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Toxicity of Glyphosate and Ethoxysulfuron to the Green Microalgae (*Scenedesmus obliquus*)

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In this study, toxic effects of two herbicides which contain glyphosate and ethoxysulfuron for the green microalgae *Scenedesmus obliquus* (Turpin) Kützing were investigated and 96 h median effective concentrations (EC_{50}) were determined. The experiments were conducted with static bioassay method by three replicates. A control group and 6 different concentrations for both herbicides (800, 400, 200, 100, 50, 25 mg L^{-1} for glyphosate and 8, 4, 2, 1, 0.5, 0.25 mg L^{-1} for ethoxysulfuron) were used. The regression equations of percentage inhibition and herbicide concentrations were calculated. The EC_{50} value of herbicides which contains glyphosate and ethoxysulfuron for *Scenedesmus obliquus* were 80.0 mg L^{-1} (95 % confidence interval = 47.63-118.19 mg L^{-1}) and 1.1 mg L^{-1} (95% confidence interval = 0.74-1.54 mg L^{-1}), respectively. The results indicated that the toxicity of ethoxysulfuron to *Scenedesmus obliquus* is 80 times more than glyphosate.

Key Words: *Scenedesmus obliquus*, Glyphosate, Ethoxysulfuron, Toxicity.

INTRODUCTION

Agricultural ecosystems are environments which are affected by additional substances such as fertilizers and pesticides. A wide range of pesticides are used in agricultural practises and there is a risk of contamination of aquatic environments. Pesticides' toxicity is usually not limited to the location where they are applied. They reach other locations and environmental compartments through various physical transport processes, adversely affecting organisms that happen to be present[1]. Assessment of human exposure to pesticides and other toxicants through biological monitoring offers one means to evaluate the magnitude of potential health risk of this chemicals[2,3].

Their increased usage has elicited extensive research into pesticide effects on non-target organisms. Algal toxicity tests are increasingly being used in bioassay

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test batteries for environmental management of chemical discharges and it has been observed in several studies that for a large variety of chemical substance, algal tests are relatively sensitive bioassay tools[4].

Microalgae are organisms which are most widely-used in pesticide bioassays to determine the toxic effects of pollutants to the ecosystems. They are used as bioassay organisms due to their sensitivity to the pollutants. There are many investigations concerning the toxicity of herbicides and various industrial chemicals on microalgae[5-14]. However, less information is available for the toxicity of glyphosate and ethoxysulfuron. The accumulation of pesticides depend on the nature of pesticide, its solubility, its concentration in the medium, the concentration of algal cells and the size of the cells[5]. Herbicides can affect the structure and function of aquatic communities by altering the species composition of an algal community[15].

The objective of this study was to determine the toxic effects of glyphosate and ethoxysulfuron to the microalgae *Scenedesmus obliquus* which was isolated from natural waters. Displaying the toxic effects of these herbicides to the growth rate of *Scenedesmus obliquus* and comparing the toxic effects of these herbicides is critically important for aquatic ecosystems.

EXPERIMENTAL

The algae *Scenedesmus obliquus* (Turpin) Kützing belongs to Chlorophyceae were selected for the toxicity tests. It was isolated from a freshwater fish aquarium with standard isolation methods[16]. *Scenedesmus obliquus* were grown in a medium recommended by the Turkish Standards[17]. The culture medium was autoclaved at 121 ºC and 15 psi for 15 min. The algae in the logarithmic growth phase were inoculated to 250 mL erlenmayer flasks, amounting to 100 mL of the culture media, the compound and the algae. The initial cell concentration of algae was *ca.* 1×10^5 cells mL^{-1}. Flasks were shaken periodically to prevent clumping of the cells. Direct microscopic counts were performed using a Neubauer counting chamber by the aid of a Nikon microscope. pH of the culture medium was adjusted at 7.1 ± 0.2. Triplicate bioassay experiments were carried out for each concentration and control. The cultures were incubated at 24 ± 2 ºC under continuous light (average light intensity of 78 µmol m^{-2} s^{-1}) produced by cool-white fluorescent lamps.

Glyphosate, N-(phosphonomethyl) glycine, is a systemic and non-selective herbicide used to kill broadleaved grass and sedge species[18]. Accordingly, glyphosate inhibits the activity of EPSP (5-enolpyruvyl shikimate-3-phospate) synthase enzyme belonging to shikimic acid metabolic pathway in plants and as a result reduction in protein synthesis and growth and death in cells can be seen[19].

The suggested effective dose for glyphosate[20] is 300-1000 mL da^{-1}. Water solubility[21] at pH 2 is 10.5 ± 0.2 g L^{-1}. In this study, soluble concentrate formulation of glyphosate-isopropylamine salt which contains 48 % of glyphosate (Knockdown 48 SL) was used.

Ethoxysulfuron is a selective herbicide used for specific weeds and it belongs to sulfonylurea chemical class. Generally ethoxysulfuron is used in cotton, rice, sugar cane, turf and wheat culture. Ethoxysulfuron is inhibiting acetolactate synthase in plants and thereby blocking the biosynthesis of branched chain amino acids. This stops cell division and plant growth[22].

The suggested application dose for this herbicide[23] is 3-5 g da^{-1}. Water solubility is 5.5 g L^{-1} at pH 3 for phosphoric acid, 1353 g L^{-1} for 0.1 M potassium dihydrogen-phosphate at pH 7 and 5452 g L^{-1} for sodium hydroxide[24] at pH 10. In this study, water dispersible granule formulation of ethoxysulfuron which contains 60 % of ethoxysulfuron (Sunrice 60 WG) was used.

The inhibition test was carried out in accordance with the Turkish Standard[17]. The organisms were exposed to 6 concentrations of herbicides for 96 h. The cells were counted at 24, 48, 72 and 96 h after the beginning of the experiment. A wide range of concentrations were developed in a previous test to determine the adequate toxicity for each herbicide. EC_{50} (0-96 h) values with 95 % confidence limits were estimated by probit analysis by using EPA software.

RESULTS AND DISCUSSION

In this study, toxicity of glyphosate and ethoxysulfuron to the green alga *Scenedesmus obliquus* (Turpin) Kützing were investigated and 96 h median effective concentrations (EC_{50}) were determined.

Toxicity of glyphosate: Toxicity of glyphosate on *Scenedesmus obliquus* was evaluated for 6 different concentrations ranging between 25-800 mg L^{-1} (Fig. 1).

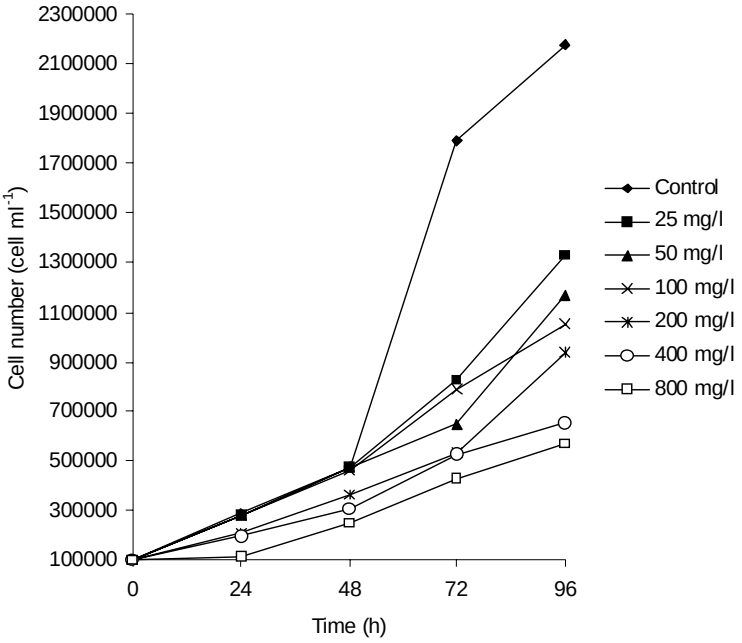

Fig. 1. Cell numbers at different concentrations of glyphosate by time

At the highest concentration which was selected by the result of range finding test, growth rate was reduced to 88 %, as compared with the control and this rate was 11 % at the lowest concentration. With 0.01 % concentration of glyphosate, the observed inhibition rate was 50 %. According to these results, the main experimental concentrations were determined from a logarithmic scale.

The results of the main test that was conducted with 6 various concentrations (800, 400, 200, 100, 50, 25 mg L^{-1} Knockdown), selected in respect of the concentration caused 50 % growth inhibition are presented in Table-1. The concentration of herbicide which contains 48 % glyphosate and led to 50 % growth inhibition on *Scenedesmus obliquus* at the end of 96 h was determined as 80.002 mg L^{-1}. 95 % lower and upper confidence limits for the EC_{50} were 47.634 mg L^{-1} and 118.186 mg L^{-1}, respectively. The toxic substances whose median effective concentrations are changing between 10-100 mg L^{-1} for the aquatic organisms are slightly toxic substances according to ecological toxicity categories[25]. In this context, it was concluded that glyphosate is a slightly toxic herbicide.

TABLE-1
DOSE RESPONSE RELATIONSHIPS OF TWO HERBICIDES TO *Scenedesmus obliquus**

Herbicide	Regression equation	Significance level	R^2	EC_{50} (mg L^{-1})	95 % Confidence limits (mg L^{-1})
Glyphosate	y=10,387x + 76.651	0.0001	0.9804	80.0	47.63-118.19
Ethoxysulfuron	y = 11,83x + 131.12	0.001	0.9448	1.1	0.74-1.54

*y = Percentage inhibition; x = Natural logarithm of herbicide concentration.

Ma[26] investigated the toxic effect of the glyphosate (95 %) on *Scenedesmus obliquus* and found that the EC_{50} value is 55.9 mg L^{-1} for this herbicide. This difference with present findings probably resulted from the different glyphosate concentration contents of the herbicides. Additionally, the different initial cell concentration, temperature and light intensity in the studies might be the other factors.

In another study, which was investigated the toxicity of the herbicide containing 95% glyphosate (TC), *Scenedesmus quadricauda* was used as test organism and it was determined that the 96 h(r) EC_{50} value[13] is 70.5 mg L^{-1}.

Chlorella pyrenoidosa used as a test organism in another study and determined that the 96 h EC_{50} value of the herbicide which contains 95 % glyphosate[3] is 3.530 mg L^{-1}. It was concluded that the 96 h EC_{50} value is 590 mg L^{-1} for the same algal species and glyphosate[27]. It was reported[8] that 20 mg L^{-1} or more of glyphosate completely inhibited algal growth, photosynthesis and chlorophyll a synthesis in *Scenedesmus quadricauda*.

Toxicity of ethoxysulfuron: Toxicity of ethoxysulfuron on *S. obliquus* was evaluated for 6 different concentrations between 0.25 and 8 mg L^{-1} (Fig. 2).

At the highest concentration which was selected by the result of range-finding test, growth rate was reduced to 86 %, as compared with the control and this rate

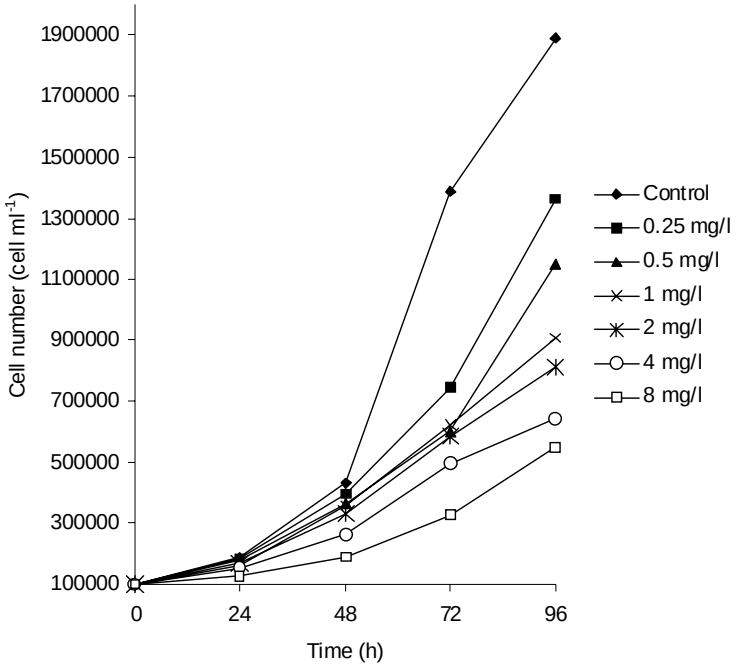

Fig. 2. Cell numbers at different concentrations of ethoxysulfuron by time

was 13 % at the lowest concentration. With 0.0001 % concentration of ethoxysulfuron, the observed inhibition rate was 53 %. According to these results, the main experimental concentrations were selected from a logarithmic scale.

The results of the main test that was conducted with 6 various concentrations (8, 4, 2, 1, 0.5, 0.25 mg L^{-1} Sunrice), selected in respect of the concentration caused 50 % growth inhibition, are presented in Table-1. The inhibition percentage values, concerning mean cell numbers for 96 h, range between 71-28 %. The concentration of herbicide which contains 60 % ethoxysulfuron and led to 50 % growth inhibition on *Scenedesmus obliquus* at the end of 96 h was determined as 1.1 mg L^{-1}. 95 % lower and upper confidence limits for the EC_{50} were determined as 0.74 and 1.54 mg L^{-1}, respectively. The toxic substances whose median effective concentrations are changing between 1-10 mg L^{-1} for the aquatic organisms are moderately toxic substances according to ecological toxicity categories while below 1 mg L^{-1} categorized as highly toxic[25]. In this context, it was concluded that ethoxysulfuron is a moderately-highly toxic herbicide.

The toxic effects of some sulfonylurea herbicides like nicosulfuron, metsulfuron-methyl, cyclosulfamuron, tribenuron and ethametsulfuron on *Scenedesmus obliquus* were investigated and determined EC_{50} values ranged[26] between 2.8 and 155.7 mg L^{-1}. The values gained in present study are similar to the lowest value of this study, but lower than it. The results demonstrated that there is a differential response of *S. obliquus* to herbicides at the same chemical class.

Ma and Liang[28], used different herbicides of sulfonylurea to examine the toxic effects on *S. obliquus* and determined the EC_{50} values as ranged between 11.8 and 56.8 mg L^{-1}. The impact mechanism of growth inhibition on algae by herbicides is related with enzyme systems and it causes damages on vital activities[29,30].

In light of the foregoing, it is concluded that the response of an algal species to the various herbicides and even various formulations of one herbicide is changed and there is a variation between the sensitivity of the organisms which live in the same environment and the different species of the same genus. Present investigation shows that impact mechanism of the herbicides shifts and determining the toxic effects of the formulations of a specific herbicide to the algae individually is considerable as a matter of assessing harmful effects to the ecosystem.

In conclusion, the EC_{50} value of ethoxysulfuron is lower than the EC_{50} value of glyphosate for *S. obliquus* and it is determined that ethoxysulfuron can be effective on microalgae at low concentrations. Ethoxysulfuron is more toxic than glyphosate and the concentration of it should be designated attentively in agricultural practices. The findings of this study are important in terms of arranging the harmless application doses of these herbicides to the aquatic environment.

REFERENCES

1. J.W. Deneer, *Pesticides Manag. Sci.*, **56**, 516 (2000).
2. V.K. Bhatnagar, J.S. Patel, M.R. Variya, K. Venkaiha, M.P. Shah and S.K. Kashyap, *Bull. Environ. Contam. Toxicol.*, **48**, 302 (1992).
3. J. Ma, W. Liang, L. Xu, S. Wang, Y. Wei and J. Lu, *Bull. Environ. Contam. Toxicol.*, **66**, 536 (2001).
4. J. Ma, R. Zheng, L. Xu and S. Wang, *Ecotoxicol. Environ. Safety*, **52**, 57 (2002).
5. S.A. Shehata, O.A. Aly and H. Farag, *Environ. Intnat.*, **10**, 225 (1984).
6. H. Geyer, I. Scheunert and F. Korte, *Chemosphere*, **14,** 1355 (1985).
7. M.I. Abdel-Hamid, *Water Sci. Technol.*, **33**, 129 (1996).
8. P.K. Wong, *Chemosphere*, **41**, 177 (2000).
9. G. Lu, X. Yuan and Y. Zhao, *Chemosphere*, **44**, 437 (2001).
10. M. Sáez, A. Gómez-Parra and E. Gónzalez-Mazo, *Fresenius J. Anal. Chem.*, **371**, 486 (2001).
11. C. Sabater, A. Cuesta and R. Carrasco, *Chemosphere*, **46**, 953 (2002).
12. I. Moreno-Garrido, M. Hampel, L.M. Lubián and J. Blasco, *Bull. Environ. Contam. Toxicol.*, **70**, 242 (2003).
13. J. Ma, F. Lin, S. Wang and L. Xu, *Bull. Environ. Contam. Toxicol.*, **71**, 594 (2003).
14. J.E. Djomo, A. Dauta, V. Ferrier, J.F. Narbonne, A. Monkiedje, T. Njine and P. Garrigues, *Water Res.*, **38**, 1817 (2004).
15. J. Ma, X. Ligen, S. Wang, R. Zheng, S. Jin, S. Huang and Y. Huang, *Ecotoxicol. Environ. Safety*, **51**, 128 (2002).
16. R.A. Andersen, Algal Culturing Techniques, Elsevier Academic Press, Amsterdam, p. 578 (2005).
17. Turkish Standards, TS EN 28692, Ankara (1996).
18. C. Cox, *J. Pesticide Reform*, **18**, 3 (1998).
19. Anonymous, Pesticides in Ontario: A Crititical Assessment of Potential Toxicity of Agricultural Products to Wildlife, with Consideation for Endocrine Disruption, Canadian Wildlife Service, Technical Report Series, 369, p. 112 (2002).
20. http://www.safatarim.com.tr. (2006).
21. EC Working Document, Glyphosate 6511/VI/99-final (2002).

22. http://avpma.gov.au/gazette 0501 p14.pdf (2006).
23. http://www.bayercropscience.com.tr. (2006).
24. EC Working Document, Ethoxysulfuron 7461/VI/98-final (2002).
25. Anonymous. Glyphosate Herbicide Information Profile: United States Department of Agriculture, Forest Service Pacific Nuorthwest Region (2000).
26. J. Ma, *Bull. Environ. Contam. Toxicol.*, **68**, 275 (2002).
27. A. Maule and S.J.L. Wright, *J. Appl. Bacteriol.*, **57**, 369 (1984).
28. J. Ma and W. Liang, *Bull. Environ. Contam. Toxicol.*, **67**, 347 (2001).
29. M. Wiegand-Rosinus and U. Obst, *Environ. Toxicol. Water Qual.*, **7**, 313 (1992).
30. M. Megharaj, I. Singleton and N.C. McClure, *Bull. Environ. Contam. Toxicol.*, **61**, 108 (1998).

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