	19.89	8.33
2,4-D 0.1 mg ml^{-1}				
+ Glucose (500 mg ml^{-1})	5.70	42.84	18.89	8.15
2,4-D 5.0 mg ml^{-1}				
+ Aspartic acid (25 mg ml^{-1})	2.15	8.24	8.31	4.57
2,4-D 5.0 mg ml^{-1}				
+Glucose (500 mg ml^{-1})	1.90	6.90	6.96	4.21

(-------) not detectable

Table 2: Effect of Aspartic acid and glucose on pigment content of *Spirulina platensis* at different concentrations of 2, 4 -D. Each value represents the mean of three closely concordant determinations.

Treatment	Chlorophyll-a in mg 4^{th} 16^{th}		Carotenoid in 4^{th} 16^{th}	
Control	20.85	192.5	9.0	77.0
2.4-D 0.05 mg ml^{-1}	24.32	201.42	9.44	80.76
2,4-D 0.05 mg ml^{-1}				
+Aspartic acid (25 mg ml^{-1})	31.25	229.32	12.5	92.76
2,4-D 0.05 mg ml^{-1}				
+ Glucose (500 mg ml^{-1})	29.72	218.16	11.4	85.72
2,4-D 5.0 mg ml^{-1}	6.95	-	3.24	-
2,4-D 5.0 mg ml^{-1}				
+Aspartic acid (25 mg ml^{-1})	11.66	48.72	4.3	16.48
2,4-D 5.0 mg ml^{-1}				
+Glucose (500 mg ml^{-1})	9.75	36.34	3.8	13.92

(-------) not detectable

2, 4-D is a synthetic growth hormone and stimulation of growth by its low concentration has been demonstrated (Tiwari and Pandey, 1981) [13]. The stimulation of growth at low concentration (0.05 mg ml^{-1}) may be due to alteration in the membrane permeability leading to increased availability of nutrients. Generallly it is associated with increase in RNA and photosynthetic pigments level (Schuter, 1979) [10].

It is expected that 2, 4-D at higher concentrations may block CO_2 fixation either by reducing photolysis of water or by interferring at the level of electron transport chain (Moreland. 1980) [7].

The physiological damage generated at higher doses of 2,4-D may be partially reduced-price buy glucose or amino acids probably because of their interference at the level of herbicide uptake by cyanobacterial cells through the formation of inactive herbicide complex with these substances which get hydrolysed to other metabolites causing relief from herbicide toxicity as reported in higher plant systems (Klambt, 1961; Feung *et al*., 1973; 1974; Pandey, 1981) [4, 3, 3,13].

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