Karboksinin gökkuşağı alabalıklarında (*oncorhynchus mykiss*) gulutatyon redüktaz enzim aktivitesi üzerine etkisi

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ÖZET

Karboksin tarımsal üretimde sıkça kullanılan bir fungusit olmasına rağmen balıklardaki toksisitesi üzerine yeterli sayıda çalışma bulunmamaktadır.
Bu çalışmada karboksin gökkuşağı alabalıkları (*Oncorhynchus mykiss*) antioksidant savunma sistemleri üzerindeki etkisinin belirlenmesi amaçlanmıştır. Balıklar 7 gün boyunca bu toksik bileşiğin 3,85 ppm' lik dozuna maruz bırakılmış ve karaciğerlerden alınan örneklerde antioksidan parametrelerinden glutatyon redüktaz (GR), enzimi ölçümü yapılmıştır. Araştırma bulguları; karboksine maruz bırakılan gökkuşağı alabalıklarının karaciğerlerinde GR aktivitesinin önemli oranda ($p< 0.01$) artırdığını ve bu türde oksidatif strese neden olduğunu göstermiştir.

Anahtar Kelimeler: karboksin, gökkuşağı alabalığı, toksisite, antioksidan enzim, GR

Effects of carboxın on glutathıone reductase enzyme actıvıty ın raınbow trout (*oncorhynchus mykiss*)

ABSTRACT

Carboxin is one of the most widely used fungicides in agriculture, but information about toxicity on fish is limited. This study assessed the effects of exposure to to carboxin on the antioxidant defence system of rainbow trout (*Oncorhynchus mykiss*). The fish were exposed to carboxin (3,85 ppm) for seven days. And antioxidant parameter (glutathione reductase, (GR)) was measured in hepar. The results indicated that carboxin exposure significantly affected the activity of GR in fish hepar ($p< 0.01$). Thus, it was assumed that carboxin caused oxidative stress in rainbow trout and GR enzyme played a role in protection against carboxin toxicity.

Keywords: carboxin, rainbow trout, toxicity, antioxidant enzyme, GR

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1. 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 [1].

Many environmental pollutants are capable of inducing oxidative stress in aquatic animals [2]. Living things are equipped with an antioxidant defense system (ADS) in order to be protected against oxidative stress. Chemical toxic pollutants are important sources of ROS in biological systems and inhibits the activity of some enzymes of the antioxidative defense system. Oxidative stress and damage to fundamental biomolecules and to antioxidant defenses of organisms are an established field in environmental toxicology and ecotoxicology[3].

Antioxidant enzymes (Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), Glutathione-S-Transferase (GST), glutathione reductase (GR), Catalase (CAT)) play a vital role in the regulation of cellular balance and their induction is the result of a reaction against contaminants [4], while antioxidant enzyme activities and lipid peroxidation are important indicators in analysing cellular damage in toxicological studies [5,6,7].

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.

2. MATERIALS AND METHODS

2.1.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 of 102mg as CaCO3/l, dissolved oxygen 8±0.5 ppm and pH 7.8. The research platforms were 780 l fiberglass circular tanks (100cm diameter, 100cm depth) with a constant and fresh water flow (1.5L/minute-1) with no recirculation and under natural light conditions.

The tanks were aerated with air pumps. Twenty-four 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 seven days. Enzyme measurement and lipid peroxidation assays were carried out by separate experiments, using three fish in each (n = 6) group.

2.2. Biochemical analysis

Glutathione reductase (GR) activity was analysed from rainbow trout tissues. Extracts from liver tissue was prepared from each individual in according to Wiegand [8] with a little modifications. To prepare the tissue homogenates, tissues were ground with liquid nitrogen in a mortar. The samples were homogenized by KH_2PO_4 (30mM, pH=7,3) buffer. And than homogenates were centrifuged at 13000 rpm, 2 hours at 4 °C. These supernatants were used for the determination of the enzymatic activities. All results were referred to the protein content in the samples. Protein content of each homogenate was measured according to Bradford with Coomassie brilliant blue G-250 using bovine serum albumin as a standard. All values were analyzed by Student's t-test at the $p < 0.01$ level.

3. 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 seven days for GR enzyme activity. Carboxin increased the GR (EU mg protein-1) activities in rainbow trout at seven days as compared to the control values. The GR enzyme activity of the fish in the treatment group (0,59±0,08EU mg protein-1) was induced more compared to the control group (0,26 ±0,03EU mg protein-1). These alterations were found to be statistically significant ($p< 0.01$). 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 [4].

The result abouth GR activity in present research were parallel to prior reports [9,10,11]. Nile tilapia (*Oreochromis niloticus*), exposed to oxyfluorfen in different concentrations (0.3 and 0.6 mg/L) 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 [12]. The other 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), the oxidative stress indicators (LPO and ROS) and antioxidant (SOD, CAT, GR and GPx) enzyme activities were analyzed, in the 7-day study, the antioxidant defense system reacted to this effect with adaptation, and in the 20-day and 30-day periods, high levels of oxidative stress indicators and inhibition of the antioxidant enzymes were noticed; long term exposure caused severe oxidative damage [13].

Figueiredo-Fernandes [14] looked into the effects of paraquate (PQ), which was applied at different temperatures 17 and 27 oC) as a single dose (0.5 mg L-1), on the antioxidant enzymes of Nile tilapia (*O. niloticus*) and it was noted that the aforementioned herbicide increased the activities of SOD, GST and GR. GR has a crucial role in the maintaining of GSH/GSSG homeostasis under stress conditions, however it is not involved in direct antioxidant defence in the same way as the SOD activity[12].

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. In the light of the present and prior studies, the parameters regarding the antioxidant defense system are considered useful in determining the effects of environmental pollution on aquatic organisms [15].

In this study, GR enzyme activitie was presented in I. National Interdisciplinary Environmental Congress, 14 - 16 MAY 2012, SAKARYA

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