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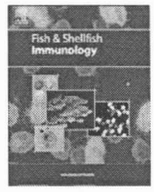

Short communication

Immunologic parameters evaluations in Nile tilapia (*Oreochromis niloticus*) exposed to sublethal concentrations of diazinon

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ABSTRACT

Fish resistance to microorganisms depends basically on the immune response. Although there are several studies on the diazinon mammalian immunotoxicity, in the case of fish there are only few. The aim of present study was to evaluate the effect of diazinon on immunological parameters (relative spleen weight, splenocytes count, lysozyme activity, respiratory burst and IgM concentration) in Nile tilapia. Diazinon at sublethal concentrations (0.39 and 0.78 mg/L) did not alter RSW, splenocytes count or lysozyme activity. However, at the highest concentration tested (1.96 mg/L) diazinon significantly increased respiratory burst and IgM concentration. In summary, diazinon (and perhaps other pesticides) could alter immunological response and induce oxidative stress.

The aquatic environment is continuously affected by pollutants, which could alter the immune response of fishes and induce alterations in host resistance [1,2]. Since organophosphorus pesticides (OPs) are widely used in agriculture [3], the aquatic environment near to fields is frequently affected by OPs such as diazinon (O,O-diethyl O-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl phosphorothioate).

Tilapia (Oreochromis spp.) fish is a teleost with a worldwide distribution, particularly in warm-water aquaculture. Therefore, it serves as a good model for ecotoxicological studies [4]. In addition, the knowledge of pesticide effects on the fish immune system might help reduce financial losses incurred by the aquaculture industry [5]. Knowledge about the effects of diazinon on other relevant immunological parameters is lacking. Hence, this study is designed to investigate the sublethal effect of diazinon on immunological parameters such as relative spleen weight, splenocytes/mm^3, respiratory burst, IgM concentration, and lysozyme activity in the Nile tilapia, *Oreochromis niloticus*.

Juvenile Nile tilapia (*O. niloticus*) (2-months-old, 60.3 ± 12 g) were transferred to an oxygenated 40-L glass aquarium, maintained at constant temperature (26 ± 2 °C) with continuous aeration for a 10-day acclimation period before experiments. A commercial formulation of diazinon (Diazinon Dragon 25 E) was used. According to the CL_{50} previously calculated [6], fish were exposed to three sublethal concentrations of diazinon: 0.39 (1/20 CL_{50}), 0.78 (1/10 CL_{50}) and 1.96 mg/L (1/4 CL_{50}). Ten fish, tested for each concentration used and control conditions (no pesticide treatment), were evaluated simultaneously. The bioassays were carried out under static conditions without solution replacement for a period of 96 h. The average values of water quality were: 26 ± 2 °C, pH 8.0 ± 0.1, 7.0 ± 0.2 mg/L of dissolved oxygen, and 85.4 ± 2.4% oxygen saturation.

The number of splenocytes/mm^3 and the relative spleen weight (RSW) were also determined [7]. A lysozyme assay was performed for the plasma, according to Parry et al. (1965), with modifications. Briefly, 25 μL of plasma from Nile tilapia were mixed with 175 μL of Micrococcus luteus in PBS, pH 5.8. The optical density was determined immediately and after 15 min at 450 nm. Units were determined using an egg lysozyme standard (41,800 Units/ml) [8].

Respiratory burst in splenocytes (phagocytic activity index) was calculated according to Fujiki and Yano (1997) [9], with some modifications [10]. Splenocytes (2×10^6) were mixed with PBS containing 0.1% nitro-blue tetrazolium (NBT) and 6×10^6 cells/mL of Candida albicans. The plasma IgM concentration was measured by ELISA [11,12]

To determine the significance between experimental and control groups, data were compared using the Mann–Whitney test and ANOVA with a subsequent Dunnett's test. All analyses were carried out using Sigma Stat 2.0 software with a significance level set at $p < 0.05$.

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