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Effect of Beta-Cyfluthrin Pesticide on Zebra Mussel (*Dressienna polymorpha*)

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Abstract

In this study, some biochemical responses of Beta-Cyfluthrin (β-CF), which is a commercial insecticide in *Dreissena polymorpha*, were investigated. The 96 hour LC50 value of β-CF on *D. polymorpha* was calculated as 509.62 µg L^{-1}. *D. polymorpha* was exposed to subletal concentrations (1/16, 1/8 and 1/4 of LC50 value: 32, 64 and 128 µg L^{-1}) of β-CF for 24 and 96 hours. Malondialdehyde (MDA) and reduced glutathione (GSH) levels and Acetylcholinesterase (AChE) enzyme activities were determined in *D. polymorpha* individuals. In *D. polymorpha* exposed to β-CF, MDA levels increased compared to control and with increasing concentration. It was determined that GSH level decreased and AChE activity was inhibited compared to control and with increasing concentration.

In conclusion, exposure to β-CF resulted in increased oxidative damage and has been found to cause neurotoxicity even at low concentrations.

Keywords: Acetylcholinesterase, β-cyfluthrin, *Dreissena polymorpha*, glutathione, malondialdehyde

Beta-Cyfluthrin Pestisitin Zebra Midye (*Dressienna polymorpha*) Üzerindeki Etkisi

Öz

Yapılan bu çalışmada, *Dreissena polymorpha*'da ticari insektisit olan Beta-Cyfluthrin (β-CF)'nin bazı biyokimyasal yanıtları araştırılmıştır. β-CF'nin *D. polymorpha* üzerindeki 96 saatlik LC50 değeri 509.62 µg L^{-1} olarak hesaplanmıştır. *D. polymorpha*, β-CF'nin subletal konsantrasyonlar (LC50 değerinin 1/16, 1/8 ve1/4'ü:32, 64 ve 128 µg L^{-1})'ına 24 ve 96 saat süre ile maruz bırakılmıştır. *D. polymorpha* bireylerinde malondialdehit (MDA) ve redükte glutatyon (GSH) düzeyleri ile Asetilkolinesteraz (AChE) enzim aktiviteleri belirlenmiştir. β-CF'ye maruz bırakılan *D. polymorpha*'da, MDA seviyeleri kontrole kıyasla ve artan konsantrasyonla artmıştır. Kontrole kıyasla ve artan konsantrasyonla GSH seviyesinin azaldığı ve AChE aktivitesi inhibe olduğu belirlenmiştir.

Sonuç olarak, β-CF'ye maruz kalan *D. polymorpha* bireylerinde oksidatif hasarda artışa, düşük konsantrasyonlarda bile nörotoksisiteye neden olduğu bulunmuştur.

Anahtar Kelimeler: Asetilkolinesteraz, β-cyfluthrin, *Dreissena polymorpha,* glutatyon, malondialdehit

INTRODUCTION

Pesticides are defined as a substance or mixture of substances used to remove, reduce, suppress or degrade any pest. The pesticide can be a chemical agent or biological agents such as viruses and bacteria.

The use of pesticides has become almost mandatory to increase yield and quality in the agricultural field. When the advantages of pesticides such as increasing product quality and being economical are added to this, pesticide consumption has increased significantly (Tiryaki, 2010).

Cyfluthrin is a synthetic pyrethroid pesticide that contaminates aquatic ecosystems as a potential toxic contaminant (Benli, 2005). Since cyfluthrin is widely used in both urban and agricultural areas, it potentially acts on the river surfaces with which it is associated, causing toxicity to susceptible aquatic organisms. Since not all insecticides are specific, they not only kill target organisms, but also affect other vertebrate and invertebrate organisms. As a result of improper application of pesticides, it is included in the food chain by causing environmental

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pollution and causes serious health problems (Güvenç and Aksoy, 2010).

Cyfluthrin, which has a wide area of use, is in motion on the river surfaces with which it is related in various ways. In this case, it causes toxicity to sensitive aquatic organisms (Dinçel et al., 2009). Enzyme systems to hydrolyze these chemicals when taken into their bodies by aquatic organisms are not sufficient (Benli, 2005).

Synapses use acetylcholine (ACh), which acts as a transmitter substance, creating targets for many types of pesticides. ACh transmits messages from the central nervous system (CNS) and sensor neurons to the CNS. Cholinergic synapses are called nicotinic and muscarinic synapses, and both are sensitive to ACh. Nicotinic receptors are located in the sodium channels of postsynaptic membranes in certain parts of the nervous system. By connecting two acetylcholine molecules, the channel opens, sodium enters and the impulse is transferred. Toxic substances affect the nervous system as they act as nicotinic and muscarinic in some parts of the nervous system (Stenersen, 2004).

Free radicals, stimulation of free radical production, initiation of lipid peroxidation (LPO), deterioration of the body's antioxidant capacity are due to the toxic mechanism of most pesticides (Mohammad et al., 2004). Pesticides have the ability to produce reactive oxygen species. The amount or proportion of reactive oxygen species produced increases due to excessive use of natural or synthetic pesticides (Livingstone, 2001). Free radicals are defined as species that have one or more unpaired electrons and are able to survive independently. Radicals are capable of reacting with other molecules in a variety of ways. So, if two radicals meet, they combine and convert their un-shared electrons into a paired electron pair structure (Halliwell and Chirico, 1993).

Oxidative stress and the reduction of the LPO antioxidant defense system or the increase in the production of reactive oxygen species (ROS) disrupt the ROS-antioxidant balance and cause oxidative stress. Disruption of the balance between pro-oxidants and antioxidants is defined as oxidative stress and causes oxidative damage to begin (Oruç, 2010). Oxidative stress may lead to disorders such as strand breaks in DNA, increase in intracellular free Ca+2, damage to the membranes of ion transporters or other special proteins, and LPO associated with cell metabolism (Halliwell and Aruoma, 1991). The increase of oxyradical products that reduce the antioxidant defense system causes harmful biochemical and physiological effects. Two biochemical disorders caused by oxidative stress are LPO and protein carbonylation (Almroth et al., 2005).

LPO causes decrease in cell functions under oxidative stress conditions (Storey, 1996). LPO is the process of oxidative degradation of polyunsaturated fatty acids (PUFA), which occurs in biological membranes, weakening membrane functions, reducing membrane permeability, and causing inactivation of many membrane-bound enzymes (El-Gendy et al., 2010).

Malondialdehyde (MDA) is a natural degradation product of LPO (Munnia et al., 2004). It is characterized by the oxidation of the production of PUFAs in lipoproteins (Halliwell and Aruoma, 1991). MDA is extremely toxic to cells due to its high affinity for nucleic acids, enzymes, amino groups of proteins and thiols (Oropesa et al., 2009). It is used as a bioindicator in determining LPO (Dinçel et al., 2009).

Antioxidant defense mechanisms cells have different mechanisms to repair damaged macromolecules and to alleviate oxidative stress. Primary defense includes enzymatic and non-enzymatic antioxidants involved in the removal of ROS and free radicals (El-Gendy et al., 2010). These antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD) and glutathione-S-transferase (GST), non-enzymatic glutathione (GSH). Vitamin E (α-tocopherol) and Vitamin C (ascorbic acid) (Bebe and Panemangalore, 2005).

Small molecule antioxidant compounds (vitamins, flavonoids, carotenoids, uric acid, and GSH) serve as the second line of antioxidant defense. Non-enzymatic antioxidants prevent uncontrolled free radical formation, restrict the reactions of biological components and free radicals, and eliminate many free radicals that are responsible for the oxidation of endogenous antioxidants (Zama et al., 2007). Glutathione and GSH-related processes play a central role in antioxidant defense.

The zebra mussel (*D. polymorpha*) is one of the most important catching and invasive creatures living in freshwater ecosystems. Disrupting or

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completely blocking the water flow with the biomass they create on the surfaces they hold, causing corrosion, blocking the water filter or sieves, causing clogging in closed irrigation systems, restricting the life of other creatures naturally found in the aquatic ecosystem, causing many important problems, both technical and It can be economically and ecologically harmful. Zebra mussels have a very wide life tolerance. They can adapt to prolonged hunger, dryness, very high and very low temperatures, quite different dissolved oxygen and calcium levels (Bobat et al., 2001).

Due to the long lifespan of freshwater mussels, limited mobility and filter feeding, biomarkers are used extensively and reliably in toxicological studies examining the pollution in aquatic ecosystems. Although *D. polymorpha* is recognized as an invasive species, it is also a suitable species as a model and biological monitoring organism and is used in aquatic ecosystems to investigate anthropogenic stress factors (Yıldırım et al., 2015; Alişer 2020).

In this study, the biochemical response of Zebra Mussel (*D. polymorpha*) against Beta-cyfluthrin (β-CF) 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 METHODS

Chemical

β-CF active ingredient pesticide used in the study Agrofarm Kimya San. ve Tic. A.S. Pointer SC 125 brand manufactured by the commercial firm was purchased.

Test Organism

The *D. polymorpha* samples used in the study were obtained from Keban Dam Lake. *D. polymorpha* was collected manually and brought alive to Munzur University Faculty of Fisheries Aquatic Toxicology Research Laboratory in plastic containers with air reinforcement.

Adaptation of *D. polymorpha* to Laboratory Conditions

D. polymorpha samples brought as alive to the laboratory were placed in 80x40x25 cm size stock aquariums. Photoperiod was applied in laboratory lighting for 12 hours light and 12 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 plankton were used to feed *D. polymorpha*.

An aquarium air motor and external filter were used to meet the oxygen demand in stock aquariums. During the adaptation, the health status of the organisms was observed and noted at certain periods.

Metric-meristic measurements of mussels were recorded before the experimental study. Individuals of similar height and characteristics were used in the studies. In order for the study results to be correct, healthy organisms with similar sizes and characteristics were used in the studies. The healthy ones of the organisms were decided by selecting individuals who react to sound and light by closing their shells.

Determination of the LC50 value

The LC50 value in test organisms was determined by a static 96 hour acute toxicity test (OECD 202, 2004). In order to determine the acute lethal concentration of β-CF, range determination tests were performed first. After the range determination study, 5 different β-CF concentration groups (0.0, 12.5, 125, 500, 1000 $\mu g.L^{-1}$ β-CF) were formed, one of which was the control group, to determine the lethal concentrations in *D. polymorpha*. At these concentrations, dead individuals were noted and removed in 96 hours. LC50 value was calculated by using probit analysis with the obtained data. From the calculated LC50 value, the following 4 groups were formed (as 3 sublethal groups and control group).

- Control group, μgL^{-1} β-CF (Group without any β-CF; Control24, 24 hour exposure group, Control96 96 hour exposure group)
- C1 group, about 1/16 of the 32 μgL^{-1} β-CF LC50 value (C1_24; exposed to β-CF for 24 hours, C1_96; exposed to β-CF for 96 hours),
- C2 group, about 1/8 of the 64 $\mu g\ L^{-1}$ β-CF LC50 value (C2_24; exposed to β-CF for 24 hours, C2_96; exposed to β-CF for 96 hours),
- C3 group is about 1/4 of the 128 $\mu g\ L^{-1}$ β-CF LC50 (C3_24; exposed to β-CF for 24 hours, C3_96; exposed to β-CF for 96 hours).

The organisms were exposed to β-CF concentrations determined in the formed groups and

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kept in a -86 C 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

Test organism individuals were separated from each other with the help of scalpel and forceps. 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 at 17000 rpm for 15 minutes in a cooled centrifuge. The supernatants obtained were stored in a -86 ° C freezer until the measurement process was completed.

Determination of Biochemical Response

In the study, MDA and GSH levels and AChE enzyme activities were determined to determine the biochemical response.

The MDA (Catalog No 10009055) and GSH (Catalog No 703002) kits used in the study were purchased from CAYMAN. AChE enzyme has been revised from the method of Elman et al. (1961).

Determination of MDA Level

Determination of MDA levels was carried out using Cayman's TBARS Test Kit, which provides a simple, reproducible and standardized tool for LPO testing in mussel tissue homogenates. These values were calculated by measuring the acidic reaction of MDA and TBA at high temperature by spectrophotometer colorimetrically.

Determination of GSH Level

Cayman branded GSH Test kit was used to measure GSH. The kit uses glutathione reductase enzymatic recycling for the GSH level. With this method, the Sulfhydryl group reacts with GSH, DTNB (5,5'-dithio-bis-2-(nitrobenzoic acid), Ellman's reagent) to produce yellow colored 5-thio-2-nitrobenzoic acid (TNB). Simultaneously produced mixed disulfide, GSTNB (between GSH and TNB) is reduced by glutathione reductase to recycle GSH and produce more TNB. The TNB production rate is directly proportional to this recycling reaction, which is directly proportional to the concentration of GSH in the sample. By measuring the absorbance of TNB at 405-414 nm, the GSH level in the sample is determined.

Determination of AChE Activity

AChE is an enzyme that catalyzes the decomposition reaction of acetylthiocholine with thiocholine into acetate. AChE activity was determined by measuring the intensity of the yellow color given by 5-thio-2-nitrobenzoic acid as a result of the reaction between thiocholine and 5.5 'dithio-bis (2-Nitrobenzoic acid) (DTNB) at 412 nm wavelength spectrophotometer (Elman et al. 1961).

Statistical analysis

In this study, probit analysis was used to calculate the LC50 value. In the evaluation of biochemical analysis data, SPSS 24.0 package program one-way ANOVA (Duncan 0.05) was used.

RESULTS AND DISCUSSION

Metric-meristic measurements

Metric-meristic measurements of mussels before biochemical analysis (length data (20.30 ± 1.92 mm length, 9.71 ± 0.99 mm height, 9.99 ± 0.94 mm width) with 0.001mm precision digital caliper and weight data (1.002 ± 0.268 g) with 0.001g precision. It was recorded by weighing with the scales.

Lethal Concentration

The 96 hour LC50 value of β-CF on *D. polymorpha* was calculated as 509.62 ± 29.7 µg L^{-1} by probit analysis.

Some biochemical responses (MDA, GSH and AChE) of test organisms at 24 and 96 hours are given in Table 1.

MDA Level

The time-dependent changes of MDA levels in the test organism in which different concentrations of β-CF were applied with the control group are given in Figure 1. It was determined that the MDA levels in organisms exposed to β-CF increased in all groups (Figure 1) compared to the control group ($p<0.05$). It was determined that the MDA levels of the exposure time in the same concentration groups increased (Figure 1) ($p <0.05$).

GSH Level

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The time-dependent changes of GSH levels in the control group and the test organism in which different concentrations of β-CF were applied are given in Table 1. It was determined that GSH levels in organisms exposed to-CF decreased in all groups compared to the control group (Figure 2) ($p < 0.05$). It was determined that the GSH level decreased during the exposure time in the same concentration groups (Figure 2) ($p > 0.05$).

AChE Activity

The time-dependent changes of AChE activity in the test organism in which different concentrations of β-CF were administered with the control group are given in Table 1. AChE activity was determined as 0.58 ± 0.01 and 0.43 U mg^{-1} protein in the groups at the 24th and 96th hours of the study. AChE activity was found to be decreased in all groups compared to the control group ($p < 0.05$). It was determined that the exposure time made a statistically significant difference in the same concentration groups only in the highest concentration group (C3_24-96) (Figure 3).

Table 1. *Changes in MDA, GSH levels and AChE activities in D. polymorpha exposed to β-CF concentrations.*

	MDA (nmol/g tissue)	GSH (nmol/g tissue)	AChE (U/mg protein)
Control 24	$21.70 \pm 2.6^{a*}$	121.23 ± 3.8^{b}	0.58 ± 0.01^{a}
Control 96	27.03 ± 0.9^{b}	$184.77 \pm 4.6^{a*}$	0.57 ± 0.00^{a}
C1_24	$28.66 \pm 0.4^{b*}$	102.09 ± 3.5^{cd}	0.54 ± 0.01^{ab}
C2_24	$29.81 \pm 1.5^{bc*}$	$114.17 \pm 2.9^{bc*}$	0.50 ± 0.01^{bc}
C3_24	$42.80 \pm 5.7^{e*}$	56.37 ± 3.6^{e}	$0.48 \pm 0.00^{c*}$
C1_96	33.51 ± 1.2^{d}	99.42 ± 3.9^{d}	0.51 ± 0.01^{bc}
C2_96	62.84 ± 9.7^{f}	90.62 ± 3.1^{d}	0.48 ± 0.03^{c}
C3_96	82.53 ± 6.0^{g}	55.37 ± 2.6^{e}	0.43 ± 0.03^{d}

a,b,c,d,e,f The difference between values with different letters in the same column was found to be statistically significant ($p<0.05$).
* The difference between the values of the concentration group in the same column at different exposure times was found to be statistically significant ($p<0.05$).

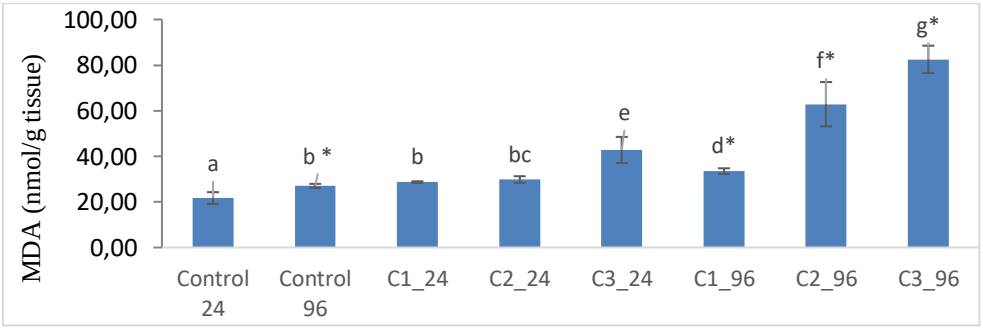

Figure 1. MDA (nmol/g tissue) levels of *D. polymorpha* exposed to different concentrations of β-CF. Different letters on the bar (a, b, c, d, e, f, g) show statistical differences in all application groups in the same exposure time and asterisk (*) shows statistical differences between different exposure time (24 and 96 h) in the same groups ($p<0.05$).

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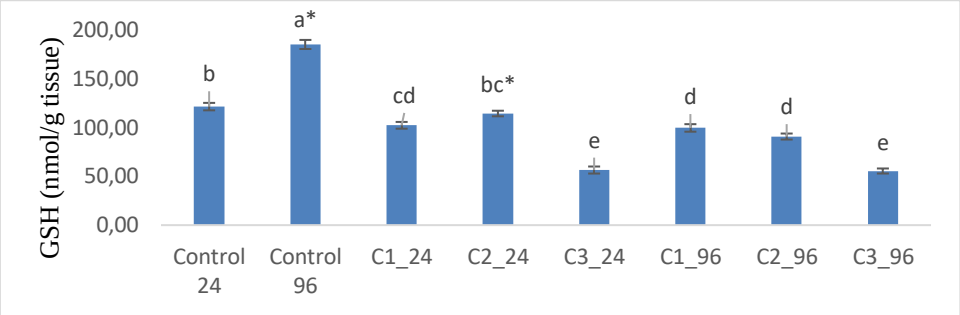

Figure 2. GSH (nmol/g tissue) levels of *D. polymorpha* exposed to different concentrations of β-CF. Different letters on the bar (a, b, c, d, e) show statistical differences in all application groups in the same exposure time and asterisk (*) shows statistical differences between different exposure time (24 and 96 h) in the same groups ($p<0.05$).

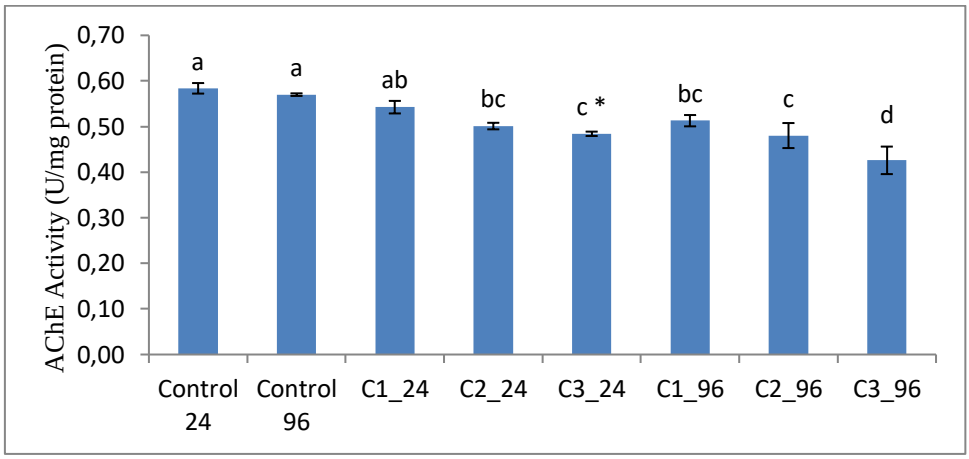

Figure 3. AChE (U/mg protein) activity of *D. polymorpha* exposed to different concentrations of β-CF. Different letters on the bar (a, b, c, d) show statistical differences in all application groups in the same exposure time and asterisk (*) shows statistical differences between different exposure time (24 and 96 h) in the same groups ($p<0.05$).

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 illuminate many subjects (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). Studies have been conducted on the acute toxicity of β-CF in various organisms. In this study, 96 hour LC50 value of β-CF was determined by static method to determine the subletal concentration to which the test organism will be exposed. It has been determined that β-CF 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. Thus, 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).

Antioxidant enzyme activities, glutathione redox states and LPO product levels (TBARS and MDA) are the most commonly used biomarkers in toxicological evaluations (Oruç et al., 2004). There are scientific studies that many pollutants cause an increase in MDA level in aquatic organisms. Tatar et

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al. 2018, reported an increase in MDA in the aquatic organism *Gammarus pulex*, which they exposed to in municipal treatment plant inflow. In another study, Serdar 2019, reported that MDA increased depending on the concentration in *G. pulex*, which exposed to the dimethoate pesticide. Serdar et al 2019, reported that in *D. polymorpha* mussels exposed to the sulfamethazine chemical, MDA increased depending on the concentration. In a different study, Tatar et al. 2019, reported that MDA increased significantly in *G. pulex*, which they exposed to Congo Red, compared to control. Yüksel et al. 2020, reported that MDA increased in concentration groups compared to control in *G. pulex*, which they exposed to malathion pesticide. In this study, it was determined that the level of MDA in organisms exposed to β-CF increased significantly compared to control and with increasing concentration. This increase in MDA level was regarded as a result of oxidative stress as the organism defends against β-CF oxidative damage.

Toxicity mechanisms in aquatic organisms exposed to pollution are stated as ROS production induced by pollutants and the resulting oxidative stress (Livingstone, 2003). Oxidative stress occurs when free radicals and non-radical ROS are generated in amounts that the antioxidant defense capacity cannot cope, and then ROS damages cellular metabolism (Nishida, 2011; Halliwell & Gutteridge, 2015). Pesticides, like other environmental pollutants, are potential factors that can create oxidative stress in aquatic organisms through ROS mechanisms (Valavanidis et al., 2006; Slaninova et al., 2009; Lushchak, 2011). Non-enzymatic antioxidant mechanisms are also responsible for protecting cellular components from oxidative damage, just like enzymatic antioxidants (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). Many studies have reported that pollutants inhibit GSH levels in aquatic organisms. Tatar et al. 2018, reported a decrease in GSH in the aquatic organism *G. pulex*, which they were exposed to municipal treatment plant inlet water. In another study, Serdar 2019, reported that GSH decreased depending on the concentration in *G. pulex*, which he exposed to the dimethoate pesticide. Similarly, a reduction in GSH content produced by anticholinesterase agents has been reported in various aquatic organisms (Della et al. 1994; Hai et al. 1997; Venturino et al. 2001; Ferrari et al. 2007; Yüksel et al.2020). In this study, it was found that there was a decrease in the GSH level in mussels exposed to β-CF, similar to the previous studies. GSH depletion has been associated with the oxidation of glutathione peroxidases due to an increase in free radicals or direct oxidation of these compounds.

AChE is an important regulatory enzyme that controls the passage of nerve impulses through cholinergic synapses by hydrolyzing the stimulating transmitter acetylcholine (ACh), and they are known as a biomarker of neurotoxicity as they are suppressed by anticholinesterase chemicals such as organophosphates, polybrominated di-phenyl ethers, and pharmaceuticals (Haase et al., 2002). It has been reported in many scientific studies that β-CF, a pyrethroid insecticide, inhibits AChE at low doses/concentrations, causing behavioral, neurological, oxidative, histopathological, endocrine and other effects. In scientific studies, it has been determined that it inhibits AChE activity in aquatic organisms exposed to substances containing various neurotoxicity (Chandrasekara and Pathiratne 2005; Xuereb et al. 2007; Guimaraes et al. 2007; Demirci et al.2018). Similarly, in this study, it was observed that AchE activity was inhibited with increasing concentration in mussels exposed to β-CF.

In addition, AChE activity is used as a biomarker in aquatic pollution (Dembele et al., 2000).

CONCLUSION

According to the results of the data obtained from the study; Toxic effect of β-CF peticide on *D. polymorpha* was determined. It was concluded that AChE, MDA and GSH2 are useful biomarkers in the investigation of the toxic effects of β-CF 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 varies with the concentration of the toxic substance and the application time.

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CONFLICT OF INTEREST

The Author report no conflict of interest relevant to this article

RESEARCH AND PUBLICATION ETHICS STATEMENT

The author declares that this study complies with research and publication ethics.

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