EFFECT OF PESTICIDES ON THE GROWTH, PHOTOSYNTHETIC OXYGEN EVOLUTION AND NITROGEN FIXATION OF *WESTIELLOPSIS PROLIFICA*

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We examined the effect of the pesticides Furadan (Carbofuran, 3%), Sevin (Carbaryl, 50%), Rogor (Dimetnoate, 30% EC) and Endotaf (Endosulfan, 35% EC) on the survival, growth, photosynthetic oxygen evolution and nitrogen fixation of the cyanobacterium *Westiellopsis prolifica*. Lower concentrations of the carbamate and organophosphate pesticides (10 ppm) increased the growth and nitrogen fixation while higher concentrations (> 20 ppm) had an inhibitory effect. The organochlorine pesticide Endotaf was toxic even at 10 ppm. Survivability and nitrogen fixation of the cyanobacterium was reduced to 72 and 93% respectively in the presence of 100 ppm of Endotaf. An exposure of the filaments to over 100 ppm of Furadan, Sevin or Rogor caused about a 40% inhibition of the photosynthetic oxygen evolution. Endotaf was more toxic than the other pesticides. Oxygen evolution was totally suppressed after incubation of the organism with over 250 ppm of Endotaf; there was similar suppression of O₂ evolution on treatment with more than 500, 1,000, and 1,500 ppm of Rogor, Sevin and Furadan respectively.

Various cyanobacterial species occur abundantly in rice-fields of tropical and sub-tropical countries, whose agricultural technologies involve extensive use of pesticides for selective elimination of pests of rice crops (1, 3, 8, 14). Therefore, the indiscriminate use of pesticides may be causing adverse effects on these rice-field nitrogen-fixing cyanobacteria which have a direct influence on the total productivity. A number of pesticides have been tested for their toxicity in microbiological systems (6, 9, 12, 13). Four commonly used pesticides—Furadan (Carbofuran, 3%), Sevin (Carbaryl, 50%), Rogor (Dimetnoate, 30% EC) and Endotaf (Endosulfan, 35%

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EC), applied extensively to control rice pests of this region, have been tested in the present work to determine their effect on the survivability, growth, photosynthetic oxygen evolution and nitrogen fixation of a locally isolated rice-field cyanobacterium *Westiellopsis prolifica* Janet.

MATERIALS AND METHODS

A pure culture of the rice-field cyanobacterium *Westiellopsis prolifica* Janet was used as the experimental organism. The cyanobacterium was grown in Allen and Arnon's nitrogen free medium (2) with micronutrients as used by Fogg (5). The cultures were maintained at $28 \pm 2^\circ\text{C}$ under 2,400 lx provided by daylight fluorescent tubes. Four commercial grade pesticides: two carbamate pesticides, Furadan and Sevin (Union Carbide Limited, Bangalore, India) and one each of organophosphate and organochlorine, Rogor and Endotaf respectively (Rallis India Limited, Bombay, India) were used in this investigation. Fresh stock solutions of these pesticides were prepared for experiments in sterilized media and added aseptically to the culture media to obtain the desired concentration. The pH of all of the test media was adjusted to 7.8. Experiments were conducted by inoculating actively growing organisms into 25 ml of culture medium in 100-ml cotton stoppered conical flasks. Growth was estimated on a dry weight basis by the procedure followed earlier (11). Growth was also measured in terms of the absorbancy of acetone extracts in a Systronics Spectrocolorimeter at 660 nm. Total nitrogen fixed by the cyanobacterium in the presence of different concentrations of the pesticides was estimated on the 15th day of incubation. The cultures were subjected to Kjeldahl digestion and total nitrogen was estimated according to Friedrich as modified by Herbert et al. (7). Corrections were made for the nitrogen present in the pesticides.

The survivability of the organism was studied on agar plates containing varying concentrations (10 to 1,500 ppm) of the pesticides. Samples of 1.0 ml of a diluted suspension of the cyanobacterium were spread aseptically onto each of the agar plates and incubated under light in the culture room. After 10 days of incubation, the colonies were counted under a binocular microscope and the survival data were plotted taking survival on the control plates as 100%. The rate of O_2 evolution was measured with a Clark type oxygen electrode (Yellow Spring, Ohio, U.S.A.) and a Hitachi 056 recorder. Cyanobacterial filaments containing approximately 2.0 to $3.0 \mu\text{g}$ of chlorophyll *a* from the 72-h-old experimental cultures, containing various concentrations of the pesticides, were transferred to a periglass vessel fitted with an outer jacket for water circulation and temperature maintenance (25°C) during the experiment. To avoid reduction at the cathode and to maintain a stable oxygen gradient across the membrane, the cyanobacterial suspension was stirred with a magnetic stirrer. Saturating light intensity of $200 \text{ W} \cdot \text{m}^{-2}$ was provided by a 150 W halogen lamp through a heat cut filter (70%). The signal from the cathode was fed through a pre-amplifier to increase the efficiency of recording. Chlorophyll *a* was

extracted from the cyanobacterial filaments with 80% acetone and the amount was determined spectrophotometrically using the absorption coefficient of Mackinney (10).

RESULTS AND DISCUSSION

The effect of four different pesticides on the survival, growth and nitrogen fixation of *Westiellopsis prolifica* was studied in experiments with 10, 20, 50, 100, 250, 500, 1,000, and 1,500 ppm of each pesticide in the culture solution. The results, Tables 1 and 2, indicate a progressive decrease in the growth and nitrogen fixation with increasing concentrations of the pesticides. The carbamate pesticides Furadan and Sevin were less toxic than organophosphate, Rogor or the organochlorine Endotaf. The presence of 1,000 ppm of Furadan and Sevin in the medium were algistatic, but that concentration of Rogor and a still lower concentration of Endotaf were algicidal (Fig. 1). With Rogor the growth remained almost unaffected at 10 ppm, but at the same concentrations, the carbamate insecticides actually stimulated the growth. Nitrogen fixation was also increased at these lower doses. Similar enhancement of growth and nitrogen fixation in various other cyanobacterial species in the presence of Furadan (8) and 2,4-D (4) have been reported. At concentrations above 20 ppm of Furadan, Sevin and Rogor, growth and nitrogen fixation progressively declined (Tables 1 and 2). Endotaf was toxic even at 10 ppm; at more than 250 ppm the growth and nitrogen fixation stopped completely.

The effect of pesticides on photosynthetic oxygen evolution is shown in Table 3. Incubation of *W. prolifica* for a period of 72 h in the presence of varying concentrations of pesticides and determination of the rate of photosynthesis thereafter (in terms of $\mu\text{mol O}_{2\text{ evolved}} \cdot \text{mg chlorophyll } a^{-1} \cdot \text{h}^{-1}$) showed that this process was inhibited by all the tested pesticides. Exposure of the filaments to Sevin at a dose of 10, 50, 100, 250, 500, and 1,000 ppm inhibited photosynthetic oxygen evolution 5.3, 25.8, 41.9, 61.0, 88.0, and 100% respectively. The commercial grade Furadan was less toxic to the organism than Sevin (Table 3). Oxygen evolution was completely suppressed on exposure to over 500 ppm of Rogor. The toxic effect of Endotaf on the photosynthetic oxygen evolution was more pronounced. Even at only 10 ppm, the oxygen evolution rate was reduced by 22.5% and it was completely suppressed at more than 250 ppm (Table 3). These results accord with the changes in the morphology of *W. prolifica* filaments exposed to pesticides. In the presence of more than 250 ppm of Furadan, Sevin and Rogor and 100 ppm of Endotaf, the erect branches of the filaments were suppressed and the number of heterocysts per filament decreased even to a larger extent. This shows that higher concentrations of pesticides decrease the survivability, growth and nitrogen fixation of rice-field nitrogen-fixing cyanobacteria. The toxic dose of organophosphate and carbamate pesticides in these experiments was > 10 ppm, so the lower concentrations may not affect the growth and nitrogen fixation of cyanobacteria under field conditions.

Table 1. Effect of different concentrations of pesticides on the growth of *Westiellopsis prolifica*.

Concentration of pesticide (ppm)/25 ml culture	Furadan		Sevin		Rogor		Endotaf	
	Dry wt. (mg)	O.D. of acetone-dissolved pigments (660 nm)	Dry wt. (mg)	O.D. of acetone-dissolved pigments (660 nm)	Dry wt. (mg)	O.D. of acetone-dissolved pigments (660 nm)	Dry wt. (mg)	O.D. of acetone-dissolved pigments (660 nm)
Control (without pesticide)	28.5	0.34	28.5	0.34	28.5	0.34	28.5	0.34
10	30.0	0.36	30.2	0.35	28.9	0.34	26.0	0.3
20	32.8	0.34	26.9	0.3	25.4	0.25	20.2	0.21
50	27.2	0.28	22.8	0.26	18.6	0.18	14.5	0.09
100	22.4	0.23	16.2	0.2	12.8	0.11	6.9	0.03
250	15.7	0.18	12.5	0.15	9.2	0.04	2.8	0.01
500	6.4	0.08	5.6	0.06	3.8	0.015	1.0	0
1,000	1.8	0	1.0	0	0.8	0	0.6	0

Harvested after 15 days of incubation.

Dry weight of the initial inoculum = 1.0 mg.

The O.D. (optical density) of acetone-dissolved pigments at 660 nm of the initial inoculum = 0.02.

Table 2. Effect of different concentrations of pesticides on the total nitrogen fixed (mg/25 ml culture) by *Westiellopsis prolifica* harvested after 15 days of incubation.

Concentration of pesticide (ppm)	Furadan	Sevin	Rogor	Endotaf
0 (control)	3.6	3.6	3.6	3.6
10	3.8 (+ 5.5)	3.5 (+ 2.7)	3.8 (+ 5.5)	1.7 (− 52.7)
20	3.4 (− 5.5)	3.2 (− 11.1)	2.7 (− 25.0)	1.1 (− 69.4)
50	2.8 (− 22.2)	2.2 (− 38.8)	2.0 (− 44.4)	0.83 (− 79.6)
100	2.0 (− 44.4)	1.5 (− 58.3)	0.9 (− 74.7)	0.25 (− 93.0)
250	1.6 (− 55.5)	0.96 (− 73.3)	0.33 (− 90.8)	0
500	0.83 (− 76.9)	0.45 (− 87.5)	0.1 (− 97.2)	0
1,000	0	0	0	0

Figures in parenthesis show the percentage increase or decrease relative to the nitrogen fixation in the control.

+, percentage increase; −, percentage decrease.

Fig. 1. Percentage survival of *Westiellopsis prolifica* in the presence of different concentrations of pesticides in the medium. Cultures were incubated at $28 \pm 2^\circ\text{C}$ under 2,400 lx light intensity for 15 days.

But at higher concentrations (> 50 ppm) the nitrogen fixing ability of cyanobacteria decreased considerably, so excessive use of these pesticides may well affect the overall nitrogen economy of rice-field soils. The organochlorine pesticides are especially more toxic; at > 10 ppm they affected *W. prolifica* adversely, so they may also adversely affect the survivability of a variety of microorganisms in nature. Therefore, caution should be taken to determine the appropriate application dosage of these environmental pollutants before applying them in rice-fields and other aquatic ecosystems.

Table 3. Effect of different concentrations of pesticides on photosynthesis in *Westiellopsis prolifica*.

Concentration of pesticide (ppm)	$\mu\text{mol of O}_2 \text{ evolved} \cdot \text{mg chl. a}^{-1} \cdot \text{h}^{-1}$			
	Furadan	Sevin	Rogor	Endotaf
0 (control)	186	186	186	186
10	182 (2.15)	176 (5.3)	170 (8.6)	144 (22.5)
50	154 (17.2)	138 (25.8)	124 (33.3)	98.5 (47.0)
100	112 (39.7)	108 (41.9)	96.4 (48.1)	33.2 (82.1)
250	76.6 (58.8)	72.4 (61.0)	48.3 (74.0)	12.2 (93.4)
500	44.1 (76.2)	22.2 (88.0)	7.4 (96.1)	0 (100)

The organism was incubated in the presence of pesticides for a period of 72 h; then the rate of photosynthesis was determined.

Figures in parenthesis show the percentage inhibition of photosynthetic oxygen evolution in the presence of pesticides.

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