Effects of Pesticides on Growth and Survival of *Euglena gracilis Z*

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Introduction

Contamination of the environment with pesticides has caused much concern recently. This contamination has been documented by a number of investigators and has been concerned primarily with the chlorinated insecticides (1, 2, 6).

Only recently has attention been given to the interaction of pesticides, especially the organophosphates and fungicides, with photosynthetic microorganisms (3, 4). These organisms are basic building blocks in the food webb and are important in producing oxygen through photosynthesis. Pesticides which affect the growth and survival of photosynthetic organisms could affect the whole ecosystem.

The effects of six pesticides on growth and survival of Euglena gracilis Z are reported here.

Methods and Materials

The test organism was Euglena gracilis Z strain (Indiana Culture Collection). The organism was grown in the Euglena medium, pH 5.5 (5) under constant illumination from fluorescent lamps with constant aggitation at 22°C. The intensity of illumination was 200 ft-C at the surface of the flasks. Cells were harvested and washed with fresh medium by centrifugation and used as an inoculum for experiments.

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concentrations did not protect cells from inactivation. Iron or copper solutions alone did not affect the survival of Euglena. Other divalent ions were not tested for their protective effect.

TABLE 1

Effect of Pesticides on Growth of Euglena sp.

	Concentration-ppm	% Inhibition	
		Light[a]	Dark[b]
Malathion	7.25	48.9	19.2
	1.45	0	5.2
	0.30	3.8	21.1
	0.15	7.1	+11.4
Parathion	1.20	14.1	+22.1
	0.24	+ 9.4[c]	+44.8
	0.05	+ 1.2	+ 8.3
	0.01	13.8	+ 7.8
Nabam	10.0	<99.9	<99.9
	5.0	97.9	26.9
	1.0	39.0	19.8
	0.1	33.7	11.5
	0.05	32.0	2.1
Vapam	10.0	95.0	86.5
	5.0	99.6	84.4
	1.0	88.7	+54.6
	0.1	+ 9.4	24.6

a As % of growth in control after 5 days which contained 6.03×10^5 cells/ml.

b As % of growth in control after 9 days which contained 4.80×10^5 cells/ml.

c Stimulation of growth.

For the growth experiments, pesticides were added to the culture medium as aqueous solutions and thoroughly mixed before addition of the cells. Cell numbers were determined after the indicated times with a Coulter Counter Model B. Pesticide solutions were made freshly and filter sterilized before each experiment. Conditions of growth were the same as above. Heterotrophic growth was under the same conditions except flasks were wrapped with aluminum foil to exclude light.

Survival of cells following exposure to pesticides was determined by plating cells on the growth medium solidified with 1.0% agar, wrapping the plates in plastic film, and incubating in the light for 14 days.

Results and Discussion

Vapam appeared to be the most inhibitory for photosynthetic and heterotrophic growth of Euglena. Only at the lower concentrations did any apparent stimulation of growth occur, and this was not consistent. At 5 and 10 ppm, almost complete inhibition of growth occurred (Table 1).

Nabam was toxic at all concentrations tested. Even at the lowest concentration, photosynthetic growth was reduced 1/3 compared with the control. Cells growing in the light with nabam appeared yellow in color for the first three days of growth. Disappearance of nabam from the culture solution could account for the normal greening of the cells during the last two days of growth. Attempts to detect nabam in cultures or stock solutions after 24 hours failed, since this compound hydrolyzed rapidly in aqueous solutions. Malathion produced inhibition only at the highest concentrations while parathion was generally non-inhibitory.

Cells at different densities exposed to parathion and nabam (Table 2) were killed after 1 and 2 hours. Nabam appeared to be the more toxic compound regardless of cell density. Compared with other fungicides (Table 3), nabam appeared to be the most toxic for Euglena. A suggested mode of action of nabam is chelation of divalent metal ions. Addition of $FeCl_2$ or $CuSO_4$ to cultures of Euglena or to nabam in equimolar

TABLE 2

Effects of Various Pesticides on the Survival of Euglena gracilis Z strain

Pesticide and Concentration	Exposure Time: 1 Hour — % Survivors	Exposure Time: 2 Hours — % Survivors
Experiment 1		
None (Control)	100[a]	100
Nabam - 10 ppm	46.20	15.52
Parathion - 10 ppm	28.62	8.96
Experiment 2		
None (Control)	100[b]	100
Nabam - 10 ppm	77.55	20.41
Parathion - 10 ppm	68.36	70.91

a As % of control which contained 2.9×10^4 cells/ml.

b As % of control which contained 9.8×10^4 cells/ml.

TABLE 3

Effects of Various Pesticides on Survival of Euglena gracilis Z

Pesticide and Concentration	Exposure Time: 1 Hour — % Survivors[a]	Exposure Time: 2 Hours — % Survivors[b]
Control	100	100
Acetone Control	93.55	104.35
Maneb (1.25 ppm)[c]	83.87	52.17
Zineb (1.25 ppm)[c]	145.16	95.65
Thiram (1.25 ppm)	77.42	108.70

a As % of control which contained 1.55×10^5 cells/ml.

b As % of control which contained 1.15×10^5 cells/ml.

c Added as acetone solutions (0.1%) final concentration.

The effects of parathion, Vapam, and nabam on respiration of Euglena was examined in the presence of 0.01 M glucose. All three pesticides, at 10 ppm, did not affect respiration over a period of 80 minutes. The mode of action of these compounds on Euglena gracilis still remains to be determined.

Summary

Vapam and nabam were more toxic to cultures of Euglena gracilis Z than malathion and parathion. Growth and survival of cells were affected by these compounds. Divalent metal ions did not offer protection against the killing action of nabam.

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