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Full Length Research Paper

Growth rate of *Scenedesmus acutus* in laboratory cultures exposed to diazinon

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The effects of the pesticide, diazinon on growth rate was examined in unialgal cultures of freshwater green algae, *Scenedesmus acutus* through 96 h acute toxicity tests. *S. acutus* was exposed to different concentrations of diazinon (1, 2, 4, 8, 16 and 32 µl) in the laboratory maintained at 23 ± 1°C and 16:8 light : dark regime. Cell numbers were determined daily and growth rates were calculated for a period of 4 days. The growth rate of *S. acutus* in the control cultures was higher at 0 to 4 days (40.000 to 276600 individuals), but the growth rate of the treated cultures with diazinon decreased at 2 to 4 days (28000 to 10320 individuals). The results demonstrated adverse effects of diazinon on freshwater green algae (*S. acutus*). Thus, the application of this pesticide for pest control in agriculture must be done carefully since any disturbance affecting algae with similar or higher sensitivity will have severe repercussions on higher trophic levels.

Key words: Acute toxicity, algae, diazinon, growth rate, *Scenedesmus acutus*.

INTRODUCTION

The total arable land is decreasing today as the competition between emerging countries grows day by day. There are attempts to obtain more products from the unit area on land and aquatic ecosystems to overcome the hunger problem which will be more obvious in the coming years due to the rapidly growing world population (Chen et al., 2007). There is a widespread application of pesticides to control pests and diseases that attack agricultural products. Today, more than 10,000 chemicals are used for industrial and agricultural purposes (Katsumata et al., 2006). During the last century, by the help of the technological developments, chemical products mostly succeeded to improve the human health and life quality, to improve agricultural production, and increase the living conditions and general quality of life (Katsumata et al., 2006). However, besides these positive effects, the pollution caused by both the production process of these chemicals, and the chemicals themselves, present many new problems.

Particularly, chemical application to increase the amount of products and protect the agricultural products from the pests and diseases are done in an uncontrolled way; and most of these chemicals are carried far away from the areas they are applied. Contamination of water bodies appears to be the unavoidable consequence of agricultural activities, mainly related to the use of plant protection products (Kyriakopoulou and Anastasiadou, 2009). The pesticides which are applied on extensive agricultural area may combine with the soil particles or reach underground water sources by mixing with the irrigation and rain water. In aquatic systems, pesticides are a common type of contaminants (Relyea, 2009). The pesticides dissolve in water, easily adhere to hydro-cycle, whereas some combine with soil particles and accumulate. The pesticides which mix with the underground and surface water, have a limit value for the living organisms which responds to their changing structures with several receptive environments (Cetin and Mert, 2006; Chen et al., 2007; Daam et al., 2009; Dewez et al., 2008). Any concentration above these limit values affects the living organisms in a negative way. The absorbed amount of pesticide from soil media to water media by a living organism is expressed as bioconcentration. Although, pesticides are designed specifically to destroy

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unwanted target organisms, their application may cause many diverse problems to non-target organisms like algae, fish, birds and even humans. The effects of pesticides on aquatic environment were frequently evaluated using organisms such as fish, water flea and algae (Yeh and Chen, 2006).

The planktonic form the first step of the bio-concentration process in water systems. Algae form the most parts of plankton in water habitats and play a functional role in the habitats they live in as the primary producers (Fairchild et al., 1998). In aquatic media, algae form the base of the organic production as the primary producers and they are extremely sensitive to the physical and chemical changes occurring in the media (Sabater et al., 2002). Some reports on the comparative sensitivity of pesticides toward various green algae have been published (Ma et al., 2004a, b; Bengitson et al., 2005). Much information on the toxicological aspects of insecticides on green algae has been complied (Ma et al., 2005; Relyea, 2009). However, there is little information about the toxicological aspect of diazinon on algae (Ma et al., 2005). 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 (Ma and Liang, 2001; Sabater et al., 2002).

It is important to expand the knowledge about harmful effects of toxic substances on population growth of planktonic algae, since they are an important component of aquatic systems. Algae are the key targets for the pesticide contaminations because of their similarity with other vegetative organisms. Diazinon is widely used in developing countries such as Turkey to control pests. Therefore, in this study, a green alga, *Scenedesmus acutus* was investigated in axenic culture exposed to different concentrations of diazinon.

MATERIALS AND METHODS

Diazinon is from a group called organophosphorus substances and a synthetic chemical also commercially known as bazudin. Its chemical name is phosphorothioate (O_2O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl).

S. acutus was collected from a fishpond using plankton ladle and grown in Jaworski liquid medium (Thompson et al., 1988). 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 temperature and 1 atmospheric pressure for 15 min. *S. acutus* was exposed to various concentrations of diazinon (1, 2, 4, 8, 16 and 32 µl). Algal toxicity test of 96 h (4 days) of exposure was conducted following the general design of Environmental Protection Protection Agency (EPA, 2000). After the inoculation, Erlenmeyer flask was incubated in a 23±2°C culture growth cabinet, on a 16 h light, 8 h dark photoperiod and triplicate samples were taken every 24 h. The cells were counted in a phytoplankton microscope.

RESULTS AND DISCUSSION

Diazinon is the most widely used pesticide by homeowners on lawns. The effects of diazinon on the changes of time dependent freshwater algae growth are shown in Figure 1. Addition of 1, 2, 4, 8, 16 and 32 µl diazinon affected algae growth differently. On the first day of inoculation, approximately, the same number of *S. acutus* was counted in the control group and cultures exposed (1 µl diazinon) to pesticide (45.000 ind./ml). However, on the second day, *S. acutus* growth was decreased to 37.9% in treated culture as compared to the control. The decrease continued and *S. acutus* population growth decreased by 54.3% on the third day, 75.2% on the fourth day as compared to the control group. When the diazinon concentrations were increased, algal growth decreased significantly. The population growth of *S. acutus* in treated cultures with 2 µl diazinon decreased significantly, 4.5% on the first day, 39.1% on the second day, 57.7% on the third day and 80.7% on the fourth day as compared to the control group (Figure 1). On the first, second, third and fourth days, the cell count of the algae was 6.7, 41, 58.4 and 85.1%, respectively, in *S. acutus* cultures treated with 4 µl diazinon. When the concentrations of the pesticide were increased to 8 µl, growth of *S. acutus* almost ceased. Culture growth of *S. acutus* with respect to control was decreased at 11.1% on the first day, 52% on the second day, 73.9% on the third day and 88.8% on the last day. *S. acutus* growth in the 18 µl diazinon treated liquid culture was decreased by 8.9, 58, 77.7 and 95% on the first, second, third and fourth days, respectively. With the highest dose of diazinon (32 µl), the growth of *S. acutus* was decreased to 12.6% on the first day, 68.2% on the second day, 85% on the third day and approximately 96% on the fourth day as compared to the control. The results of this study indicated that widely used organic phosphate compounds containing diazinon as active substance affected the growth of *S. acutus*. Increased pesticide concentrations significantly decreased *S. acutus* growth. Different concentrations of diazinon in liquid cultures (1 to 32 µl), reduced algal growth by 5 to 96% of the growth of *S. acutus* population as compared to the control group.

The effects of different pesticides on photosynthesis reproduction and growth and metabolic activities of algae had been investigated by different workers. According to these studies, the high variability of responses exhibited by different species to the same chemical substance can be explained by the morphology, cytology, physiology and genetics of the organisms. The study of Grahl et al. (1981) on phytoplankton colony, especially *Scenedesmus* and *Pediastrum* species showed that 0.325 mg/L pesticide concentration slightly changed the growth of phytoplankton. According to our study, the lowest concentration (1 µl) of diazinon did not affect *S. acutus* growth at the first day, but in the following days, it

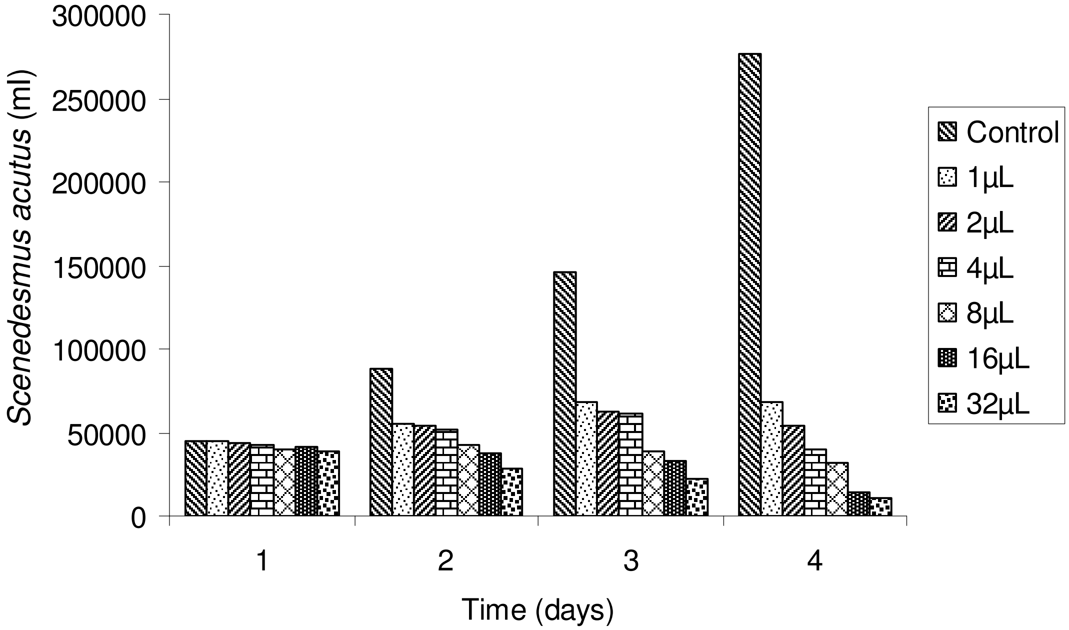

Figure 1. Total cell number of *S. acutus* in the cultures exposed to different concentration of diazinon.

decreased gradually. Relyea (2009) reported that diazinon inhibited the growth of aquatic communities. Hydrophilic organisms, particularly benthic and planktonic algae have been reported in many researchers as very susceptible organisms to physical and chemical environmental changes (Kaur et al., 2002). In a research on the effect of organophosphorus compound on algae, three species of blue-green algae were shown to tolerate 300 to 400 mg/L diazinon concentration (Singh, 1973). In a study by Clegg and Koevening (1974) on the effect of organophosphorus compound, diazonin on algae, three species of blue-green algae were inhibited by 0.01 to 0.1 mg/L diazinon concentration and also inhibited many species of Chlorophyta and Cyanophyta. Wong and Chang (1988) reported that 5 to 10 mg/L diazinon decreased but 20 to 40 mg/L diazinon completely inhibited the growth of *Chlamydomonas reinhardtii*, a member of Chlorophyta. Doggett and Rhodes (1991) investigated the effect of diazinon at various concentrations such as 1, 5, 10, 20 and 40 mg/L on phytoplankton population. They reported that 1 and 5 mg/L concentrations supported the growth of *Selenastrum capricornutum* and *Chlorella* algae but higher concentrations such as 10, 20 and 40 mg/L inhibited the growth of these algae completely. Our results are similar to those found by several investigators with other pesticides. We found that the growth of *S. acutus* was reduced significantly at diazinon concentrations. It has been reported from the study on the effect of diazinon on *S. capricornutum* that 6,400 µg/L diazinon in 7 days reached EC50 concentration (Anonymous, 2005). Firchild et al. (1998) reported that the sensitivity of algae to various pesticides was different and pesticides inhibited the growth of algae by suppressing the biosynthesis of lipid, protein and flavonoids in different physiological ways. The diazinon, an organophosphorus compound, used in our study, prevents the growth of algae in parallel to the concentration and duration of effect, which supports the findings of Firchild et al. (1998). 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 works in mixed culture of algae showed that application of pesticide resulted in the elimination of sensitive species (Ma et al., 2004a, 2005).

Most of the work in mixed culture of algae showed that application of pesticide resulted in elimination of sensitive species (Kyriakopouluo and Anastasiadou, 2009; Daam et al., 2009). The aquatic ecological systems are very complicated. Single-species toxicity tests have historically been the source of biological data for hazard evaluation. Yet it has been discussed as to whether the information from these standard tests alone is enough for predicting the effects at the ecosystem level. The toxicity of pesticides to algal species has been generally ignored by researchers. Cyanophyta, Chlorophyta, Cryptophyta and Bacillariophyta may be good organisms for toxicity tests due to the character of natural aquatic systems (Ma et al.., 2005). As a result, in our modern world where human health and environment is extremely important, chemicals used to increase the fertility in agricultural production, affect directly non-chemical target organisms. Although, the pesticide amount used in Turkey is below the world average, in the intensive farming regions, the usage of pesticides is high. Although, in aquatic media, algae which form the primary production are not directly the target organisms, they are adversely affected by pesticide contamination. Diazinon which is used intensely around our region significantly affected the development (in high concentrations) and almost stopped the growth of *S. acutus* which is an important member of the freshwater phytoplankton.

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