



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.

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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/mm³, 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 CL₅₀ previously calculated [6], fish were exposed

to three sublethal concentrations of diazinon: 0.39 (1/20 CL₅₀), 0.78 (1/10 CL₅₀) and 1.96 mg/L (1/4 CL₅₀). 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/mm³ 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 × 10⁶) were mixed with PBS containing 0.1% nitro-blue tetrazolium (NBT) and 6 × 10⁶ 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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Table 1
Relative spleen weight and lysozyme activity in Nile tilapia after a 96-h exposure to diazinon.

Parameters	Diazinon concentration (mg/L)			
	control	0.39	0.78	1.96
Splenocytes ($10^6/\text{mm}^3$)	8.78 ± 5.29	6.81 ± 2.77	12.83 ± 10.42	26.28 ± 24.30
Relative Spleen Weight (g)	0.128 ± 0.06	0.173 ± 0.116	0.108 ± 0.04	0.114 ± 0.03
Lysozyme (IU/min/mL)	10.2 ± 2.9	10.3 ± 2.9	14.9 ± 7.5	15.7 ± 5.8

Our results showed no significant differences in relative spleen weight, plasma lysozyme activity or splenocytes/ mm^3 ($p > 0.05$) between experimental and control groups (Table 1). In contrast, the highest diazinon concentration (1.96 mg/L) had an effect on respiratory burst activity and IgM concentration (Fig. 1).

Previously, our group showed that 7.83 and 3.91 ppm diazinon induced significant decreases in RSW [6]. The results presented here show that sublethal concentrations of diazinon (0.39, 0.78 and 1.96 mg/L) did not affect RSW or the splenocyte count. However, in mice, diazinon (25 mg/kg for 28 days) was shown to have a negative effect on the same parameters [13]. At higher doses (300 mg/kg for 45 days), diazinon also induced morphological abnormalities such as inflammation, fibrosis in the capsular region, and atrophy of the red pulp [14].

Lysozyme plays an important role in the innate immune system, effectively protecting against Gram-positive bacterial infections [15]. None of the diazinon doses tested here affected lysozyme activity. To the best of our knowledge, this is the first study evaluating the effect of diazinon on lysozyme activity.

Fish phagocyte cells engulf invading foreign agents and normally kill them by releasing reactive oxygen species (ROS), such

as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^\cdot). In this study, when fish were exposed to 1.96 mg/L of diazinon, an overproduction of ROS was observed. Since a large amount of ROS released by phagocytes could induce oxidative damage [10,16], our results suggested that diazinon may provoke oxidative stress. As some biochemical events involved in fish phagocytic respiratory burst are almost identical to those in mammals, comparison between these very different taxa could shed some light on diazinon toxicity and its mechanism. In PC12 cells exposed to 30 μM diazinon, an increase in lipoperoxidation, measured by TBARS, was found [17]. ROS overproduction can lead to oxidative damage in cell suspensions [10] and in vivo. The latter could depend on the water level within an organ and antioxidant mechanisms like glutathione or enzymatic systems. Since the antioxidant systems of the spleen and thymus are less efficient than that of the liver, it may be that these organs experience greater oxidative damage. ROS are not the only free radicals produced by macrophages. Reactive nitrogen species could be produced during the bactericidal activity of macrophages [18]. These could mediate neutrophil adhesion and the subsequent pathologies. Furthermore, gene regulation by diazinon itself or via reactive intermediates (oxygen or nitrogen) is worth investigating. The upregulation of genes encoding antioxidant enzymes stimulated by diazinon has recently been reported [19]. Evidently, there are highly complex mechanisms involved in the production of the respiratory burst that should be investigated further.

In fish, IgM is the most important antibody class [20,21]. The IgM levels in plasma from Nile tilapia exposed to 0.39 and 0.78 mg/L diazinon were not affected. At 1.96 mg/L, diazinon increased IgM concentrations. This finding is in agreement with a previous study in which intermediate doses were tested [7].

This study is one of the few to date that has examined the effect of diazinon on immunological parameters in Nile tilapia. In view of diazinon's extensive use in agriculture, the indirect, but potentially immunotoxic, hazard to fish should be investigated. Fish could be exposed to concentrations of diazinon for long periods or in combination with another pesticide; these effects should be investigated.

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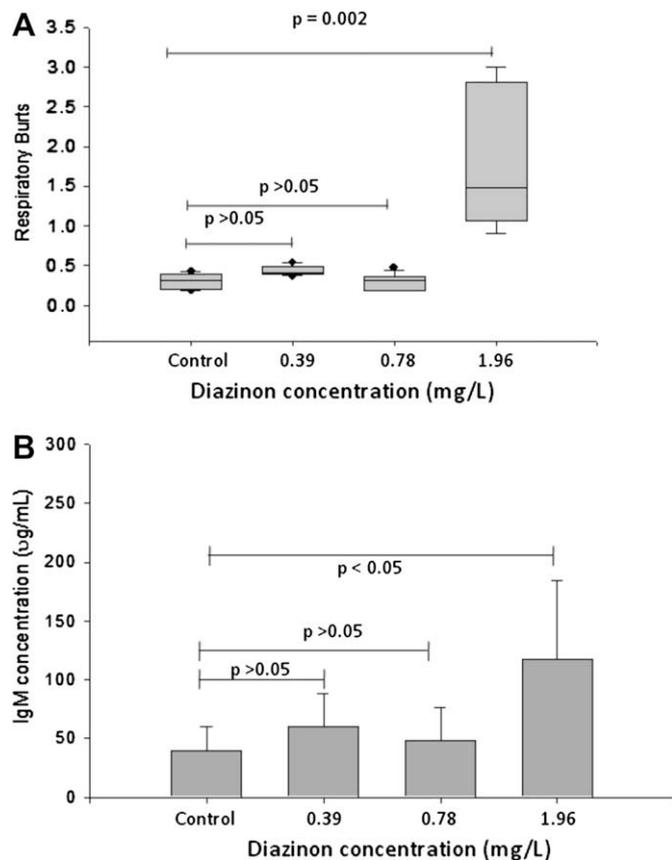


Fig. 1. (A) Respiratory burst in splenocytes and (B) IgM concentration from Nile tilapia exposed to diazinon (1/20 of CL_{50} , 1/10 of CL_{50} and 1/4 of CL_{50}) for 96 h and control fish. The results are represented as the median (A) and the mean (B).

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