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Comparative Acute Toxicity Assessment of Organophosphate and Avermectin Insecticides on a Freshwater Fish *Oreochromis niloticus*

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Abstract

Oreochromis niloticus was exposed to 10.0 ppb of organophosphate insecticide chlorpyrifos (CPF) and avermectin insecticides abamectin (ABM) and emamectin benzoate (EB) for 48 and 96 h. RBC and Hb decreased in CPF- and ABM-exposed fish after 96-h. Plasma ALT, AST, cortisol, and glucose increased in 96-h CPF-, ABM- and EB-exposed fish, while plasma ions declined in 96-h CPF-exposed ones. Insecticides caused alterations in liver oxidative stress parameters. In fish exposed to CPF, CAT increased after 48-h whereas it decreased after 96-h. Also, CAT declined in 48- and 96-h ABM-exposed fish, whereas it elevated in 48-h EB-exposed ones. Insecticides caused decreases in SOD at 48- and 96-h and in GR after 96-h. GSH elevated in CPF-exposed fish after 48-h, while it decreased in all the tested insecticide exposures after 96-h. Malondialdehyde of fish exposed to insecticides for 96-h increased. Consequently, toxic effects of insecticides on *O. niloticus* were generally as CPF > ABM > EB.

Keywords *Oreochromis niloticus* · Chlorpyrifos · Abamectin · Emamectin benzoate · Oxidative stress · Plasma biochemistry

Pesticides from agricultural fields enter the aquatic environment through surface run-off or spray drifting and have been a serious threat to aquatic organisms including fish (Ullah et al. 2018). Chlorpyrifos (CPF), one of the most widely used organophosphate pesticides, has been more toxic to fish than organochlorinated pesticides. This insecticide used to control foliar insects and subterranean termites (Venkateswara Rao et al. 2005). Abamectin (ABM) and emamectin benzoate (EB) are avermectin insecticides, which are the natural fermentation products of the soil-dwelling actinomycete *Streptomyces avermitilis* (Raftery and Volz 2015). Avermectins are widely used as antihelminthic and antiparasitic agents against many pests in agriculture and domestic animals depending on their broad-spectrum effectiveness. ABM has low toxicity to mammals whereas it can cause severe toxic effects in fish (Ma et al. 2014). EB is considered as Toxicity Category II by WHO (Wang et al. 2012).

Investigating the blood cells, biochemistry and hormones is crucial in monitoring the physiological status of fish and in diagnosing diseases (Fırat et al. 2011). Pesticides are well known to produce reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical etc., which may lead to oxidative stress, showing the role of ROS in pesticide toxicity (Narra 2016). During oxidative stress, the ROS production overwhelms the capacity of antioxidant defence systems, causing adverse biological consequences (Sharma 2009). The oxidative stress responses such as alterations in antioxidant system and lipid peroxidation have been used as biochemical indicators to evaluation the toxic effects of environmental toxicants on fish (Nasr et al. 2016).

Oreochromis niloticus is one of the most widely cultured freshwater fish in the world because of its economic importance for fisheries and aquaculture (Tunçsoy et al. 2017). Because of its easy handling, culture, and maintenance in the laboratory, and because it responds promptly to environmental alterations, *O. niloticus* is also a well-established model for toxicologic research (Garcia-Santos et al. 2006). So aquatic environments are considered to be the ultimate receiving medium for a wide variety of pesticides used against agricultural pests, the fish in natural habitats can simultaneously be exposed to more than one pesticide. A review of the literature reveals that there is a paucity of information on toxic effects of more than one pesticide on

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fish. Also, comparative studies on effects of organophosphate and avermectin insecticides in these organisms seem to be very limited. Therefore in this research it was compared toxicities of an organophosphate (CPF) and two avermectins (ABM and EB) on the hematology (RBC, WBC and Hb), the plasma biochemistry (ALT, AST, cortisol, glucose, Na and Cl), and the liver oxidative state (SOD, CAT, GR, GSH and MDA) of *O. niloticus*.

Materials and Methods

All procedures used in the animal experiment were carried out in accordance with current Turkish legislation and approved by Animal Experiments Local Ethics Committee of the Cukurova University (Protocol 8/2014). Nile tilapia (*O. niloticus*), with body mass of 42.11 ± 0.6 g and total length of 13.49 ± 0.7 cm, were obtained from Fish Culture Farm of Cukurova University and were then transferred to research laboratuary and were acclimatized for two months in 120 L glass aquariums containing clean water dechlorinated by intense aeration, static system, conditioned under laboratory conditions and natural photoperiod (12 h dark/12 h illumination). The mean values of the water quality parameters throughout the experiment were: temperature: 21.42 ± 0.17°C, pH 8.02 ± 0.04, dissolved oxygen: 7.32 ± 0.02 mg/L, alkalinity: 209 ± 4.7 mg/L $CaCO_3$, and total hardness: 323 ± 3 mg/L $CaCO_3$. During the acclimatization and experimentation period, the fish were fed once daily at the same hour with commercial fish feed, in an amount equivalent to 2% of their body weight. All the experiments, including the controls, were set up in duplicate considering different times points. In each repeat set the fish were divided into four groups and six fish were randomly distributed in each tank. Group I fish were used as control group and were not treated at all. Group II, III and IV fish were used as treatment groups and treated with 10 ppb CPF (Korban 4, 480 g/L, Koruma companies-Agrochemicals), ABM (Agrimec® 18 EC, 18 g/L, Syngenta) and EB (Proclaim Opti UV 5 WG, 50 g/kg, Syngenta) for 48 and 96 h. The insecticide concentration applied in the present research was selected as sub-lethal concentration based on available literature data and has been found in the aquatic environment highly contaminated by these pollutants (Ma et al. 2014; Xing et al. 2015). Mean CPF, ABM, and EB levels in exposure media were determined as 9.93 ± 0.07, 9.95 ± 0.05 and 9.96 ± 0.04 µg/L, respectively by gas chromatography–mass spectrometry (GCMSQP-2010 ULTRA). For determination of these insecticide concentrations, the chromatographic analyses were performed according to the methods described by Li et al. (2010) and Matos et al. (2012). Analysis was applied in triplicate. Calculated the limit of detection (LOD) and the limit of quantification (LOQ) values for all insecticides were 0.22 µg/L and 0.67 µg/L, respectively. Pesticide solutions in treatment groups were replaced every 24 h. At the end of each duration six fish were removed from aquaria and used as replicates. After 48- and 96-h blood samples from the control group and the insecticide treatment groups were drawn from the fish caudal vein using the heparinized plastic disposable syringe. Values of RBC, WBC, and Hb were determined using a hematology analyser (Beckman Coulter LH 750 Miami, FL). For the biochemical analyses of plasma, the remaining blood samples were centrifuged at 5000 rpm over 5 min at 4°C. Biochemical parameters in the plasma samples were determined using biochemical otoanalyzers (Modular Roche E170, Modular Roche DPP, Hitachi Ltd, Tokyo, Japan). After blood sampling fish were killed by transection of the spinal cord and were dissected. The liver tissues were immediately homogenized in Tris buffer (20 mM, pH 7.8) containing sucrose (0.25 M) with a ratio of 1/10 in using a steel homogenizer at 10,000 rpm for 2–3 min. The homogenate was centrifuged at 9500×*g* for 30 min at + 4°C. The activities/levels of SOD, CAT, GR, GSH, MDA and protein were measured by the methods of Sun et al. (1988), Lartillot et al. (1988), Carlberg and Mannervik (1975), Beutler (1975), Dubovskiy et al. (2008), and Lowry et al. (1951), respectively. For statistical evaluation of data one-way analysis of variance (ANOVA) followed by the SNK test and independent samples *t* test were performed to compare treatment groups and exposure times, respectively. Significant differences were statistically considered at $p < 0.05$.

Results and Discussion

Alterations in the liver oxidative stress parameters of *O. niloticus* in response to CPF, ABM and EB exposures were presented Table 1. In fish exposed to CPF, CAT activity increased after 48-h whereas it decreased after 96-h. It was observed CAT activity declined in 48- and 96-h ABM-exposed fish, whereas it elevated in 48-h EB-exposed ones. In 96-h CPF- and ABM-exposed fish the decreases of CAT activity were approximately 35% and 20%, respectively. SOD activity showed a decrease in all the tested pesticide exposures after both periods. Also, CPF, ABM and EB caused a decline in GR activity after 96-h. Following 96-h CPF, ABM and EB exposures the SOD activity was declined by 48%, 36% and 20%, respectively, while GR activity was decreased by 35%, 23% and 21%. In CPF-exposed fish GSH level elevated after 48-h, while it decreased at the end of the exposure period. At the same time in 96-h ABM- and EB-exposed fish, GSH level declined. It was found an elevation in MDA levels of fish exposed to CPF, ABM and EB for 96 h. After CPF, ABM and EB exposures for 96 h the decreases of GSH levels were approximately 36%, 19%, and

Table 1 Alteration in liver oxidative stress parameters of *O. niloticus* exposed to CPF, ABM and EB

Parameter	48 h	96 h
CAT activity (U/mg protein)		
Control	391 ± 19ax	401 ± 15ax
CPF	513 ± 18bx	260 ± 23by
ABM	290 ± 27cx	320 ± 21cx
EB	482 ± 11bx	397 ± 18ay
SOD activity (U/mg protein)		
Control	30.23 ± 0.77ax	29.50 ± 0.25ax
CPF	15.87 ± 0.52bx	21.47 ± 0.20by
ABM	19.47 ± 0.78cx	21.78 ± 0.23bx
EB	24.23 ± 0.30dx	24.61 ± 0.21cx
GR activity (U/mg protein)		
Control	0.074 ± 0.001ax	0.077 ± 0.002ax
CPF	0.070 ± 0.003ax	0.049 ± 0.002by
ABM	0.073 ± 0.003ax	0.059 ± 0.003cy
EB	0.075 ± 0.004ax	0.061 ± 0.002cy
GSH level (μmol/g protein)		
Control	2.28 ± 0.11ax	2.37 ± 0.14ax
CPF	3.06 ± 0.22bx	1.52 ± 0.15by
ABM	2.43 ± 0.24ax	1.91 ± 0.09cy
EB	2.17 ± 0.18ax	1.95 ± 0.12cx
MDA (nmol/mg protein)		
Control	1.94 ± 0.02ax	1.90 ± 0.03ax
CPF	1.97 ± 0.03ax	2.77 ± 0.04by
ABM	1.95 ± 0.04ax	2.37 ± 0.03cy
EB	1.92 ± 0.03ax	2.33 ± 0.05cy

Values are expressed as mean ± standard error ($N=6$). Letters a, b and c show the differences between groups at the same time and letters x and y show differences between time for the same group ($p<0.05$)

18%, respectively, while the elevations of MDA levels were 46%, 25%, and 22%.

SOD and CAT are two key antioxidant enzymes responsible for elimination of cellular ROS induced by toxicants (Ma et al. 2014). The inhibition of SOD activity in 48- and 96-h CPF-, ABM- and EB-exposed fish may be showed a reduction in the ability of cells to protect against superoxide radicals and consequently cellular structures becoming more susceptible to the effects of these radicals. In our study increased CAT activity in 48-h CPF-exposed fish might be in response to H_2O_2 produced by insecticide. On the other hand decreased CAT activities in 96-h CPF- and ABM-, and 48-h EB-exposed fish might be a consequence of high production of superoxide anion radicals induced by insecticides. In agreement with our findings, Kavitha and Venkateswara Rao (2008) reported the fish, *Gambusia affinis* after exposure to 297 μg/L CPF for 96-h indicated significant decreases in activites of CAT (77%) and SOD (71%). The results found by Liu et al. (2015) revealed that 0.5 mg/L triazophos, an organophosphorus pesticide, caused a significant inhibition of SOD and CAT activities in tissues of *Carassius auratus* and that decreased these enzymes activities can show toxicity associated with ROS stress caused by pesticide. GR is another important enzyme protecting cells against oxidative injury (Jos et al. 2005). In our research reduced the liver GR activities in *O. niloticus* were observed after 96-h exposure to CPF, ABM and EB, which might be a result of toxic effects of these insecticides on the enzyme. The declined GR activity was also reported in the liver of *O. niloticus* exposed to 200 μg/L concentration of methomyl, carbamate insecticide, for 30 days (Meng et al. 2014) and in brain of *G. affinis* following exposure to 297 μg/L concentrations of CPM for 96-h (Kavitha and Venkateswara Rao 2008).

The antioxidant GSH, the most abundant non-protein thiol is involved in many cellular processes in fish, including protects cells against oxidative damage (Sinhorin et al. 2014). The GSH level in *O. niloticus* was found to significantly increase following exposure to CPF for 48-h, indicating an adaptation response to this insecticide. On the other hand the exposures of *O. niloticus* to 96-h CPF, ABM and EB resulted in a decline in GSH levels, which might be depending on its utilization to challenge the prevailing oxidative stress caused a consequence of the toxicity of ROS induced by insecticide (Dar et al. 2015). Declined GSH levels were found in brain and kidney of rat after CPF, ABM and CPF + ABM exposures (Nasr et al. 2016). MDA is the product of the reaction between ROS and unsaturated fatty acids in cellular membrane and its content alternation in tissue indirectly reflects the damage to cellular membrane caused by excess ROS (Li et al. 2013). Our research indicated, 96-h exposure of CPF, ABM and EB caused significant elevations in MDA levels in liver tissue of *O. niloticus*, probably by the result of damaged cellular membrane. Increased MDA level might be a clear indication of lipid peroxidation by induced insecticides in the liver of fish. Our results were similar to findings found in *G. affinis* acutely exposed to CPF (Kavitha and Venkateswara Rao 2008). The researchers also noted the increased lipid peroxidation as ROS-induced injury can be one of the main toxic effects of CPF.

The data presented here indicate that the increases/decreases in SOD, CAT and GR activities and GSH and MDA levels were higher in CPF-exposed fish than ABM- and EB-exposed fish. Nasr et al. (2016) reported that declined SOD and CAT activites and GSH level and elevated MDA levels in rat exposed to CPF were higher than those in ABM-exposed ones and that CPF had the capability to cause lipid peroxidation, disturbances in antioxidant defence system more than ABM.

The changes in hematology induced by insecticides in *O. niloticus* were shown in Table 2. No significant alterations were observed in WBC count after all the tested

Table 2 Alteration in hematological and plasma biochemical parameters of *O. niloticus* exposed to CPF, ABM and EB

Parameter	48 h	96 h
RBC count (10^6/μL)		
Control	1.67 ± 0.03ax	1.69 ± 0.02ax
CPF	1.62 ± 0.05ax	1.28 ± 0.04by
ABM	1.72 ± 0.04ax	1.34 ± 0.03by
EB	1.64 ± 0.05ax	1.59 ± 0.04ax
WBC count (10^3/μL)		
Control	45.13 ± 1.03ax	43.59 ± 1.15ax
CPF	43.72 ± 0.87ax	41.11 ± 1.33ax
ABM	46.54 ± 1.39ax	42.63 ± 1.24ax
EB	44.28 ± 2.01 ax	44.47 ± 0.95ax
Hb content (g/dL)		
Control	8.09 ± 0.27ax	8.14 ± 0.58ax
CPF	8.31 ± 0.41ax	6.02 ± 0.39by
ABM	7.88 ± 0.60ax	6.56 ± 0.21by
EB	7.93 ± 0.48ax	8.07 ± 0.27ax
Plasma ALT activity (U/L)		
Control	12.61 ± 0.14ax	12.29 ± 0.25ax
CPF	13.63 ± 0.43ax	19.38 ± 0.41by
ABM	13.01 ± 0.75ax	15.64 ± 0.22cy
EB	12.26 ± 0.31ax	15.40 ± 0.19cy
Plasma AST activity (U/L)		
Control	120 ± 3.10ax	114 ± 2.73ax
CPF	128 ± 2.43ax	213 ± 5.30by
ABM	117 ± 3.57ax	162 ± 3.18cy
EB	115 ± 5.24ax	158 ± 2.86cy
Plasma cortisol level (ng/dL)		
Control	5.13 ± 0.08 ax	5.07 ± 0.05ax
CPF	6.29 ± 0.10bx	8.53 ± 0.14by
ABM	6.08 ± 0.05bx	6.12 ± 0.07cx
EB	5.03 ± 0.07ax	6.09 ± 0.06cy
Plasma glucose level (mg/dL)		
Control	43.79 ± 0.74ax	42.86 ± 0.49ax
CPF	71.81 ± 0.55bx	63.93 ± 0.38bx
ABM	53.74 ± 0.58cx	57.60 ± 0.52bx
EB	43.85 ± 0.37ax	55.14 ± 0.27by
Plasma Na level (mmol/L)		
Control	147 ± 2.18ax	149 ± 1.57ax
CPF	152 ± 1.72ax	112 ± 1.40by
ABM	143 ± 2.51ax	147 ± 1.28ax
EB	141 ± 1.63ax	150 ± 2.21ax
Plasma Cl level (mmol/L)		
Control	126 ± 1.17ax	129 ± 1.41ax
CPF	132 ± 2.34ax	93 ± 1.29by
ABM	124 ± 2.57ax	135 ± 1.88ax
EB	128 ± 1.68ax	130 ± 1.52ax

Values are expressed as mean ± standard error ($N = 6$). Letters a, b, and c show the differences between groups at the same time and letters x and y show differences between time for the same group ($p < 0.05$)

insecticide-exposed fish at the both periods. On the other hand, the RBC count and Hb content decreased in CPF- and ABM-exposed fish after 96-h. CPF and ABM exposures caused significant decreases of 24% and 21% in the RBC count and 26% and 19% in the Hb content, respectively. The findings found in present investigation indicated a significant reduction in RBC count and Hb content of *O. niloticus* exposed to CPF and ABM. The decreases in these hematological values might be resulted from erythropoiesis and hemosynthesis dysfunction due to the insecticide toxicity. The present findings are in agreement with the results of Harabawy and Ibrahim (2014) who reported the significant decreases were observed in RBC count and Hb concentration in fish, *Clarias gariepinus,* exposed to 0.16 and 0.49 mg/L carbofuran for 35 days. The researchers suggested that these declines in hematological parameters could indicate the toxic effects of carbofuran pesticide on hematology of fish.

The responses of plasma biochemistry in *O. niloticus* in exposed to insecticides were given Table 2. Exposures of 96-h CPF, ABM and EB caused significant elevations in ALT and AST activities and in cortisol and glucose levels, while sodium and chlorine levels indicated significant decreases in 96-h CPF-exposed ones. After exposures of 96-h CPF, ABM and EB, elevations of 58%, 27% and 25% in the ALT activity, 87%, 42% and 36% in the AST activity, 68%, 21% and 20% in the cortisol level and 49%, 34% and 29% in the glucose level, respectively, were determined. On the other hand, exposure of fish to 96-h CPF resulted in decreases of 25% and 28% in sodium and chlorine levels, respectively.

ALT and AST are sensitive responders to the pollution and represent the key enzymes in assessment of hepatocellular damage and many hepatic diseases (Ibrahim and Mahmoud 2005). Treatment of *O. niloticus* with all tested insecticides induced significantly elevations in activities of plasma ALT and AST. Increased these enzymes activities might be a result of toxicities of CPF, ABM and EB on the liver tissue of *O. niloticus*. Similarly, Fırat et al. (2011) and Jee et al. (2005) recorded that elevations in serum ALT and AST activities of *O. niloticus* and *Sebastes schlegeli* exposed to insecticides.

Alterations in levels of serum/plasma cortisol and glucose are important indicators to assessment stress conditions caused by toxicants in fish (Fırat et al. 2011). In our work levels of plasma cortisol and glucose might increase to cope with the elevated energy demand during CPF-, ABM- and EB-induced stress. These results are similar to the study conducted by Fırat et al. (2011) who reported serum cortisol and glucose levels increased in 4- and 21-days cypermethrin-exposed *O. niloticus*. The researchers noted elevated cortisol and glucose levels were important pathways for the recovery from stress by insecticide.

In our work exposure of *O. niloticus* to CPF caused a decline in plasma sodium and chlorine levels indicating the osmoregulatory disturbances, which may be due to the decrease in activity of Na^+/K^+- ATPase and/or inhibition of active ion uptake by insecticide. Fırat et al. (2011) reported alterations in the hydromineral balance of fish may be a result of the effects of toxicants on tissues involved in osmoregulation or on active transport processes.

The present investigation provides important evidences on the induced responses in *O. niloticus* after organophosphate and avermectin insecticides exposures. Our results showed the effects of insecticides on hematological, plasma biochemical and liver oxidative stress parameters were generally as CPF > ABM > EB and these insecticides caused both the alterations in blood profiles and oxidative stress in the liver of *O. niloticus*. Therefore, the findings of this study allow us to conclude the blood and liver tissues of fish can be used as good target tissues to reflect the toxic effects of insecticides and oxidative stress is one of the mechanisms responsible for insecticide toxicity to fish. Also, this research confirms blood and oxidative stress parameters may be used as biochemical indicators to assessment the toxicities of organophosphate and avermectin insecticides on fish.

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