Determination of optimum concentration of Diuron for the growth and bloom of the algae (*Scenedesmus obliquus*) in in Vitro condition

Fallahi M.[1]*; Rahbary S.H.[2]; Shamsaii M.[2]

Abstract

Growth rate of the green algae, *Scenedesmus obliquus*, influenced by Diuron, with the trade name of (Karmex) and chemical name of (N'-(3,4-Dichlorophenyl)-N,N-Dimethylurea), was examined in the present study. This study is performed in the laboratory of National Inland Waters Aquaculture Institute in Iran during 96 hours in 6 treatment and 3 replicates, using 500 cc Erlenmeyer Flasks. Measurement of growth rate in the same period of time was performed by three simultaneous methods of cell counting, measurement of turbidity and determination of the rate of chlorophyll *a*. Quantities of EC_{10}, EC_{50}, EC_{90} and allowable concentration level of this poison for the studied algae were obtained as 0/0019, 0/1, 0/05, 0/02 milligram per liter, respectively. Achieved quantities of EC were used in four 500cc Erlenmeyer flasks each containing more than one million algae cells and in 24h periods adding up to be 96 hours, concentration of the algal cells was evaluated by cell counting. Results of the present experiment showed that the frequency of *S. obliquus* was significantly different in presence of concentrations obtained from EC indicators and decreased sharply in EC_{90} quantities.

Keywords: *Scenedesmus obliquus*, Diuron, Growth rate, Chlorophylla

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1-National Inland Water Aquaculture Institute, P.O. Box 66, Bandar Anzali

2- Islamic Azad University Science and Research branch, Tehran, Iran

* Corresponding author's email: m_fallahi2011@yahoo.com

Introduction

Research on food chain in water ecosystems is very important. Algae contain protein, fats, essential amino-acids, and other nutritions required by aquatics; it has many important roles in growth of fish as a natural food. The Green-algae *S. obliquus* is a phytoplankton of warm water ecosystems, which is used for feeding zooplanktons and filter feeding fishes (Carmicnael and Falconer, 1992). Scenedesmus is used in pools and water ecosystems for feeding different kinds of aquatic larvae and some fishes like silver carps. Also different species of *Scenedesmus* are suitable food for *Daphina* valuable zooplankton (Maosen, 1978). Without a strong management in aquaculture farms, many problems may occur due to phytoplankton blooms (Smiths, 1985).

One of the methods for preparing bio-genes specially N and P is using fertilizers that should be in a small amount in ponds however this appears to be the limiting factor for production (Maoson, 1980). One of the materials which is used for controlling this phenomenon in warm water fish culture is Diuron (FAO, 2001). Diuron has a ring of phenylurea; it is used in control of waste grasses around buildings, railroads, and routes (Feber *et al.*, 2004).

Flecher and Addision (1972) exposed *S. acutus* to Diuron during 14 days in static method. They obtained 0.05mg/l of Diuron as EC50 for growth of this alga. Petrocelli *et al.* (1973) exposed *selenastram carpicronatum* to Diuron. The 50 % effects of this material on the frequency of the algae based on cell counting during 96

hours was estimated to be 0.002 mg/l. Frequency of this algae in presence of Simazin was studied by De vries *et al.* (1991) during 96 hours. This experiment that performed in static method showed 1mg/l Simazin as indicator of EC_{50} for population growth of this alga. Riley and Weil (1987) put *S. obliquus* in exposure of Diuron during 96 hours. They used cell counting method for measurements. They obtained 0.004mg/l as EC50. Geoffrey et al. in 2002 examined the effect of Exiphloron and Diuron on *S. obliquus*. Measurement of changing activities in proximity of the compounds showed that these two poisons inhibit growing of this alga. The effect of Diuron on growth rate of the green algae *S. obliquus* is examined in the present study.

Also EC_{10}, EC_{50}, EC_{90}, and allowed level of Diuron on *S. obliquus* are calculated.

Materials and methods

Effect of Diuron on the algae *S. obliquus* is experimented based on standard method (OECD, 1987). Rate of cell growth of the algae was the criterion in estimating measurement of Diuron poison effects. First Stock of the green algae, *S. obliquus*, was 500cc, prepared in National Inland Waters Aquaculture Institute in Iran. Zinder (Z-8) media was used for culturing this alga. 6 treatments with different densities and control treatment were considered. The medium culture was placed in several 250 ml Erlenmeyer flasks. This experiment had 3 replications, performing static method. Diuron concentrations were selected based on

Wasserweschadstoff-Katalog, 1975, and are presented in table 1. The concentrations used for each treatment were based on logarithmic calculations.

This was between 0.005 -01 mg/l for the experimental treatments (Piri and Ordog, 1997).

Table 1: Table of different toxicity level.

Level	LC50 (mg/l)	Poisonous Degree
A	>500 mg/l	No poisonous
B	100-500 mg/l	Little poisonous
C	10-100 mg/l	Medium poisonous
D	1-9 mg/l	Poisonous
E	<1 mg/l	Very poisonous

(Wasserweschadstoff-Katalog,1975)

Dry algae were added to the medium culture to make a concentration of 1 mg/l (Pirri and Ordog, 1997). Before the experiment, 2 ml of each Erlenmeyer flask treatment was fixed by 37% formalin for primary algae counting. Meyers were aerated and transferred on the Culture table which was equipped with Florcent lamps. A 3500±350 lux light was set for 14 hours light period and 10 hours dark mode. The temperature was 25±2 degrees centigrade. After 6 hours of algae culture, the experiment for determining acute toxicity was run during 96 hours and then operation of aerators was stopped for counting and examination of the algae using a 2 ml subsample for each treatment fixed by 37% formalin (TRC, 1984).

Growth rate was studied by 3 methods: cell counting, measuring turbidity and measuring the rate of chlorophyll a. For cell counting method toma lam and light microscopy (Nikon) were used. For counting number of algae cells in primary (before 96 hours) and secondary (after 96 hours) samples, 1 drop of each sample was placed on the big square of toma (Piri and Ordog, 1997). After 1 minute, numbers of strings for *S. obliquus* were counted. Each of 10 samples was counted and then averages were calculated. The size of each square is 10^{-4} square centimeters and number of algae was calculated in 1 ml according to number of algae in second method. A 10 ml sample was collected from each Erlenmeyer flask for measuring rate of turbidity by spectro-photometers (model 2000-Dr in a wavelength of 750 nanometer).

In the third method chlorophyll a was measured based on standard method (ASTM D3731-87, 1998). Phytoplankton was filtered by filter paper (GFF). A filter paper was used to filter phytoplankton in laboratory tube, and then 10cc ethyl alcohol was added to it. Then the tube was placed in a shaker. After homogenization the samples were transferred to bain-marie. The temperature of Bain-marie after 20 minutes was set at 75-78°C. Then chlorophyll was extracted in the alcohol. Finally chlorophyll samples were centrifuged and measured by

spectrophotometer (Hitachi, 2000uv) once by 2N hydrochloridric acid and once without acid. Spectrophotometer was done with absorption in length waves of 750 and 665 using the following formula. Rate of achieved chlorophyll *a* based in μg:

$$(b665\text{-}b750)\text{-}(A665\text{-}A750)\times \frac{V}{V_2}$$

b665 = absorption of liquid before adding acid.
b750 = absorption of liquid before adding acid.
A665 = absorption of liquid after adding acid.
A750 = absorption of liquid after adding acid.
V = Mass of Alcohol in CC
V₂ = mass of filtered water in litre

EC_{10}, EC_{50}, EC_{90} and Minimum Allowable Concentrations (MAC values) of Diuron on this algae base on cell counting method and rate of chlorophyll *a* were determined. In next stage of the experiment, in 3 Erlenmeyer Flasks containing the green-algae, *S. obliquus,* which were abundant and in the boundary of bloom (clear change color), experimental concentrations (MAC values) were prepared. Each Erlenmeyer was sampled for cell counting in each of the two algae treatments during 24, 48, 72, 96 hours of the experiment.

Results
Results of the experiment showed that survival of the green-algae, *S. obliquus*, in presence of Diuron decreased quantitatively in EC_{10}, EC_{50} and EC_{90} results using cell counting method and determination of the rate of chlorophyll *a* calculated in the method of measuring turbidity. The reducing process for the population of the algae in each of treatments was observed.

Results of different methods used in the present study were compared.

Results of cell count method
Increasing percentage of algae cell numbers showed no normal distribution by Shapiro-Wilk test. So Kruskal Wallis test was used for comparing increasing percentages between different treatments. Results showed that percentage of increasing growth was significantly different between treatments ($p<0.05$). (Chi-Square = 15.633, df = 6, $p = 0.016$)

Table 2: Effective quantities of Diuron poison on *S. obliquus* based on cell counting.

Quantities in mg/l	MAC values $EC_{50}/10$	EC_{10}	EC_{50}	EC_{90}
Diuron	0.0019	0.005	0.019	0.006

Correlation between probit value and logarithm of algal concentration was: ($r = 0.97$, $p = 0.002$, $n = 6$) Results suggested that correlation probit value and logarithm of algal concentration was highly significant at 0.01 level and below linear correlation between is presented ($p<0.01$). Mann-Whitney test showed the treatments were significantly different ($p<0.01$).

Figure1: Equation of line and correlation coefficient and logarithms of algal concentrations with p.v levels for effect of Diuron on concentration of *S. obliquus* base on cell counting.
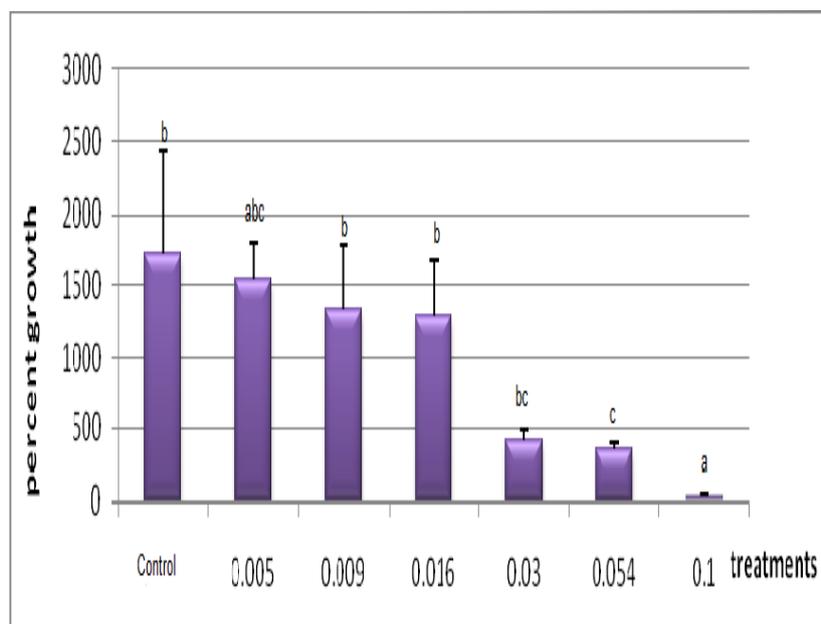
Figure 2: Percentages of increasing growth of the algae (*S. obliquus*) in presence of Diuron based on cell counting.

Results of the method based on the chlorophyll a

Table 3: Effective quantities of the poison, Diuron, on *S. obliquus* based on measuring chlorophyll *a*.

Quantities in mg/l	MAC value $EC_{50}/10$	EC_{10}	EC_{50}	EC_{90}
Diuron	0.005	0.02	0.05	0.01

Correlation between probit value and logarithm of algal concentration was:
($r = 0.97$, $p = 0.0017$, $n = 6$)
Results suggested that correlation between probit value and logarithm of concentrations was highly significant at 0.01 level that created a linear correlation as below ($p<0.01$):

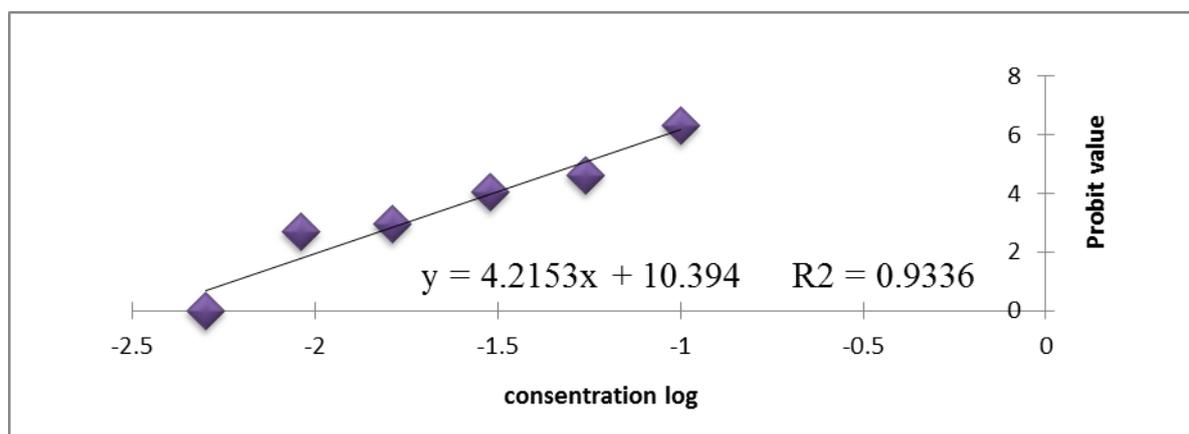$$y = 4.2153x + 10.394 \quad R2 = 0.9336$$

Figure 3: Equation of line and correlation coefficient and logarithms concentration with p.v levels for the effect of Diuron on concentration of *S. obliquus* based on chlorophyll *a*.
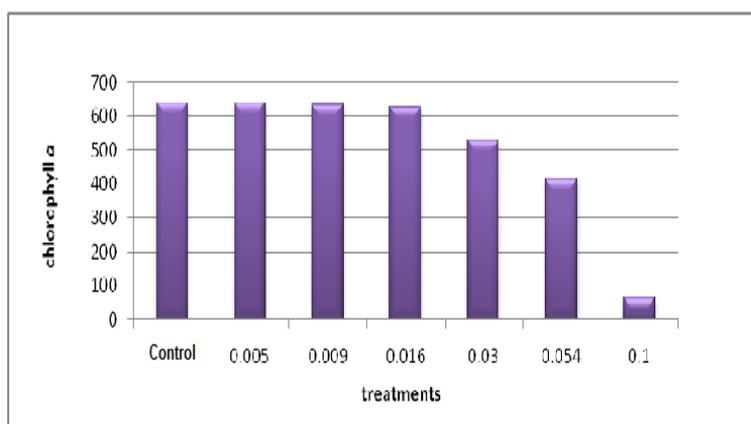

Figure 4: Percentages of increasing growth of the algae (*S. obliquus*) in presence of Diuron based on chlorophyll *a* (µg/L).
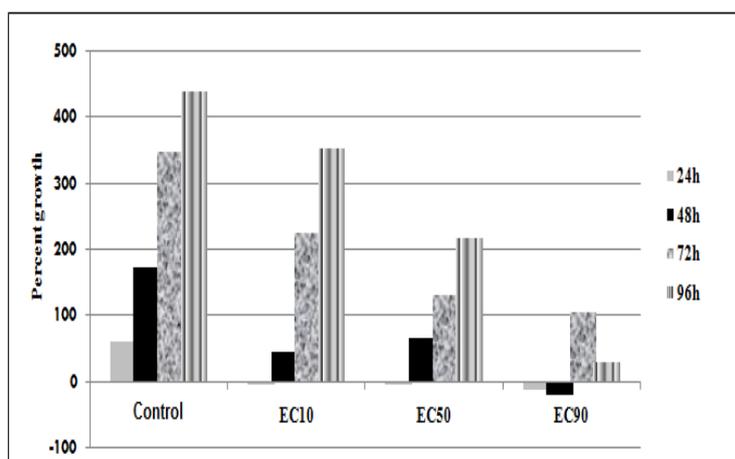

Figure 5: Evaluating of the growth of the *S. obliquus* in each 24 hours period, during 96 hours, based on cell counting method.

Discussion

Comparing achieved quantities of EC_{10} with numbers of toxicity levels showed that Diuron is very toxic. So, greater amounts of EC_{10}, would seriously damage fresh water ecosystems. But in the present study it seems that Diuron has a positive

effect in bloom control of *S. obliquus* population.

Linear regression diagram emphasized lower growth with increasing of Diuron condensation. Also more growth of the algae was observed in presence of Diuron. Treatment 1 had more growth and treatment 6 had less growth. Results of the two methods used for measuring were both acceptable. Comparison of the resulted quantities showed that more growth of the algae was observed by EC_{10} and EC_{50} indicators. An increase in growth in the last day caused considerable reduction in the obtained EC_{10} indicators. However, the two other indicators still showed increase in growth of the algae.

Riley and Weil (1987) exposed the green algae, *Chlorella vulgaris,* to Diuron for 96 hours. They obtained an EC_{50} indicator level of 0.004 mg/l based on cell counting, which was much closed to results of the present study. The obtained EC_{10} in the present study for the green-algae, *S. obliquus,* based on cell counting was 0.005 mg/l. The 50% effect of Diuron on population based on cell counting during 96 hours was 0.002 mg/l, the achieved rate of EC_{50} in the present study for the algae, *S. obliquus,* based on cell counting was similarly 0.0019 mg/l. Impacts on population of green-algae in the presence of Simazin is studied by De Vries et al. (1991) during 96 hours . This experiment performed in static method and expressed 0.1 mg/LEC_{50}, as indicator for growth population of the algae.

Comparing effective rate of Diuron with results of using Simazin showed that toxic effect of Diuron for the green-algae, *S. obliquus*, is less than that of Simazin and caused less prevention of growth and cell increase. In other words, Diuron is better to control harmful algal bloom (such as some species of blue – green algae) than Simazin because less damage to green algae.

If the rate of this poison in water ecosystem decreases, it will be useful for other live components of the ecosystem. Of course the algae, *S. obliquus,* is one useful species for warm water fish nutrition and contains chlorophyll *a*. As this material has the most impact on chlorophyll *a*, it may be resulted in controlling blooming of this green-algae and can be used in control of algal bloom. *S. obliquus* has chlorophyll *a* and Diuron causes serious damages to chlorophyll.

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