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THE INFLUENCE OF CARBAMATE INSECTICIDES ON SOME PHYSIOLOGICAL PROCESSES IN SCENEDESMUS QUADRICAUDA (Turp./Breb.)

1. INTRODUCTION

Problems concerning the use of insecticides are topical world over. The significance of the fact arises from the extensive application of these compounds and persistent contamination they cause in the environment. Insecticides constitute, among pesticides, the biggest group of substances of varied utility. The most popular are polychlorinated insecticides — e.g. DDT. The application of DDT yielded some positive results in hygiene, agriculture and pomology, but on the other hand there is evidence of the obvious polluting of the biosphere by DDT and its derivatives. Such effects are due to both the prolonged residence of these compounds in the natural environment and their accumulation in organisms [6, 8, 9, 15]. This forced man to search for DDT substitutes which would be as effective as DDT, but quickly degradable.

Derivatives of carbamic acid suited these requirements excellently, but the application of new biologically active substances requires prolonged and detailed studies of their impact on the natural environment. As regards carbamates, their influence on several animal species has been examined thouroughly, but there are only a few reports concerning the influence of these compounds on plants, especially aquatic plants. However, it is well known that the application of insecticides for plant protection on land leads to the eventual pollution of water, thus disturbing the ecological balance. In literature, there are very few and often very rough reports dealing with new carbamate insecticides, their stability, metabolization and toxicity in aquatic environments. Little is known, in particular, about their effect on algae — one of the most important links in the food chain.

This paper presents studies of the influence of widely used insecticides, carbaryl and propoxur, on some physiological processes in the green alga Scenedesmus quadricauda. The impact of insecticides on growth, chlorophyll and dry weight production as well as photosynthesis intensity have been examined.

2. MATERIAL AND METHODS

2.1. Cultivation of algae

Basal culture was carried out in thermoluminestate in luminescent light of about 4500 lx intensity with retained normal photoperiodical conditions, at a temperature of 21±2°C. The algae were cultivated on Lefévre [7] medium modified by Schlüter [17]. After the elapse of 7 - 8 days the algae were transplanted on a medium containing pesticides; as control, medium with no pesticides added was used. The transplantation was made in such way that the initial number of cells amounted to 30 000, the cells counted as 4-cell cenobias per cm^3. The experiments lasted 9 days. Chlorophyll and the number of cells were measured on the 1st, 3rd, 5th, 7th and 9th day of the culture. Dry weight was determined on the 7 and 9th days.

2.2. Applied pesticides

In experiments, two carbamate pesticides were examined:
- — carbaryl — α-naphthol N-methylcarbamate: colourless, flavourless crystalline powder, sparingly soluble in water (about 0.1% at 20°C), but well soluble in organic solvents, extremely toxic to fish and bees,
- — propoxur — 2-isopropoxyphenyl N-methylcarbamate: colourless and flavourless crystalline powder, sparingly soluble in water (about 0.2% at 20°C), but well soluble in all polar organic solvents.

In carbaryl tests a compound of 99.5% purity, as well as a commercial product — Karbatox — containing 75% of carbaryl in suspension, were used. In the case of propoxur — a 98% purity compound and also a commercial product — Unden 50 — containing 50% of propoxur as active substance, were used.

Some metabolites of carbaryl and propoxur were also examined:
- — α-naphthol, carbaryl metabolite: colourless and flavourless crystalline substance well dissolved in alcohol and ether, but sparingly in water, of 100% purity,

Table 1. The influence of propoxur and Unden 50 on the growth activity and chlorophyll „a” production in cultures of Scenedesmus quadricauda

Tab. 1. Wpływ propoksuru i Undenu 50 na dynamikę wzrostu i produkcję chlorofilu „a” w kulturach Scenedesmus quadricauda

Kind of substance Rodzaj substancji	Days of cultivation Dni hodowli	Concentration – stężenie [mg SA/dm³]								
		0.0			10.0			18.0		
		cenobium/cm³ ·10⁴ cenobii/cm³ ·10⁴	μ	chlorophyll „a” chlorofil „a” [mg/dm³]	cenobium/cm³ ·10⁴ cenobii/cm³ ·10⁴	μ	chlorophyll „a” chlorofil „a” [mg/dm³]	cenobium/cm³ ·10⁴ cenobii/cm³ ·10⁴	μ	chlorophyll „a” [mg/dm³] chlorofil „a” [mg/dm³]
Propoxur	0	3.0± 0.3*		0.43±0.11	3.0± 0.3		0.43±0.11	3.0± 0.3		0.43±0.11
	1	5.5± 1.6	0.61	0.66±0.24	3.5± 0.5	0.15	0.43±0.11	3.2± 0.2	0.06	0.35±0.13
	3	32.6± 8.2	0.89	2.57±0.14	9.5± 3.9	0.50	1.06±0.56	3.6± 0.4	0.06	0.44±0.09
	5	50.1± 5.8	0.21	5.45±0.82	38.4± 3.8	0.70	3.63±0.84	8.3± 3.7	0.42	0.76±0.38
	7	61.8± 6.2	0.10	6.93±0.52	53.3± 5.6	0.16	5.93±0.56	22.4±10.0	0.52	2.38±1.00
	9	71.5±12.0	0.07	8.43±0.72	75.3± 8.8	0.17	8.55±0.55	80.6±11.4	0.62	6.99±2.22
Unden 50	0	3.0± 0.3		0.43±0.04	3.0± 0.3		0.43±0.04	3.0± 0.3		0.43±0,04
	1	5.8± 1.6	0.66	0.81±0.27	3.4± 0.8	0.12	0.48±0.09	2.7± 0.4	0	0.37±0.00
	3	25.5±11.1	0.74	2.54±1.10	9.9± 2.6	0.53	1.26±0.36	3.1± 0.9	0.07	0.45±0.00
	5	40.0± 7.8	0.22	4.27±0.88	34.7± 3.0	0.63	3.79±0.38	11.8± 3.8	0.67	1.24±0.28
	7	50.9± 9.6	0.12	5.64±0.68	46.8±11.1	0.15	5.50±0.50	25.9± 4.2	0.39	2.76±0.13
	9	60.1± 3.8	0.08	8.50±1.12	60.8± 4.2	0.13	8.55±1.42	61.8± 3.6	0.43	6.02±0.56

* – standard error (p=0.05); odchylnie standardowe (p=0.05) μ – specific growth ratio; właściwa szybkość wzrostu SA – active substance; substancja aktywna

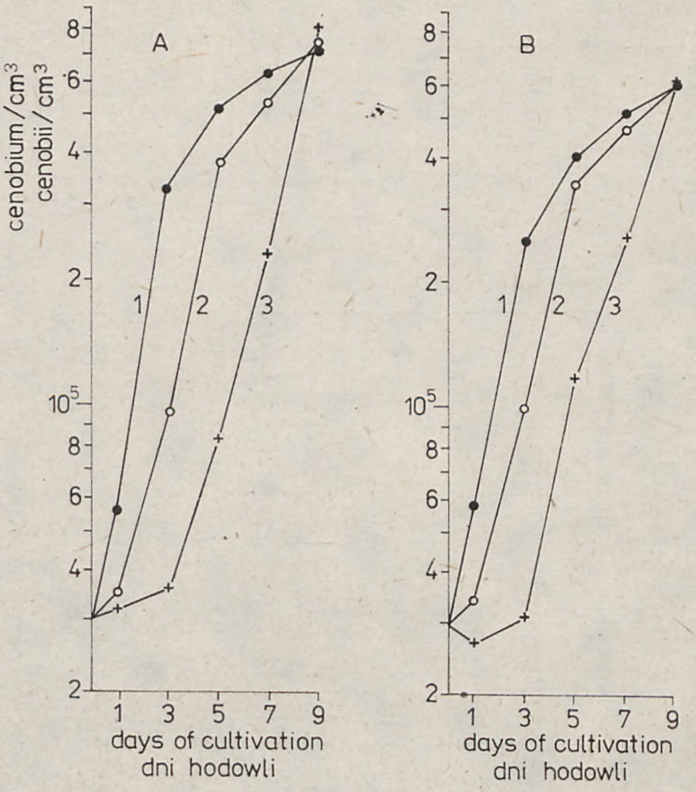

Fig. 1. Growth responses of Scenedesmus quadricauda to different concentrations of propoxur (*A*) and its commercial product Unden 50 (*B*)

Rys. 1. Reakcja wzrostowa Scenedesmus quadricauda na różne stężenia propoksuru (*A*) i produktu handlowego Unden 50 (*B*)
1 — control, kontrola; *2* — 10.0 mg/dm³; *3* — 18.0 mg/dm³

— 2-isopropoxyphenol, metabolite of propoxur: yellow, oily substance with characteristic sharp flavour, sparingly dissolved in water, but well soluble in toluene and alcohol, of 94.7% purity.

2.3. Measurements

The algae cells were counted under a microscope in a Bürker chamber. On the basis of the cell number the maximum growth rate (μ) was determined [1, 4, 14].

Chlorophyll was measured by spectrophotometric method [20]. Extraction of chlorophyll was conducted into 10 cm³ of 90% acetone over a period of 20 h. Results were expressed in terms of chlorophyll "a" — the major indicator of algae biomass accumulation.

Dry weight increase was determined gravimetrically [1, 3]. Algae suspension was filtered through membrane filters HA Millipore; results were expressed in mg/dm³ to show the general productivity in a given volume of the cultivation medium.

Photosynthesis and respiration tests lasted 1 day. The intensity of these processes was examined by the oxygen method with transparent and blackened bottles [22, 12, 13]. Oxygen content was measured electrochemically. Results were related to the respective control samples.

All investigations were conducted in 2 or 3 separate experiments, and each variant of these experiments was repeated three times. The results were analyzed statistically for standard deviation and least significant difference (LSD) by t-test at $p=0.05$ [19, 1, 18, 11].

3. RESULTS AND DISCUSSION

The first part of the experiment concerned the influence of carbaryl and propoxur on the growth of Scenedesmus quadricauda in various doses from 1.0 to 100.0 mg/dm³ added to the medium. It was found that the concentration range could be split into three divisions: 1 — doses with no significant effect on algae growth or slightly promoting activity, 2 — concentrations which temporarily inhibited the development of algae populations and also caused temporary morphological alterations

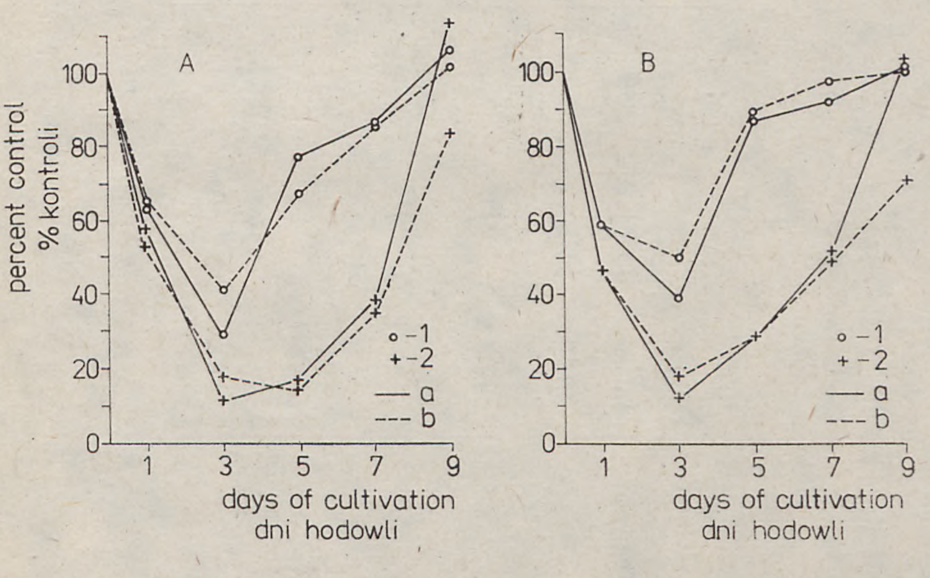

Fig. 2. The influence of propoxur (*A*) and its commercial product Unden 50 (*B*) on cell number and chlorophyll production in cultures of Scenedesmus quadricauda

Rys. 2. Wpływ propoksuru (*A*) i produktu handlowego Unden 50 (*B*) na liczebność komórek i produkcję chlorofilu w kulturach Scenedesmus quadricauda
1 — 10.0 mg/dm³; *2* — 18.0 mg/dm³; *a* — cell number, liczebność; *b* — chlorophyll, chlorofil

Table 2. The influence of carbaryl and karbatox on the growth activity and chlorophyll ,,a" production in cultures of Scenedesmus quadricauda

Tab. 2. Wpływ karbarylu i karbatoxu na dynamikę wzrostu i produkcję chlorofilu ,,a" w kulturach Scenedesmus quadricauda

Kind of substance Rodzaj substancji	Days of cultivation Dni hodowli	Concentration – stężenie [mg SA/dm³]								
		0.0			3.2			10.0		
		cenobium/cm³ ·10⁴ cenobii/cm³ ·10⁴	μ	chlorophyll ,,a" chlorofil ,,a" [mg/dm³]	cenobium/cm³ ·10⁴ cenobii/cm³ ·10⁴	μ	chlorophyll ,,a" chlorofil ,,a" [mg/dm³]	cenobium/cm³ ·10⁴ cenobii/cm³ ·10⁴	μ	chlorophyll ,,a" chlorofil ,,a" [mg/dm³]
Carbaryl Karbaryl	0	3.0± 0.3*		0.37±0.04	3.0± 0.3		0.37±0.04	3.0± 0.3		0.37±0.04
	1	3.8± 1.1	0.24	0.47±0.16	2.5± 0.6	0	0.30±0.00	1.9± 0.4	0	0.18±0.06
	3	18.6± 7.1	0.79	1.81±0.34	8.1± 0.8	0.57	0.86±0.00	1.9± 0.5	0	0.21±0.08
	5	37.4± 4.1	0.35	3.91±0.30	27.5± 5.4	0.61	2.85±0.55	4.2± 0.8	0.40	0.58±0.20
	7	51.8± 7.3	0.16	5.61±1.00	41.8± 7.7	0.21	4.59±1.22	19.0± 5.7	0.75	1.65±0.90
	9	65.2±11.1	0.11	7.43±0.94	64.4±12.5	0.22	6.56±1.34	70.0±16.7	0.65	5.38±0.88
Karbatox Karbatox	0	3.0± 0.3		0.38±0.10	3.0±0.3		0.38±0.10	3.0±0.3		0.38±0.10
	1	5.0± 1.0	0.51	0.55±0.22	3.8±0.5	0.24	0.35±0.00	2.1±0.7	0	0.20±0.08
	3	17.5± 6.2	0.60	2.06±1.25	9.6±2.6	0.46	1.24±0.38	2.6±0.8	0	0.37±0.12
	5	33.0± 6.7	0.34	4.42±1.19	24.4±5.3	0.47	2.93±0.50	9.0±1.3	0.73	1.10±0.12
	7	46.0± 4.7	0.17	5.46±1.08	37.0±7.9	0.21	4.44±0.54	22.8±4.0	0.46	2.34±0.50
	9	54.6±10.8	0.08	7.00±0.44	50,2±5.4	0.15	6.19±0.54	48.5±6.0	0.38	4.58±0.33

* – as in Tab. 1; jak w Tab. 1 μ – as in Tab. 1; jak w Tab. 1 SA – as in Tab. 1; jak w Tab. 1

of cells, 3 — concentrations producing partial agregation of cells or their total disintegration.

Two concentrations of carbaryl, 3.2 and 10.0 mg/dm³, and two of propoxur — 10.0 and 18.0 mg/dm³ — were chosen for further studies. These concentrations did not yield lethal effects, but the algae visibly reacted to their presence in the medium.

The investigations proved that carbaryl, as well as propoxur inhibited some physiological functions of Scenedesmus quadricauda and the algae response depended on the exposure time, dose and the compound used. Inhibition of cell division was one of the harmful effects. Both low and high concentrations of carbaryl and propoxur visibly altered the cell morphology as early as 24 h after addition. Apart from this, the number of cells remained at the same level, while in the control variant a considerable enhacement of cell multiplication was observed (Tab. 1, 2 and Fig. 1). At low pesticide concentrations the strongest inhibitory effects occurred in 3-day cultures. The symptoms of inhibition caused by higher doses remained for 5 days and then gradually disappeared; this situation demonstrated for propoxur is presented in Table 1 and Fig. 2. Generally, the maximum growth rate (μ) in control cultures was observed between the first and the third day, while that of cultures exposed to pesticides was distinctly delayed to between the third and the fifth day, depending on the concentration of the pesticide.

Table 3. The effect of different pesticides on dry matter production in Scenedesmus quadricauda cultures

Tab. 3. Wpływ wybranych pestycydów na pro dukcję suchej masy w kulturach Scenedesmus quadricauda

Kind of substance Rodzaj substancji	Concentration Stężenie [mg SA/dm³]	Dry matter — sucha masa			
		Days of cultivation — dni hodowli			
		7		9	
		mg/dm³	%	mg/dm³	%
Carbaryl Karbaryl	0.0	318	100.0	563	100.0
	3,2	262	82.4	528	93.8
	10.0	169	53.1	387	68.7
Karbatox Karbatox	0.0	293	100.0	514	100.0
	3.2	250	85.3	463	90.1
	10.0	162	55.3	274	53.3
Propoxur Propoksur	0.0	309	100.0	534	100.0
	10.0	243	78.6	490	91.8
	18.0	157	50.8	336	62.9
Unden 50 Unden 50	0.0	241	100.0	461	100.0
	10.0	233	96.7	447	97.0
	18.0	171	70.9	288	62.5

SA — as in Tab. 1; jak w Tab. 1

Significant differences were noticed in the activity of pure compounds and commercial products (Table 1, Fig. 2). The inhibitory effect of commercial products was noticably weaker, and the maximum growth rate was found between the third and the fifth day irrespective of the concentration applied.

The experiments indicated that the impact of some carbamate concentrations was only temporary. The examined substances did not produce any permanent toxic changes in the cells, the only result of their activity was a prolonged adaptation phase and delayed phase of intensive growth (Fig. 1). The stage preceding intensive growth was extended to three or five and even seven days (e.g. carbaryl — pure substance), while in control it was limited to 24 h (Tab. 2). Similar observations were made by Batterton et al. [2] for certain Cyanophyta

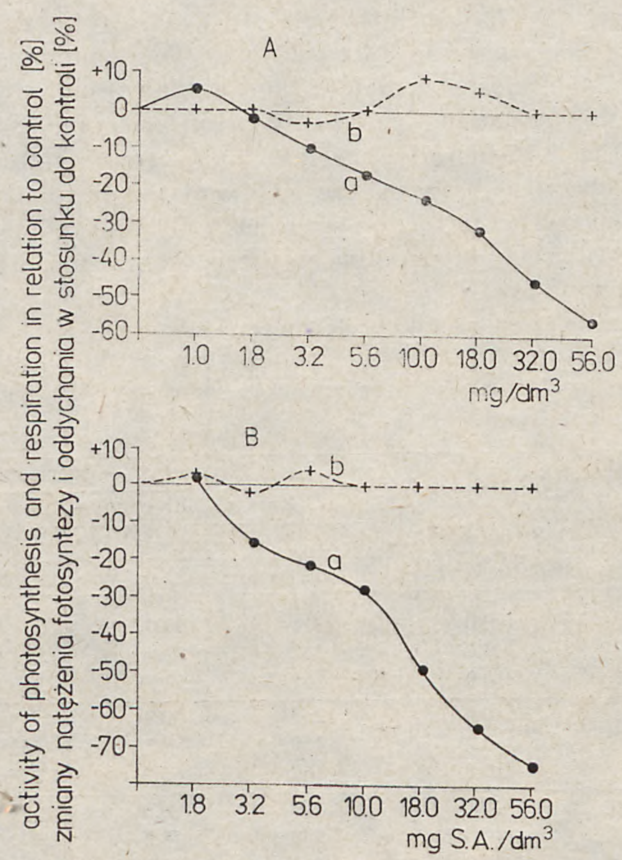

Fig. 3. The influence of carbaryl (*A*) and its commercial product karbatox (*B*) on photosynthesis and respiration activity of Scenedesmus quadricauda

Rys. 3. Wpływ karbarylu (*A*) i produktu handlowego karbatox (*B*) na aktywność fotosyntezy i oddychania Scenedesmus quadricauda

a — photosynthesis, fotosynteza; *b* — respiration, oddychanie; *SA* — active substance, substancja aktywna

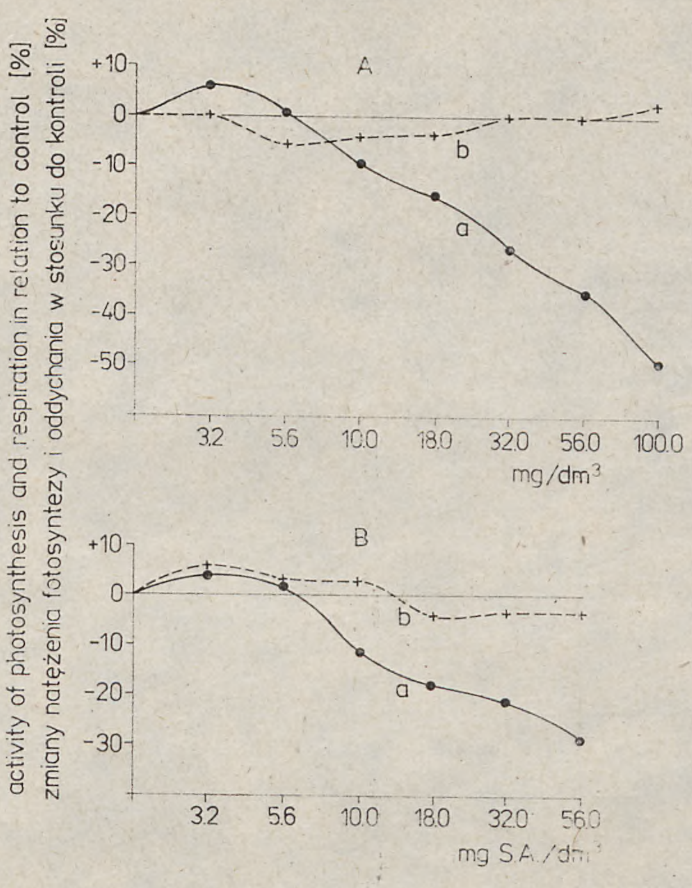

Fig. 4. The influence of propoxur (*A*) and its commercial product Unden 50 (*B*) on photosynthesis and respiration activity of Scenedesmus quadricauda

Rys. 4. Wpływ propoksuru (*A*) i produktu handlowego Unden 50 (*B*) na aktywność fotosyntezy i oddychania Scenedesmus quadricauda
a, *b* — as in Fig. 3, jak na Rys. 3; *SA* — as in Fig. 3, jak na Rys. 3

exposed to pesticides from the group of polychlorinated hydrocarbons. According to Myers [10] and Schlegel [16] the temporary decrease of growth rate during the adaptation phase especially in the presence of harmful substances, results from the necessity for synthetizing of a specific RNA and adaptive enzymes, which are not produced in normal conditions. In consequence, a considerable amount of cell energy is transferred to the process of these syntheses with simultaneous depletion of growth rate.

A detailed analysis of 7- and 9-day cultures revealed specific activity of both studied compounds: after 7 days of exposure to carbaryl or propoxur as well, the changes in chlorophyll content were parallel to those in the cell number (Fig. 2). Simultaneously the dry matter production was visibly inhibited (Tab. 3). On the other hand, it was found, however, that the changes in dry matter and chlorophyll "a" contents in cells treated with pesticides were similar to those noted in control cultures. Thus the depletion of the total amount of chlorophyll and dry matter should be attributed mainly to the reduction of cell number.

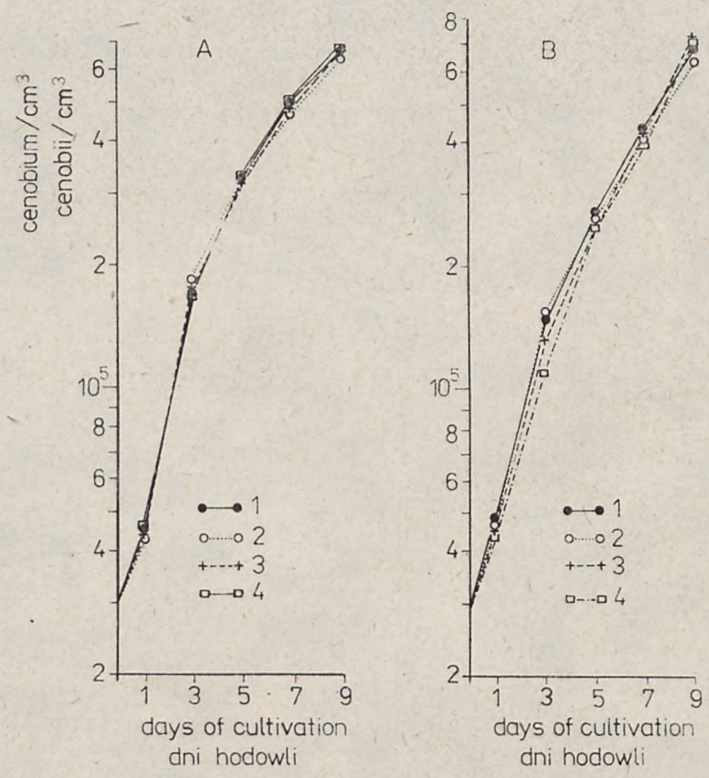

Fig. 5. Effect of some metabolites of carbaryl (*A*) and propoxur (*B*) on the growth of Scenedesmus quadricauda

Rys. 5. Efekt działania metabolitów karbarylu (*A*) i propoksuru (*B*) na wzrost Scenedesmus quadricauda

A: α-naphthol, α-naftol; *B:* 2-isopropoxyphenol, 2-izopropoksyfenol; 1 — control, kontrola; *2* — 1.0 mg/dm³, *3* — 3.2 mg/dm³, *4* — 10.0 mg/dm³

No significant variations of chlorophyll "a" level in cells were observed, thus, the decrease found in the production of dry matter probably ensued from the lessening of the pigment photosynthetic effectiveness.

Carbaryl produced much stronger harmful effects than propoxur, although the mechanisms of their activity were similar. It should be stressed that the effect of commercial products on algae proved to be much weaker than that of pure substances. This might be due to the presence of some accessory components, especially carriers. The fact of different biological activity of various products was previously stated by Jara [5] and Solski [21], who pointed out that the effect of pesticides and the organism's reaction largely depended on the form in which the compound was applied.

The intensity of photosynthesis was studied in the presence of the concentrations ranging from 1.0 to 56.0 mg/dm³ and 3.2. to 100.0 mg/dm³ for carbaryl and propoxur, respectively. The results manifested a gradual decrease of photosynthesis intensity according to the increasing pesticide

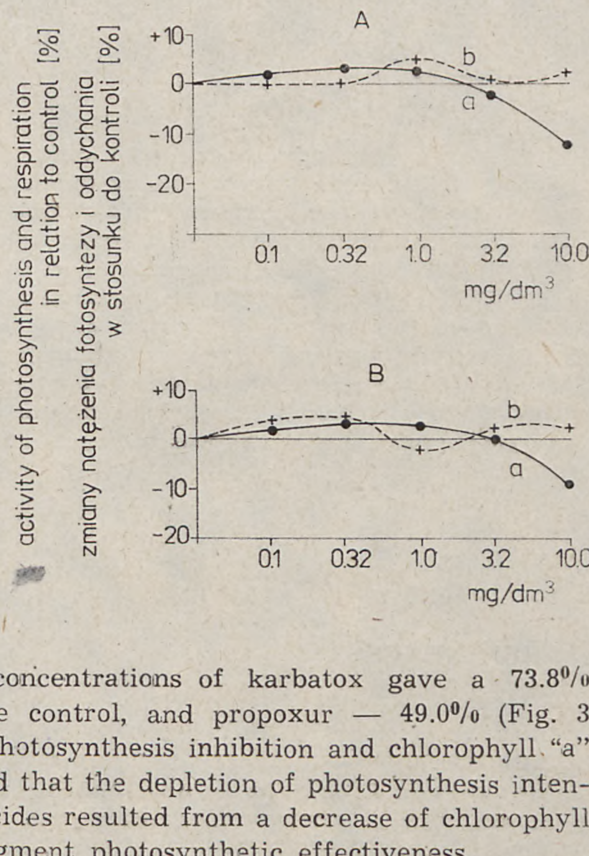

Fig. 6. Effect of some metabolites of carbaryl (*A*) and propoxur (*B*) on photosynthesis and respiration activity of Scenedesmus quadricauda

Rys. 6. Efekt działania metabolitów karbarylu (*A*) i propoksuru (*B*) na aktywność fotosyntezy i oddychania Scenedesmus quadricauda

A, *B* — as in Fig. 5, jak na rys. 5; *a* — photosynthesis, fotosynteza; *b* — respiration, oddychanie

concentration. The highest concentrations of karbatox gave a 73.8% reduction in relation to the control, and propoxur — 49.0% (Fig. 3 and 4). The comparison of photosynthesis inhibition and chlorophyll "a" content in the cells suggested that the depletion of photosynthesis intensity in the presence of pesticides resulted from a decrease of chlorophyll synthesis, as well as the pigment photosynthetic effectiveness.

No significant influence of any of the studied compounds on the respiration of algae was observed.

Tests with α-naphthol and 2-isopropoxyphenol showed neither noticeable effect on the growth (Fig. 5) and photosynthesis (Fig. 6) of Scenedesmus quadricauda, nor did the compounds influence the chlorophyll and dry matter production. It could then be assumed that the main metabolites of carbaryl and propoxur are biologically neutral as regards the examined range of concentrations.

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BADANIA NAD WPŁYWEM WYBRANYCH INSEKTYCYDÓW KARBAMINIANOWYCH NA NIEKTÓRE PROCESY FIZJOLOGICZNE U SCENEDESMUS QUADRICAUDA (Turp./Breb.)

Streszczenie

Badano reakcję glonu Scenedesmus quadricauda na działanie insektycydów karbaminianowych, karbarylu i propoksuru w formie substancji czystych i preparatów handlowych. Prześledzono także wpływ głównych metabolitów obu pestycydów, -α-naftolu i 2-izopropoksyfenolu. Wyniki badań wykazały, że w określonym zakresie stężeń zarówno karbaryl jak i propoksur powodują okresowe zahamowanie wzrostu komórek, a w konsekwencji także produkcji biomasy. Związki te wpływają również hamująco na natężenie fotosyntezy obniżając poziom chlorofilu „a" lub też wydajność fotosyntetyczną pigmentu. Najsilniejsze działanie hamujące insektycydów ujawniało się z reguły w okresie 3 - 5 dni ich działania. Hamujące działanie uzależnione było od rodzaju i formy oraz stężenia stosowanego związku. Metabolity karbarylu i propoksuru, w zakresie badanych stężeń, nie wykazywały aktywności biologicznej.

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