THE INFLUENCE OF THE ISOPROTURON HERBICIDE ON SOME PHYSIOLOGICAL PROCESSES IN *CHLORELLA VULGARIS* BEIJERINCK

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ABSTRACT

The reactions of *Chlorella vulgaris* Beij. cells treated with an urea compound, isoproturon, were investigated. The effects on cell numbers, growth, assimilatory pigments content and gas exchanges were recorded. Isoproturon was added in Arnon nutritive medium of algal suspension in increasing concentrations from 0.03 µM to 0.3 µM. The concentration of 0.03 µM isoproturon decreased the growth rate at 50% from control level. This concentration did not affect significantly the photosynthesis and respiration rate. These processes were significantly inhibited by concentrations higher than 0.08 µM. Assimilatory pigments content showed a sharp decrease from control at all used concentrations and in all sampling days.

Key words: assimilatory pigments, Chlorella vulgaris Beij., growth, isoproturon herbicide, photosynthesis, respiration.

INTRODUCTION

The study of the effects of herbicide applications on algae culture represents an easier way for the understanding of herbicide interference with physiological processes and a considerable amount of resulting information may be extrapolated for higher plants (Böger and Sandmann, 1990, 1993).

Chlorella vulgaris, which is an unicellular green alga, represents an useful model for the study of the herbicide impact on plant physiology.

In this article are presented the effects of an ureic compound, isoproturon (N-4-isopropylphenyl) N', N'-dimethylurea) on growth, gas exchanges and assimilatory pigments content in *Chlorella vulgaris*.

The present study is part of a series of researches concerning the effects of different herbicides on algae physiology (Atanasiu and Dumitrescu, 1996).

MATERIALS AND METHODS

Chlorella vulgaris alga was cultivated on Arnon nutritive medium (Boldor et al., 1983). The experiments were carried out in a chamber with artificial illumination of 8 000 lux. The ambient temperature varied between 24-25^{O}C. To avoid mutual shadowing, the algal suspensions, in cylindrical glass recipients of 1 000 ml, were bubbled with a steady stream of air produced by vibrator devices. The culture medium was inoculated with an amount of algal biomass producing a 100 000 cells/ml suspension in all experiment variants. The results represent average values of two repetitions for each variant.

The isoproturon herbicide was introduced in the culture medium before inoculation and the concentrations of the tested herbicide solutions varied from 0.03 µM to 0.3 µM.

The commercial product Izoguard was used as a source of isoproturon. The Izoguard herbicide has 50% active substance (Şarpe, 1987).

The growth of algae was observed at each two days within the culture cycle (established at 21 days) with a "Cecil 1020" spectrophotometer at l = 676 nm. A strong quadratic relationship (R^2=0.998) was found between extinction and the number of cells counted with a Thoma haemocytometric mount (Figure 1).

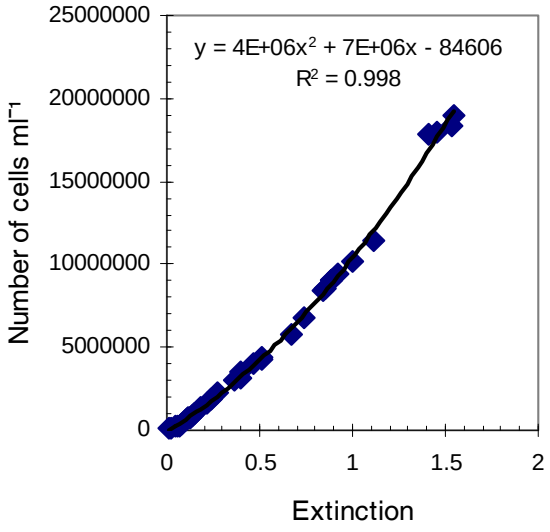

Figure 1. The relationship between extinction and the cells number/ml

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The areas (A) encompassed under the graphs for the number of cells/ml (X) from each concentration (c) at different days (t) were calculated with the following formula:

$$A_{(c,\ t_n)} = \sum_{i=0}^{n} (t_{i+1} - t_i) \times \frac{(x_{(c,\ t_i)} + x_{(c,\ t_{i+1})})}{2}$$

for t = 2, 14 and 21 days.

For each replicate, several simply non-linear regressions were performed between the reductions (% from control) of these areas (X values) and the doses of herbicide as Y values. The equation: $Y = a + bX^3$ was preferred because the confidence intervals were narrower than those obtained by other types of equations which produced better values for R^2. The equations for each replicate were used to interpolate the LD 0, 25, 50, 75, 100% for these replications in order to calculate the standard deviations. The regressions for all the replicates from a given day were used to calculate a common R^2. The interpolated values with these equations were similar with those obtained from replicates. This mathematical treatment integrates the influence of each herbicide concentration over the whole duration of experiment.

The photosynthesis rate was investigated with Warburg method using the treated algal suspension and the Warburg buffer solution number 9 (15 ml Na_2CO_3 0.1 M + 85 ml $NaHCO_3$ 0.1M) (Boldor et al., 1983).

The respiration rate was established with Warburg method using the treated algal suspension and a solution of KOH 30% to capture the CO_2 resulted in the respiration process (Boldor et al., 1983).

The absorbance of the solutions extracted with 100% acetone were measured at 661.6, 644.8 and 470 nm and the calculation of the content in a, b chlorophylls and carotenoids was performed according to Lichtenthaler (1987); the values were expressed in mg/g dry matter (DM).

RESULTS AND DISCUSSIONS

The influence of isoproturon on growth in each treatment was observed from the second day after herbicide application. The growth depression was proportional to the applied dose and there were clear cut differences between treatments with the exception of 0.3 µM treatment which did not differ significantly from 0.18 µM treatment. These last two concentrations prevented any significant growth till the 9th day. After this, a small increase in the extinction of these variants was noticed. The treatments with 0.03 µM, 0.08 µM and 0.13 µM isoproturon induced an increasing growth rate from the 7th till the 11th day of the culture cycle. After that, these three variants maintained themselves at an almost constant level (Figure 2).

The procedure described in "Material and methods" was used to fit the a and b empirical parameters for equation $Y = a + b * X^3$, where the X values are the differences from control for the areas under the growth curves (%) and Y is the herbicide dose which is expected to produce this effect. The values for 0.3 µM isoproturon concentration were excluded from calculation because this concentration is obviously over the lethal level and its effect is not significant different from that produced by 0.18 µM in growth of cell number of *Chlorella*.

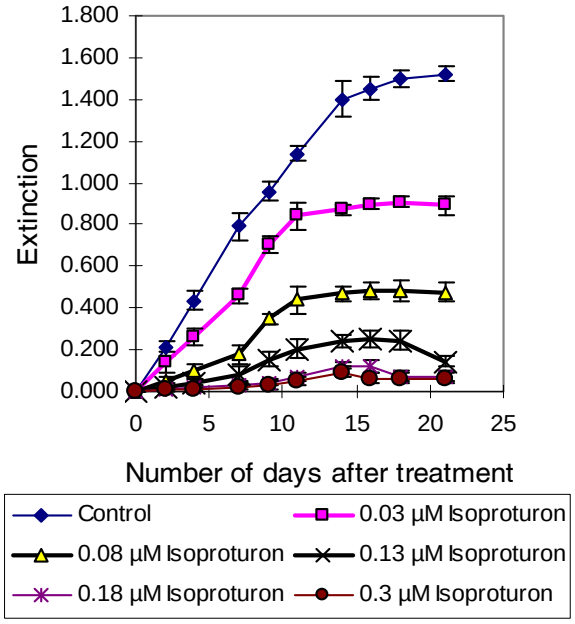

Figure 2. The influence of isoproturon on extinction of culture of *Chlorella vulgaris*[*]

[*] The vertical bars represent standard deviations

Table 1. Isoproturon growth inhibitory concentrations (μM) for *Chlorella vulgaris*

Days	2		14		21	
Inhibition %	Average	SD*	Average	SD*	Average	SD*
0	0.0111	0.0082	0.0085	0.0039	0.0051	0.0029
25	0.0135	0.0086	0.0113	0.004	0.0080	0.0031
50	**0.0304**	**0.0114**	**0.0309**	**0.0049**	**0.0279**	**0.0039**
75	0.0762	0.0188	0.0841	0.0074	0.0820	0.0062
100	0.1654	0.0332	0.1878	0.0122	0.1873	0.0106

*) SD = standard deviation

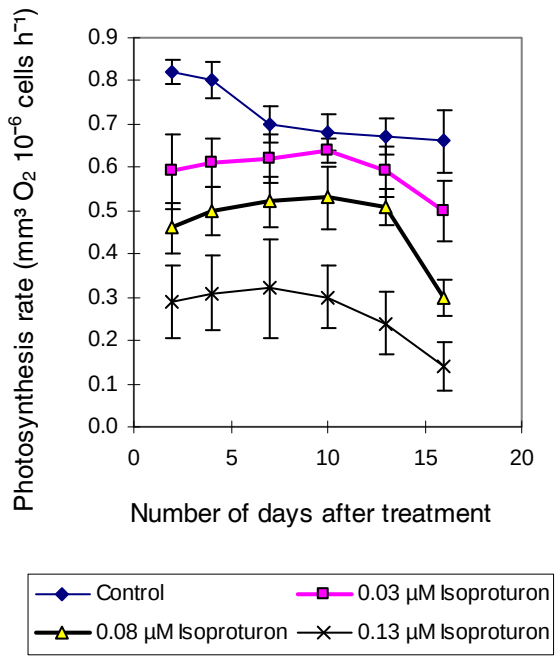

Figure 3. The influence of isoproturon on photosynthesis rate of *Chlorella vulgaris*

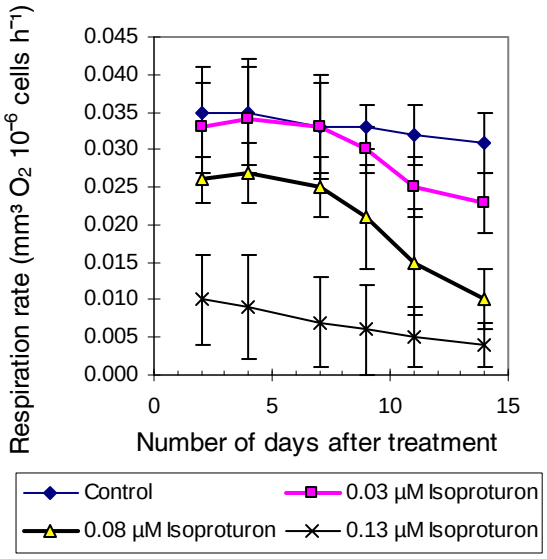

Figure 4. The influence of isoproturon on respiration rate of *Chlorella vulgaris*

The lethal concentrations of isoproturon for 2, 14 and 21 days calculated with the presented method are shown in Table 1. The concentration reducing growth at 50% of control level is about 0.03 μM and the concentration over 0.18 μM isoproturon reduces growth at the minimal level.

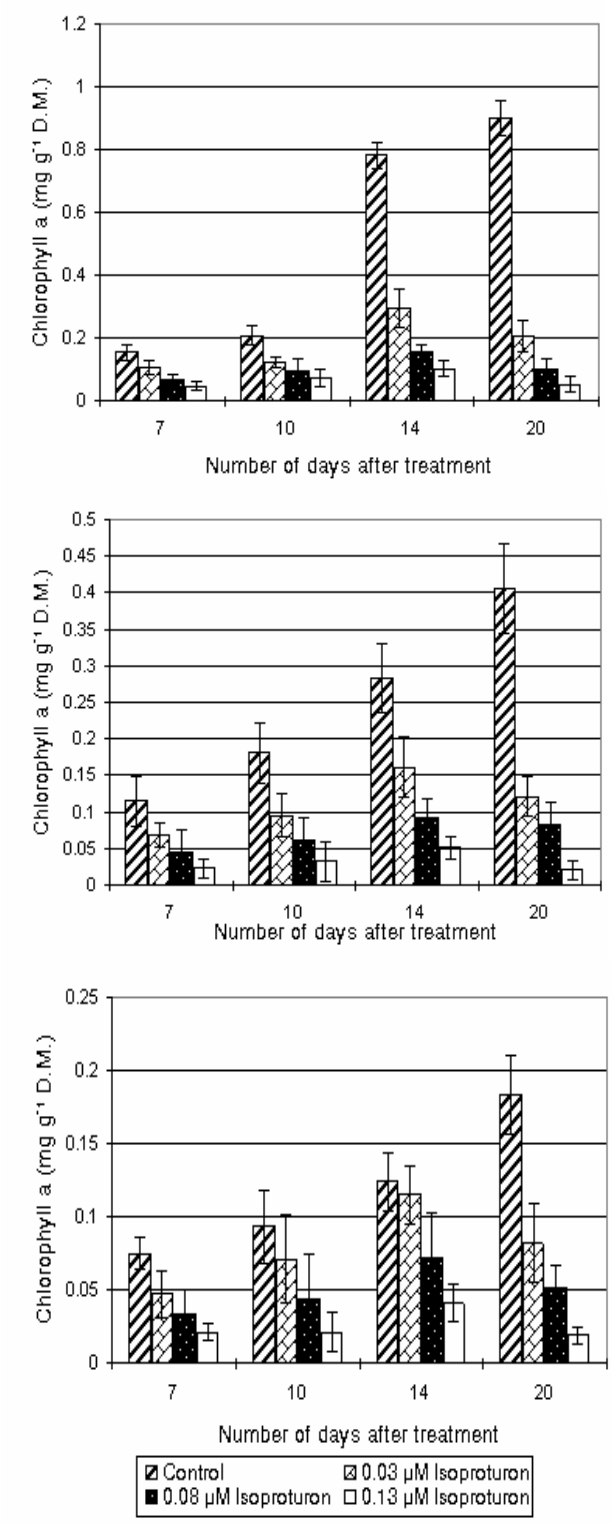

Figure 5. The influence of isoproturon on chlorophyll a, b and carotenoid pigments contents in *Chlorella vulgaris*

The effect of isoproturon on photosynthesis rate is obvious for the variants treated with 0.08 μM and 0.13 μM concentrations. The process in algal cultures treated with 0.03 μM was significant different from control for the first 4 days but for the second week the differences were lower due to lower rate of photosynthesis in control (maybe as a result of an unavoidable mutual shading that occurred in high cell densities cultures). For each treatment the observations from the 16th day were somehow lower than the averages of the other observations (Figure 3).

The respiration rate for the 0.03 μM treatment is not significant different from control and the 0.08 μM dose produced a difference from control, larger than two standard deviations only from the 11th day. The inhibitory effect of the 0.13 μM isoproturon dose was clear from the second day of treatment. One may notice a decreasing in respiration rate from the 9th day of the culture cycle (Figure 4).

The assimilatory pigments content for each treatment apart increased until the 14th day of the experiment and than it was observed a decrease of the chlorophylls and carotenoids content. But the assimilatory pigments content showed a sharp decrease from control at all used concentrations and in all sampling days (Figure 5).

The results show that the isoproturon herbicide induced a quick and sharp limitation of the physiological activity related with algal cell growth in liquid suspension.

CONCLUSIONS

Growth rate in *Chlorella vulgaris* decreased at 50% and 100% from control level by concentrations of 0.03 μM and 19 μM isoproturon respectively.

In the first days of the experiment photosynthesis was inhibited by the dose of 0.03 μM isoproturon and respiration rate was significantly inhibited by concentrations higher than 0.08 μM isoproturon.

Isoproturon in all tested concentrations showed an apparently inhibitory effect on the assimilatory pigments content in all sampling days.

Chlorella vulgaris proved to be an useful biological model for the study of the effect of isoproturon on some physiological processes.

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