EFFECTS OF HERBICIDES ON THE GROWTH OF MARINE PHYTOPLANKTON

by

M. ÜNSAL

Middle East Technical University
Institute of Marine Sciences
P.O. Box-28, 3731 Erdemli-Içel, Turkey

A B S T R A C T

The effect of two herbicides; 2,4-D and Trifluralin on the growth of two marine phytoplankton species, Phaeodactylum tricornatum and Skeletonema costatum has been studied. The growth of P. tricornatum was stimulated by 1 and 5 ppm of the herbicides while it was inhibited at higher test concentrations. During the 9 day recovery period, the division rate of P. tricornatum decreased at 1 and 5 ppm of 2,4-D and Trifluralin as did the control while it increased at the other test concentrations even at 30 ppm.

The effect of 2,4-D on the growth of S. costatum was the same as that observed for P. tricornatum. However, the effect of Trifluralin was completely different: this herbicide inhibited the growth of S. costatum at all the concentrations (0.05-1.0 ppm) tested.

1. INTRODUCTION

Pesticide use has increased greatly in recent years and there has long been concern that these compounds could have wider environmental effects.

Among the pesticides, herbicides have been used widely in agriculture and have entered the marine environment either directly or indirectly, where they may affect non-target organisms.

The annual usage of herbicides in the Cukurova region (south of Turkey) ranged between 300 to 400 tons (Ünsal, in press). Of these herbicides, Trifluralin was most commonly used in cotton fields and 2,4-D in cereal fields in order to control unwanted vegetation (Özgür, Personal communication).

Although, there have been many investigations (Wurster, 1968; Fisher, 1974; Powers et al., 1977; Ünsal and Kideyş, 1987) of the toxic effects of various insecticides on the growth of phytoplankton, only a few studies have been conducted on the effects of herbicides on these unicellular organisms which form the foundation of the marine food web (Sullivan et al., 1981; Wong and Chang, 1988). Therefore, two herbicides, 2,4-D and Trifluralin which have been widely used in the Cukurova region, were chosen as the test material for this study.

The aim of this study was to evaluate the impact of these herbicides on two marine phytoplankton species; Phaeodactylum tricornatum and Skeletonema costatum.

2. MATERIAL AND METHODS

Two marine phytoplankton species, Phaeodactylum tricornatum and Skeletonema costatum, which were obtained from the axenic stock cultures in our laboratory, were used in this study. The culture conditions have been described in a previous study (Ünsal and Kideyş, 1987).

Trifluralin and 2,4-D were purchased in emulsifiable concentrated forms from commercial sources in Içel (Turkey). The manufacturer's description of Trifluralin is "Trifilin E.C.", active ingredient 480 g l^{-1} Trifluralin. The proper chemical name is α,α,α- trifloro-2,6 dinitro-N, N-dipropyl-P-toluidine. The manufacturer's name of 2,4-D was "Weed Killer D", active ingredient 500 g l^{-1} dimethyl amine salt of 2,4 dichlorophenoxyacetic acid.

The working solutions of herbicides were prepared by dilution with sterile distilled water. Different concentrations (1, 5, 10, 15, 20, 25, and 30 ppm) of Trifluralin and 2,4-D were added to 250 ml Erlenmayer flasks containing 50 ml of a culture medium of P. tricornatum enriched with Provasoli nutrient medium (Provasoli et al., 1957). Based on the results of preliminary tests, S. costatum was exposed to the same concentrations of 2,4-D but only to 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm of Trifluralin. Three replicates were used at each test concentration and for each control. Experiments were repeated three times. After 96 h of treatment the phytoplankton was fixed with formalin and counted under the microscope. The results were analysed statistically by Student Newman-Keuls Test (Reish and Oshida, 1986). After the toxicity test, experiments continued for 9 additional days in fresh test medium without the toxicant, in order to determine the recovery of the contaminated cells according to the method described in UNEP/FAO/IAEA (1989).

The growth rate (k) of the phytoplankton, during the recovery period, in the test medium and in the control was calculated by the following equation:

$$k = \frac{\log_2 \frac{N_2}{N_1}}{t}$$

where N_1 and N_2 are the numbers of cells at the beginning and at the end of the recovery period and t is its duration (9 days).

3. RESULTS AND DISCUSSION

Two marine phytoplankton species, Phaeodactylum tricornatum and Skeletonema costatum were exposed to different concentrations of Trifluralin and 2,4-D. The mean reduction in cell numbers showed that the effects of the herbicides tested were species dependent (Tables 1 and 2).

Table 1

The mean cell number ± S.D. of Phaeodactylum tricornatum per ml of test medium after 96 h.

Test Conc. (ppm)	2,4-D				Trifluralin			
	n	Mean	SD	Mean % change comp. W/Cont	n	Mean	SD	Mean % change comp. W/Cont
Contr.	9	224400	89837	-	9	187000	40454	-
1	9	359040	56473	+60.0	9	325795	67631	+74.2
5	9	316653	7785	+41.0	9	203206	21266	+ 8.7
10	9	144613	60112	-35.5	9	128712	33660	-31.2
15	9	73553	21914	-67.2	9	69813	13485	-62.6
20	9	33660	7480	-85.0	9	36539	2159	-80.5
25	9	26180	3740	-88.3	9	12051	720	-93.5
30	9	22440	7480	-90.0	9	11220	5713	-94.0

Table 2

The mean cell number ± S.D. of Skeletonema costatum per ml of test medium after 96 h.

Test Conc. (ppm)	2,4-D				Test Conc. (ppm)	Trifluralin			
	n	Mean	SD	Mean % change comp. W/cont.		n	Mean	SD	Mean % change comp. W/Cont
Contr.	9	683173	96813	-	Contr.	9	264605	4675	-
1	9	1176853	259986	+72.3	0.05	9	84441	11220	-68.1
5	9	1259133	136360	+84.3	0.1	9	49770	23874	-81.2
10	9	1011046	132879	+48.0	0.2	9	31582	6150	-88.1
15	9	285487	54498	-43.7	0.4	9	24795	2505	-90.6
20	9	259307	43347	-55.2	0.6	9	21193	3740	-91.9
25	9	98487	24902	-74.3	0.8	9	19946	2150	-92.9
30	9	69813	7785	-92.0	1.0	9	18700	2493	-92.9

The growth of P. tricornatum was stimulated by 1 and 5 ppm of 2,4-D and Trifluralin (Table 1). This stimulation was statistically significant ($P < 0.05$) at the 1 ppm level but insignificant at the 5 ppm level (Tables 3 and 4). Boyle (1980) showed that the photosynthetic uptake of ^{14}C by natural phytoplankton was stimulated by treatment with 2 mg l^{-1} 2,4-D DMA. Poorman (1973, cf. in Boyle, 1980) reported enhancement of algal growth at 1, 5 and 10 mg l^{-1} 2,4-D during 24 h by comparison with a control culture.

Table 3

Comparison of test concentrations with control by Student Newman-Keuls Test (at $P < 0.05$). (Pollutant: 2,4-D; Species: P. tricornatum).

Comparison	Q	P	$Q^{0.05,16p}$	Conclusion
Control- 1 ppm	5.273	2	3.00	Different
Control- 5 ppm	3.624	3	3.65	Not different
Control- 10 ppm	3.140	4	4.05	Not different
Control- 15 ppm	5.927	5	4.34	Different
Control- 20 ppm	7.484	6	4.56	Different
Control- 25 ppm	7.785	7	4.74	Different
Control- 30 ppm	7.942	8	4.90	Different

Table 4

Comparison of test concentrations with control by Student Newman-Keuls Test (at $P < 0.05$). (Pollutant: Trifluralin; Species: P. tricornatum).

Comparison	Q	P	$Q^{0.05,16p}$	Conclusion
Control- 1 ppm	7.574	2	3.00	Different
Control- 5 ppm	0.894	3	3.65	Not different
Control- 10 ppm	3.886	4	4.05	Not different
Control- 15 ppm	6.332	5	4.34	Different
Control- 20 ppm	8.249	6	4.56	Different
Control- 25 ppm	9.600	7	4.74	Different
Control- 30 ppm	9.308	8	4.90	Different

The other test concentrations (10, 15, 20, 25 and 30 ppm) inhibited the growth of P. tricornatum (Fig. 1). This inhibition was not significant at the 10 ppm level of both pollutants but significant ($P < 0.05$) at 15, 20, 25 and 30 ppm levels (Tables 3 and 4). Wong and Chang (1988) found also that algal cultures (Chlamydomonas reinhardtii) treated with 1 ppm of 2,4-D showed increases in their chlorophyll-α contents over that of the control. However, 10 ppm 2,4-D did not affect algal photosynthesis though at high concentrations (20 and 40 ppm) it inhibited growth, chlorophyll-α synthesis and photosynthesis of the algal cells.

The response of S. costatum to herbicides was different from that of P. tricornatum; 2,4-D had a stimulating effect on this species at 1, 5 and 10 ppm while it inhibited growth at higher concentrations (15, 20, 25 and 30 ppm) (Fig. 2).

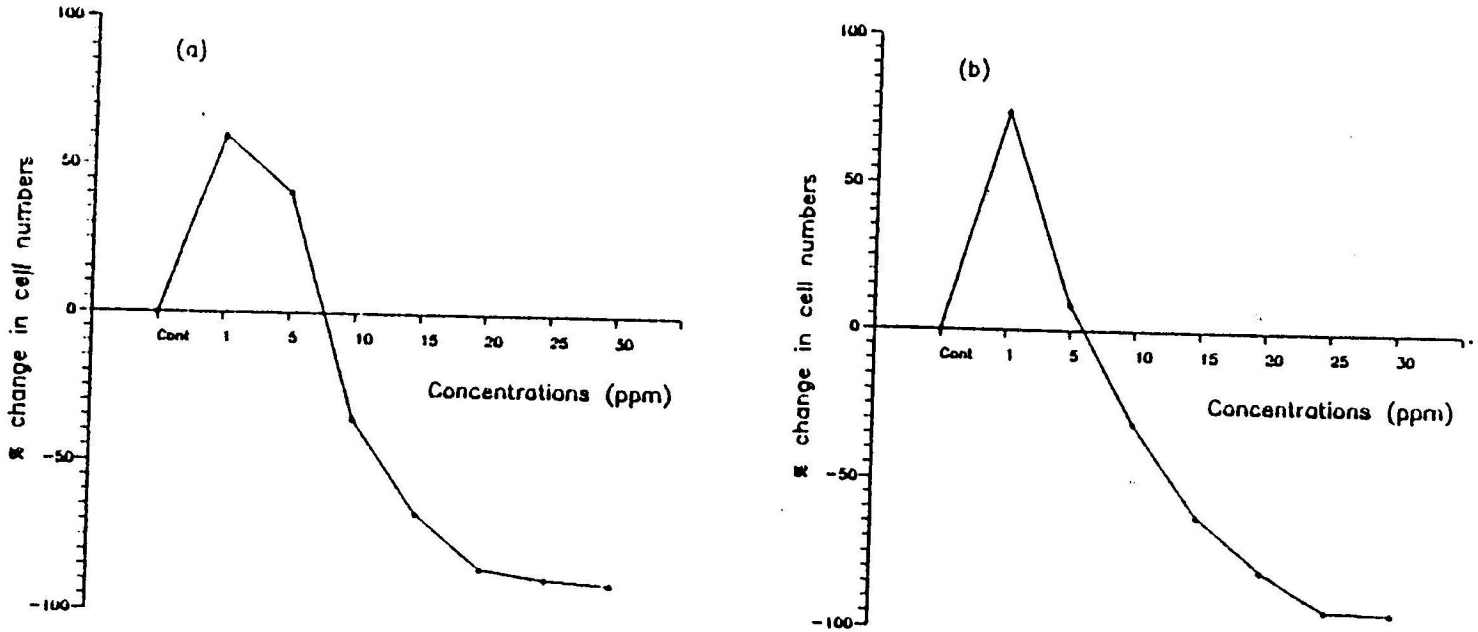

Fig. 1 Mean percent change, compared to the control, of the cell numbers of Paeodactilum tricornatum exposed to 2,4-D (a) and Trifluralin (b) for 96 h

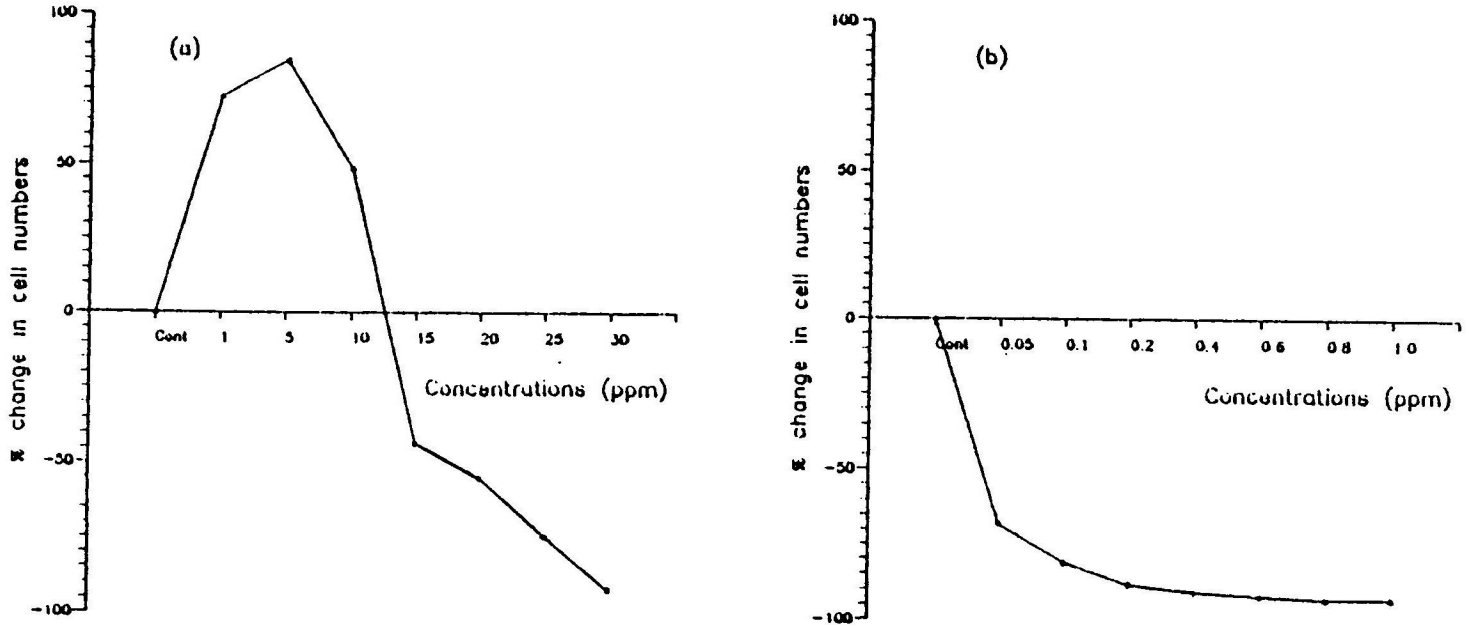

Fig. 2 Mean percent change, compared to the control, of the cell numbers of Skeletonema costatum exposed to 2,4-D (a) and Trifluralin (b) for 96 h

A contrasting result was obtained for S. costatum grown in Trifluralin-containing medium; this herbicide was much more toxic to S. costatum than it was to P. tricornatum. For example, the lowest test concentration of Trifluralin (1 ppm) had a stimulating effect on the growth of P. tricornatum while it completely inhibited the growth of S. costatum (Table 2).

The results of Student Newman-Keuls test obtained for S. costatum showed that, all test concentrations gave results which were significantly different from those of the control (Tables 5 and 6). This difference was due to the stimulating effect of 2,4-D at 1, 5 and 10 ppm and to its inhibiting effect at other test concentrations.

In the case of Trifluralin, the difference between control and test concentrations resulted from the inhibitory effect of this pollutant at all test concentrations.

Table 5

Comparison of test concentrations with control by Student Newman-Keuls Test (at $P < 0.05$).
(Pollutant: 2,4-D; Species: S. costatum).

Comparison	Q	P	$Q^{0.05,16p}$	Conclusion
Control- 1 ppm	7.017	2	3.00	Different
Control- 5 ppm	8.187	3	3.65	Different
Control- 10 ppm	4.619	4	4.05	Different
Control- 15 ppm	5.652	5	4.34	Different
Control- 20 ppm	6.022	6	4.56	Different
Control- 25 ppm	8.310	7	4.74	Different
Control- 30 ppm	8.718	8	4.90	Different

Table 6

Comparison of test concentrations with control by Student Newman-Keuls Test (at $P < 0.05$).
(Pollutant: Trifluralin; Species: S. costatum).

Comparison	Q	P	$Q^{0.05,16p}$	Conclusion
Control- 0.05ppm	31.32	2	3.00	Different
Control- 0.1 ppm	37.41	3	3.65	Different
Control- 0.2 ppm	40.55	4	4.05	Different
Control- 0.4 ppm	41.71	5	4.34	Different
Control- 0.6 ppm	42.34	6	4.56	Different
Control- 0.8 ppm	42.52	7	4.90	Different
Control- 1.0 ppm	42.75	8	4.90	Different

After 96 h of exposure to 1 and 5 ppm of 2,4-D and Trifluralin, the specific growth rate (division per day) of P. tricornatum was higher than the control but growth decreased markedly in the presence of 10 to 30 ppm of 2,4-D and Trifluralin (Table 7).

The growth rates of S. costatum showed also the stimulating and inhibiting effect of 2,4-D on this species. As it can be seen from Table 7, the division rate of S. costatum exposed to different concentrations of 2,4-D for 96 h was higher at all test concentrations than that of P. tricornatum exposed also to the same concentrations of the same pollutant.

Table 7

Growth rate (division per day) of P. tricornatum and S. costatum grown in different concentrations of 2,4-D and Trifluralin.

Species	Pollutants	Concen. (ppm)	96 h	9 day
P. tricornatum	2,4-D	Control	1.12	1.05
		1	1.29	1.07
		5	1.24	1.06
		10	0.96	1.05
		15	0.71	0.99
		20	0.43	0.91
		25	0.35	0.86
		30	0.29	0.80
	Trifluralin	Control	1.05	0.79
		1	1.25	0.84
		5	1.08	0.96
		10	0.92	0.97
		15	0.70	0.97
		20	0.46	0.93
		25	0.07	0.76
		30	0.04	0.75
S. costatum	2,4-D	Control	1.52	
		1	1.72	
		5	1.74	
		10	1.66	
		15	1.21	
		20	1.17	
		25	0.83	
		30	0.70	
	Trifluralin	Control	1.18	
		0.05	0.76	
		0.1	0.58	
		0.2	0.41	
		0.4	0.32	
		0.6	0.27	
		0.8	0.24	
		1.0	0.22	

During the recovery period (9 days), the division rate decreased at the two lowest concentrations (1 and 5 ppm) of 2,4-D and Trifluralin as it did in the controls; it increased considerably in the presence of the higher concentrations (10, 15, 20, 25 and 30 ppm) (Table 7). This may be due to the fact that, the herbicides had accumulated in the phytoplankton cells at high concentrations during the 96 h. experiments and had a stimulating effect throughout the recovery period.

Trifluralin also stimulated the growth of P. tricornatum at low concentrations, but higher concentrations inhibited growth. This herbicide inhibited the growth of S. costatum at very low concentrations.

In conclusion, the effects of the two herbicides tested were species dependent. 2,4-D had a stimulating effect at low concentrations but it inhibited the growth of both phytoplankton species at high concentrations.

The effect of Trifluralin on P. tricornatum was similar to that observed for 2,4-D. However, it was an inhibitant for S. costatum at all test concentrations.

More experiments should be conducted including the investigation of more phytoplankton species in order to be able to evaluate the effects of herbicides on marine phytoplankton.

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