Journal of Animal and Veterinary Advances 11 (15): 2716-2720, 2012
ISSN: 1680-5593

Effects of Carboxin on Glutathione-S-Transferase Enzyme Activite in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Carboxin is one of the most widely used fungicides in agriculture but information about toxicity on fish is limited. The aim of this study was to determine the effects of exposure to carboxin on the Antioxidant Defense System of rainbow trout (*Oncorhynchus mykiss*). The fish were exposed to carboxin (3.85 ppm) for 7 days. The antioxidant parameter (Glutathione-S-Transferase (GST)) was measured in fish liver samples. The results indicated that carboxin exposure significantly affected the activity of GST in rainbow trout liver ($p<0.01$). Thus, it was assumed that carboxin caused oxidative stress in rainbow trout and GST enzyme played a role in protection against carboxin toxicity.

Key words: Carboxin, rainbow trout, toxicity, antioxidant enzyme, GST, MDA

INTRODUCTION

Carboxin (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin -3-Carboxamide) is a member of the oxathiin class of systemic fungicides. It is applied to seed prior to planting for control of various fungi that cause seed and seedling diseases (smut, rot and blight). Carboxin may be used to prevent the formation of these diseases or may be used to cure existing plant diseases. Its mode of action is to selectively concentrate in fungal cells where it inhibits succinic dehydrogenase a respiratory enzyme in the mitochondria. It is available in a variety of formulations including wettable powder, dust, flowable concentrate, emulsifiable concentrate and ready-to-use liquid. Carboxin is applied both by commercial seed treaters and on-farm applicators.

Living things are equipped with an Antioxidant Defense System (ADS) in order to be protected against oxidative stress. Oxygen is a very reactive molecule due to its electron affinity and more reactive intermediate compounds are formed during the reduction of O_2 into H_2O (Liedias *et al*., 1998). Antioxidant enzymes are components which are induced by oxidative stress (Oruc *et al*., 2004) and consist of endogenous enzymes (Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px), Glutathione-S-Transferase (GST), Catalase (CAT), Mitochondrial Cytochrome Oxidase System, hydroperoxidase) and exogenous enzymes (Vitamin E and C, some drugs).

The presence of an O_2 rich atmosphere allowed the growth of an Endogenous Antioxidant System against Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). The reduction of the products of O_2 metabolism is controlled by enzymatic (SOD), CAT and GSH-Px cellular defense mechanisms (Wohaieb and Godin, 1987; Wickens, 2001). Glutathione conjugate GST which is considered as the first step in the detoxification of contaminants is produced in the liver of some animals (Sureda *et al*., 2006).

The most important characteristic of ADS is that all components of the system function mutually against (ROS) (Chaudiere and Ferrari-Lliou, 1999). Therefore, antioxidant enzymes play a vital role in the regulation of cellular balance and their induction is the result of a reaction against contaminants (Doyotte *et al*., 1997) while antioxidant enzyme activities and lipid peroxidation are important indicators in analysing cellular damage in toxicological studies (De Zwart *et al*., 1999; Oruc *et al*., 2004). This study was planned with the aim of determining the effect of carboxin on the antioxidant defense systems of rainbow trout (*Oncorhynchus mykiss*) and contributing to such studies.

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MATERIALS AND METHODS

Fish and carboxin: The rainbow trout (*Oncorhynchus mykiss*) were obtained from Ataturk University, Fisheries Faculty (with an average weight of 125±15 g). They were acclimatized for 28 days before the experiments. The Spring water used for the experiments had a temperature of 10±1°C, total hardness 102 mg as $CaCO_3$, free O_2 8±0.5 ppm and pH 7.8.

The research platforms were 780 L fiberglass circular tanks (100 cm diameter, 100 cm depth) with a constant and fresh water flow (1.5 L min^{-1}) and were kept in natural light conditions.

The tanks were aerated with air pumps. About 24 fish were placed into three tanks, two tanks for testing the carboxin (seven fish per tank) and the other one for the control group with ten fish.

Fish were exposed to a liquid form of carboxin at concentrations of 3.85 ppm for 7 days. Enzyme measurement and lipid peroxidation assays were carried out by separate experiments using three fish in each (n = 3).

Preparation of liver homogenates: Extracts from each tissue were prepared from each individual in according to Wiegand *et al*. (2001) with modifications. Sample were homogenized by a KH_2PO_4 (30 mM, pH = 7.3) buffer and then homogenates were centrifuged at 13000 rpm, 2 h, 4°C. Antioxidant enzyme activities GST and MDA concentrations were determined on the supernatants. All results were referred against the protein content in the samples.

Assay of antioxidant enzyme activities: The activity of Glutathione S-Transferase (GST) was determined at 340 nm using reduced Glutathione (GSH) and 1-chloro-2, 4-Dinitrobenzene (CDNB) as substrate according to the modified method of Habig *et al*. (1974).

Measurement of MDA level: MDA levels of rainbow trout liver were estimated according to Gulcin *et al*. (2009). About 800 μL phosphate buffer (50 mM, pH 7.4), 25 μL BHT and 500 μL of 30% TCA were added to 200 μL hemolysate followed by further mixing and incubation at -20°C for 2 h and centrifugation at 2000 rpm for 15 min. Then, 1.0 mL supernatant was separated. Following this, 75 μL EDTA-Na_2H_2O, 250 μL TBA were added to each sample and control and placed in a boiling water bath for 15 min, cooled to room temperature and measured at 532 nm. Total thiobarbituric acid-reactive materials were expressed as MDA using a molar extinction coefficient for MDA of 1.56×105/cm/M. The protein content of each homogenate was measured according to Bradford with Coomassie Brilliant Blue G-250 using bovine serum albumin as a standard.

Statistics: All values were analyzed by Student's t-test at $p<0.01$ level.

RESULTS AND DISCUSSION

In the present study, it was observed that there were significant differences ($p<0.01$) between the enzyme activities determined in the control group at 7 days for GST enzyme activity and MDA levels. Results are shown in Table 1. Carboxin increased the GST (μmol mg^{-1} prot) activities and MDA (nmol mg^{-1} prot) levels in rainbow trout at 7 days as compared to the control values. These differences were found to be statistically significant ($p<0.01$ using t-test).

When the organism is exposed to oxidative stress, ADS can react by increasing the synthesis of antioxidant enzymes in this system. Within the parameters regarding ADS, GSH level and the activities of GR and GST are useful indicators for determining the environmental pollution in aquatic organisms (Doyotte *et al*., 1997). GST catalyzes the reaction of the glutathione group with CDNB:

$$GSH + CDNB \xrightarrow{GST} CDNB\text{-}S\text{-}glutathione$$

As can be understood from Table 1, the GST enzyme activity of the fish in the treatment group was induced more compared to the control group. In similar enzymatic studies, the results were parallel to the findings (Rao, 2006; Seth *et al*., 2001; Jos *et al*., 2005). In one study, the GST activity of silver goldfish (*C. auratus*) which was exposed to 2,4-dichlorophenol in different concentrations, increased in small concentrations while there was no difference in bigger concentrations (Zhang *et al*., 2005). Stephensen *et al*. (2002) reported that the liver GST activity of rainbow trout (*O. mykiss*) which was exposed to paraquat, a herbicide, increased depending on time and concentration. In another study, two chemicals in different doses were mixed (27 ppm 2, 4-D + 0.003 ppm asynphos methyl) and applied to *O. niloticus* in 24, 48, 72 and 96 h periods and their hepatic antioxidant enzyme

Table 1: GST enzyme activity in the liver of rainbow trout tissues exposed to carboxin

Samples	GST	MDA
Exposed carboxin	0.36±0.02**	3.99±0.16**
Control	0.22±0.02	3.01±0.16

The results are expressed as the mean of 3 fishes ±SD; **Significantly different from control ($p<0.01$)

activities and lipid peroxidation levels were analyzed. It was reported that there was a higher increase in the Glucose-6-Phosphate Dehydrogenase (G6PD) and Glutathione Reductase (GR) activities compared to the control group and the MDA level did not change (Oruc and Uner, 2000). Sayeed *et al*. (2003) reported that increased the enzymatic activity in the liver and kidneys by applying a single-dose deltamethrin (0.75 μg L^{-1}) for 48 h in spotted murrel (*Channa punctatus*). It was reported that deltamethrin enhanced the activity of catalase and induced the lipid peroxidation. In a study with *C. punctatus*, deltamethrin was applied for 48 h and lipid peroxidation, protein levels and GST activity were analyzed. The lipid peroxidation, protein levels and GST activity increased significantly in the liver tissue (Sayeed *et al*., 2003). In a study with eels (*Anguilla anguilla*), an organophosphorus insecticide was applied to the fish for 96 h and it was reported that the GST activity increased in the liver (Pena-Llopis *et al*., 2003). In another study where the effect of contaminants on antioxidant enzyme activity was searched, endosulfan was applied to the rainbow trout (*O. mykiss*) and it was noted that GST activity and lipid hydroperoxid amounts increased significantly (Dorval and Hontela, 2003). Nile tilapia (*Oreochromis niloticus*), the fish were exposed to oxyfluorfen in different concentrations (0.3 and 0.6 mg L^{-1}) and for different periods (7, 14 and 21 days) and the CAT, SOD, GR and GST activities were analysed and it was reported that there was an increase in all groups (Peixoto *et al*., 2006). In a study with common carp fries (*Cyprinus carpio*) (Sepici *et al*., 2009), the fries were exposed to a sublethal dose (10 μg L^{-1}) of cyfluthrin for 48 h and it was noted that the MDA levels increased in the brain tissue of *C. carpio*. In a study with Propiconazole (PCZ), rainbow trout (*O. mykiss*) were exposed to the aforementioned fungicide for different periods (7, 20 and 30 days) and at sublethal concentrations (0.2, 50 and 500 μg L^{-1}), the oxidative stress indicators (LPO and ROS) and antioxidant (SOD, CAT, GR and GPx) enzyme activities were analyzed in the 7 days study, the Antioxidant Defense System reacted to this effect with adaptation and in the 20 and 30 days periods, high levels of oxidative stress indicators and inhibition of the antioxidant enzymes were noticed; long term exposure caused severe oxidative damage (Li *et al*., 2010).

In their study with Nile tilapia (*O. niloticus*), Figueiredo-Fernandesa *et al*. (2006) looked into the effects of Paraquate (PQ) which was applied at different temperatures 17 and 27°C) as a single dose (0.5 mg L^{-1}), on the antioxidant enzymes and it was noted that the aforementioned herbicide increased the activities of SOD, GST and GR. In a study with common roach (*Rutilus rutilus*), the effect of diazonin which is applied for different periods on the CAT activity was analyzed, the increase in the CAT activity in the 24 h was stated as an adaptation and the decrease in CAT activity at the 48 and 96 h was reported (Keramati *et al*., 2010). The same chemical was used in the study with common carp (*C. carpio*) (Oruc and Usta, 2007) and it was reported that the insecticide which was used for different periods (15 and 30 days) affected antioxidant enzyme activities in several tissues and increased the SOD, CAT and GPx activities and MDA level. In another study where diazonin was applied, *O. niloticus* was exposed to the aforementioned chemical for different periods (1, 7, 15 and 30 days) at the end of the trial an increase in SOD, CAT and GPx activities and MDA level (Durmaz *et al*., 2005). Parves and Rasiuddin (2006) noted that the non-enzymatic antioxidant structures of *C. punctatu* which was exposed to deltamethrin were affected and the amount of glutathione decreased. In his study, Dorval and Hontela (2003) had researched into the effect of different application levels of endosulphane an organochlorine pesticide, on the enzyme activities of *O. mykiss* and they reported that this pesticide increased the activity of CAT, GST and GPx and the MDA level.

Antioxidant enzymes which convert the well-recognized reactive oxygen radicals into less toxic products are the enzymes of SOD, CAT and GSH cycles (glutathione peroxidase, glutathione reductase and Glutathion S-transferase). In the study, emphasis was put on the GST enzyme activity and MDA levels of the liver. In the organisms which are chronically exposed to contaminants, the contaminants may accumulate in the tissues and organs and in the long term cause irreversible molecular changes which have harmful effects (Lopes *et al*., 2001).

CONCLUSION

In the light of the studies being conducted, the parameters regarding the Antioxidant Defense System are considered useful in determining the effects of environmental pollution on aquatic organisms (Doyotte *et al*., 1997).

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