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EFFECT OF BAYLUSCIDE ON GROWTH AND METABOLIC ACTIVITIES OF SOME FRESHWATER PHYTOPLANKTERS

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ABSTRACT

The present study entails effect of the commonly used molluscicide Bayluscide on growth and biochemical constituents of the freshwater chlorophytes **Scenedesmus dimorphus** (Trup.) Kuetzing, **Scenedesmus quadricauda** (Trup.) de Brebisson and **Ankistrodesmus falcatus** (Corda) Ralfs. Although the three test algae are phylogenitically close, they varied greatly in their responses to Bayluscide. The EC_{50} values of Bayluscide for inhibition the growth were: 270, 355 and 250 ppb for **S. dimorphus**; 175, 125 and 168 ppb for **S. quadricauda**; and 176, 190 and 165 ppb for **A. falcatus** after 48, 72 and 96 h exposure, respectively. The chlorophyll a, b, carotenoid, carbohydrate and protein contents of **S. quadricauda** and **A. falcatus** slightly increased when treated with low doses of Bayluscide; a phenomenon that was reversed at high doses. The growth and chemical constituents of **S. dimorphus** cells decreased with increasing Bayluscide concentrations.

INTRODUCTION

Bayluscide registered trade mark (Bayer, 73) proved to be more effective per unit concentration against harmful aquatic snail **Biomophlaria alexandrina.** This snail is the intermediate host in which the parasites of **Schistosomes** pass through certain stages in their life cycle and finally causes the most serious disease in our country **Schistosomiasis** or **Bilharziasis.**

Bayluscide acts as inhibitor to the citric acid cycle in the snail **Biomophalaria alexandrina** (Ishak et al., 1970). The effect of Bayluscide on growth and metabolism of some freshwater and marine algae has been reported by Ibrahim (1978 and 1983). He found that low concentrations of Bayluscide stimulated the growth and metabolism of some blue-greens

and marine diatoms while the high doses caused severe drop in growth and metabolic activity of the studied algae. The toxic action of Bayluscide on the egg, juvenile and adult stages of **Tilapia leucostrica** and **Herotilapia multispinosa** has been investigated by Robert (1979).

Since Bayluscide is widely used in our country, the determination of its effect on the aquatic organisms is of great importance. The present study, however, was initiated in order to determine the effect of this molluscicide on some phytoplankters, which represent the vital link in the aquatic food chain.

MATERIALS AND METHODS

The freshwater chlorophytes **S. dimorphus, S. quadricauda** and **A. falcatus** were isolated from Lake Wadi El-Rayan. The steps of isolation and purification recommended by Pringsheim (1946) were followed to obtain unialgal cultures.

Artificial medium for stock algal cultures and tests with Bayluscide was prepared by dissolving the following major salts in 1 liter galss distilled water: 467 mg Na NO_3, 18.5 mg Ca Cl_2, 25 mg Mg SO_4, 31 mg K_2 HPO, 10.5 mg Na HCO_3 and 6.95 mg Fe SO_4.

To these major salts 1 ml of the following trace elements was added: 39 mg Cu SO_4, 43 mg Zn SO_4, 13 mg K I, 15 mg K Br, 40.6 mg Mn SO_4, 56 mg Co Cl_2, 10 mg H_3BO_3, 18.4 mg Na_2MoO_4, 16.7 mg Na_2WO_4 and 91 mg $Na_2B_4O_7$. They were dissolved in 1 L glass distilled water.

All glassware and media were sterilized by autoclaving at 121°C and 1.5 Kg/cm^2 for 15 minutes. Media were prepared on the day before a test was to be conducted to allow for pH stabilization at 8.1.

Bayluscide is a 70% formulation of ethanolamine salt of 5, 2^1-dichloro-4^1-nitro-salicylic anilide. The sample used in this study was a Bayer Product.

Algae were grown in 250 ml Erlenmeyer flasks with 100 ml of medium. After autoclaving, each flask was inoculated with 5000 algal cells/ml in logarithmic phase under sterilized condition (U.V. champer). Cultures were incubated with hand shaking once a day under 4000 lux illumenation. The test duration was 96 h, the temperature was 25 $\pm$ 1 °C and the lighting cycle was of 14 L : 10 D.

After 48, 72 and 96 h incubation, the population growth of the control and treated cultures was determined by haemocytometer at 300 X. The growth rate was calculated according to the equation reported by Kratz and Mayers, (1955). The EC_{50} (the calculated concentration of Bayluscide which inhibits growth of the test algae by 50 % of the control) was calculated by straight line graphical interpolation (APHA, 1975).

After 96 h incubation, dry weight, chlorophyll a, b, carotenoid, carbohydrate and protein contents of the control and treated algal cells were determined. The dry weight was measured using Millipore filter paper 0.45 µm pore diameter, (APHA, 1975). Chlorophyll a, b and carotenoid contents were extracted with 90% acetone according to Gliterman and Clymo (1971). The chlorophyll a was calculated according to Lorenzen's equation (1967), and chlorophyll b and carotenoid contents were calculated according to the method of Parsons and Strickland (1963). Total carbohydrate was determined by the phenol sulphuric acid method using glucose as standard (Myklestad and Haug, 1972 and Dubois et.al., 1956). Total nitrogen was determined by the micro-Kjeldahl method (Hiller et al., 1948). Portein content was calculated by multiplying the value of total nitrogen by 6.25.

Using the total cell number at the end of the experiments (96 h) the dry weight, chlorophyll a, b, carotenoid, carbohydrate and protein content were caluclated on an average per cell basis.

RESULTS

Table (1) and Fig. (1) show that the population growth of the test algae decreased when treated with Bayluscide, a phenomenon that was enhanced at high concentrations. The inhibitory effect of Bayluscide increased with increasing the exposure tim. The EC_{50} values of Bayluscide for depressing the growth were: 270, 255, and 250 ppb for **S. dimorphus**; 175, 125 and 168 ppb for **S. quadricauda**; and 176, 190, 165 ppb for **A. falcatus** after 48, 72 and 96 h exposure, respectively.

The dry weight of the test algal cells increased under the influence of low doses of Bayluscide except the lowest two concentrations in case of S. dimorphus (Table 2 and Fig. 2). Conversely, the highest dose reduced the dry weight gain by the test algal cells below the control level.

Table (3) and Fig. (2) show that chlorophylls and carotenoid contents of **S. dimorphus** cells decreased when treated with Bayluscide. Contrariwise, low doses of Bayluscide slightly stimulated the chlorophylls and carotenoids of **S. quadricauda** and **A. falcatus**; a phenomenon was reversed at high concentrations.

The stimulatory effect of low doses of Bayluscide was also observed with the carbohydrate and protein content of **S. quadricauda** and **A. falcatus** cells, whereas the high doses caused significant drop. The carbohydrate and protein content of **S. dimorphus** decreased with increasing Bayluscide concentrations (Tables 4 and 5), (Figs. 4 and 5)

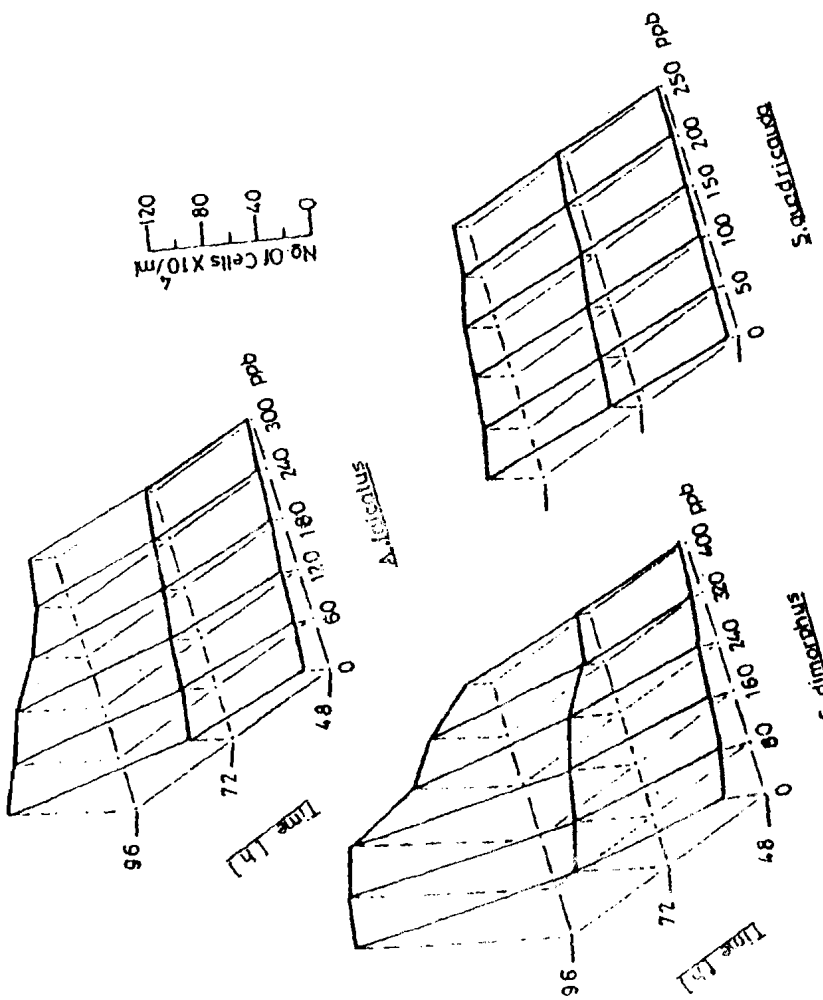

Fig. (1)
Effect of Baylusclde on growth of the test algae.

TABLE 2
Effect of Bayluscide on the dry weight of the test algal cells.

Test algae	Bayluscide conc. (ppb)	µg Dry weight of 10^6	Decrease (%)	Increase (%)
S. dimorphus	0	29.8	-	-
	80	26.9	9.7	-
	160	28.4	4.7	-
	240	33.5	-	12.4
	320	37.6	-	26.2
	400	28.4	4.7	-
S. quadricauda	0	96.6	-	-
	50	117.7	-	21.8
	100	124.6	-	29.0
	150	139.5	-	44.4
	200	169.5	-	75.5
	250	93.6	3.1	-
A. falcatus	0	27.6	-	-
	60	32.2	-	16.7
	120	37.2	-	34.6
	180	30.0	-	8.7
	240	28.8	-	4.4
	300	19.0	31.2	-

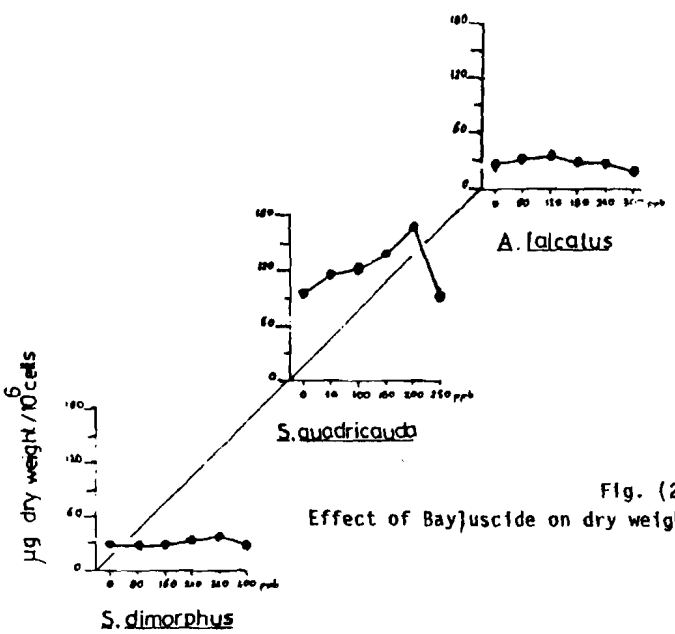

Fig. (2)
Effect of Bayluscide on dry weight of the test algal cells.

TABLE 3
Effect of Bayluscide on Chlorophyll a, b and carotenoid contents of the test algal cells.

Test algae	Concentrations of Bayluscide	Chlorophyll a			Chlorophyll b			Total Carotenoids		
		µg/10^6 cells	Dec. %	Inc. %	µg/10^6 cells	Dec. %	Inc. %	µg/10^6 cells	Dec. %	Inc. %
S. dimorphus	0	0.35	-	-	0.1	-	-	0.17	-	-
	80	0.35	-	-	0.09	10	-	0.12	29.4	-
	160	0.35	-	-	0.09	10	-	0.09	47.1	-
	240	0.34	2.9	-	0.08	20	-	0.08	52.9	-
	320	0.28	20	-	0.06	40	-	0.06	64.7	-
	400	0.18	48.6	-	0.04	60	-	0.03	82.4	-
S. quadricauda	0	0.45	-	-	0.14	-	-	0.23	-	-
	50	0.44	2.2	-	0.17	-	21.4	0.26	-	13
	100	0.43	4.4	-	0.19	-	35.7	0.17	26.1	-
	150	0.39	13.3	-	0.21	-	50	0.15	34.8	-
	200	0.29	35.6	-	0.15	-	7.1	0.12	47.8	-
	250	0.12	73.3	-	0.04	71.4	-	0.05	78.3	-
A. falcatus	0	0.52	-	-	0.13	-	-	0.25	-	-
	60	0.61	-	17.1	0.14	-	7.7	0.27	-	8
	120	0.64	-	23.1	0.14	-	7.7	0.29	-	16
	180	0.32	38.5	-	0.07	46.2	-	0.22	12	-
	240	0.24	53.9	-	0.04	69.2	-	0.19	17.4	-
	300	0.15	71.2	-	0.01	92.3	0	0.11	52.2	-

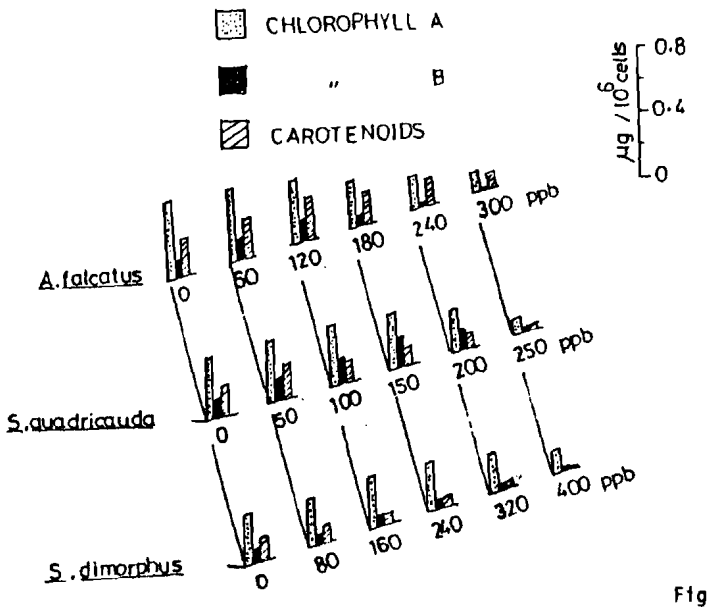

Fig. (3)
Effect of Bayluscide on chlorophyll A, B and carotenoids.

TABLE 4
Effect of Bayluscide on total cellular carbohydrate of the test chlorophytes.

Test algae	Bayluscide Conc. (ppb)	µg Carbohydrate/10^6 cells	Decrease (%)	Increase (%)
S. dimorphus	0	4.4	-	-
	80	4.0	9.9	-
	160	3.7	16.7	-
	240	3.5	21.2	-
	320	1.7	61.7	-
	400	1.1	75.2	-
S. quadricauda	0	19.1	-	-
	50	19.5	-	2.1
	100	20.1	-	5.2
	150	14.9	22	-
	200	11.8	38.2	-
	250	6.1	68.1	-
A. falcatus	0	6.6	-	-
	60	6.7	-	1.2
	120	7.3	-	10.6
	180	4.3	34.9	-
	240	3.1	53	-
	300	1.5	77.3	-

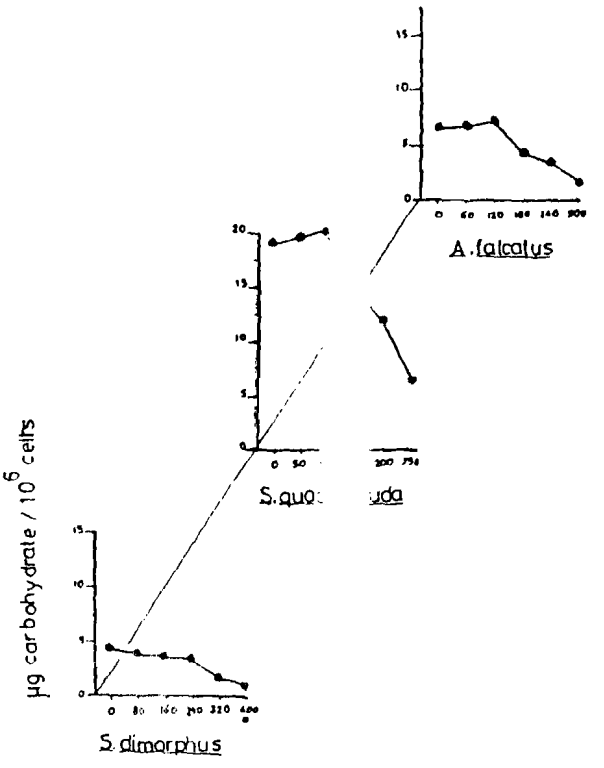

Fig. (4)
Effect of Bayluscide on cellular carbohydrate.

TABLE 5
Effect of Bayluscide on total cellular protein of the test algae.

Test Algae	Bayluscide Conc.(ppb)	µg Protein/10^6 cells	Decrease (%)	Increase (%)
	0	16.2	-	-
	80	13.6	16.1	-
	160	11.8	27.2	-
S. dimorphus	240	9.7	40.1	-
	320	6.7	58.6	-
	400	4.9	69.8	-
	0	56.3	-	-
	50	56.7	-	0.7
	100	52.6	6.6	-
S. quadricauda	150	44.6	20.8	-
	200	30	46.7	-
	250	12.8	77.3	-
	0	15.4	-	-
	60	16.6	-	7.8
	120	17.3	-	12.3
A. falcatus	180	9.	41.6	
	240	7.6	50.7	-
	300	3.8	75.3	-

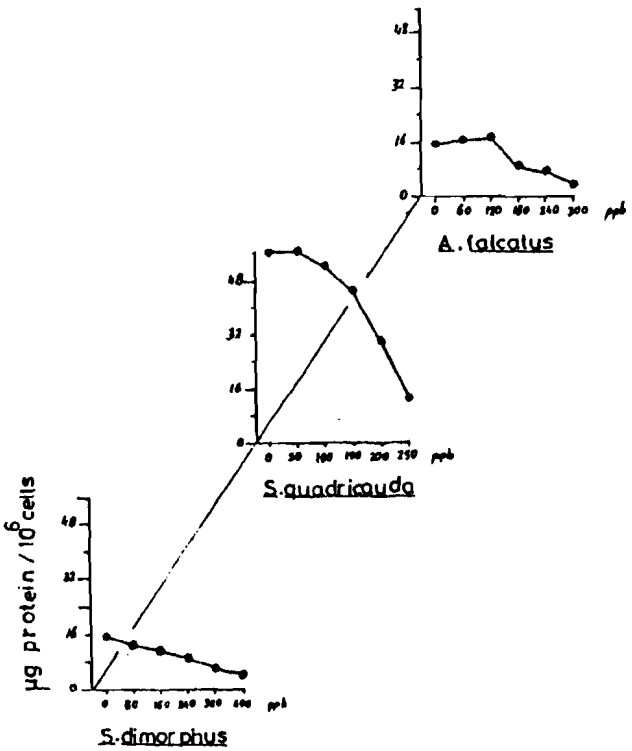

Fig. (5)
Effect of Bayluscide on protein content

DISCUSSION

The present results reveal that the inhibitory effect of Bayluscide on population growth of the test algae increased with increase in the exposure time, while in case of **S. quadricauda** the maximum inhibitory effect of Bayluscide was observed after 72 h exposure. The 96 h EC_{50} values of Bayluscide for depression population growth of the test algae in ppb were: 250 for **S. dimorphus**; 168 for **S. quadricauda**; and 165 for **A. falcatus**. Ibrahim (1983) studied effect of Bayluscide on growth of the marine diatoms **Skeletonema costatum, Amphiprora paludosa** and **Phaeodactylum tricornutum.** He found that the 96 h EC_{50} value of Bayluscide for depressing the growth

of these diatoms in ppb were 71 for **S. costatum**; 1450 for **A. paludosa**; and 41.5 for **P. tricornutum**. This indicates the high susceptibility of the marine diatoms **P. tricornutum** and **S. costatum** to Bayluscide as compared with the test freshwater chlorophytes.

The obvious increment in the dry weight gain by the test algal cells is mainly due to abnormal enlargement of the treated cells and consequently increased their dry weight. The highest concentration of Bayluscide caused significant drop in the biochemical constituents of the test algal cells and finally depressed the dry weight.

The present results indicate that the chlorophyll a, b and carotenoid content of **S. dimorphus** cells decreased when treated with Bayluscide. On the other hand, chlorophylls and carotenoid contents of **S. quadricauda** and **A. falcatus** cells slightly increased only at low doses of Bayluscide. This phenomenon was also observed with some blue-greens and marine diatoms when treated with low doses of Bayluscide (Ibrahim, 1978 and 1983). This can be related to the cabability of the treated algal cells to absorb and metabolize low doses of Bayluscide that consequently stimulated chlorophyll and carotenoid synthesis. Also, low concentrations of Bayluscide increased the cell volume of the treated algae that consequently increased the biochemical constitutents of the cells.

Bayluscide has the same sequence of inhibitory and/or stimulatory effect on carbohydrate and protein contents of the test algal cells as that on chlorophyll and carotenoid contents. This leads to the conclusion that low doses of Bayuscide slightly stimulated the enzyme activity responsible for carbohydrate and protein synthesis. Contrariwise, high concentrations of Bayluscide caused severe drop in the carbohydrate and protein contents of the test algae. In this connection, Ishak et. al., (1970) concluded that Bayluscide acts as inhibitor to the enzyme responsible for carbohydrate synthesis in the snail **Biomophalaria alexandrina.**

The obvious inhibitory effect of Bayluscide on growth and metabolic activity of the three test chlorophytes has also been observed with other aquatic organisms. Robert (1979) recorded the toxic effect of Bayluscide on eggs, juveniles and adult stages of cichlids **Tilapia leucostrica** and **Herotilapia multispinosa.** He pointed out that the application of Bayluscide should not take place during the early development of the fry in region where the breading of Tilapia is carried out on a commercial basis.

The present results reveal the inhibitory effect of Bayluscide on growth and metabolic activities of the three test phytoplankters which represent the freshwater phytoplankton. Since phytoplankton represent the vital link in the aquatic food-chain, Bayluscide has deleterious effect on the productivity of the aquatic areas.

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