ARAŞTIRMA MAKALESİ   RESEARCH PAPER

Determination of the Effect of Cyfluthrin Pesticide on Zebra Mussel (*Dreissena polymorpha*) by Some Antioxidant Enzyme Activities

Osman SERDAR
Munzer University, Faculty of Fisheries Tunceli, Turkey

*⊕: https://orcid.org/0000-0003-1744-8883

*Corresponding author's:
Osman SERDAR
Munzer University, Faculty of Fisheries
Tunceli, Turkey.
✉: oserdar@munzur.edu.tr

Abstract: In this study, some biochemical responses of Cyfluthrin (CFT), a commercial insecticide in *Dreissena polymorpha* were investigated. The 96 hour LC50 value of CFT on *D. polymorpha* was calculated as 553.22 ± 27.3 µg/L. *D. polymorpha* was exposed to sublethal concentrations (1/20, 1/10 and 1/5 of LC50 value) of CFT for 24 and 96 hours. The enzyme activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined in *D. polymorpha* individuals. In *D. polymorpha* exposed to CFT, SOD activity increased compared to control. It was determined that CAT and GPx activities were inhibited compared to the control.
As a result, it was determined that there was an increase in the oxidative damage of *D. polymorpha* individuals with CFT exposure.

Keywords: Catalase, cyfluthrin, *Dreissena polymorpha*, glutathione peroxidase, superoxide dismutase.

Cyfluthrin Pestisitinin Zebra Midyesi (*Dreissena polymorpha*) Üzerindeki Etkisinin Bazı Antioksidan Enzim Aktiviteleriyle Belirlenmesi

*Sorumlu yazar:
Osman SERDAR
Munzer Üniversitesi, Su Ürünleri Fakültesi,
Tunceli, Türkiye.
✉: oserdar@munzur.edu.tr

Öz: Yapılan bu çalışmada, *Dreissena polymorpha*'da ticari insektisit olan Cyfluthrin (CFT)'nin bazı biyokimyasal yanıtları araştırılmıştır. CFT'nin *D. polymorpha* üzerindeki 96 saatlik LC50 değeri 553,22 ± 27,3 µg/L olarak hesaplanmıştır. *D. polymorpha*, CFT'nin subletal konsantrasyonlar (LC50 değerinin 1/20, 1/10 ve 1/5)'ına 24 ve 96 saat süre ile maruz bırakılmıştır. *D. polymorpha* bireylerinde superoksit dismutaz (SOD), katalaz (CAT) ve Glutatyon peroksidaz (GPx) enzim aktiviteleri belirlenmiştir. CFT'ye maruz bırakılan *D. polymorpha*'da, SOD aktivitesi kontrole kıyasla artmıştır. CAT ve GPx aktivitelerinin kontrole kıyasla inhibe olduğu belirlenmiştir.
Sonuç olarak, CFT'ye maruz kalan *D. polymorpha* bireylerinin oksidatif hasarında artış olduğu belirlenmiştir

Anahtar kelimeler: Cyfluthrin, *Dreissena polymorpha*, glutatyon peroksidaz, katalaz, superoksit dismutaz.

INTRODUCTION

Aquatic organisms constitute a large part of the living potential in the ecosystem. Aquatic environment is the final stop of environmental pollution and is the environment most affected by pollution compared to other environments. In case of direct and indirect exposure of aquatic organisms to any pollutant, it is important to know the possible toxic effects of the pollutants, the amount of accumulation on the organism, the biochemical and histopathological damage and the trophic transfer

interaction with the food chain, directly and indirectly, affect the organisms in the ecosystem.

In order to determine the toxicity level of any substance on living things, acute toxicity tests are required. The most common acute toxicity used is the lethal dose/concentration (lethality) test. The purpose of this test is to determine the toxic symptoms that may arise on a living being exposed to a chemical, the degree of affection of certain organs such as the brain, kidney, liver, or the lethal dose/concentration level. Lethal dose/concentration value is also considered as an indicator of how safe that substance can be used (Saygı, 2003).

The production of reactive oxygen species (ROS) stimulated by pollutants and the resulting oxidative stress are stated as toxicity mechanisms in aquatic organisms exposed to pollution (Livingstone, 2003). Oxidative stress occurs when an intolerable amount of free radicals and non-radical ROS exceed the antioxidant defense capacity of the cell and subsequently, ROS causes damage to cellular metabolism (Halliwell & Gutteridge, 2015; Nishida, 2011). Xenobiotics are potential factors that can create oxidative stress in aquatic organisms through the activation of ROS mechanisms (Lushchak, 2011; Slaninova et al., 2009; Valavanidis et al., 2006). In organisms, enzymatic (catalase, glutathione peroxidase, superoxide dismutase, etc.) and non-enzymatic antioxidant mechanisms (ascorbic acid, tocopherols, glutathione, etc.) are responsible for the protection of cellular components against oxidative damage (Uluturhan et al., 2019). Exposure to increased ROS production can also lead to the induction of certain antioxidant enzymes by interacting with antioxidant-responsive gene elements and increased transcription (Livingstone, 2003). Lipid peroxidation (LPO) causes an increase in ion permeability and loss of membrane fluidity by disrupting the membrane structure. LPO is one of the most researched topics regarding ROS-related damage. Phospholipids found in membranes are the most abundant targets that ROS can affect. Antioxidant enzyme activities, glutathione redox states and LPO product levels are the most frequently used biomarkers in toxicological evaluations (Oruç et al., 2004).

Acetylcholine (ACh) is a major neurotransmitter in the somatic and autonomic nervous system. ACh is involved in the transmission of nerve impulses by neuromuscular chemical pathways and activates muscles. This neurotransmitter is broken down by the acetylcholinesterase (AChE) enzyme. AChE, which is frequently used as a biomarker in biomonitor studies, is an enzyme that inactivates acetylcholine in the synaptic cleft (Pohanka, 2011). Acetylcholinesterase functions as a regulatory enzyme of cholinergic neurotransmission by hydrolyzing acetylcholine to choline and acetate (Müller et al., 2019).

Choosing the appropriate test organism as well as the appropriate test type to obtain up-to-date and meaningful results in toxicological tests is an important factor affecting the research (Rand, 1995).

Since mussels are fed by filtering, they absorb dissolved and particulate pollutants. Due to their non-selective nutrition in their nutritional intake (Wesch et al., 2016), mussels that accumulate pollutants in high concentrations are among the leading biological indicators reflecting pollution since they keep them in their bodies for a long time (Bilgin & Uluturhan, 2015; Viarengo et al., 2007).

Zebra mussel (*Dreissena polymorpha*) is one of the two freshwater shelled species distributed in Eastern Europe, North America and Western Asia (Kinzelbach, 1992). The invasive behavior of the zebra mussel is seen as a disadvantage in its widespread use. This distinct disadvantage may represent one of the important reasons to ensure the protection of native species by sampling *D. polymorpha*, which is both invasive and widely used in biomonitoring and toxic effect assessment studies (Binelli et al., 2015). For example, a worldwide decline in the population of Unionid species (Unio spp., Anodonta spp.), etc. has been reported for several years (Binelli et al., 2015; Bogan, 1993). Another reason is that Zebra mussel (*D. polymorpha* Pallas, 1771) was chosen as living material in the study because it is not selective in food intake.

Substances that pollute water are of great importance because they cause toxic effects in aquatic creatures and threaten the health of all organisms in the ecosystem by accumulating in the food chain (Tokatlı et al., 2016; Wildi et al., 2004).

Cyfluthrin (CFT) is a synthetic pyrethroid pesticide that can contaminate water ecosystems and cause pollution in aquatic environments (Benli, 2005). Widely used, CFT causes toxicity to susceptible aquatic organisms by acting on river surfaces with which it is associated. Like other insecticides, it also has toxic effects on non-target organisms (Güvenç & Aksoy, 2010). CFT, which has a wide range of uses, contaminates water, which is the final stop of pollution, in various ways. Thus, it causes toxicity for aquatic organisms (Dinçel et al., 2009).

In this study, the biochemical response of Zebra mussel *(D. polymorpha)* against CFT pesticide with some biomarkers was investigated, since it is suitable as a scientific study material with its suitability to these features, economic value and accessibility.

MATERIAL AND METHOD

Test organism: *D. polymorpha* samples used in the study were obtained from Keban Dam Lake. *D. polymorpha* was collected by hand and brought as alive to

Munzur University Faculty of Fisheries Aquatic Toxicology Research Laboratory in plastic containers with air reinforcement.

Adaptation of the test organism to laboratory conditions: *D. polymorpha* samples brought as alive to the laboratory were placed in prepared aquariums. Photoperiod was applied in laboratory lighting with 14 hours light and 10 hours dark. The ambient temperature was kept constant by adjusting to 18 ° C during both the adaptation and test stages, thanks to the thermostat air conditioner. Cultured phytoplanktons were used to feed *D. polymorpha*.

In order to meet the oxygen need in stock aquariums, the air motor and external filter are used in the aquarium.

Determination of LC50 value and experimental design: The LC50 value in test organisms was determined by a static 96-hour acute toxicity test. For this, 5 different CFT concentration groups (0, 2, 10, 50, 250, and 1250 μg / L CFT) were formed, one of which was the control group. Dead individuals were noted at these concentrations due to CFT exposure, and the LC50 value was calculated using probit analysis with the data obtained. Ten individual organisms were used for each bioassay group, including recurrences. About 400 organism individuals were used in the study together with the LC50 experiments. The following 4 groups were formed from the calculated LC50 value (3 sublethal groups and control group).

Control group, 0 mg/L CFT, (Control24 24th hour control group, Control96 96th hour control group),

Group A, 28 μg/L CFTapproximately 1/20 of the LC50 value, (A_24; 24th hour, A_96; 96th hour),

Group B, 56 μg/L CFT approximately 1/10 of the LC50 value, (B_24; 24th hour, B_96; 96th hour),

Group C, 112 μg/L CFT approximately 1/5 of the LC50 value (C_24; 24th hour, C_96; 96th hour).

Organisms were exposed to CFT concentrations determined in the formed groups and stored in a -86 ºC freezer to determine their biochemical responses at 24 and 96 hours. All experimental studies (range determination, LC50 value, and biochemical responses) were performed in triplicate.

Dissection procedures and preparation of supernatants: The test organism individuals were opened with a scalpel and the dissection process was performed. 0.5 g of the organism was weighed and homogenized using a homogenizer with ice, adding PBS buffer (phosphate buffered salt solution) at a ratio of 1/5 w / v. These homogenized samples were centrifuged in a refrigerated centrifuge at 17000 rpm for 15 minutes and the supernatants obtained were stored in a -86 °C deep freezer until the measurement process was completed.

Determination of biochemical response: In this study, SOD, CAT and GPx enzyme activities were determined for the biochemical response of *D. polymorha* mussel individuals exposed to CFT pesticide.

The SOD (Catalog No 706002), CAT (Catalog No 707002) and GPx (Catalog No 703102) kits used in the study were purchased from CAYMAN.

Statistical analysis: The statistical difference between application concentrations was determined according to Duncan's multiple comparison tests in the same application period, and the difference between the application times in the same application group was determined by Independent T-test**.**

RESULTS

Metric-meristic measurements: Metric-meristic measurements of mussels before biochemical analysis (length data (27.43 ± 1.51 mm length, 10.62 ± 1.13 mm height, 11.09 ± 1.24 mm width) with 0.001 mm precision digital caliper and weight data (1.53 ± 0.33 g) with 0.001 g precision. It was recorded by weighing with the scales.

LC50 Value: In order to determine the lethal concentration of CFT, first range determination tests were carried out. After the study, 6 different CFT concentration groups (0, 2, 10, 50, 250, 1250 μg/L) were formed, one of which was the control group, to determine the lethal concentrations of CFT in *D. polymorpha*. With these concentrations, the LC50 value was calculated as 553.22 ± 27.3 μg/L by probit analysis.

SOD Activity: The time-dependent changes of SOD activities in the test organism in which different concentrations of CFT were applied with the control group are given in Figure 1. It was found that the SOD activities of organisms exposed to CFT increased in all groups compared to the control group ($p < 0.05$). It was determined that SOD activities increased ($p < 0.05$) during the exposure time (A and B group) in the same concentration groups (Figure 1).
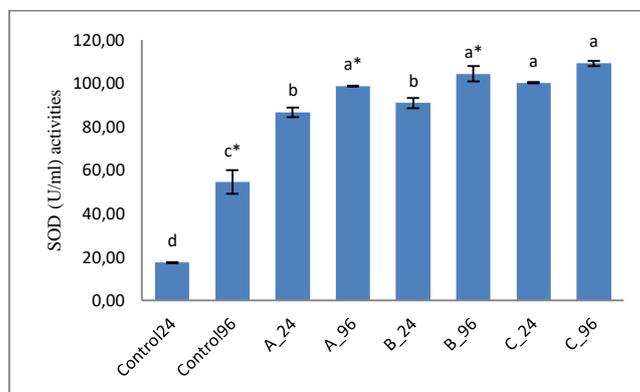

Figure 1. Changes in SOD activities in *D. morpha* exposed to different sublethal concentrations of CFT.

Asterisk (*) shows statistical differences according to the two-tailed independent T test between different exposure time (24 and 96 h) in the same groups; *p < 0.05. Different letters on bar (a, b, c, d) show statistical differences of Duncan's multiple range test among all application groups in the same exposure time; abc p<0.05.

CAT Activity: Time-dependent changes of CAT activities in the test organism where different concentrations of CFT were applied with the control group are given in Figure 2. It was determined that CAT activities decreased in organisms exposed to CFT compared to the control group (p <0.05). It was determined that CAT activities increased (p <0.05) during the exposure time (B and C groups) in the same concentration groups (Figure 2).
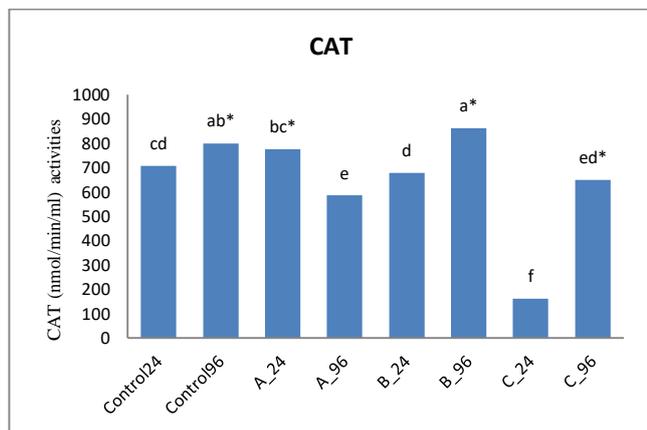

Figure 2. Changes in CAT activities in *D. morpha* exposed to different sublethal concentrations of CFT.

Asterisk (*) shows statistical differences according to the two-tailed independent T test between different exposure time (24 and 96 h) in the same groups; *p < 0.05. Different letters on bar (a, b, c, d, e, f) show statistical differences of Duncan's multiple range test among all application groups in the same exposure time; abc p<0.05.

GPx Activity: The time-dependent changes of GPx activities in the test organism where different concentrations of CFT were applied with the control group are given in Figure 3. The changes in GPx activities in organisms exposed to CFT decrease compared to the control group (Figure 3) were determined (p <0.05). It was determined that GPx activities increased (p <0.05) during the exposure time in the same concentration groups (B and C group) (Figure 3).
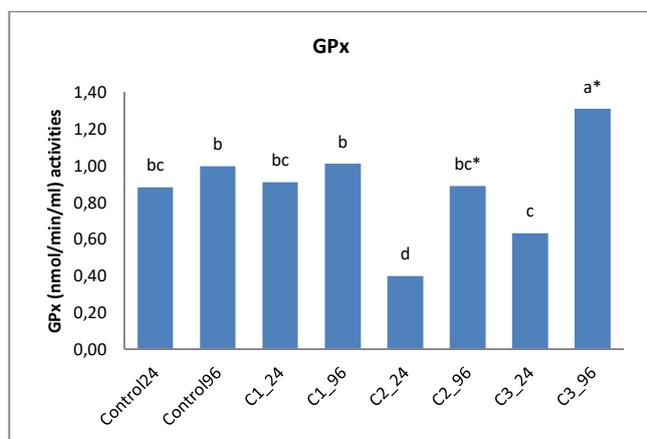

Figure 3. Changes in GPx activities in *D. morpha* exposed to different sublethal concentrations of CFT

Asterisk (*) shows statistical differences according to the two-tailed independent T test between different exposure time (24 and 96 h) in the same groups; *p < 0.05. Different letters on bar (a, b, c, d) show statistical differences of Duncan's multiple range test among all application groups in the same exposure time; abc p<0.05.

DISCUSSION

The main purpose of toxicity tests on aquatic organisms is to determine at what concentration a substance is harmful to the organism. Bioassays today are the physiology, pathology, nutrition, behavior patterns of organisms. It is used as a tool to enlighten many issues (Çetinkaya, 2005).

LC50 value is very important in terms of evaluating the acute toxic effects of chemical substances due to short-term applications (Saygı, 2003). In this study, 96 hour LC50 value of CFT was determined by static method to determine the sublethalconcentration to which the test organism will be exposed. It has been determined that CFT values in acute toxicity on *D. polymorpha* are very low and if the wrong and unconscious use of pesticides continues to increase, it can harm non-target organisms. Behavioral changes that occur in toxicity tests can provide predictions of endpoints for non-fatal toxicity and serve as a tool for environmental risk assessment and analysis of toxicological impact. Therefore, measuring the behavior of an organism after exposure to contaminants provides a better prediction of the potential environmental consequences of toxic contamination rather than simply measuring lethal effects (Khalil et al., 2013).

SOD, a metalloenzyme group, is the primary defense against the toxic effects of superoxide radicals in aerobic organisms and catalyzes the conversion of superoxide radicals to H_2O_2 and O_2, which play an important role in the antioxidant system (Kappus, 1985; Kohen & Nyska, 2002). It is not a general rule that an increase in xenobiotic concentrations induces antioxidant activity (Cheung et al., 2001). In some cases, O_2^- alone or after conversion to H_2O_2 causes strong oxidation of the cysteine in the enzyme and reduces SOD activity (Dimitrova et al., 1994; Durmaz et al., 2006). In the presence of xenobiotics, the initial decreased response in the antioxidant system may be followed by an induction. Therefore, the presence of an inducible antioxidant system may reflect an adaptation of the organism (Doyotte et al., 1997; Oruç & Usta, 2007). The response of the antioxidant system to oxidative stress in various tissues differs from one species to another due to the differences in the antioxidant potential of these tissues (Ahmad et al., 2000; Oruç & Usta, 2007). In this study, an increase in SOD activity was determined in *D. polymorpha* after CFT exposure. Similarly, studies have reported increased SOD activity in some aquatic organisms exposed to various pollutants (Ergüven et al., 2020; Serdar et al., 2018; Tatar et al., 2018; Yildirim et al., 2019).

CAT is a very common enzyme found in almost all living organisms that use oxygen. It plays a role in the formation of water and oxygen by catalyzing the

decomposition of hydrogen peroxide (Chelikani et al., 2004). CAT activity in contaminated environments may increase or decrease depending on the substance (Sobjak et al., 2017). In this study, CAT enzyme activity is inhibited by CFT exposure by organisms under stress compared to control. Similar to the current study, reductions in CAT activity have been reported in aquatic organisms exposed to various contaminants (Crestani et al., 2007; Hasspieler et al., 1994; Sayeed et al., 2003; Serdar, 2019; Thomas & Murthy, 1976; Yildirim et al., 2018; Zhang et al., 2004). These unexpected results in the literature on the activity of this enzyme, species, habitats, sex, etc. this can be explained by the fact that the differences can lead to antioxidant changes. (Glusczak et al., 2007).

Considered as a protective enzyme against lipid peroxidation at the expense of GSH, GPx catalyzes the reduction of hydrogen peroxide and lipid peroxides (Moreno et al. 2005). Monteiro et al. (2006) showed that the activity of this enzyme is reduced due to damage caused by negative feedback or oxidative modification caused by an excess of substrate. Inhibition of GPx activity may reflect the failure of the antioxidant system in contact with pesticides (Ballesteros et al., 2009) or may be related to the direct effect of superoxide radicals or pesticides on enzyme synthesis (Bainy et al., 1993). The GPx activity in *D. polymorpha* exposed to CFT was significantly lower than that obtained for control organisms in this study. This decrease may indicate that the antioxidant capacity is exceeded by the amount of hydroperoxide product synthesized through lipid peroxidation (Remacle et al., 1992). In this regard, the GPx inhibition observed in this study may reflect a possible antioxidant defense failure responsible for the observed increase in MDA levels. Similar to the current study, many studies reported that GPx activity was triggered by pollutants (Almeida et al., 2002; Fatima et al., 2000; Oliveira et al., 2012; Sayeed et al., 2003; Serdar, 2019; Tatar et al., 2018; Zhang et al., 2004).

CONCLUSION

According to the results of the data obtained from the study; the toxic effect of CFT pesticide on *D. polymorpha* has been determined. It was concluded that SOD, CAT and GPx are useful biomarkers in the investigation of the toxic effects of CFT on the test organism *D. polymorpha*, which is fed by filtering water, has no selective nutrition and has limited mobility. The results obtained depending on the concentration and time at the levels of these biomarkers show that the response of the test organism to the toxic substance changes with the concentration of the toxic substance and the application time.

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