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The protective effect of vitamin E against changes in fatty acid composition of phospholipid subclasses in gill tissue of *Oreochromis niloticus* exposed to deltamethrin

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HIGHLIGHTS

- The effects of deltamethrin on the phospholipid subclasses were studied.
- The protective effect of vitamin E against deltamethrin was determined.
- The fatty acid compositions were analysed by gas chromatography.
- Deltamethrin induced changes on the fatty acid composition of phospholipid subclasses.
- Vitamin E did not show complete protective effect.

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ABSTRACT

The effects of deltamethrin on the fatty acid composition of phospholipid subclasses (phosphatidylchlonine (PC), phosphatidylethanolamine (PE), phosphatidylinositole (PI) and phosphatidylserine (PS)) in gill tissue of *Oreochromis niloticus* (Perciformes: Cichlidae) and the possible protective effect of vitamin E against deltamethrin were determined by gas chromatography. The changes in the fatty acid profile were analysed after 14 d of exposure. Treatments included Group I (fed with basal diet only), Group II (fed with vitamin E-supplemented diet), Group III (fed with basal diet and exposed to deltamethrin) and Group IV (fed with vitamin E-supplemented diet and exposed to deltamethrin). The effects of deltamethrin on PI, PE and PS were valid for the total saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The effect on PC was detected in total SFAs and total PUFAs. The vitamin E-supplemented diet did not show complete protective effect on fatty acid composition of the fish exposed to deltamethrin. However, the protective effect was observed in total SFAs, total MUFAs and total PUFAs in PC. In PI, protective effect was only recorded on total PUFAs. There was no protective effect in PS and PE. The results of the present study demonstrated that deltametrin exposure had harmful effects on cell membrane and treatment with vitamin E could only partially protect fish gills.

1. Introduction

Pesticides are chemical compounds or biological agents produced and used to prevent plant damage by harmful insects, eliminate these hazardous organisms, repulse and decrease the number of these pests.

Pyrethroids are synthetic derivatives of pyrethrins produced by the flowers of *Chrysanthemum cinerariaefolium* (Luty et al., 2000). Pyrethroid pesticides are preferred due to their strong insecticidal properties and lower toxicity to the non-target animals and specifically mammals. Since the pyrethroids are simply metabolized and they have a short term life in most of the animals. However, there is a different situation in case of the fish. Fish seem to be deficient in the enzyme system that hydrolyses pyrethroids (Haya, 1989). Synthetic pyrethroids are neither fully metabolized nor lose their toxicity quickly in the body of fish. Therefore, residual and deposit of pyrethroids cause quite serious problems. Furthermore,

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the use of pyrethroid pesticides in the program of aquatic larvicide constitutes a potential danger for fish.

Deltamethrin is one of the most effective and commonly used pyrethroid pesticides. Deltamethrin is heavily consumed for the household, agricultural and veterinary applications. The over use of deltamethrin causes the mixing of this pesticide with the water, which in turn affects the aquatic organism life in a negative manner or kills these living creations (Mittal et al., 1994).

Most of the insecticides are hydrophobic molecules that bind extremely strong to the biological membrane especially phospholipid layer (Lee et al., 1991). Due to the lipophilic structures of the pyrethroids, biological membranes and tissues can take the pyrethroids.

Phospholipids have an important role in the biochemistry of all the living cells. Phospholipids constitute the lipid double layer starting the outer border of a cell and also they are structural units for the delimitation of organelles in the cell. The naming of the phospholipids is made in terms of polar head groups. The main phospholipid subclasses of organisms are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS).

Free radicals are important components for the toxic effects of the pesticides and the other environmental chemicals. Pesticides cause the occurrence of free radicals and these free radicals trigger the oxidative stress in the cells (Almeida et al., 1997). It was proved that the oxidative stress occurs by the action of pyrethroids namely deltamethrin (Amin and Hashem, 2012).

Antioxidants are a type molecules that fight with the free radicals harmful for the macromolecules. Vitamin E (α-tokoferol) is a very strong antioxidant and constitutes the first defense line for the protection of polyunsaturated fatty acids located in the cellular membrane phospholipids from negative effects of free radicals (Lucy and Dingle, 1964).

The changes in the composition of the phospholipids in membranes in response to environmental factors, temperature and pesticides, have been reviewed previously (Hazel and Williams, 1990; Kotkat et al., 1999; Zhong et al., 2012).

No studies were found about pesticide effects on the fatty acid composition of phospholipid subclasses in gill tissue. The aim of the current study was to evaluate whether deltamethrin induced changes on the fatty acid composition of phospholipid subclasses in gill tissues in *Oreochromis niloticus* and to investigate the possible protective effects of vitamin E on deltamethrin-induced changes.

2. Material and methods

2.1. Fish

Nile tilapia (*O. niloticus* L.) (32.14 ± 8.27 g and 12.80 ± 1.50 cm) were obtained from the fish ponds in the University of Cukurova (Adana, Turkey). The fish were acclimated to the laboratory conditions for 30 d prior to the experiment. During the adaptation and exposure period, fish were kept under a photoperiod of 14 h light: 10 h darkness at 26 ± 1 °C.

2.2. Feeding

During acclimation period, fish were fed with basal diet (Golden Fish Food) (35% protein) at a rate of 2% body weight/d. In order to prepare the vitamin E-supplemented diet (α-tocopherol content 100 mg kg^{-1}), the basal diet was milled by a feed producer, the vitamin E added and the diets repelletized in a pellet mill. Vitamin E (α-tocopherol acetate) was supplied by Sigma–Aldrich®.

2.3. Experimental design

The LC50 (96 h) value of deltamethrin for Nile tilapia was determined by Golow and Godzi (1994) and 10% of the LC50 (1.45 μg L^{-1}) was selected for the study.

The study was performed in three replicates in order to ensure the reproducibility of the results. All fish used in the experiment were female. Fish were distributed in four different groups, as follows:

Group I (n: 10): Fish were fed with basal diet and kept in pesticide free water.

Group II (n: 10): Fish were fed with vitamin E-supplemented diet and kept in pesticide free water.

Group III (n: 10): Fish were fed with basal diet and exposed to deltamethrin.

Group IV (n: 10): Fish were fed with vitamin E-supplemented diet and exposed to deltamethrin.

After exposure period of the 14 d, three fish from each replicate were anesthetized with MS-222 and sacrificied. Gills were rapidly removed.

2.4. Lipid extraction and separation of phospholipid subclass

The lipids were extracted in chloroform/methanol (2/1 v v^{-1}) according to method of Folch et al. (1957). Butylated hydroxyl toluene (BHT) in chloroform was added as an antioxidant. The homogenate was filtered through Whatman No. 1 filter paper (Cat. No. 1001 150). The filtrate was placed in a separatory funnel. 0.88% KCl solution was added to the filtrate up to 1/4 of the total volume and then the solution was rinsed and left to rest until two clear phases occur. Two ml chloroform was added into the solution taken into volumetric flask after the solvent of the solution was evaporated. Before use, TLC plates were thoroughly wetted with a solution of 1.8% (w v^{-1}) boric acid in ethanol, drained for 5 min at room temperature and dried for 15 min at 100 °C in an oven. The homogenates were rapidly deposited on the TLC plates, which were dried under nitrogen and rapidly placed in the chromatography tank containing the following solvent: chloroform/ethanol/water/triethylamine (30/35/7/35, v v^{-1}) (Leray and Pelletier, 1987; Vaden et al., 2005).

The plates were then dried at room temperature. The PL (phospholipid) fractions were detected under UV light after spraying with 2′, 7′- dichlorofluorescein on TLC plates. The fatty acid composition of the phospholipids was determined after methanolysis. Each phospholipid was converted to fatty methyl ester by transesterification with acidified methanol for 120 min at 85 °C.

2.5. Chromatographic condition

The fatty acid methyl esters were separated by gas chromatography (Shimadzu GC 2010 PLUS; Kyoto, Japan) with a flame ionization detector (FID) and a fused silica capillary column (BP-21) (Bonded Polyethylene Glycol (TPA treated)) (J & W Scientific, Folsom, CA, USA, 25 m × 0.32 mm x 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 0.50 ml min^{-1}. The temperature profiles were as follows: initial temperature, 170 °C (initial time, 2 min), heating rate, 2 °C min^{-1}; final temperature, 220 °C; injector and the detector temperatures, 250 °C. The split ratio was 1/20. The fatty acid esters were identified by comparison with the standard certificate, Supelco 37 Component FAME Mix (Sigma–Aldrich, Bellefonte, PA, USA).

The results were expressed as FID response area relative percentages. The levels of fatty acids were given as percentages.