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Growth Rate of *Ankistrodesmus falcatus* and *Scenedesmus bijuga* in Mixed Culture Exposed to Monocrotophos

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The organophosphorus insecticides, a very effective and widely used group, have gained the attention of agriculturists all over the world due to their non-persistent nature (La1 1982; Wright 1978). Monocrotophos [Dimethyl (E) -I-methyl-2-(methylcarbamoyl) vinyl phosphate], an organophosphorus compound, is a broad spectrum insecticide and acaricide with excellent activity against target pests. It is registered effective for more than 20 crops in over 50 countries and is extremely well tolerated in all crops. However, besides these desired benefits of controlling target pests, the insecticide may also show side effects on non-target organisms like mammals, birds, soil organisms, beneficial arthropods and aquatic organisms (Guth 1994), which play an important role in the ecosystem and the food chain by way of their involvement in many biological processes. A major portion of non-target organisms is constituted by algal cells (Netrawali and Gandhi 1990). The significance of phytoplankton as primary producers as well as their ability to intrinsically alter the balance of aquatic ecosystem has warranted greater concern due to the toxic effects of widely accepted insecticides.

The effects of monocrotophos and other organophosphorus pesticides on growth, photosynthesis, survival, reproduction, membrane permeability and other metabolic activities of algae were studied by different workers (Maly and Ruber 1983; Megharaj *et al.* 1986). A number of studies on the effects of pesticides and other toxicants in single algal cultures have demonstrated a differential algal sensitivity. A shift in composition with more resistant species replacing sensitive ones has been documented (Maly and Ruber 1983).

In the present study, two different green algae *Scenedesmus bijuga* and *Ankistrodesmus falcatus* have been studied both in axenic and mixed cultures exposed to different concentrations of monocrotophos. Since these two experimental species dominate the local aquatic environment and thereby represent the main primary producer of the ecosystem, it is of interest to know their behaviour in response to environmental pollution.

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MATERIALS AND METHODS

Axenic cultures of *Scenedesmus bijuga* and *Ankistrodesmus falcatus* isolated in this laboratory were maintained in liquid sterilised medium (USEPA, 1985) at pH 7.0. Aliquots of the cultures were transferred weekly to maintain algal growth in log phase. Stock and experimental cultures were grown in 250 mL and 100 mL borosilicate flasks containing 100 mL and 50 mL of medium, respectively. All the cultures were incubated at 27±2° C under an illumination of photosynthetic photon flux density (PPFD) of 70µ mole/m^2 from white fluorescent tubes with 12 hr light and 12 hr dark photoperiod and relative humidity of 75%.

Monocrotophos (36EC), a generous gift of Pesticides India Limited, Udaipur, was used as the test chemical. Single species experiment was carried out to know the change in the total cell number (TCN) and growth rate of both the algae in presence of monocrotophos. Exponentially growing cultures (7d old) containing 150 x 10^2 cells/ml for *Scenedesmus* and 75 x 10^2 cell/mL for *Ankistrodesmus* were used as initial inocula. Pesticide dissolved in acetone was added to the algal cultures just at the end of the lag phase which was 26 hr after inoculation for *Scenedesmus* and 28 hr after inoculation *for Ankistrodesmus* to attain final concentrations of 0 to 300 mg/L and 0 to 50 mg/L, respectively. Multispecies experiment was carried out to compare the change in the growth rate in mixed and axenic cultures for both the algae. The initial inocula for mixed culture experiment consisted of 50 x 10^2 cells/ml of each species. Test chemical was added after 28 hr of inoculation (lag phase for mixed culture) to obtain final concentrations of 0 to 300 mg/L. Culture only with 0.2 % acetone and without pesticide served as the control.

The experimental sets were run in triplicate, and all the cultures were hand shaken twice daily. The cultures were incubated for 12d, and cell counts were made in a hemocytometer (improved Neubauer ruling) on 0, 4, 8 and 12d of pesticide application. Exponential growth rate (R_E) was calculated for the time intervals 0 to 4d, 4 to 8d and 8 to 12d by using the following formula.

$$R_E = \frac{\text{Log}_2 X_2 - \text{Log}_2 X_1}{t_2 - t_1}$$

Where X_1 is the average number of cells (mean) at the beginning (time t_1) and X_2 is the average number of cells (mean) at the end (time t_2) of the interval. The results were statistically tested with least significant difference (LSD) at confidence limit $P \leq 0.05$

RESULTS AND DISCUSSION

In the axenic culture experiments *Scenedesmus* proved to be more resistant than *Ankistrodesmus.* The effects of monocrotophos on TCN of both these species in axenic as well as mixed culture are presented in Table 1 and Table 2, respectively.

Table 1. Effect of monocrotophos on Total cell number (in X x l0² cells/mL of culture) of *Scenedesmus bijuga* and *Ankistrodesmus falcatus* in axenic culture.

Concentration (mg/L)	*Scenedesmus bijuga* 4d	8d	12d	Concentration (mg/L)	*Ankistrodesmus falcatus* 4d	8d	12d
0	2956.0[a] ± 315.0	33118.0[a] ± 1973.0	17125.0[b] ± 481.0	0	450.0[b] ± 32.0	1000.0[b] ± 43.0	2625.0[b] ± 94.0
25	3206.0[a] ± 176.0	19875.0[b] ± 2163.0	19937.0[a] ± 1336.0	10	600.0[a] ± 38.0	1968.0[a] ±77.0	3162.0[a] ± 88.0
50	1381.0[c] ± 139.0	16600.0[b] ± 861.0	10750.0[c] ± 769.0	15	612.0[a] ± 23.0	1956.0[a] ±70.0	3012.0[a] ± 85.0
75	781.0[d] ± 98.0	1900.0[c] ± 129.0	1950.0[d] ± 169.0	20	387.0[c] ±35.0	987.0[b] ± 61.0	2212.0[c] ±82.0
100	500.0[d] ±59.0	1137.0[c] ± 118.0	750.0[d] ±71.0	25	68.0[d] ± 17.0	331.0[c] ± 41.0	300.0[d] ± 22.0
200	412.0[d] ± 83.0	787.0[c] ± 44.0	706.0[d] ± 68.0	30	56.0[d] ± 18.0	112.0[d] ± 23.0	50.0[e] ± 12.0
300	400.0[d] ± 72.0	375.0[c] ± 21.0	212.0[d] ± 39.0	40	43.0[d] ± 12.0	50.0[d] ± 12.0	31.0[e] ± 11.0
				50	37.0[d] ± 12.0	12.0[d] ± 08.0	12.0[c] ± 08.0

Table 2. Effect of monocrotophos on total cell number (in X x 10² cells /mL of culture) of mixed culture of *Scenedesmus* and *Ankistrodesmus.*

Concentration(mg/L)	*Scenedesmus bijuga* 4d	8d	12d	*Ankistrodesmus falcatus* 4d	8d	12d	*Mixed culture* 4d	8d	12d
0	987.5[c] ± 48.1	2231.2[d] ± 44.4	12637.5[b] ± 170.0	437.0[a] ± 37.0	718.7[a] ± 30.5	1456.2[a] ± 68.2	1425.0[b] ± 50.41	2943.7[b] ± 47.9	14093.7[b] ± 194.8
10	1262.0[b] ± 53.0	5675.0[b] ± 119.0	14487.0[a] ± 190.0	381.0[a] ± 34.0	756.0[a] ± 31.0	1506.0[a] ± 47.0	1643.0[b] ± 55.0	6431.0[a] ± 115.0	16087.0[a] ± 231.0
25	2106.0[a] ± 67.0	6262.0[a] ± 128.0	9868.0[c] ± 257.0	243.0[b] ±.24.0	293.7[b] ±.34.7	420.0[c] ± 40.0	2350.0[a] ± 61.0	6493.0[a] ± 114.0	10318.0[c] ± 260.0
50	300.0[d] ± 20.0	2806.0[c] ± 103.0	6156.0[d] ± 122.0	87.0[c] ± 22.0	200.0[b] ± 25.0	600.0[b] ± 35.0	387.0[c] ± 17.0	3006.0[c] ± 28.0	6756.0[d] ± 136.0
75	112.0[e] ± 12.0	468.0[e] ± 28.0	581.0[e] ± 36.0	81.0[c] ± 13.0	112.0[c] ± 22.0	200.0[d] ± 34.0	193.0[c] ± 17.0	606.0[c] ± 28.0	781.0[e] ± 33.0
100	75.0[e] ± 11.0	200.0[f] ± 22.0	362.0[e] ± 27.0	75.0[c] ± 11.0	112.0[c] ± 22.0	150.0[d] ± 30.0	150.0[c] ± 12.0	312.0[c] ± 23.0	512.0[e] ± 28.0
200	62.5[e] ± 12.0	156.0[f] ± 18.0	250.0[e] ± 25.0	62.0[c] ± 12.0	62.0[c] ± 15.0	93.0[d] ± 17.0	125.0[c] ± 11.0	218.0[c] ± 13.0	343.0[e] ± 24.0
300	50.0[e] ± 12.0	97.0[f] ± 17.0	200.0[e] ± 20.0	50.0[c] ± 12.0	43.0[c] ± 12.0	50.0[d] ± 12.0	87.0[c] ± 15.0	137.0[c] ±.15.0	250.0[e] ± 27.0

Note. Values represented by same letters within a column are not significantly different from each other at $P \leq 0.05$.

The data on the 4th d observation show that the TCN of *Scenedesmus* increased significantly (LSD = 4.0959) up to 25 mg/L of the pesticide and decreased later, significantly, with increase in the concentration. On the 8th d the TCN also increased up to 25 mg/L of the pesticide followed by significant reduction beyond this concentration. In a similar experiment, Megharaj *et al.* (1986) reported significant stimulation of growth of *Scenedesmus* up to 50 mg/L monocrotophos, but the stimulatory action was later confined to the lower concentrations like 5 and 10 mg/L. In the present experiment, in case of *Ankistrodesmus* significant increase in the TCN was noticed up to 15 mg/L of monocrotophos. Concentrations > 15 < 40 mg/L of the pesticide proved to be toxic as reduction in the TCN was evident. Concentrations ≥ 40 mg/L were lethal to the alga as there was decrease in the TCN in comparison with initial inoculum. However, in case of *Scenedesmus all* the tested concentrations did not decrease the TCN in comparison with initial inoculum.

In mixed culture, also the TCN increased significantly up to 25 mg/L of monocrotophos and decreased at higher concentrations. It was comparatively less in *Ankistrodesmus* than in *Scenedesmus.* This may be due to the faster growth rate of *Scenedesmus.* In general, the slow growing organisms are more affected by any kind of environmental stress. In this mixed culture experiment the contribution to the TCN is mainly by the fast growing species *Scenedesmus.* In contrast to axenic culture, *Ankistrodesmus* could survive up to 200 mg/L of monocrotophos in mixed culture. It could be due to the presence of *Scenedesmus* which was able to survive even at 300 mg/L of monocrotophos. The ability of the alga to survive in such a high concentration of pesticide may be attributed to the following reasons. Firstly, due to easy metabolism of pesticide by the alga and secondly because of its characteristic tolerance. These are evidenced by the fact that *Ankistrodesmus* could live at 200 mg/L of monocrotophos only in presence of *Scenedesmus* in the mixed culture but not in axenic culture. So it is plausible that *Scenedesmus* produces certain enzymes which facilitate degradation of the pesticide to simpler compounds along with general hydrolytic process. However, we have not identified any such enzymes or degradation product.

P-dimethyl-phosphate, a hydrolytic degradation product of monocrotophos and N-hydroxymethyl and N-dealkylated derivatives were found from cotton plants treated with monocrotophos (Beynon and Wright 1972). The initial metabolism pathways evidenced in the degradation of monocrotophos in plants are (a) cleavage of the O-P-CH_3 linkage (b) hydrolysis of P-O vinyl bond and (c) dehydroxylation of N-methyl group followed by N-dealkylation. Route (a) and (b), which are essentially detoxification reactions, represent the major pathways in most of the crops investigated whereas route (c), leading to potent ChE inhibitors, is a minor metabolic pathway (Donzel 1994). Organophosphorus hydrolase (OPH), a new enzyme isolated from *Pseudomonas diminuata* MG and *Flavobacterium* sp. ATTCC, is a broad spectrum organophosphate anhydrolase (EC 3.1.8.1) which catalyses the hydrolysis of P-F and P-CN bonds (Dave *et al.*

Table 3. Effect of monocrotophos on growth rate (R_E) of *Scenedesmus, Ankistrodesmus* and mixed cultures of both algae.

Organism	Time interval in d	Concentration of monocrotophos in mg/L											
		0	10	15	20	25	30	40	50	75	100	200	300
Ankistrodesmus	0-4	0.646	0.750	0.757	0.592	-0.031	-0.103	-0.194	-0.250	-	-	-	-
axenic culture	4-8	0.288	0.428	0.126	0.337	0.567	0.048	0.048	-0.396	-	-	-	-
	8-12	-0.348	0.170	0.155	0.290	-0.035	-0.169	-0.169	0	-	-	-	-
Ankistrodesmus from	0-4	0.781	0.732	-	-	0.321	-	-	0.159	0.175	0.146	0.080	0.000
mixed culture	4-8	0.179	0.247	-	-	0.067	-	-	0.289	0.117	0.146	0.000	-0.048
	8-12	0.254	0.248	-	-	0.128	-	-	0.396	0.207	0.103	0.146	0.048
Scenedesmus axenic	0-4	1.075	-	-	-	1.104	-	-	0.853	0.595	0.434	0.364	0.353
culture	4-8	0.871	-	-	-	0.658	-	-	0.843	0.320	0.296	0.233	-0.023
	8-12	-0.237	-	-	-	0.001	-	-	-0.156	0.009	-0.150	-0.039	-0.035
Scenedesmus from	0-4	1.075	1.164	-	-	1.349	-	-	0.646	0.292	0.146	0.080	0.000
mixed culture	4-8	0.294	0.542	-	-	0.393	-	-	0.806	0.514	0.353	0.330	0.226
	8-12	0.625	0.248	-	-	0.164	-	-	0.283	0.077	0.214	0.169	0.273
Mixed culture	0-4	0.958	1.009	-	-	1.138	-	-	0.488	0.238	0.146	0.080	-0.048
	4-8	0.261	0.492	-	-	0.366	-	-	0.738	0.411	0.267	0.201	0.163
	8-12	0.568	0.330	-	-	0.167	-	-	0.292	0.091	0.178	0.049	0.215

1993; Serdar *et al.* 1989; Dumas *et al.* 1990; Caldwell *et al.* 1991; Ashani *et al.* 1991). The reaction mechanism of bacterial phosphotriesterase has been examined thoroughly and shown to proceed with inversion of configuration about the phosphorus of the substrate (Lewis *et al.* 1988). It is presently believed that the hydrolysis of phosphotriesters occur via SN_2 mechanism whereby an active site base of the protein abstracts a proton from a water molecule. This "activated" water serves as the nucleophile and directly attacks the electrophilic phosphorus of the substrate in a single in-line displacement reaction (Lewis et al. 1988). No such enzyme has yet been detected in plant or algal system.

Most of the work in mixed culture of algae showed that application of pesticide resulted in elimination of sensitive species. Simetryne application to natural habitat changed the species composition and relative abundance of algae (Kasai *et al.* 1993). Application of diquot changed the algal community by decreasing species diversity without affecting the final biomass (Melendez *et al.* 1993). In the present case, higher concentrations of monocrotophos did not eliminate the population of *Ankistrodesmus* in the mixed culture. Rather, the alga could grow at higher concentrations of monocrotophos which were lethal to it in axenic culture.

The growth rate (R_ε) rose rapidly at 0 to 4d interval and declined quickly at 4 to 8d and 8 to 12d intervals both in control and treated experiments where enhancement of TCN was observed (Table 3). The negative growth rate beyond 4d interval was probably due to nutrient depletion through faster growth as observed by Hersh and Crumpton (1987) in case of green algae treated with atrazine. In axenic culture of *Ankistrodesmus* at concentrations ≥ 25 mg/L of monocrotophos growth rate was negative at 0 to 4d interval which later on became positive at 4 to 8d interval. It could be due to the degradation of the pesticide with increase in incubation time. Growth rate of *Scenedesmus* in axenic culture was always high at 0 to 4d interval and decreased in subsequent intervals at all the tested concentrations. However, during 8 to 12d interval at concentrations ≥ 50 mg/L of monocrotophos, it attended a negative value. In contrast, in the mixed culture the growth rate of *Scenedesmus* was always positive. However, at higher concentrations (≥ 50 mg/L) of monocrotophos the growth rate at 4 to 8d interval was higher than earlier which clearly indicates the extension of lag phase of the alga by the pesticide. Growth rate of the mixed culture was like that of *Scenedesmus* being highest at 0 to 4d interval and lowest at 8 to 12d interval up to 25 mg/L of monocrotophos. At 4 to 8d interval it was more than that of the earlier. Higher growth rate during 0 to 4d interval at lower concentrations might be due to the utilisation of phosphorus present in the pesticide.

It seems in view of algae not occuring in isolation in nature, mixed species culture is more meaningful than axenic culture. Moreover, experiment comprising of algae of related forms is interesting in order to know, under the stress condition, the differential sensitivity and tolerance. Besides, the interspecific competition along with relative abundance can also be known in the changed environmental condition.

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