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SHORT PAPER

Effects of Deltamethrin on Lipase Activity in Guppies (*Poecilia reticulata*)

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Abstract

Effect of the deltamethrin, which is extensively used synthetic pyrethroid, was investigated on the lipase activity of *Poecilia reticulata* in the present study. One control and five experimental groups were exposed to different concentrations of deltamethrin. Three experiments, including 60 guppies each, were conducted. Titration method was used in order to determine the lipase activity. Lipase activity level in control group was 5 U/min, while it was 2.5 U/min in the highest concentration of deltamethrin exposed fish. These results revealed that deltamethrin inhibited the lipase activity which may have a negative effect on nutrition and physiological condition of fish.

Keywords: *Poecilia reticulata*, deltamethrin, lipase, pyrethroid insecticides.

Deltamethrinin Lepistes (*Poecilia reticulata*)'deki Lipaz Enzimi Üzerine Etkisi

Özet

Yaygın kullanıma sahip bir sentetik pyrethroid olan deltamehtrinin *Poecilia reticulata*'daki lipaz enzim aktivitesi üzerine etkisi bu çalışma kapsamında incelenmiştir. Kontrol grubu ve 5 farklı deney grubu, deltamethrinin farklı konsantrasyonlarına maruz bırakılmışlardır. Deneyler 3'er defa tekrar edilmiş ve her bir deneyde 60 balık kullanılmıştır. Lipaz aktivitesi titrasyon yöntemi kullanılarak saptanmıştır. Kontrol grubunda lipaz aktivitesi 5 u/dak bulunurken, en yüksek konsantrasyonda deltamethrin verilen grupta 2,5 u/dak olarak bulunmuştur. Deney sonuçları deltamethrinin lipaz aktivitesini inhibe ettiğini göstermektedir. Lipaz aktivitesinde meydana gelen bu değişimin, balığın besin alımını ve fizyolojik şartlarını negatif olarak etkilemesi muhtemeldir.

Anahtar Kelimeler: *Poecilia reticulata*, deltamethrin, lipaz, pyrethroid insektisit.

Introduction

Deltamethrin is an insecticide belonging to the pyrethroid family. Pyrethroids are the man-made versions of pyrethrins, natural insecticides from chrysanthemum, *Chrysanthemum cinerariaefolium*, flowers. It has become much more popular than other insecticides in recent years due to its high photostability, low toxicity on mammals and long persistence in the natural medium (Leake *et al*., 1985; Reddy and Philip, 1994). The pyrethroids used against mosquitos in lake and riverside areas, are carried into wetlands by rainwater and flood from where they were applied (Dhawan and Kaur, 1996; Nemcsok *et al*., 1987). Pollutants can react with each other and develop new forms when entering the water, and they have undesirable toxic effects on living organisms such as fish and arthropods (Moore and Waring, 2001). Pyrethroids are generally safe in mammals and birds but are highly toxic to fish and aquatic invertebrates. Low solubility in water and high lipophillic properties of pyrethroids, cause fast seizing by gills, and lack of hydrolithic enzymes can cause serious harm to fish (Hill, 1989).

Deltamethrin is the most powerful pyrethroid and is used more commonly than other synthetic pyrethroids. Toxic effects of deltamethrin on fish has been covered in the literature (L'Hotellier and Vincent, 1986; Viran *et al*., 2002). Deltamethrin causes severe morphological alterations in the gills and liver (Yildirim *et al*., 2006) and causes toxic effects on the haemobiotic organs, liver and gills (El-Sayed and Saad, 2008).

The fate of chemicals in the aquatic environment depends on the manner of physical transport, physicochemical distribution and transformation.

Because of the low oxygen content of water compared to air, fish and many other aquatic organisms need to imbibe large quantities of water, and the equilibration of chemicals between body and water by passive transport can be very fast. The principal sites of metabolism of chemicals by vertebrate enzymes are the liver and the gastrointestinal tract, followed by lesser activity in the gills, the kidney and the skin (Simon *et al*., 1999). For this reason investigation of the enzyme activity in the gastrointestinal system may help to explain pyrethroid toxicity. Lipase, which is one of the digestion enzymes, is responsible for breaking down fats into fatty acids in the lipid metabolism of living organism. Pyrethroid insecticides may inhibite enzymes in gastrointestinal system, such as lipase. This study was conducted to examine the effects of deltamethrin on the lipase activity of *Poecilia reticulata* and to evaluate its potential impact. Inhibition of lipase activity and other digestion enzymes may affect the utilization of food. Therefore, it may change the physiological state of the fish and leads to an important problem in the feeding of fish (Simon *et al*., 1999).

Materials and Methods

Poecilia reticulata is regarded as the standard test fish commonly used in the bioassay experiments (Greenberg *et al*., 1985). Guppies were obtained from a local breeder in Ankara and brought to the laboratory one month before the study. The fish were kept in glass aquaria and all the aquaria were aerated by air compressors.

Test chambers were filled with 25 L of dechlorinated tap water. The temperatures of the aquaria was regulated at 22±1°C with heaters (Greenberg *et al*., 1985; Viran *et al*., 2002). Some characteristics of this aquarium water were: dissolved oxygen concentration 7.2-7.9 mg/L, pH 7.5-7.7 and conductivity 0.212-0.260 mS.

After concentration determination experiments, tests were conducted in 3 repetition. Randomly selected 10 adult fish with a length of 2.4±0.3 cm were put into each aquarium. Feeding was done once a day and was stopped 48 h before the experiment. After 48 h of adaptation, fish were placed in separate aquaria and were exposed to a series of nominal deltamethrin concentrations.

Technical grade deltamethrin (98%) was supplied from the Insecticide Testing Laboratory of Hacettepe University, Ankara, Turkey. Stock solution was prepared by weighing a certain amount analytical grade deltamethrin, stored at +4ºC, dissolved in acetone. Test concentrations were prepared by using appropriate amount of stock solution. The fish were exposed to 0; 0.02; 0.04; 0.06; 0.08; 0.1 g/L of deltamethrin for 1 hour. The control group received acetone at the maximum acetone volume used in the dissolving of the dosing concentration. The bioassay system was as described in standardized methods (Eaton *et al*., 1995). No mortality was observed in control or treated samples. The statistical analysis of data was made using SPSS 15.0 for Windows using one-way analyses of variance (ANOVA). Differences between means were determined using Duncan's multiple range test in which the significance level was defined as $P<0.05$.

Preparation of the Supernatant

After the exposure, the gastrointestinal tract of the fish was cut out and homogenised in 0.1 M Tris/HCl buffer (pH 7.4). After gentle stirring on ice for 30 min, the homogenate was centrifuged at 2.000 rpm for 20 min, and supernatant was transferred to another tube and it was further centrifuged at 4.000 rpm for 30 min. The supernatant was kept at 50ºC for 10 min to remove proteins. Finally, remaining supernatant was centrifuged at 4.000 rpm again for 20 min, and the clear supernatant was used in the experiment (Simon *et al*., 1999).

Measurement of Enzyme Activity

The experiment mixture contained 1 mL olive oil, 4.5 mL, 50 mM acetic acid (CH_3COOH), 0.1 M calcium chloride ($CaCl_2$) and 1 mL supernatant. The mixture was incubated for 30 min at 30 ºC with continuous stirring at 500 rpm. The enzyme reaction was stopped by adding 20 mL of ethanol (Sugihara *et al*., 1991).

Lipase (EC 3.1.1.3) activity was assayed by titrating fatty acids extracted from olive oil with KOH. The enzyme activities were calculated in accordance with the KOH amount read from the burette. One unit of lipase activity was determined as the amount of enzyme that releases 1 ml of fatty acids free (Sugihara *et al*., 1991).

Results and Discussion

Various concentrations of deltamethrin inhibited the lipase activity in *Poecilia reticulata* as represented in this study. Significance decrease of lipase activity

Table 1. Changes in the activity of lipase in *Poecilia reticulata* following exposure to deltamethrin

Enzyme	Control	0.02 g/L	0.04 g/L	0.06 g/L	0.08 g/L	0.1 g/L
Lipase Activity (u/min)	5.0±0.17	4.32±0.10	3.61±0.10	3.51±0.17	2.82±0.10	2.50±0.11

* The values are expressed as means±SD of three replicates. Doses have been expressed as final concentration in aquarium water.

was observed as the concentration of deltamethrin increased ($P<0.05$) (Table 1). Exposure to deltamethrin for 1 h caused significant reduction in the lipase activity. Lipase activity in the control group was 5 u/min while it was found 2.5 u/min in the group of fish that had been exposed to the highest concentration of deltamethrin (0.1 g/L). Exposure of fish to 0.02 g/L; 0.04 g/L; 0.06 g/L; 0.08 g/L and 0.1 g/L concentration of deltamethrin for the same period reduced the lipase activity by 14%, 28%, 30%, 44%, 50% of the controls, respectively (Figure 1).

Liver and gastrointestinal system are the most important regions, where chemicals are metabolised by the vertebrates enzymes. The chemicals are metabolised at a much lower rate in gills and kidneys. Pesticides are fused to the active center of the enzyme or near it, and might modify (inhibit or activate) the enzyme catalysis. The effects depend on the chemical structure of the pesticide (Simon *et al*., 1999).

There are a few studies that investigate the effect of deltamethrin on digestive enzymes of fish. Simon *et al*. (1999) examined the effects of deltamethrin, permethrin and cypermethrin, which are pyrethroid insecticides, on the digestion enzymes of carp, *Cyprinus carpio*. In their study, the carp was exposed to 10 µg/ml deltamethrin for 5 min and the lipase activity of carp inhibited by 30%. Same concentrations were used in this study with *Poecilia reticulata* however fish were exposed to deltamethrin for 1 hour and 50% lipase inhibition was observed. The results of this study is higher than the findings of Simon *et al*. (1999) in terms of inhibition level of lipase. Lipase inhibition differences between these two studies may be related to exposure times and more than 50% inhibition may be observed if fish exposed to deltamethrin more than 1 hour.

To summarize, in this work we found that deltamethrin has an adverse inhibitory effect on the lipase enzyme activity in *Poecilia reticulata*. Because of the widespread use of deltamethrin that may lead to the exposure in natural ecosystems is likely to have a negative effect on nutrition and physiological condition in wild fish.

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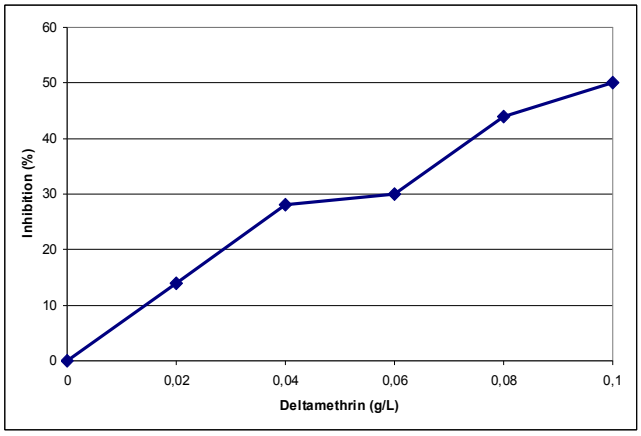

Figure 1. The effect of deltamethrin on inhibition of lipase activity in *Poecilia reticulata*.

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