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Letter to Editor

Growth Rate of *Scenedesmus acutus* (Meyen) in Cultures Exposed to Trifluralin

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Abstract

The objective of this study was to determine the effect of trifluralin on the growth of *Scenedesmus acutus* in comparison to growth without trifluralin during 5 days. For growth in trifluralin 20-40 μg/L, four days of *S. acutus* growth occurred compared to growth in the absence of trifluralin. This decrease in growth in *S. acutus* was correlated with an increased amount of trifluralin concentration. At trifluralin concentration of 60 μg/L, growth was maintaned for three days. *S. acutus* in the presence of at 80 μg/L trifluralin showed one-day growth. It was concluded that increasing trifluralin concentration decreased growth of *S. acutus* in a dose-dependent manner.

Keywords: trifluralin, algal sensitivity, *Scenedesmus acutus*

Introduction

In the present world environmental problems are multiple and complex, especially those arising from the disposal of identifiers and the assessment of the toxicity of such substances [1]. The wide use of pesticides in recent years has caused concern about the fate of the chemicals in the environment. There is little evidence that herbicide residues are accumulating in aquatic environments, but the amount of herbicides used yearly has been increasing faster than any other group of pesticides. Many herbicides are applied to the soil and thus may eventually enter aquatic systems [2]. In all parts of the world pesticides have been found in the aquatic ecosystem and often information of how these pesticides affect inhabiting organisms is missing. The use of herbicides has increased substantially in the last four decades and has contributed to both increased crop yields and decreased production costs. Although the instructions for use of these chemicals are aimed at minimizing the risk of contamination of aquatic environments, residuals of pesticides can be detected in water courses draining agricultural areas.

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Among the living organisms in aquatic ecosystems, phytoplankton communities are key targets for herbicide contamination because of their ecophysiological similarities with terrestrial plants (i.e., a potent sensitivity of the same metabolic processes) and their function of primary producers (i.e., a change in quality and quantity of primary producers will lead to a global ecosystemic perturbation) [3]. However, herbicides also pose a threat to both the environment and human health. Much of the research has been focused on phytoplankton (microalgae), because these organisms form the basis of food chains in the aquatic environment. Effects of herbicides in the ecosystem do not remain restricted to target organisms but rather extend to non-target organisms such as algae, which play an important role in the primary production of the aquatic ecosystem [4]. The significance of phytoplankton as primary producers as well as their ability to intrinsically alter the balance of aquatic ecosystems has warranted greater concern due to the toxic effects of widely accepted pesticides [5].

Algae are known to be comparatively sensitive to many chemicals, and the inclusion of these organisms in test batteries has been shown to improve the capac-

ity of the battery to predict the most sensitive ecosystem responses [6]. The toxicity of some pesticides on algae is higher than toxicity reported by several authors on organisms like zooplankton, filter-feeding invertebrates and fishes [7-9]. This is an important fact because in the freshwater ecosystem, algae are important primary producers in the food chain, with phytoplankton providing food for a diverse community of invertebrates and fishes. Depending on pesticide toxicity, wetland contamination could result in a die-off of many present algal species, decreasing this food source [10].

Contamination of surface waters by herbicides has been reported to have direct toxic effects on populations of phytoplankton. Herbicides can affect the structure and function of aquatic communities by changing the species', composition of an algal community.

The effects of herbicides on growth, photosynthesis, survival, reproduction, membrane permeability and other metabolic activities of algae have been studied by different authors [5]. On the other hand, freshwater algae often are used as test organisms in laboratory ecotoxicological investigations. Most of the information on the ecotoxicity of pesticides is related to the chemical tests either under laboratory conditions or separately in experimental ecosystem studies. Single species laboratory tests may indicate the approximate concentration at which particular species are likely to be directly affected by pesticide contamination in natural waters [11].

Material and Methods

Trifluralin, an emulsifiable concentrate and its percent of 480 g/l, was obtained from Koruma Tarim Inc. (Turkey). Trifluralin (α,α,α-trifluoro-2.6-dinitro-N,N-dipropyl-p-toluidine) was used as a test chemical, which is a dinitroalinine herbicide used to control a wide spectrum of annual grasses and broadleaf weeds in agriculture, horticulture, viticulture, amenity and home garden.

The green alga *Scenedesmus acutus* was isolated from plankton samples in a fishpond. This alga was grown in sterilized Jaworski's Medium. The media was composed of distilled water and the following chemical ingredients $Ca(NO_3)_2.4H_2O$, KH_2PO_4, $MgSO_4.7H_2O$, $NaHCO_3$, EDTA FeNa, EDTA Na_2, H_3BO_3, $MnCl_2.4H_2O$, $(NH_2)6Mo7O_2H_2O$, $NaNO_3$, Na_2HPO_4, Cyanocobalamin, Thiamine and Biotin. The culture medium was sterilized at 121°C, 1.05 kg cm^{-2} for 30 min. Single species experiment was carried out to know the change in the total cell number. *S. acutus* was exposed to various concentrations of trifluralin. The incubation time chosen to investigate the effect of trifluralin on growth of *S. acutus* was 5 days (120 hours), which is comparable to previous studies on the effects of other chemicals on algae growth.The stock and experimental cultures were grown in the same liquid medium at a temperature of 23± 1ºC and a light intensity of 2000 lux on a 16 h light and 8 h dark photoperiod. The inocula was prepared from these culture to provide an initial cell density 12000 cell/mL of *S. acutus* in treated and control culture. Trifluralin was added to the *S. acutus* culture just after inoculums. Control cultures were incubated in the same medium without trifluralin.

The experimental sets were run in triplicate, and all cultures were hand shaken twice daily. After the cultures were incubated, *S. acutus* cells were counted with an inverted microscope at 0, 24, 48, 72, 96 and 120 h (1, 2, 3, 4, 5 days).

Results and Discussion

The objective of this study was to determine the effects of trifluralin on the growth of *Scenedesmus acutus.* The effect of trifluralin was investigated after alga *S. acutus* was exposed to various concentrations at 12, 24, 36, 48, 96 and 120 hours. Control cultures with the same cellular density as the treated ones were prepared, in order to determine the effect of trifluralin on population growth of *S. acutus*. Green alga was affected in a different manner from each concentration of trifluralin. Fig. 1 shows the population growth of the cultures exposed to different concentrations of trifluralin.

The observation of toxic effects of trifluralin on *S. acutus* indicated a significant decrease in population growth, with respect to the control in the treated cultures at all assayed concentrations on the first day. *S. acutus* in control culture was counted as 26000 cells/mL. But in treated culture to 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/L of trifluralin, *S. acutus* was counted as 23983, 23250, 23666, 21166, 20583, 18066, 14933, 13916, 13900 and 11500 cells/mL, respectively. Especially, growth rate of *S. acutus* decreased in treated cultures with 120, 140, 160, 180 and 200 µg/L trifluralin.

On the second day, *S. acutus* was counted in control culture as 32766 cells/mL. The effect of trifluralin on population growth of *S. acutus* was noticed when growth of all treated cultures significantly decreased with respect to the control at second day of inoculation. *S. acutus* de-

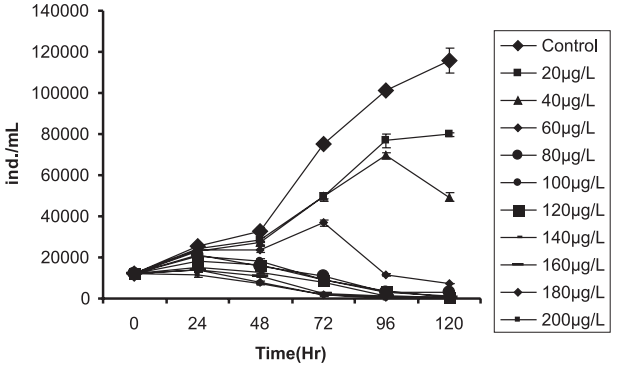

Fig. 1. Effect of trifluralin to population growth of *Scenedesmus acutus.*

clined by 28,500, 27,000, 18,750, 15,583, 18,166, 16,566, 12,600, 10,966, 7,900 and 7,066 cells/mL at 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200µg/L, respectively. The effects of trifluralin on *S. acutus* were noticed when growth ration of some treated cultures decreased significantly with respect to the control at 48 hr of exposure. On the contrary, at some treated cultures, there was a growth recovery of those cultures exposed to 20, 40 and 60 µg/L, giving no significant decrease in growth when compared to the control. The cultures exposed from 80 µg/L and higher showed a significant inhibition growth respect to the control.

However, on the third day, there were continued decreases at growth of *S. acutus* in treated cultures but green algae was a significant increase in control culture. On the fourth and fifth days, population growth of *S. acutus* were significantly decreased in treated cultures with trifluralin. At concentrations of 180-200 µg/L trifluralin, growth of *S. acutus* was completely finished.

The effects of herbicides on growth, reproduction, photosynthesis and other metabolic activities of algae were studied by different workers [12-14]. Investigations with different green algal species have shown that algae vary greatly in their response to chemicals. Differential sensitivity of the green algae to the compounds could induce species shifts within communities [15]. The loss of a few particularly sensitive phytoplankton species from a community containing hundreds of species may not be considered significant, as long as the function of the community remains unchanged. Most of the work in mixed culture of algae showed that application of pesticide resulted in elimination of sensitive species [5]. Sensitivity to toxicants is important in determining the suitability of a test for adoption into chemical regulations. The toxicities of some pesticides to species of phytoplankton and toxicity data published for several species of phytoplankton with other herbicides have shown that the variations in sensitivity may be considerable [1, 6]. In the present study, different concentrations of trifluralin decreased the population growth of *S. acutus*.

The results of this study indicate that sensitivities to trifluralin of *S. acutus* began in first day of inoculation and in this time, the algae decreased in treated culture with trifluralin.

Growth rates of *S. acutus* were always high between 0 to 5 days in control cultures but treated cultures with trifluralin continuously decreased at all concentrations between 1 to 5 days. In contrast, in the control culture the growth rate of *S. acutus* was always positive at intervals of 1 to 5 days. In general, the growth rate of *S. acutus* was found to be negative correlated with high concentrations of trifluralin.

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