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Influence of herbicides on the growth and composition of the yellow-green alga *Trachydiscus minutus*

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ABSTRACT

The yellow-green alga *Trachydiscus minutus* has not been studied in detail prior to the first decade of the 21st century due to the fact it has been easily confused with the algae of the genus *Chlorella*. It is known that the cells of the algae have a high amount of lipids and proteins and they lack chlorophyll-b. Currently, there is no published data available to describe the effects of herbicides on growth and biochemical composition. The aim of this work is to study the effects of the glyphosate-containing herbicide Roundup and the effects of atrazine on the growth of *Trachydiscus minutus*, its pigment and protein composition, its lipid composition and fatty acid profile. Both herbicides are able to significantly inhibit the growth of the algal cultures if they are treated with concentrations higher than 20 mg/l. The protector spermine was unsuccessfully used to overcome the inhibitory effects of atrazine on *Trachydiscus minutus*. Both glyphosate and atrazine are shown to lower the content of pigments in the algal cells. The influence of both atrazine and glyphosate on the content of eicosapentaenoic acid is insignificant, while spermine enhances its percentage.

Key words: algae, atrazine, glyphosate, spermine, *Trachydiscus*

Introduction

The photosynthesizing microorganisms, being a part of the soil microflora, concern the qualities of the soil as a whole and particularly its fertility. They, evolving O_2, aid the roots and, bind CO_2 of bacterial activity. Many of microalgae or cyanobacteria excrete substances, for example polysaccharides, taking part at the forming of the soil structure.

Herbicide treatment of higher plants is usually accompanied with a non-aimed but unavoidable effect on the soil algae. An undesirable aftermath could be long lasting for the photosynthesizing microorganisms. It is reasonable, that the experiments concerning plant protection have to be conducted with both higher plants and soil algae. There are well documented studies in the area which have been carried out using the green algae *Chlorella* and *Scenedesmus*, as well as the cyanobacteria *Anabaena* and *Nostoc* (Lipok et al., 2010). Experiments with cyanobacteria and microalgae, enhancing the seed germination (Petkov, 2010, 2011) or similar experiments with mineral oil polluted soils have shown that photosynthetic microorganisms facilitate the growth and development of higher plants. Biotransformation of harmful chemicals and effectors in the soil is boosted by the presence of cyanobacteria and microalgae. Their total mass in fertile soils is a pretty big amount, thus it means that they contribute considerably to the biological transformation of harmful chemicals, or herbicides as in this particular case.

The yellow-green alga *Trachydiscus minutus* has not been studied in detail prior to the first decade of the 21st century due to the fact it has easily been mistaken with the algae of genus *Chlorella*. *Trachydiscus minutus* was first isolated from cooling water of Temelin Nuclear Power Plant, where it represented nearly mono culture, however it was later estimated that it is widespread all over the world, albeit in low concentrations.

Microalgae are both an important part of ecosystems and a subject of biotechnology. That is why we have accepted to follow a complex approach to their understanding and possible applications. The photoautotrophic biotechnology of

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algae is in search of new prospective objects. According to recent publications (Iliev et al., 2010; Řezanka et al., 2010) and our experiments *Trachydiscus minutus* proves to be one of them. It is known that the cells of the algae have a high amount of lipids and proteins, lack of chlorophyll b, and some useful biotechnological qualities (Lukavský et al., 2010). Currently, there is no published data available to describe the effects of herbicides on its growth and biochemical composition.

The aim of this work is to study the effects of the glyphosate-containing herbicide Roundup and the effects of atrazine on the growth of *Trachydiscus minutus*, its pigment and protein composition, its lipid composition and fatty acid profile.

Materials and Methods

The alga *Trachydiscus minutus*, strain Lukavský and Přibyl 2005/1, was grown in laboratory as mono-algal culture under 24h continuous light from luminescent tubes. The light intensity was 8000 lx ($180.m^{-2}.s^{-1}$). The culture was bubbling with 3 $cm^3.s^{-1}$ air enriched with 0.5% CO_2. The temperature of the culture was 26°C. The alga was cultivated in nutrition medium of Zehnder (Staub, 1961), in vessels with a volume of 200 cm^3. The initial algal density was 0.4 g/l. Control culture and three concentrations, namely 5 mg/l, 20 mg/l, 40 mg/l, of herbicide Roundup with an active ingredient isopropylamine salt of glyphosate (GIPA) were applied. Atrazine was applied at concentration 20 mg/l, spermine at 35 mg/l, and their mixture at the same concentrations. Algal biomass was centrifuged 20 min at 3000 g, washed and dried at 105°C to constant weight, and the growth was measured gravimetrically every 24 h.

Proteins were analyzed according to Lowry's method, chlorophyll and carotenoids were analyzed spectrophotometrically after methanol extraction (McKinney, 1941). Lipids were extracted with chloroform/methanol (2:1), 3 times for 0.5 h under reflux, evaporated under vacuum, and reextracted with chloroform. The total lipids were estimated gravimetrically, converted to fatty acid methyl esters with methanol containing 6% anhydrous HCl for 1.5 h under reflux. Methyl esters were extracted with hexane and purified by TLC on silica gel with hexane/diethyl ether (10:1). Fatty acids were analyzed GC on a Perkin-Elmer instrument (Iliev & Petkov, 2006).

Results and Discussion

The influence of glyphosate on growth of *Trachydiscus minutus* was studied in detail. The control cultures lacking herbicide are able to grow exponentially for at least 192 hours. The algal biomass, calculated as dry weight increases six-fold for this period. All of the cultures with herbicide were showing signs of dying after the 168th hour. The growth of the cultures with concentrations of GIPA 20 mg/l and 40 mg/l was fully inhibited, while the growth of the cultures with 5 mg/l was only slightly influenced (Figure 1).

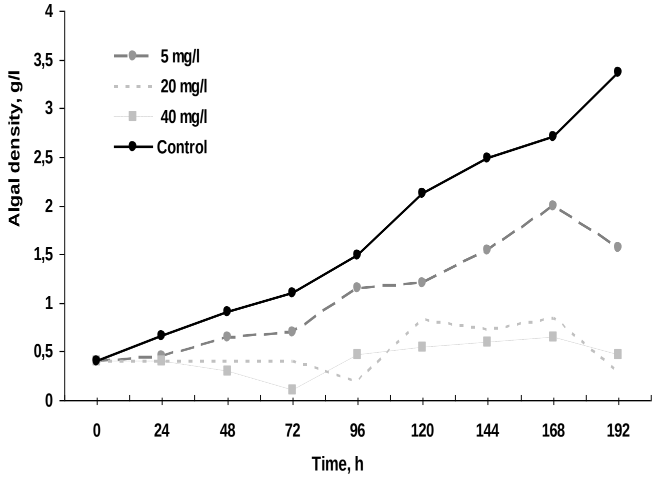

Figure 1. *Influence of glyphosate on the growth of Trachydiscus minutus*

Microscopic observations showed that nearly 70 % of the cells in cultures with concentrations of GIPA 20 mg/l and 40 mg/l were granulated, but merely 10 % at GIPA 5 mg/l. The influence of glyphosate on the protein and total lipid content is insignificant. The effect of the herbicide on the pigment content at 192 h is significant, especially on carotenoids (Figure 2). The concentrations are chosen after a set of experiments in order to find the statistically lowest acceptable one, which is detectable spectrophotometrically 48 h after treatment (Figure 3). Glyphosate poorly changes fatty acid percentage. Polyunsaturated fatty acids arachidonic and eicosapentaenoic remain unchanged and this could be mentioned as a lack of oxidative damage (Table 1).

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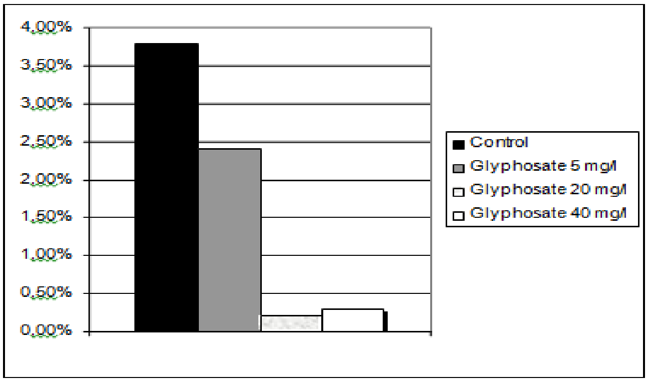

Figure 2. *Carotenoids percentage (% w/w) of glyphosate treated T. minutus.*

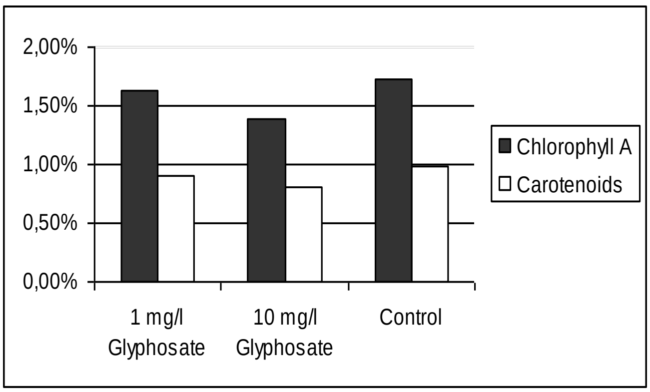

Figure 3. *Pigment percentage (% w/w) of glyphosate treated T. minutus.*

Recent studies with higher plants have proved that it is possible to prevent the herbicide action after treatment with atrazine if the plant is sprayed with both the herbicide and a polyamine which acts as a protector (Zheleva et al., 1994; Stoynova et al., 1999). A question arises whether it is possible to achieve the same effect with the alga *Trachydiscus minutus* if a protector was added to the nutrition medium together with the herbicide. Our studies show that the growth of the cultures with concentrations of atrazine 20 mg/l is fully inhibited (Figure 4).

The growth of the culture, containing atrazine of the same concentration and spermine as a protector with concentration 35 mg/l was also inhibited. The pigment composition decreases twice after atrazine treatment (Figure 5). Taking into consideration that the growth of treated cultures is marginal or absent the real yield of pigments is multiple time lower.

Table 1. *Fatty acid percentage (% w/w) of glyphosate treated T. minutus.*

Fatty acid	5 mg/l	20 mg/l	40 mg/l	Control
12:0	1.8	0.4	6.7	2.1
14:0	16.5 ± 0.2	12.6	19 ± 1	19 ± 6
16:0	11.6 ± 0.6	11.7	13 ± 4	16 ± 1
16:1	8 ± 2	5.4	6.5 ± 0.7	8 ± 2
18:0	1.3 ± 0.9	1.6	2.4 ± 0.5	2.2 ± 0.6
18:1	6 ± 4	8.6	7.7 ± 0.5	12 ± 2
18:2	12 ± 1	18.4	14.4 ± 0.9	11 ± 2
γ-18:3	0.2 ± 0.05	tr.	0.2	0.3
α-18:3	3.3	1.0	6 ± 3	3.0 ± 0.1
20:4	5 ± 1	2.5	3 ± 1	3 ± 1
20:5	35 ± 4	37.9	24 ± 9	30 ± 1

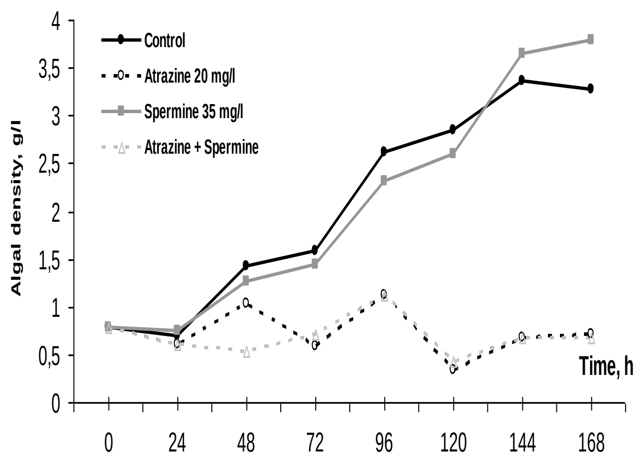

Figure 4. *Influence of atrazine on growth of T. minutus*

Our studies confirm that spermine is not able to reverse herbicide damage if the algae *Trachydiscus minutus* is treated with atrazine. Obviously results and experience obtained by higher plants cannot be applied to algae and vice versa.

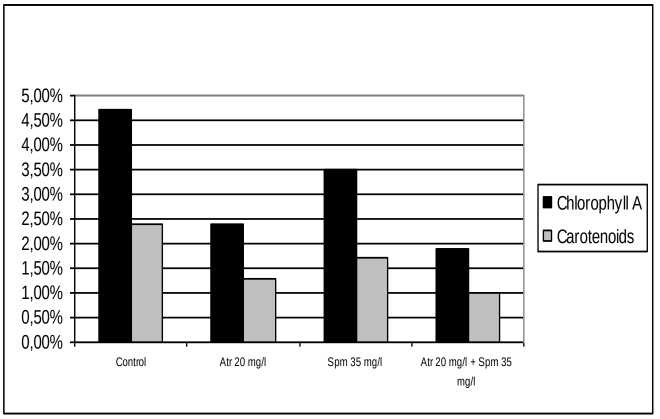

Figure 5. *Atrazine percentage (% w/w) of atrazine treated T. minutus.*

Whether spermine is unable to enter the cell of the alga, which might be due to the anatomical and morphological differences between higher plants and algae, or the alga assimilates spermine without being protected, are assumptions that have to be proved. The changes of fatty acid percentage (Table 2) tend to show that the second proposal is more likely to be correct. It is visible that the herbicide has no negative effect on the membrane and storage lipids (Table 2).

Table 2. *Fatty acid percentage of T. minutus (% w/w)*

Fatty acid	Control	20 mg/l atrazine	35 mg/l spermine
12:0	2.3	tr.	1.5
14:0	48.4	32	16.4
16:0	5.7	13	4.0
16:1	4	2.1	5.2
18:0	1.7	0.2	1.5
18:1	0.1	0.3	0.2
18:2	1.4	6	4.5
γ-18:3	tr.	tr.	tr.
α-18:3	0.1	3.6	0.2
20:4	1.5	0.7	3.5
20:5	35	42.7	63

The percentage of eicosapentaenoic acid significantly increases when add spermine in the medium, and which is more, the average growth of spermine treated culture remains the same as of the control (Table 2). That means an increase of the yield of the acid. Eicosapentaenoic acid is one of the target products of *Trachydiscus* and it is worth studying the algal behaviour at different concentrations of spermine.

Acknowledgement

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