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Araştırma Makalesi

2,4-Diklorofenoksiasetik Asit'e Maruz Kalan Capoeta umbla Karaciğer Dokusunda Malondialdehit Seviyesi, Süperoksit dismutaz ve Katalaz Aktivitesindeki Değişimlerin İncelenmesi

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Öz

Çalışmada, Capoeta umbla balıklarına 2,4-diklorofenoksiasetik asit (2,4-D) herbisitinin iki dozu 72 saat uygulanarak, balık karaciğer dokusundaki malondialdehit seviyesi (MDA), süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitesindeki değişimler incelenmeştir. MDA seviyesi, CAT ve SOD aktivitesindeki değişiklikler, spekrofotometrik yöntemlerle tespit edilmiştir. Çalışma sonucunda, herbisitin farklı dozlarına maruz kalan C. umbla karaciğer dokusunda MDA seviyesi, CAT ve SOD aktivitesinde istatistiksel olarak önemli bir artış olduğu belirlenmiştir ($p<0,05$). 2,4-D herbisitinin balık karaciğer dokusunda oksidan/antioksidan dengesini bozduğu görülmüştür. Bununla beraber, birincil savunma mekanizması olan bu iki antioksidan enzimin, 2,4-D toksik etkisine karşı direnç gösterdiği görülmektedir.

Anahtar kelimeler: *Capoeta umbla,* antioksidan enzimler, karaciğer, herbisit, oksidan/antioksidan dengesi

Investigation of Changes in Malondialdehyde Level, Superoxide Dismutase and Catalase Activity in Liver Tissue of Capoeta umbla exposed to 2,4-Dichlorophenoxyacetic Acid

Abstract

This study investigated the changes that occurred in malondialdehyde levels (MDA), superoxide dismutase (SOD) and catalase (CAT) activity in the liver tissue of Capoeta umbla fish after administration of two doses of 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide for 72 hours. The changes in the MDA levels, CAT and SOD activity were measured using spectrophotometric methods. The study result showed that there was a statistically significant increase in the MDA level, CAT and SOD activity in C. umbla liver tissue exposed to different doses of the herbicide ($p < 0.05$). The 2,4-D herbicide was observed to disrupt the oxidant/antioxidant balance in fish liver tissue. However, these two antioxidant enzymes, which are the primary defence mechanism, appear to be resistant to the toxic effect of 2,4-D.

Key words: *Capoeta umbla,* antioxidant enzymes, liver, herbicide, oxidant/antioxidant balance

Introduction

Pesticides are chemicals that are used to target and eradicate a selected population of pests living on or around humans, animals, and plants. However, they also reduce or impair the nutritional value of food supplies and cause harm to living creatures that have not been targeted by contaminating the air, water, and soil during their

production, storage, marketing and use, especially their unconscious and careless use, and depending on their concentrations, even cause death among living organisms (Yonar et al., 2012; Kirici et al., 2016; Özkan, 2017). 2,4-Dichlorophenoxy acetic acid (2,4-D) is an herbicide of the chlorinated phenoxy acid group which has been widely used in the world for about fifty years (WHO, 2016). Since the acid form of 2,4-D resides quite a long time in water, it accumulates easily in living tissues. This bioaccumulation of 2,4-D, especially along the food chain, may cause a decrease and sometimes even a complete cessation in the ability of aquatic species to reproduce, survive and grow (Oliveira et al., 2017).

Many environmental pollutants and primarily pesticides can cause oxidative stress in fish. Studies have shown that the increase in reactive oxygen species (ROS) due to pesticide toxicity leads to oxidative damage in fish (Yonar et al., 2011; Yonar and Sakin, 2011; Kirici et al., 2017). By causing genotoxicity, lipid peroxidation and enzyme inhibition, oxidative stress adversely affects the vitality of cells and organisms. As in higher organisms, fish have effective defence mechanisms to cope with oxidative stress. This antioxidant defence mechanism plays a major role in the survival of fish allowing them to adapt to chemical stress. Antioxidant defence systems consist of enzymatic components such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione reductase (GR) as well as non-enzymatic components such as glutathione (GSH) and metallothionein (Zirong and Shijun, 2007; Ispir et al., 2017). SOD and CAT are considered to be the top two antioxidant enzymes that form the first defence mechanism against pesticides. Lipid peroxidation is another toxic effect of pesticides. Lipid peroxidation leads to impairment of the structural and functional integrity of cell membranes. It is one of the widely used markers of oxidative stress and its magnitude can be demonstrated by measuring malondialdehyde (MDA) levels (Toroser et al., 2007; Yonar et al., 2016).

The liver is the main detoxification organ, and when fish are exposed to pesticides, it undergoes major morphological changes. Changes in the liver can be considered as markers of prior exposure to environmental stress factors (Velmurugan et al., 2009). Monitoring of biochemical and histo-cytological changes in fish liver in laboratory and natural settings is a highly sensitive and accurate way of evaluating the effects of xenobiotic compounds. The liver is the primary organ for the storage of pollutants in fish (Kirici et al., 2017; Taysı et al., 2021). Using spectrophotometric methods, this study aimed at investigating the extent to which 2,4-D herbicide can cause oxidative stress in the liver of Capoeta umbla.

Materials and Methods

The fish used in the study were caught from Murat River and brought to the laboratory of the Aquaculture Department of Bingöl University, Faculty of Agriculture and kept in 600 L tanks for 14 days to calm down. The fish were then divided into three groups, one control group and two treatment groups. The study was conducted in 60 L aquariums using 21 fish in total, 7 from each group. The 72-hour LC50 value (82.2759 ppm/L) found by Gül et al. (2005) was used as the basis for administration doses. The fish were administered 25% and 50% of the LC50 value (41.14 and 20.57 ppm/L) for 72 hours. The dissolved oxygen, pH, temperature, alkalinity and total hardness (CaCO3) values of the water used were 7.26 ± 1.11 mg/L, 7 ± 1.6, 17 ± 2 ºC, 128 ± 3 mg /L and 147 ± 5 mg/L, respectively. Considering that the concentrations of pesticide solutions used in the experimental aquariums may change in time, the solutions were replaced daily by newly prepared solutions diluted at desired proportions. At the end of each predetermined period, the fish that were randomly selected from the experimental aquariums were washed under tap water, the droplets on their surfaces were removed with blotting paper, and their height and weight were measured to prepare them for a dissection. The fish were sacrificed by spinal intervention before their dissection. Dissected liver tissue samples were put on ice with sterile instruments, washed with 0.59% NaCl, and after being weighed, stored at -80 °C until biochemical analysis. The samples with a weight/volume ratio of 1/10 were then homogenized at 10000 rpm for 3 minutes in an ultra-turrax homogenizer in ice with 0.05 M chilled Na-P buffer (pH: 7.4) containing 0.25 M sucrose. The homogenates were centrifuged at 10000 rpm at +4 °C for 30 minutes, and CAT and SOD activities and MDA levels in the supernatants were measured using spectrophotometric methods.

The Ledwozyw method (1986) was used to find the MDA level. 250 µL of tissue homogenate was mixed with 1250 µL of Trichloroacetic acid (TCAA) solution (in 1.22 M, 0.6 M HCl). After 15 minutes, it was incubated with 750 µL of Thiobarbituric acid (TBA) solution (0.047 M) in a boiling water bath for 30 minutes. With the addition of 2000 µL of commercial n-Butanol, the mixture was then centrifuged at 1560 g for 10 minutes. After taking the butanol phase, the absorbance rates were

recorded at 532 nm and calculated as nmol MDA/g protein (Ledwozyw et al., 1986). CAT was found using the Aebi (1974) method. The catalase enzyme catalyses the conversion of H_2O_2 to H_2O. This conversion can be traced with a decrease in absorbance at 240 nm. During the experiment, 0.2 mL of H_2O_2 solution (30mM) + phosphate buffer was added for each sample on 0.4 mL of tissue homogenate and it was calculated in U/mg of protein (Aebi 1974). The SOD activity was measured at 560 nm and 20 °C using the method of Sun et al. (1988).

One-way ANOVA was used to analyse the data and significant differences between the control and experimental groups were identified using the Duncan Test. The SPSS 17.0 program was used for statistical analysis. Any value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Fish are sensitive to water pollution. They can be exposed to toxic effects of various contaminants including pesticides in their living environment at almost every moment of their life cycle. Pesticides alter the basic physiological and biochemical processes in many organs and tissues of fish resulting in their death in severe cases. The liver, gills and intestines of fish play major roles in the biotransformation, detoxification and storage of pollutants (Topal et al., 2014; Kirici et al., 2015; Kirici, 2021). In the present study, no mortality was observed in *Capoeta umbla* under the influence of 2,4-D herbicide at the end of the 72-hour period when the experiment was terminated. However, this herbicide showed sublethal effects that were reflected by changes in antioxidant enzyme activities and MDA levels. The absence of death under the influence of 2,4-D suggests the presence of a strong detoxification and defence mechanism in these fish. In response to 2,4-D interaction, significant changes occurred in the oxidative stress parameters that were studied in *C. umbla* depending on the tissue in question and the concentration of the medium. Similar to our research results, previous studies have also reported that the changes in the antioxidant defence systems in fish tissues are associated with the type of the pesticides being studied as well as the length of exposure to, and respective concentrations of, these chemicals (Yan et al., 2015; Tutuş, 2016; Piancini et al., 2015; Golombieski et al., 2016).

The effects of 2,4-D on CAT enzyme activity in the liver tissue of *C. umbla* in two different medium concentrations are shown in Figure 1. CAT is an important intracellular antioxidant enzyme involved in the defence mechanism and a peroxidase that enables the detoxification of H_2O_2 by disintegrating it into oxygen and water using the formed H_2O_2 as a substrate (Adeyemi et al., 2015; Tabassum et al., 2016). When exposed to 2,4-D, especially a high concentration of it, a significant increase in CAT activity was observed in the liver tissue ($P<0.05$). This increase in the CAT activity was believed to occur as a cellular response to H_2O_2 in the present study. Similar to our study, a study exploring the oxidative stress responses in Danio rerio when exposed to thiamethoxam for different lengths of time found that the CAT activity increased at the end of 7 and 14-day periods and decreased after 21 and 28-day periods (Yan et al., 2016). Oruç et al. (2004) investigated the effects of azinphosmethyl, 2,4-D and combinations of these two pesticides on the antioxidant systems in the gill, kidney and brain tissues of Oreochromis niloticus (Tilapia fish) and *Cyprinus carpio* (Carp fish). The researchers reported that when these pesticides were administered alone or in combination, the CAT activity did not change in the brain tissue of *O. niloticus* but increased in the kidney tissue of *C. carpio*. Contrary to some studies showing increases in catalase levels, some other studies on the toxic effect of pesticides have reported decreases in the CAT activity (Zhang, 2005; Vasylkiv et al., 2011; Xing et al., 2012).

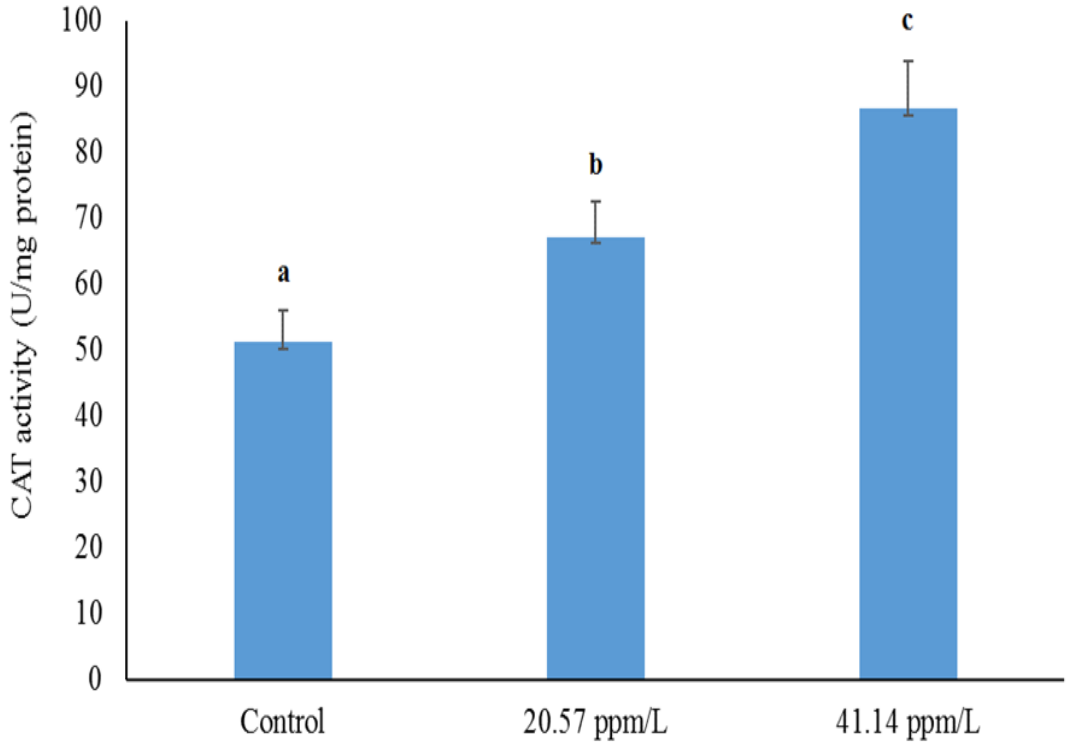

Figure 1. The effect of 2,4-D on CAT activity (U / mg protein) in the liver tissue of Capoeta umbla. Lowercase superscripts (a, b, c) indicate significant statistical differences among the experimental treatment groups, $P < 0.05$.

In the present study, significant increases were found in the antioxidant enzyme SOD activity in the liver tissues of *C. umbla* at the end of 72 hours of being exposed to 2,4-D in both medium concentrations (Figure 2, $P<0.05$). This increase in enzyme SOD activity was believed to occur as a defence response against higher levels of superoxide anion radical caused by 2,4-D. This type of a response may be important for the protection of cellular structure and components against direct harmful effects of superoxide and its indirect harmful effects arising from its conversion to hydroxyl radical as a result of some reactions. Consistent with our study results, Yan et al. (2016) found that SOD activity increased significantly at the end of 7 and 14-day periods in D. rerio that were exposed to 0.30, 1.25 and 5.00 mg/L of thiamethoxam for four weeks. Husak et al. (2016) investigated some enzyme activities in the gill tissue of *Carassius auratus* fish after administering different concentrations of Sencor herbicide Metribuzin for 96 hours. They observed an increase in the SOD activity in the gill tissue. In their study investigating the effect of carboxin on superoxide dismutase enzyme activity in Rainbow Trout (*Oncorhynchus mykiss*), Uçar et al. (2012) showed that the SOD activity increased significantly ($p<0.01$) in the livers of rainbow trout exposed to carboxine, causing oxidative stress in this species. Contrary to our study result, a study investigating the effects of cypermethrin administered to Israeli Carps on oxidative stress parameters (Uslu et al., 2016), found that SOD levels decreased significantly in liver and muscle tissues and serum samples.

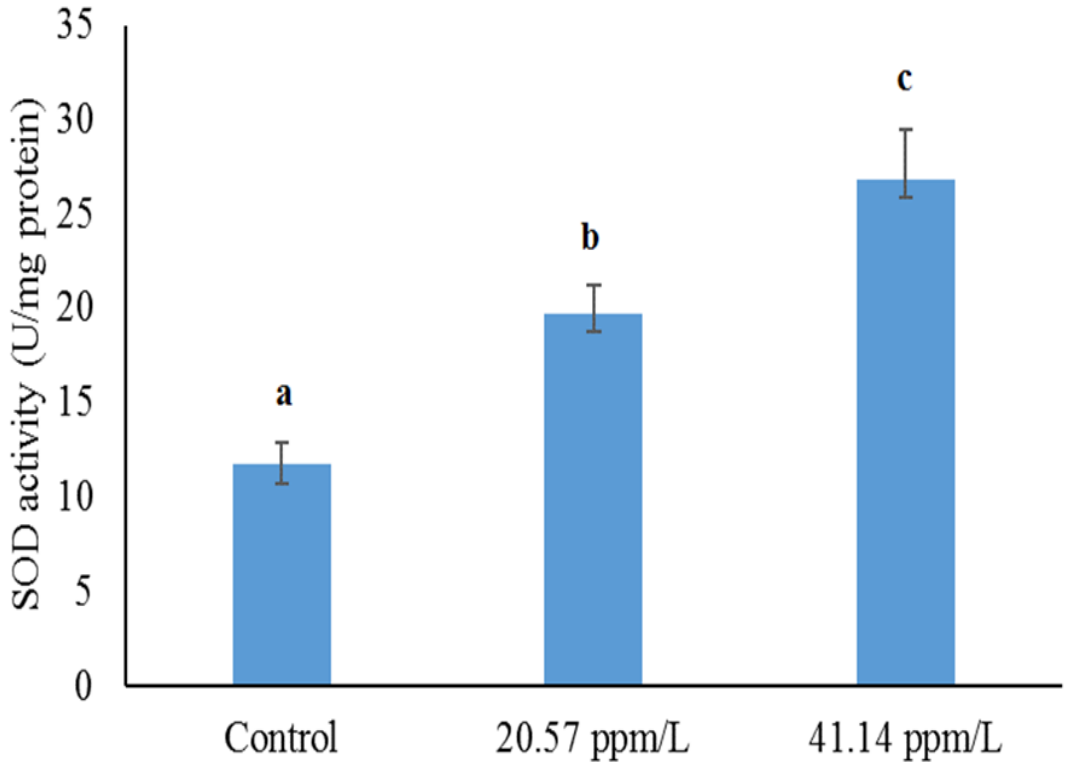

Figure 2. The effect of 2,4-D on SOD activity (U / mg protein) in the liver tissue of Capoeta umbla. Lowercase superscripts (a, b, c) indicate significant statistical differences among the experimental treatment groups, $P < 0.05$.

In this study, the MDA level of 2,4-D in the liver tissue of *C. umbla* was found to increase significantly after 72 hours, especially at high medium concentrations (Figure 3, $P<0.05$). One of the major toxic effects of pesticides at the molecular level is that they cause lipid peroxidation. As one of the end products of lipid peroxidation that occurs under the influence of toxicants, MDA is an important indicator showing that the stable structure of cell membranes has been impaired. Lipid peroxidation is a serious condition causing impairment of cell membrane structure and loss of selective permeability properties of membranes and thereby leading to emergence of processes resulting in cell death (Toroser et al., 2007; Fırat and Aytekin, 2018). Lipid peroxidation is an early indicator of pesticide-induced damage in cellular membranes (Koç and Akbulut 2012). In the present study, the MDA levels in *C. umbla* were believed to have increased as a result of the toxic effects of 2,4-D, and lipid peroxidation occurring due to oxidative stress in the liver tissue under the influence of this herbicide was the main reason for the increase in MDA levels. Another study has also found significant increases in the MDA levels in the tissues of *C. carpio* that were exposed to deltamethrin (Yonar and Sakin 2011). Similar to our study results, significant increases in MDA levels were reported with lipid peroxidation, which occurred as a result of increased ROS production due to the gradual decrease in CAT and SOD activity in D. rerio that were exposed to nitenpyram, a kind of neonicotinoid insecticide (Yan et al., 2015). Persch et al., (2017) have reported that Clomazo herbicide increased MDA levels in the liver, brain and muscle tissues of R. qualen (Silver Catfish) under oxidative stress conditions. In a study exploring acute effects of atrazine and chlorpyrifos pesticides separately and in combination on the liver and gill tissues of *C. carpio*, Xing et al., (2012) have argued that reactive oxygen products impair cell membrane lipids and cause an increase in the MDA levels.

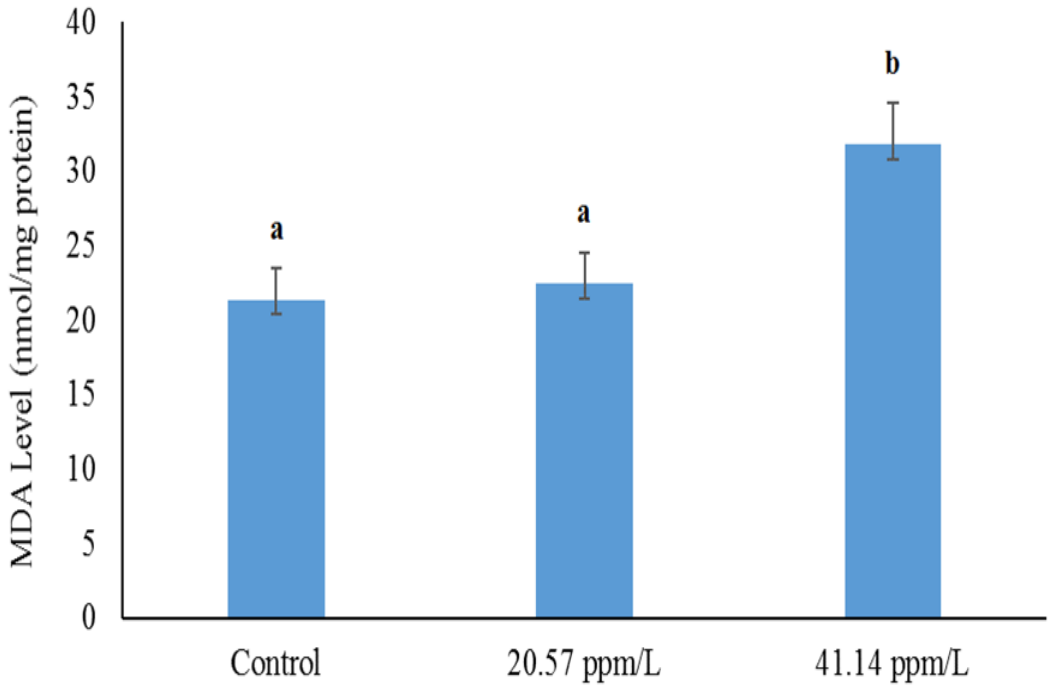

Figure 3. The effect of 2,4-D on MDA level (nmol / mg protein) in the liver tissue of Capoeta umbla. Lowercase superscripts (a, b) indicate significant statistical differences among the experimental treatment groups, $P < 0.05$.

In the present study, oxidative stress parameters in the liver tissue of C. umbla were found to have been negatively affected by 2,4-D. Increasing CAT and SOD activity and increasing MDA levels under the influence of 2,4-D led to emergence of a significant oxidative stress situation in *C. umbla*. Our study has also shown that 2,4-D should be considered as an herbicide capable of causing significant toxic effects on off-target organisms. Finally, it can be emphasized that 2,4-D is toxic to fish and the oxidative stress parameters studied here may be useful biomarkers when assessing the effects of this herbicide.

Conflict of Interest: The authors declare no conflict of interest.

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