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THE INFLUENCE OF THE HERBICIDE PARAQUAT "GRAMAXON" ON GROWTH AND METABOLIC ACTIVITY OF THE CHLOROPHYTES SCENEDESMUS DIMORPHUS, SCENEDESMUS QUADRICAUDA AND ANKISTRODESMUS FALCATUS.

EZZAT A. IBRAHIM

National Institute of Oceanography and Fisheries.
Laboratory of Aquatic Plants, El-Zamalek Fish Garden,
Cairo, Egypt.

ABSTRACT

The acute toxicity of the commercial herbicide paraquat was determined by 96-h static bioassay on the freshwater chlorophytes Scenedesmus dimorphus (Trup.) Kuetz., Scenedesmus quadricauda (Trup.) de Brebisson and Ankistrodesmus falcatus (Cord) Ralfs. The 96-h EC50 values of paraquat for reducing growth and metabolic products of the three algae were determined. In addition, the number of days required for division of control and all test cultures were calculated and these were seen to increase with increasing paraquat concentration. The three algae and their test parmaters respond differently to Paraquat. Scenedesmus dimorphus was the most susceptible alga and the chlorophll a was the most sensitive reponse parameter. On the other hand, the dry weight of the test algae was the most resistant parameter. It was observed that paraquat has a dangerous inhibitory effect on the primary producers.

INTRODUCTION

Paraquat (methyl viologen), 1,1-dimethyl-4,4-dipyridylium dichloride, is widely used herbicide effective against broad leaf weeds and grasses. In recent years, there has been an increase in the use of bipyridilium compounds, Paraquat and diaquat, for controlling aquatic weeds. The application of Paraquat to a fishing reservoir was successful in controlling the growth of macrophytic algae (Brook and Edwards, 1973).

The effects of Paraquat on growth and survival of aquatic organisms have been investigated (Benijts-Claus and Persoone 1975; Hendrich et al., 1976; Rao et al., 1980; Walsh 1972; Kapur and Yadav 1982; and Naqvi et al., 1981)

According to the available data, the toxicity of Paraquat and Diaquat is relatively low to man and to some fish species. Our knowledge, on the contrary, of the influence of these chemicals on the lowest levels of the aquatic food chain is extremely scarce.

In Egypt, Paraquat is widely used for controlling a variety of weeds associated with cotton, fruit crops, tomatoes and turf grasses. Recently, a trial will be made to use Paraquat in freshwater fish farms for controlling the macrophytes Potamogeton pectinatus, P. crispus and Ceratophyllum demersum.

The present study, however, was initiated in order to determine the effect of this herbicide on the freshwater green algae Scenedesmus dimorphus, Scenedesmus quadricauda and Ankistrodesmus falcatus representatives of the primary producers. The toxicity test experiments are urgently needed if we want to use Paraquat in safe concentrations which do not interfere with the more sensitive links of the aquatic ecosystem.

MATERIALS AND METHODS

Paraquat is dipyridylium derivative. It is a quaternary ammonium salt (double positive cation with chloride as anion). It is soluble in water and the commercial name is Gramaxon. The sample used in this study contains 40% paraquat and 10% of a mixture of the two detergents, Lissapol NX and DS 4392 or Ethomene S 25. The exact percentage of each of the detergent is a fabricate secret.

The chlorophytes Scenedesmus dimorphus, Scenedesmus quadricauda and Ankistrodesmus falcatus were isolated from the freshwater Lake Wadi El-Rayan.

The medium for stock algal cultures and test with Paraquat was prepared by dissolving the following major salts in 1l glass distilled water: 35 mg $MgSO_4$, 31 mg $NaNO_3$, 18.5 mg $CaCl_2$, 31 mg K_2HPO_4, 10.5 mg $NaHCO_3$, and 56 ug Fe^{2+} as $FeSO_4$. To these major elements, 1 ml of the following mixture of trace elements was added: 59 mg $CuSO_4$, 43 mg $ZnSO_4$, 13 mg KI, 15 mg KBr, 18.4 mg $NaMoO_4$, 40 mg $MnSO_4$, 56 mg $CoCl_2$, 91 mg $Na_2B_4O_7$ and 16.7 mg Na_2WO_4. All were dissolved in 1l glass distilled water. The medium was sterilized by autoclaving for 15 min. at 1.5 kg/cm. The medium was prepared on the day before the test and began to allow the pH to stabilize at 8.1.

The bioassay was performed in 250 ml Erlenmeyer flasks containing 100 ml of the medium and sealed with cotton plugs. Treatment and control flasks were inoculated with 5000 algal cells ml^{-1} in logarithmic growth phase. The experiments were carried out at 25 ± 1^{o} C and 4000 lux from overhead "cool white" flourescent tubes, in cycles of 14 h light followed by 10 h darkness. The cultures were shaken once a day.

At the end of 96 h testing period the flasks were removed from the growth chamber, the cultures were thoroughly mixed to insure homogeneity for an accurate count, and the cells

were counted using brightline hemocytometer counting chamber. Replicate counts were made for each flask to insure counting accuracy. The dry weight of the control and treated cultures was measured gravimetrically after filtration onto millipore filter paper 0.45 um pore diameter (Ibrahim 1984).

Chlorophyll a & b and carotenoids were extracted with 90% acetone according to the method of Golterman and Clymo (1971). The chlorophyll a was calculated according to Lorenzen's equation (1967), while chlorophyll b and total carotenoids were calculated according to Parson's and Strickland equations (1963).

Cellular carbohydrate was extracted according to Myklestad and Haug method (1972). The total amount of carbohydrate in the extract was determined by the phenol sulphuric acid method using glucose as standard (Dubois et al., 1956).

Algal nitrogen was determined by the micro-Kjeldahl method (Hiller et al., 1948). Protein-N was calculated by multipling the value of total nitrogen by 6.25.

To construct dose response curves and calculate the Paraquat EC50 values for reducing growth, dryweight, chlorophyll a & b, carotenoids, carbohydrate and protein contents of the test chlorophytes, the pooled data were treated according to the statistical method of probit analysis (Bliss, 1952; Finney 1964a & 1964b). In addition to the EC50 values, the growth rate (k) and the number of days per division (Td) were calculated for each test concentration (Guillard, 1973 and Honig et al., 1980).

RESULTS

The regression lines representing the dose responses of Scenedesmus dimorphus, Scenedesmus quadricauda and Ankistrodesmus falcatus to the selected Paraquat concentrations are presented in Fig. 1 and the data from which these curves were drawn are given in Tables 1, 2 and 3. In addition Table 1 presents also the calculated days per division of the algae under the various test conditions. Since the percentage of control growth diminishes for each algal species as concentrations of paraquat increases, the respective Td values increase in a coinciding pattern. It can be observed that under their respective optimal control conditions S. dimorphus with Td value of 0.2 is a faster-growing alga than either S. quadricauda 0.62 and A. falcatus 0.53. The calculated EC50 values for reducing the growth indicate that S. dimorphus at 39.8 ppb (ug l^{-1}) is the most sensitive of the three algal species and A. falcatus with a value of 93.3 ppb being the least sensitive. On the other hand the dry weight gained by each of the test algal cells was less affected by Paraquat. The EC50 values of Paraquat for reducing the dry weight in ppb were: 73.9 for S. dimorphus; 132 for S. quadricauda and 114.2 for A. falcatus.

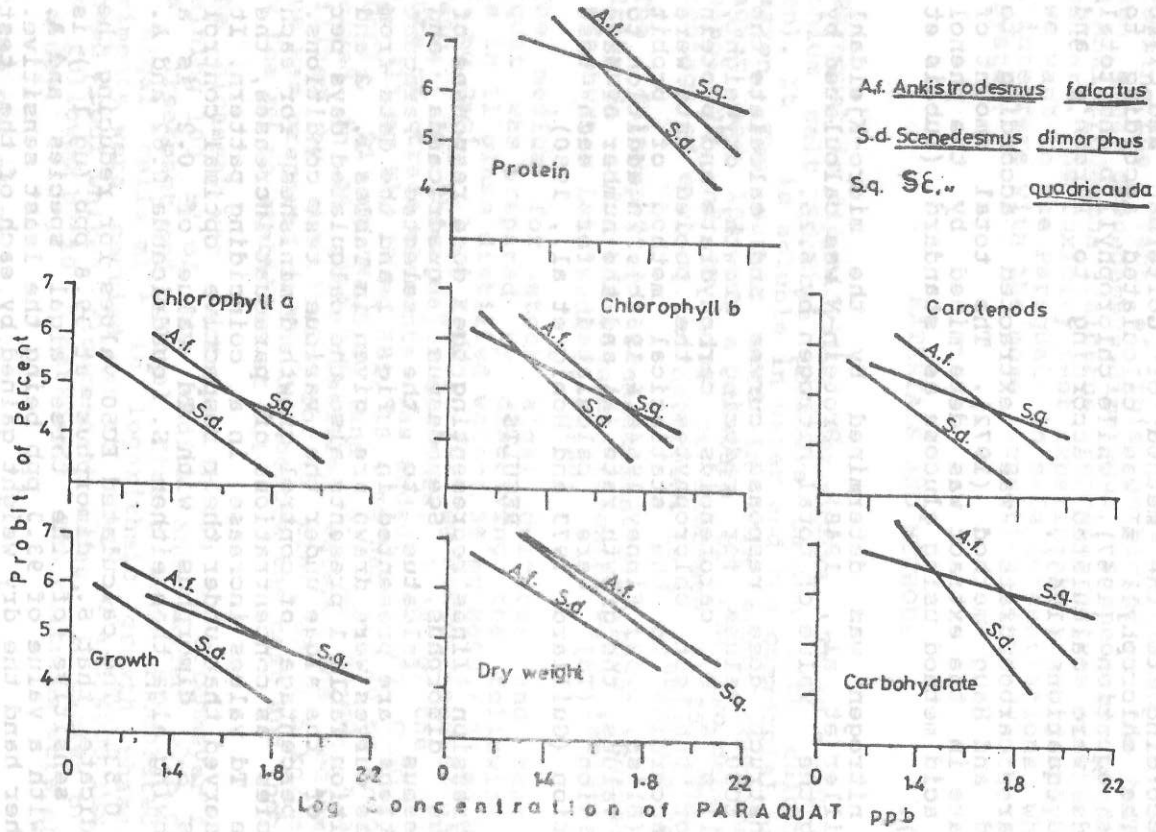

Fig. 1
The regression lines of the responses of the test Parameters to the selected doses of Paraquat.

Table 1

Effect of Paraquat on growth of the three test chlorophytes

Test algae	Log dose (ppb)	Average count No.x 10^4/ml	% of control	Emperical probit	Td
S. dimorphus	Control	170±2.94	----	----	0.20
	1.3	132.2±82.9	82.9	5.9502	0.5
	1.6	81.77±1.71	48.1	4.9529	0.55
	1.8	52.5±1.2	30.9	4.5013	0.6
	1.9	26.33±0.68	15.5	3.9848	0.71
	2	16.43±0.36	9.7	3.7012	0.81

b=-3.18, a=10.09, Y=-3.18x + 10.09 Log EC50=1.6,
EC50=39.8 ppb

S. quadricauda	Control	44.4±1.3	----	----	0.62
	1.5	34.53±1.2	77.2	5.7655	0.66
	1.8	26.1±0.9	58.8	5.2224	0.71
	1.95	20.45±0.4	46.1	4.9021	0.76
	2.08	16.96±0.30	38.2	4.6998	0.80
	2.18	13.72±0.3	30.9	4.5013	0.85

b=-1.86, a=8.56, Y=-1.86x + 8.56, Log EC50=1.91
EC50= 81.6 ppb

A. falcatus	Control	98.2±2.3	----	----	0.53
	1.5	85.93±2.0	87.5	6.1503	0.54
	1.8	64.5±1.4	65.7	5.4043	0.58
	1.95	50.08±1.6	51	5.0251	0.61
	2.08	39.1±1.2	39.8	4.7415	0.64
	2.18	30.34±0.50	30.9	4.5013	0.68

b=-2.43, a=9.78, Y=-2,43x + 9.78, Log EC50=1.97
EC50= 93.3 ppb

Table 2

Estimation of the EC50 for Paraquat from the percent response of Chlorophyll a & b and carotenoid contents of the three chlorophytes after 96 h exposure, with respective regression equations

Algal species	Log dose (ppb)	Chl. a ug l^{-1}	% of control	Probit of percent	Chl. b Ug l^{-1}	% of control	Probit of percent	Carotenoids ug l^{-1}	% of control	Probit percent
S. dimorphus	Control	590±4.2	----	----	172±2.6	----	----	282±3.4	----	----
	1.3	418±3.8	70.9	5.5505	163±1.8	94.6	6.6072	214±2.2	75.9	5.7031
	1.6	182±2.2	30.9	4.5013	97±1.4	56.4	5.1611	96±1.6	34	4.5875
	1.8	86±1.2	14.6	3.9463	36±0.8	20.9	4.1901	37±0.8	13.1	3.8783
	1.9	40±0.8	6.8	3.5091	15±0.4	8.9	3.6405	19±0.6	6.7	3.5015
	2.0	22±0.3	3.7	3.2134	8±0.2	4.7	3.3253	9±0.2	3.2	3.1478
		a=9.86,b=-3.33, Log EC50=1.46, EC50=29ppb Y=-3.33X+9.68			a=12.8,b=-4.78,Log EC50=1.63 Y=-4.78X +12.81 EC50=43 ppb			a=10.44,b=-3.65,Log EC50=1.49 Y=-3.65X +10.44, EC50=31 ppb		
S. quadricauda	Control	197±3.6	----	----	61 ±1.4	----	----	104 ±3.4	----	----
	1.5	135±2.4	68.6	5.4845	45.3±0.8	74.2	5.6495	68.7±2.3	66.1	5.4152
	1.8	86±1.2	43.7	4.8414	30 ±0.6	49.2	4.9799	49 ±1.4	47.1	4.9272
	1.95	67±1.4	34	4.5875	20 ±0.4	32.8	4.5546	35 ±0.8	33.7	4.5793
	2.08	48±0.6	24.4±	4.3065	15 ±0.4	24.6	4.3129	28 ±0.6	27.6	4.4082
	2.18	38±0.4	19.3	4.1331	11 ±0.2	18	4.0846	24 ±0.4	22.9	4.2579
		a=8.45, b=-1.99, Log EC50=1.74, EC50=54.5 Y=-1.99X +8*45 EC50=54.5 ppb			a=9.13,b=-2.32,Log EC50=1.78 Y=-2.32X +9.13, EC50=60.2ppb			a=8.02, b=-1.73, Log EC50=1.74 Y=-1.73X + 8.02, EC50=54.9 ppb		
A. falcatus	Control	512±5.2	----	----	125±2.6	----	----	248±4.4	----	----
	1.5	443±3.8	86.5	6.1031	114±1.8	91.2	6.3532	219±3.6	88.5	6.2004
	1.8	230±2.4	44.9	4.8718	66±2.2	52.8	5.0702	130±2.8	52.4	5.0602
	1.95	198±1.8	28.7	4.4378	37±1.4	29	4.4524	81±2.4	32.7	4.5518
	2.08	70±2.2	13.6	3.9015	18±0.6	14	3.9197	49±1.6	19.8	4.1512
	2.18	38±1.2	7.4	3.5534	10±0.4	8	3.5949	22±1.8	8.9	3.6531
		a=11.66, b=-3.72, Log EC50=1.79 Y=-3.72X + 11.66. EC50=61.3 ppb			a=12.5,b=-4.1,Log EC50=1.82 Y=-4.1X + 12.5, EC50=66.6ppb			a=11.68,b=-3.66,Log EC50=1.83 Y=-3.66X +11.68, EC50=67 ppb		

Table 3

Estimation of the EC50 for Paraquat from the percent response of dry weight, carbohydrate and protein contents of the test algal cells, with respective regression equations.

Algal species	Log dose ug l^{-1}	Dry weight mg l^{-1}	% of control	Probit of percent	Carbohydrate mg l^{-1}	% of control	Probit percent	Protein mg l^{-1}	% of control	Probit of percent
S. dimorphus	Control	50.2±1.4	----	----	7.6±0.4	----	----	28.0±2.1	----	----
	1.3	48.4±2.2	96.4	6.7991	7.5±0.6	98.2	7.1015	27.2±1.2	97.2	6.9110
	1.6	40.3±2.6	80.3	5.8524	4.6±0.4	60.5	5.2663	18.4±1.4	65.6	5.4016
	1.8	31 ±2.4	61.8	5.3002	1.4±0.2	18.4	4.0998	7.4±0.6	26.4	4.3689
	1.9	22.3±1.8	44.4	4.8592	0.5±0.04	5.9	3.4368	3.1±0.6	11.1	3.7241
	2.0	16.5±1.8	32.9	4.5570	0.1±0.02	1.4	2.8027	1.4±0.2	5	3.3551
	a=10.97,b=-3.19,Log EC50=1.87 Y=-3.19X + 10.97,EC50=73.9 ppb				a=15.06,b=-6.11,Log EC50=1.65 Y=-6.11X + 15.06, EC50=44.2 ppb			a=13.64,b=-5.17,Log EC50=1.67 Y=-5.17X+13.64, EC50=47 ppb		
quadricauda	Contro	42.6±1.4	----	----	8.5±0.4	----	----	26.9±2.2	----	----
	1.5	42.5±1.6	99.7	7.7065	6.25±0.3	73.5	5.6280	18.8±1.4	75.5	5.6903
	1.8	39 ±0.8	91.5	6.3722	5.3±0.4	62.1	5.3081	15.7±1.4	63	5.3310
	1.95	32 ±1.2	75.1	5.6776	4.14±0.08	48.7	4.9674	12 ±1.6	48.2	4.9549
	2.08	24 ±0.6	56.3	5.1586	3.74±0.4	44	4.8490	10.5±0.8	42	4.7981
	2.18	17.9±0.4	42.1	4.8007	3.26±0.06	38.3	4.7024	9.5±0.6	38.3	4.7024
	a=14.2,b=-4.32,Log EC50=2.12 Y=-4.32X + 14.2,EC50=132 ppb				a=7.74,b=-1.39,Log EC50=1.97 Y=-1.39X + 7.74,EC50=92.7 ppb			a=8, b=-1.53, Log EC50=1.97 Y=-1.53X + 8, EC50=92.2 ppb		
A. falcatus	Contro	27.2±1.6	----	----	6.5±0.4	----	----	15.1±1.4	----	----
	1.5	25 ±1.4	91.9	6.3984	6.2±0.6	95	6.7060	14.3±1.2	94.6	6.6072
	1.8	20.2±0.8	74.3	5.6526	4.0±0.7	61.8	5.3002	9.6±0.8	63.7	5.3505
	1.95	16.9±1.2	62	5.3055	2.29±0.2	35.2	4.6201	6 ±0.4	39.7	4.7389
	2.08	13.1±0.6	48	4.9498	1.22±0.08	18.8	4.1147	2.6±0.3	17.2	4.0537
	2.18	10 ±0.8	36.8	4.6628	0.44±0.04	6.8	3.5091	1.2±0.4	7.9	3.5882
	a=10.2,b=-2.53,Log EC50=2.06 Y=-2.53X + 10.2,EC50=114.2 ppb				a=13.62,b=4.61,Log EC50=1.87 Y=-4.61X + 13.62,EC50=74 ppb			a=13.3, b=-4.43, Log EC50=1.87 Y=-4.43X + 13.3, EC50=74.5 ppb		

Chlorophyll a & b and total carotenoids of the test algae progressively decreased with increasing Paraquat concentrations. The EC50 values of Paraquat for reducing chlorophyll a & b and carotenoids in ppb were: 29, 43, and 31 for S. dimorphus; 54.5, 60.2 and 54.9 for S. quadricauda; and 61.3, 66.6 and 67 for A. falcatus.

Carbohydrate and protein contents of each of the three test algal cells were more or less similar in their response to Paraquat. Their respective EC50 values in ppb were: 44.2 and 47 for S. dimorphus; 92.7 and 92.2 for S. quadricauda; and 74 and 74.5 for A. falcatus.

DISCUSSION

Previous studies on the effects of Paraquat have concentrated on its effects on growth of cultures of the primary producers. Whereas the present study provides a precise information not only on its effect on growth but also on the metabolic products of the three chlorophytes.

The results revealed the obvious inhibitory effect of Paraquat on growth and metabolic activity of the three test algal. The EC50 values of Paraquat for reducing growth of the chlorophytes indicate that S. dimorphus at 39.8 ppb was more susceptible than both S. quadricauda 81.6 ppb and A. falcatus 93.3 ppb. With EC50 for reducing growth of s. dimorphus taken as one, the relative tolerance of S. quadricauda 2.05 and A. falcatus 2.34 times as more tolerant as S. dimorphus. This agrees with the work of Thomas et al. (1973) who observed the remarkable decrease in growth of Chlorella pyrenoidosa, Chlorella vulgaris and Bacillus sp. when treated with Paraquat. Moreover, Hendrich et al. (1976) reported that Paraquat reduced cell size and caused morphological changes of Scenedesmus quadricauda cells. On the other hand, Benijts-Claus and Persoone (1975) reported that the cladocerun, as representative to the primary consumers was more tolerant to Paraquat as compared with the primary producers, while benthic meiofauna were the most sensitive.

The inhibitory effect of Paraquat on the dry weight of the test algae was mainly attributed to its depilatory effect on population growth that reduced the number of cells of the treated cultures as compared with the control. The dry weight, with EC50 values of 73.9 ppb for S. dimorphus, 132 ppb for S. quadricauda and 114.2 ppb for A. falcatus was more resistant to Paraquat than the growth.

Paraquat had the same sequence of inhibitory effect on chlorophyll a & b, carotenoids, carbohydrate and protein contents of the test chlorophytes as on growth and dry weight. The EC50 values indicated that chlorophyll a was the most sensitive response parameter whereas dry weight was the least sensitive.

The present results lead to the conclusion that the three chlorophytes varied greatly in their responses to Paraquat, S. dimorphus was more susceptible as compared with S. quadricauda and A. falcatus. These variations were also observed between the test parameters of the same species. This confirms the previous work of Naqvi et al (1981) and Ibrahim (1983) who reported that phytoplankton organisms and their metabolic products respond differently to the same insecticide.

Since the effective dose of Paraquat for controlling macrophytes is 1 mg l^{-1} (1 ppm), the application of this herbicide will cause a severe inhibitory effect on the primary producers.

The present study provided a predictive statement to the toxicity of paraquat based on its effects on unialgal cultures of the three chlorophytes under laboratory conditions. The precise and actual predictive statements should be made bearing on the problem of community response to Paraquat. This approach will be applied in future toxicological studies.

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