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Growth Pattern Changes of *Chlorella vulgaris* and *Anabaena doliolum* Due to Toxicity of Dimethoate and Endosulfan

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Effects of pesticides in the ecosystem do not remain restricted to target organisms but rather extend to non-target organisms like microorganisms which play an important role in the food chain vis-a-vis biological processes such as biogeochemical cycling, production, decomposition, interaction with other organisms, etc. Agrochemicals contaminate surface waters of agricultural regions and effectively inhibit growth, pigment biomass and survival rate of freshwater phytoplankton (Lal 1984; Adhikary 1989; Mandal and Mohanty 1990; Netrawali and Gandhi 1990). However, other toxicity method such as effect on growth pattern is useful to achieve standardization. The present paper describes the effects of two pesticides, viz., dimethoate and endosulfan on the growth patterns of Chlorella vulgaris and Anabaena doliolum.

MATERIALS AND METHODS

The axenic cultures of Chlorella vulgaris Beij. and Anabaena doliolum Bhar. of this laboratory were maintained in a liquid and sterilized modified Chu No.10^{+} medium (Safferman and Morris 1964) with A_6 micronutrients of Allen and Arnon (1955) for Anabaena and of Gerloff et al. (1950) for Chlorella. The stock cultures were grown in 250 -mL borosilicate conical flasks containing 100 mL of the culture maintained in a culture room at 27 ± 2°C with light/dark 12/12 hr, photosynthetic photon flux density (PPFD) 70 $\mu mol/m^2$ sec, photosynthetically active radiation (PAR: 400-700 nm), and relative humidity (RH) 75%. The experimental cultures were also grown under the same conditions in 100-mL borosilicate conical flasks containing 25 mL of medium and using exponentially grown cultures (7 d old) as initial inoculum.

An organophosphorus pesticide dimethoate [0, 0-

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dimethyl-S(-N-methyl-carbamoylethyl)-dithiophosphate]and an organochlorine pesticide endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide), made available from Rallis India Ltd., Bombay, were used as the test chemicals. The stock solutions (1 g/L) of these (acetone dissolved) were prepared in the sterilized medium under aseptic conditions for repeated use at predetermined concentrations.

The survival test of both the organisms was conducted in nutrient agar plates with certain varying concentrations of the pesticides. Sample of 0.1 mL of the exponentially grown culture (12 filaments /cm^2 and 15 cells /cm^2 in case of Anabaena and Chlorella, respectively) was spread evenly and aseptically on each plate and incubated in the culture room for 10 d. The survival rate was calculated as described in an earlier report (Mohapatra et al. 1990). The test chemicals were applied just at the beginning of log phase (8 hr and 30 hr after inoculation in case of Anabaena and Chlorella, respectively). The growth patterns were studied incubating the cultures separately with initial cell numbers 1.40 x 10^6 cells/mL (Chlorella) and 0.59 x 10^6 cells/mL (Anabaena), for 16 d and measuring the light transmission of the homogenized cultures at 660 nm every 4 d by a Carl Zeiss SPEKOL spectrophotometer. The transmission values were then converted to $\Delta \log_2$ OD (Sorokin 1975).

RESULTS AND DISCUSSION

None of the pesticides had any inhibitory effect on Chlorella at low concentrations. However, reduction in survival rates of the alga was observed at nominal concentration of 10 mg/L of both pesticides, endosulfan being more toxic than dimethoate. The sublethal doses (LC50) for the alga were 51.0 ± 0.79 mg/L and 41.5 ± 1.39 mg/L of dimethoate and endosulfan, respectively (Table 1). Growth of Chlorella completely ceased on the agar plates at 125 mg/L of dimethoate and at 100 mg/L of endosulfan.

On the other hand, reduction of survivability of Anabaena was found at all the tested concentrations of both the pesticides (Table 2). The LC50s for this cyanobacterium were 28.50 ± 0.61 mg/L and 2.15 ± 0.07 mg/L of dimethoate and endosulfan, respectively. The organism tolerated a maximum concentration of 40 mg/L of dimethoate and 3 mg/L of endosulfan, while all other higher doses were lethal.

In case of Chlorella, dimethoate at a concentration of 1 mg/L did not cause any significant change in the

Table 1. Survival rate of Chlorella vulgaris after 10 d at different nominal concentrations of the pesticides (Cultures in triplicate).

Dimethoate			Endosulfan		
Conc. in mg/L	Survival %	SE	Conc. in mg/L	Survival %	SE
0	100.0	1.80	0	100.0	2.98
1	100.0	1.41	0.1	100.0	2.63
10	93.1	1.48	1	100.0	2.51
25	84.8	3.42	10	84.4	3.08
50	55.4	2.56	30	74.7	1.02
75	19.6	2.44	40	54.6	1.26
100	11.4	1.70	50	31.6	1.20
125	0.0	0.0	75	13.4	0.33
			100	0.0	0.0
LC50 = 51.0±0.79 mg/L (p=0.05)			LC50 = 41.5±1.39 mg/L (p=0.05)		

Table 2. Survival rate of Anabaena doliolum after 10 d at different nominal concentrations of the pesticides (Cultures in triplicate).

Dimethoate			Endosulfan		
Conc. in mg/L	Survival %	SE	Conc. in mg/L	Survival %	SE
0	100.0	1.12	0	100.0	1.12
1	94.6	2.63	0.1	96.4	1.18
10	71.8	1.71	0.5	93.9	0.45
20	62.9	1.92	1.0	92.8	1.41
30	48.5	1.50	1.5	60.9	0.70
40	4.8	0.73	2.0	52.1	0.70
50	0.0	0.0	2.5	5.1	1.42
			3.0	1.0	0.0
			3.5	0.0	0.0
LC50 = 28.5±0.61 mg/L (p=0.05)			LC50 = 2.15±0.07 mg/L(p=0.05)		

SE = Standard error

growth pattern whereas endosulfan, at all the tested concentrations, had some kind of effects on it (Fig. 1 and 2). Dimethoate at 10 and 25 mg/L also did not show any change in the sigmoidal pattern of growth, while at 0.1 and 1.0 mg/L of endosulfan shortening of log phase and change in sigmoidal growth curve were observed after 8 d. Dimethoate at 40 and 50 mg/L caused slight delaying of lag phase and also shortening of log phase, while more irregularities in logistic growth pattern of the alga were seen at other higher concentrations of both the pesticides.

Unlike Chlorella, the growth pattern of Anabaena was affected from initial stage of the culture by the pesticides. Dimethoate, however, did not show any change in the growth pattern at concentrations $\leq$ 10 mg/L (Fig.3). At 20 and 30 mg/L of the pesticide, partial

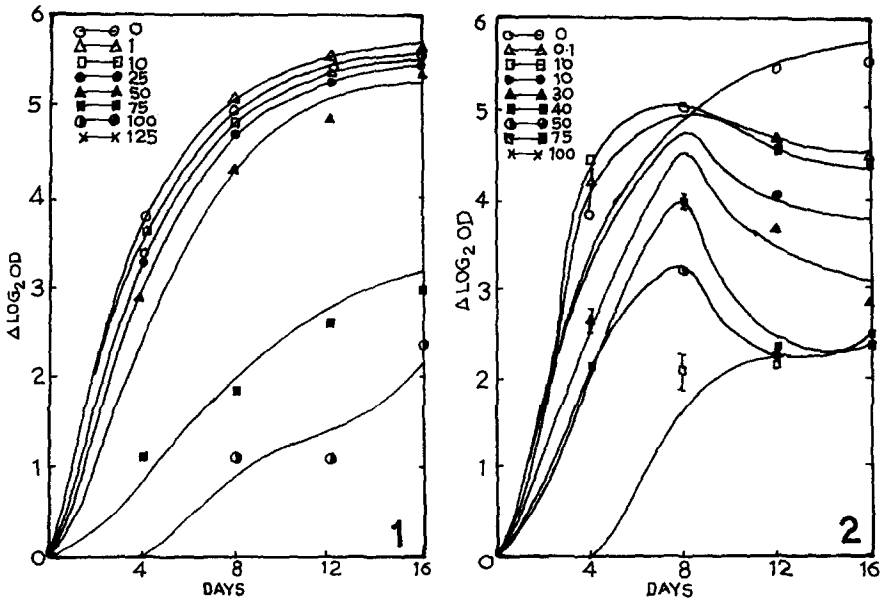

Figure 1. Effect of dimethoate concentrations (mg/L) on growth pattern of C. vulgaris

Figure 2. Effect of endosulfan concentrations (mg/L) on growth pattern of C. vulgaris

growth inhibition during the initial stage (up to 4 d) resulted in a pseudo-lengthening of lag phase. Delayed growth coupled with lengthening of lag phase of the cyanobacterium was found at all other higher doses of the pesticide ($\geqslant$ 40 mg/L).

Endosulfan also at 0.1 and 0.5 mg/L did not show any remarkable change in the growth pattern of Anabaena though reduction in the growth rates was observed at such concentrations (Fig.4). At 1 mg/L of the pesticide, partial lengthening of lag phase resulted due to growth inhibition at initial level of the culture but this concentration did not affect the rest of the logistic phase. However, while the pesticide at 2 mg/L resulted in a totally different growth pattern by affecting both lag and log phases, with application of other higher concentrations ($\geqslant$ 2.5 mg/L), the cyanobacterium was found to be at lag phase for the entire 16 d.

Endosulfan was found to be more effective in reducing the survivability of both the test organisms than dimethoate and this finding supports the view that organochlorines are more effective than organophosphates. Since both the groups of the pesticides have cell destructive action (Antunes-Madeira et al.

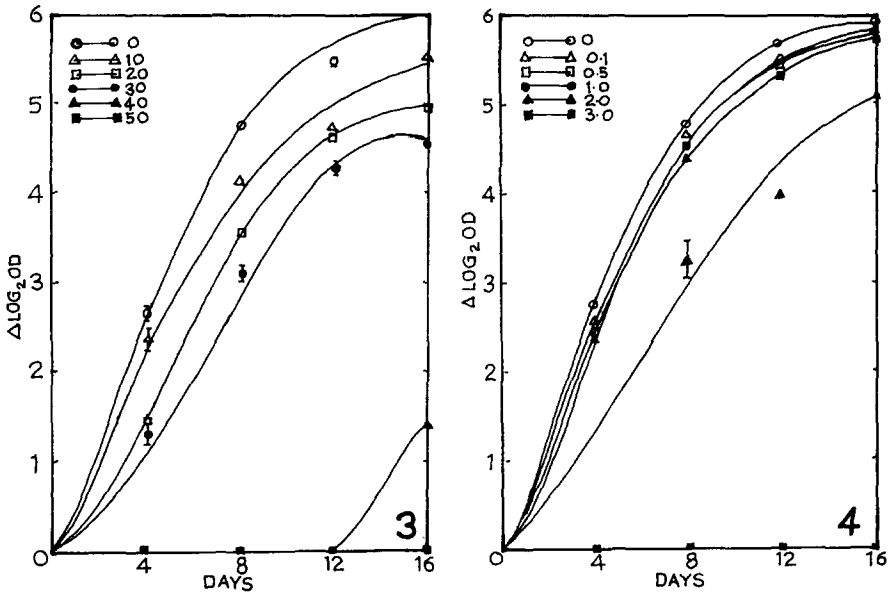

Figure 3. Effect of dimethoate concentrations (mg/L) on growth pattern of A. doliolum

Figure 4. Effect of endosulfan concentrations (mg/L) on growth pattern of A. doliolum

1980; Netrawali and Gandhi 1990), the reduction rate, in the present study, at higher doses might be due to killing of some cells from the beginning.

Four different types of growth patterns, in response to the pesticides, of the test organisms were observed viz. i) partial inhibition and slight change in log phase, ii) delayed inhibition and shortening of log phase, iii) initial growth inhibition with subsequent growth resumption and lengthening of lag phase, and iv) complete inhibition of growth. The delayed inhibition of growth was responsible for the shortening of log phase as noted in case of Chlorella in response to endosulfan (Fig.2). Probably the alga was very efficient in accumulating the organochlorine pesticide as a result of which the concentration inside the cell increased steadily causing delayed effect (Hansen 1980).

On the other hand, in both the test organisms the lengthening of lag phase, due to initial growth inhibition and resumption afterwards, might be because of (i) biodegradation of the pesticide, (ii) autodegradation of the chemicals, (iii) modification by the organisms, and/or (iv) decrease influx of the

chemicals into the cells, caused by cell wall modification (Hansen 1980; Thomas and Shanmugasundaram 1986; Netrawali and Gandhi 1990).

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