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Toxicity Determination of Plant Growth Hormones on Aquatic Alga—*Scenedesmus quadricauda*

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The use of pesticides is increasing very rapidly in agriculture and pisciculture in order to control unwanted insects and weeds. The unrestricted use of these chemicals is detrimentally affecting the aquatic biota. The universal use of pesticides in agriculture creates a necessity to study the effect of these chemicals on biological subjects in various parts of environment. Phenoxyalkanoic acids, introduced in the middle of the 1940s, are even today the most important selective herbicides, manufactured an used in the largest quantity. Phenoxyalkanoic acids and their derivatives are synthesized mainly in meristematic tissues. The first to be isolated was indole-3-acetic acid (IAA). Following the discovery of this heteroauxin several related compounds of indole-3-acetic acid were synthesised and studied. Among them was also 1-naphthylacetic acid (NAA), 2-naphthoxyacetic acid (NOXA), and 4-(indol-3-yl)butyric acid (IBA). The derivatives of substituted phenoxyacetic acids are in practice the most important and most widely used of the hormone-type herbicides. Three of these derivatives, 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methyl-phenoxyacetic acid (MCPA), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are of particular importance (Matolcsy et al. 1988). From these mentioned herbicides, four were used in our toxicity tests (IAA, NAA, 2,4-D, MCPA) and they were completed by cytokinin β-indolylpropionic acid (IPA) (Wareing and Phillips 1970). At present, there are few reports about the toxicity of these herbicides on aquatic algae and cyanobacteria. Reports of particular importance are 2,4-D and MCPA (Mishra et al. 1989; Butler et al. 1975; Singh 1973; Abou-Waly et al. 1991). Also very little information is available about about S. quadricauda as a toxicological subject. This organism is frequently found in all freshwater and is important in the freshwater food chains.

MATERIAL AND METHODS

Scenedesmus quadricauda /TURP./BRÉB. strain Greifswald 15 was supplied by the Department of Botany, Třeboň, Czech. During the tests the culture was incubated on a permanent light at a temperature of 25±1 ^{o}C and a light intensity of three 40 W white fluorescent lamps was used. The culture was maintained in a liquid medium (Báslerová and Dvořaková 1962) containing (g/L): KNO_3 0.1; $K_2HPO_4.7H_2O$ 0.01; $MgSO_4.7H_2O$ 0.001; $FeCl_3.6H_2O$ 0.001; soil extract 50 mL; pH=7.18 supplemented with various concentrations of hormone-type herbicides (nominal concentrations (mg/L) for 20 days exposure are introduced into Figure 1 and Figure 2). Each concentration was duplicated three times. All used herbicides were pure products of Lachema Brno. Approximately 25 000 coenobia (four cells connected into one unit) were grown in 100 mL Erlenmayer flasks with 50 mL of cultivation media. Growth was monitored counting the cells with a hemocytometer every 48 h during a 20-day period. Besides growth, the first unfavorable effect (FUE) and very strong unfavourable effect (VSUE) were observed. We considered a FUE when production of coenobia was decreased about 30 % and as VSUE when 50 % of coenobia were disintegrated on three-, two- or one-cell units and cultures were pallid and changed its colour from green to yellow-green. The results of quantitative determinations are valued in table and graphs and EC50 values were estimated by using probit analysis on growth data at 20 days. The 95 % confidence limit were calculated by the moving average angle method (Harris 1959). Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The effects of the hormone-type herbicides (IAA, NAA, 2,4-D, MCPA, IPA) used in 6 concentrations (the used concentrations are introduced on Figure 1 and Figure 2) on growth rates of alga S. quadricauda after 20 days of testing are summarized in Table 1 and Figure 1 and 2. From Table 1 we can see that the highest EC50 value of tested hormones is for IAA. It is nearly 100-times higher than 2,4-D LC50 value, which is the lowest. The rank order toxicity for S. quadricauda by our tests is IAA>IPA>NAA>MCPA>2,4-D. In this table there are also values for first unfavourable effect, very strong unfavourable effect, EC95, and pH values of liquid cultivation media with the highest concentrations of hormone-type herbicides. The values of very strong unfavourable effects are very near to EC50 values. When MCPA and 2,4-D herbicides were used in low concentrations (5 mg/L) they stimulated the growth of the alga. For MCPA in this case the number of coenobia

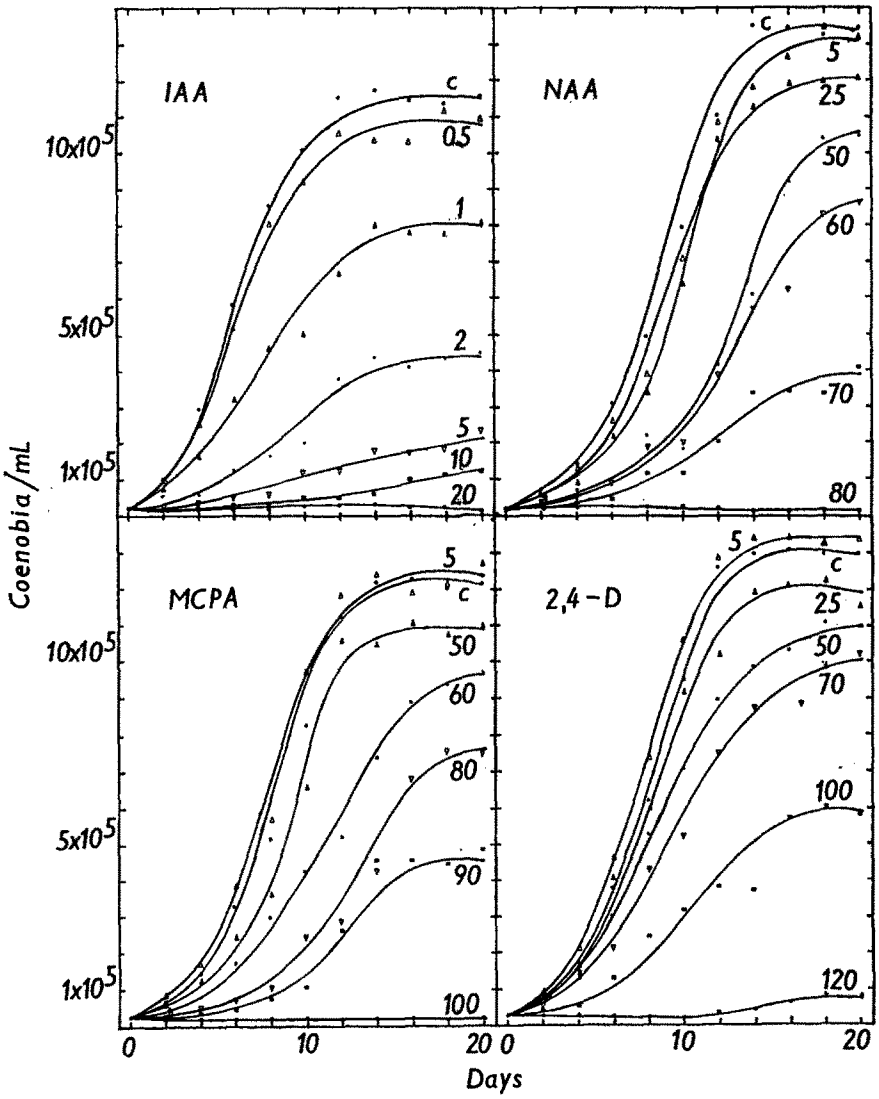

Figure 1. Individual growth curves for various concentrations of hormone-type herbicides and control (c) (mg/L)

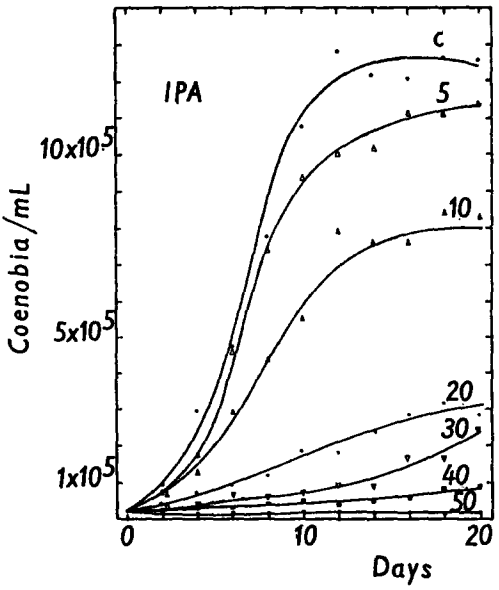

Figure 2. Individual growth curves for various concentrations of hormone-type herbicide IPA and control (c) (mg/L)

Table 1. The values of unfavourable effects, EC95 and EC50 values of hormone-type herbicides on growth rates of S. quadricauda (mg/L)

Substance	FUE	VSUE	EC95	EC50 + 95 % CL	pH
NAA	50.0	70.0	78.4	66.00 (63.21-86.73)	4.96[x]
MCPA	60.0	90.0	97.8	85.10 (79.86-98.53)	4.68
2,4-D	70.0	100.0	112.0	98.00 (92.48-115.70)	4.18
IAA	1.0	5.0	19.5	1.76 (1.69-1.93)	6.35
IPA	10.0	20.0	48.2	13.50 (12.73-15.26)	5.80

FUE - first unfavourable effect; VSUE - very strong unfavourable effect; [x] - pH value of liquid cultivation medium with the highest concentration of hormone-type herbicide, CL - confidence limit

was increased about 5 % after 20 days of cultivation and for 2,4-D about 2,4 % compared with the control (see Figure 1.). Individual growth curves for various concentrations of hormone-type herbicides and controls are shown in Figure 1. and Figure 2.It is possible that during 20 days cultivation the actual concentrations of these compounds likely changed but we considered at the end of our tests only the nominal concentrations used at the beginning of the tests.

The pathway especially for 2,4-D and MCPA has been largely elucidated mainly for bacteria (Kilpi 1980; Lappin et al. 1985), but little has been published about the toxic effect of these compounds on aquatic organisms, mainly alga S. quadricauda. This species is described in literature as very resistant to water pollutants when it is compared with other groups of phytoplankton (Palmer 1958). Also Martínez et al.(1991) found out that the three Chlorophyceae (Chlorella vulgaris, Chlamydomonas reinhardtii, and Scenedesmus quadricauda) assayed are quite resistant to pesticide trichlorfon. It did not affect their growth, in spite of using concentrations of insecticide (25, 50, 100 mg/L) far higher than those occurring under natural conditions. Neither was growth affected in experiments conducted with 300 mg/L for 6 days of exposure. Among the tested herbicides most attention is devoted to 2,4-D and MCPA (Oh and Tuovinen 1991; Sandmann and Loos 1988; Mishra and Pandey 1989). The herbicide 2,4-D, which has auxin activity, is used primarily as a post-emergence agent to control broad-leaved weeds in a variety of crops. It is also used to control aquatic weeds, and is thus directly applied to water supplies as well as being introduced in agricultural run-off. Butler et al. (1975) found that concentrations less than or equal to 1 ppm 2,4-D did not change the growth pattern of 36 isolates of planktonic algae. However, when the concentration was increased to 4 ppm, there was some inhibition of growth as indicated by the 10 % increase in the number of replicates which showed poor growth as compared with the control. In our case these lower concentrations did not inhibit the growth but slightly stimulated it. Walsh (1972) reported that greater than 75 ppm was required to reduce the growth of four marine unicellular algae by 50 % when 2,4-D as butoxy ethanol ester was used. These studies have suggested that use of the butoxy ethanol ester of 2,4-D in nature will not cause any large decrease in algal growth. These conclusions coincide with our results in which a very low level toxicity of 2,4-D for S. quadricauda was proved. Discussion of toxicity of other tested herbicides on S. quadricauda or another algae or cyanobacteria is difficult due to the very few reports regarding them.

The results of the present study indicate that the hormone-type herbicides were, except for IAA and IPA, very low in toxicity to aquatic alga S. quadricauda. On the other hand, our experiments demonstrated that S. quadricauda is a very resistant phytoplanktonic alga.

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