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# Determination of Toxic Effect of Gamma Cyhalothrin in *Dreissena polymorpha* by Some Biomarkers

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## ABSTRACT

In this study, the effects of pesticides on the freshwater mussel *Dreissena polymorpha*, a non-target organism, were aimed to be determined through biochemical responses. For this purpose, first, the acute toxicity value (LC50) of Gamma Cyhalothrin (GCH) pesticide on *D. polymorpha* was determined by standard static method and the LC50 value was calculated as 13.64 mg/L using probit analysis. Then, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities, malondialdehyde (MDA) and glutathione (GSH) level responses of *D. polymorpha* organism exposed to GCH sublethal concentrations for 24 and 96 hours were determined. In addition to biochemical biomarkers, histopathological responses were evaluated. In conclusion, this study demonstrated the abilities of GCH pesticides to induce oxidative stress. Moreover, MDA, GSH levels, SOD, CAT, GPx activities and histopathological responses could be used as an effective biomarker in *D. polymorpha*.

**Keywords:** *Dreissena polymorpha*, gamma cyhalothrin, biomarker, oxidative stress, histology

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**Gamma Cyhalothrin'in *Dreissena polymorpha*'daki Toksik Etkisinin Bazı Biyobelirteçlerle Belirlenmesi**

**Öz:** Bu çalışmada, pestisitlerin hedef olmayan organizmalar olan tatlı su midyesi *Dreissena polymorpha* üzerindeki etkilerinin biyokimyasal tepkilerle belirlenmesi amaçlandı. Bu amaçla öncelikle Gamma Cyhalothrin (GCH) pestisitinin *D. polymorpha* üzerindeki akut toksisite değeri (LC50) standart statik yöntemle belirlenmiş ve probit analizi ile 13,64 mg/L hesaplanmıştır. Daha sonra 24 ve 96 saat boyunca ölümcül olmayan GCH konsantrasyonlarına maruz kalan *D. polymorpha* türünün süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx), katalaz (CAT) aktiviteleri, malondialdehit (MDA) ve glutatyon (GSH) düzeyi tepkileri belirlendi. Biyokimyasal biyobelirteçlerin yanı sıra histopatolojik yanıtlar da değerlendirildi. Sonuç olarak, bu çalışma GCH pestisitlerinin oksidatif stresi tetikleme yeteneklerini ortaya koydu. Ayrıca MDA, GSH düzeyleri, SOD, CAT, GPx aktiviteleri ve histopatolojik yanıtlar da *D. polymorpha*'da etkili bir biyobelirteç olarak kullanılabilir.

**Anahtar kelimeler:** *Dreissena polymorpha*, gamma cyhalothrin, biyobelirteç, oksidatif stres, histoloji

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## Introduction

The combined impact of the Green Revolution allowed global food production to double over the past 50 years. Since 1960, the human population has more than doubled to approach eight billion people. It is estimated that the population will increase by 30% in 2050 and reach approximately 9.2 billion people (Popp et al. 2013). Demand for food production is expected to increase by 70% due to a growing global population and changing diets for meat and dairy products in developing countries (FAO 2009).

Globally, an average of 35% of potential crop yields are lost due to preharvest pests (Oerke 2005). In addition to pre-harvest losses, food chain losses are also relatively high. At the same time, agriculture meets the growing demand for food, feed, fiber, biofuels, and other bio-based products globally. The provision of additional farmland is limited, as agricultural expansion will often have to take place at

the expense of forests and natural habitats of wildlife, wild relatives of crops, and natural enemies of crop pests (Altıkat et al. 2009). Given these limitations, sustainable production and increased productivity on existing land are by far the better choice. The use of pesticides is inevitable for sustainable agriculture, the highest product yield per unit area, and the fight against pests. The increase in production has occurred simultaneously with a changing and increasingly unpredictable climate changing and becoming less predictable, cutting greenhouse gas emissions from agriculture, and shrinking or deteriorating soil and water resources (Popp et al. 2013). Reducing current yield losses caused by pests, pathogens, and weeds is the biggest challenge for agricultural production. The intensity of crop protection has increased significantly, as exemplified by a 15-20-fold increase in the number of pesticides used worldwide (Oerke 2005). While the use of pesticides increases agricultural production on the one hand, they also cause human and environmental health problems directly or indirectly as a result of unconscious and incorrect use. They can cause acute or chronic poisoning in people fed with foods containing high doses of pesticide residues and other living things in the environment, and they can cause aroma and quality changes, especially in some products. Widespread pesticide applications in agriculture and industry's pollution of the natural environment have a negative impact on living organisms. Pesticides that are withdrawn from use but remain in the environment produce serious and still unresolved ecotoxicological effects (Lew et al. 2009).

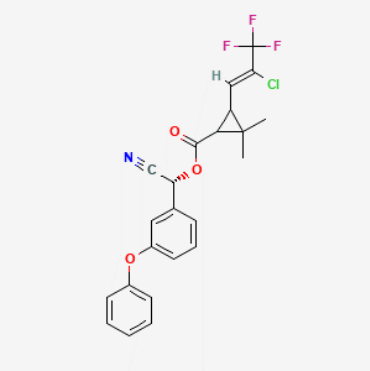
These agricultural pesticide products may reach water bodies due to drift, runoff, rainwater, and seepage (Geoffroy et al. 2004). Some organisms could be used as biological indicators for the presence of pollutants in the environment (Costa et al. 2008). To provide a basis for these assessments, organisms representing various levels of the food chain are used in acute and chronic toxicity experiments. These studies are easy-to-use materials and provide results on the possible ecotoxicological effects of pesticides on non-target organisms in aquatic environments (Florencio et al. 2014).

Aquatic invertebrates involve the effects of multiple stressors, potentially involving complex mixtures of pollutants, particularly affecting organism-sensitive developmental life stages such as growth, development, and reproduction (Oros and Werner 2005; Geist 2011; Brooks et al. 2012; Connon et al. 2012).

Pesticides entering the aquatic ecosystem can cause the reduction of aquatic organisms, such as shrimps, frogs, turtles, waterfowl, and fish in form of pathology or death. Aquatic animals are the main source of natural food chains. If other animals and humans consume these creatures, pesticides affect them. Using pesticides disadvantages people, animals, beneficial plants, and the persistence of some of these chemicals in the environment (long life) poses a serious danger to both human health and the environment (Lakhani 2015).

Gamma Cyhalothrin (GCH) is an insecticide used in agriculture in various fields such as apple, pear, quince, pistachio, tomato, hazelnut, cabbage, corn, cotton, potato, olive, and vineyard.

• **GCH Structure;**




• **GCH Molecular Formula;** $C_{23}H_{19}ClF_3NO_3$

• **Synonyms;**

- GAMA-CYHALOTHRIN
- 91465-08-6
- Cyclopropanecarboxylic acid,3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethyl-,(R)-cyano(3-phenoxyphenyl)methyl ester, (1S,3S)-rel-
- CHEMBL2270530
- SCHEMBL13408920

• **Molecular Weight;** 449.8 g/mol (National Center for Biotechnology Information 2024)

There are some organisms that are used as indicators to determine the pollution and pollutants in the waters. Among these indicators, mussels have an important place due to their non-selective feeding and sessile life.

Zebra mussel (*Dreissena polymorpha*) is economically damaging to the structures in the aquatic environment due to its nutritional characteristics, sessile (host) growth and high reproductive characteristics and can cause changes in the freshwater environment. Due to its water filtering feature, zebra mussels cause a decrease in phytoplankton in the environment. *D. polymorpha*

may cause the imbalance of the very sensitive food chain to deteriorate and the aquatic ecosystem may be adversely affected by them (Serdar 2021). In addition, the need for mussels to stick to hard surfaces causes problems. Clogging of pipes, coating of piers, corrosion of steel and concrete, in addition rotting mussels cause bad odor on beaches (Pet İhtiyaç 2013).

The longevity of freshwater mussels, limited mobility and filter feeding, and biomarkers enable their widespread and reliable use in toxicological studies in the examination of pollution in aquatic ecosystems (Serdar et al. 2021). Since *D. polymorpha* has a strong oxidative defense and a relatively high resistance to xenobiotics, it is widely used to conduct ecotoxicological experiments (Faria et al. 2009).

In this study, the effect of SOD, CAT, GPx, TBARS and GSH biomarkers on *D. polymorpha* exposed to pesticides with GCH active ingredient was investigated.

## Materials and Methods

*D. polymorpha* individuals were collected from the Euphrates River (38º 48' 25 ”N, 38º 43' 51” E). Samples taken from the Euphrates River were brought to Munzur University, Aquaculture Research Center in plastic bottles and placed in stock tanks. Before being used in the experiments, they were fed microalgae for at least 15 days for adaptation during to laboratory conditions. Then stocked in 500 L ventilated tanks with a temperature adjusted to 18 ºC, in a 12:12 hour light:dark cycle in an environment similar to natural living conditions. Healthy organisms (living things that perform siphoning) at similar developmental stages were selected for the study and were not fed during the experimental study (Serdar et al. 2021).

Gamma Cyhalothrin was obtained from a company selling agricultural chemicals with the number GCH CAS 76703-62-3 and a molecular weight of 449.8 g/mol.

A standard test value was used to determine $LC_{50}$. For this purpose, interval determination tests were applied. After range testing, *D. polymorpha* individuals were exposed to five determined concentrations of GCH (0.0 (control), 0.05, 0.25, 1.25, 6.25, 31.25 mg/L) for 24, 48, 72, and 96 hours. In acute toxicity experiments, organisms were checked every 24 hours and dead individuals were noted and excluded from the experimental groups. Acute toxicity ($LC_{50}$) values were calculated by performing probit analysis with the data obtained. Sublethal trial concentrations were established in proportion to the calculated $LC_{50}$ values (1/8, 1/4, and 1/2). The following experimental groups were formed to expose *D. polymorpha* to GCH sublethal concentrations at the mentioned concentrations at 24 and 96 hours.

Control - 24 Group: Group not exposed to any treatment for 24 hours,

Control - 96 Group: Group not exposed to any treatment for 96 hours,

C1 - 24 Group: 24-hour exposure to GCH active ingredient at the rate of 1/8 of the $LC_{50}$ value,

C1 - 96 Group: 96-hour exposure to GCH active ingredient at the rate of 1/8 of the $LC_{50}$ value,

C2 - 24 Group: 24-hour exposure to GCH active ingredient at 1/4 of the $LC_{50}$ value,

C2 - 96 Group: 96-hour exposure to GCH active ingredient at 1/4 of the $LC_{50}$ value,

C3 - 24 Group: 24-hour exposure to GCH active ingredient at the rate of 1/2 of the $LC_{50}$ value,

C3 - 96 Group: 96-hour exposure to GCH active ingredient at 1/2 of the $LC_{50}$ value.

**Biochemical response**

All application experiments were carried out in 3 repetitions and 7 pieces of *D. polymorpha* were used for each experimental group. The samples, whose trial phase was finished, were kept at -80 degrees until analysis (Aydın and Serdar 2024a). To determine the biochemical response, SOD (Catalog No 706002), CAT (Catalog No 707002), and GPx (Catalog No 703102) enzyme activities, MDA (Catalog No 10009055) and GSH (Catalog No 703002) levels were determined in a microplate reader. Assay kits were purchased from CAYMAN Chemical Company.

**Dissection procedures and preparation of supernatants**

Test organism individuals were the shells were separated with a scalpel and the body tissue inside the shell was taken. 0.5 g of organisms was weighed and homogenized. Using an iced homogenizer, PBS (phosphate buffered saline) buffer was added. These homogenized samples were centrifuged at 17000 rpm for 15 minutes in a chilled environment. The resulting supernatants were kept in a -80 ºC freezer until measurement (Aydın and Serdar 2024b).

**Histopathological evaluation**

*D. polymorpha* samples left in the Bouin’s tissue fixation solution were fixed for 24 hours. Afterward, the samples passed through 50 %, 70 %, 80 %, 96 I %, 96 % II, 100 % I, and 100 % II alcohol series (ethyl alcohol) were passed through xylol I, xylol II and xylol III series and kept in a xylol-paraffin mixture in a 45 ºC oven for 1 night (Parlak Ak et al. 2022). Following this, paraffin blocks were prepared by keeping them in three different paraffin series for one hour. Samples were embedded in paraffin. Serial sections were taken from the prepared paraffin blocks on a microtome on 4 μm thick. These sections were

dried at room temperature for 24 hours or more. *D. polymorpha* sections were stained according to Hematoxylin-Eosin staining method and examined under a light microscope (Olympus BX-51, Olympus Optical Co., Ltd., Tokyo, Japan) for histological examinations Histopathological changes in the samples were scored according to a four-stage semi-quantitative assessment (0; normal appearance, 1; mild changes, 2; moderate changes, 3; severe changes) (Mantecca et al. 2006).

**Statistical analysis**

The $LC_{50}$ value was calculated using SPSS 24.0 package program probit analysis. SPSS 24.0 package program one-way ANOVA was used for the evaluation of biochemical analyzes (Serdar et al. 2024).

The statistical analysis of the biochemical data was performed using SPSS 24.0 package programs. Changes in values of GCH in the biochemical parameters of the control and exposure groups were tested by Duncan's ($p<0.05$) multiple range test. Application times were compared using a two-way analysis of variance (ONEWAY–ANOVA) and independent ($p<0.05$) t-tests (Aydın et al. 2022).

## Results

**Determination of acute ($LC_{50}$) value**

$LC_{50}$ values obtained by applying standard static acute toxicity tests were calculated as 13.64 mg/L by the SPSS package program probit analysis.

**Determination of biochemical response**

**MDA level**

It was determined that the MDA level in the test organism exposed to GCH at different concentrations and durations increased statistically significantly ($p<0.05$) compared to the control group (Figure 1).

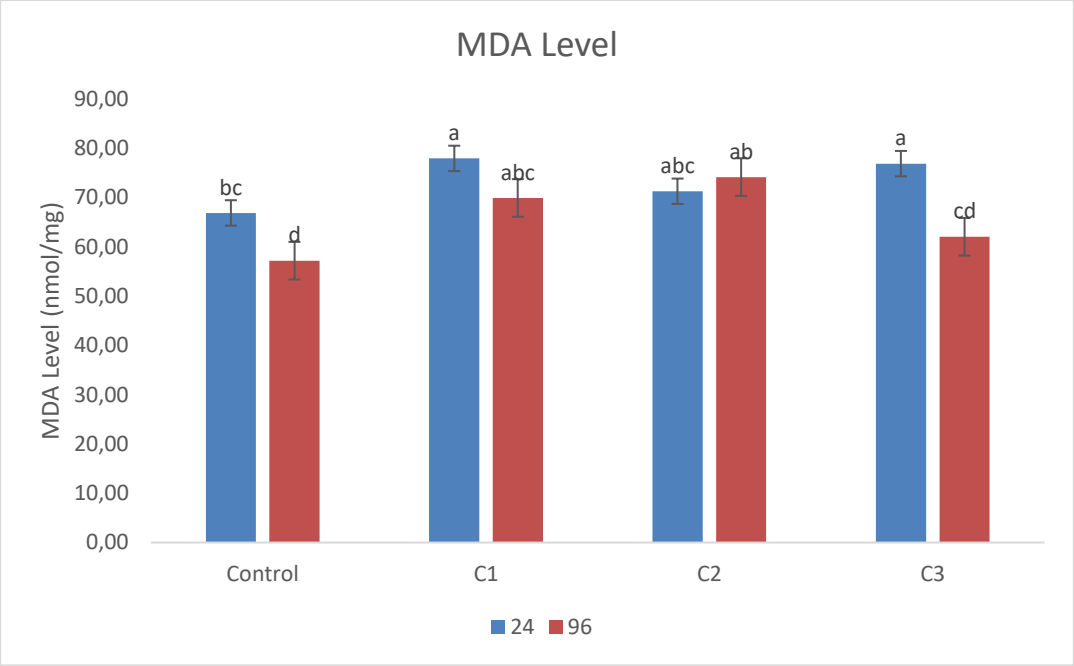



**Figure 1.** Changes in MDA (pg/mL) enzyme activity in *D. polymorpha* treated with different doses of GCH. Different letters on the bars indicate a statistically significant difference between groups in the same treatment period, $^{abc}p<0.05$ (according to Duncan's multiple comparison test) shows the statistical difference between 24$^{th}$ and 96$^{th}$ hours in the same treatment group (Independent T-test).

**GSH level**

It was determined that the GSH level in the test organism exposed to GCH at different concentrations and durations increased statistically significantly ($p<0.05$) compared to the control group (Figure 2).

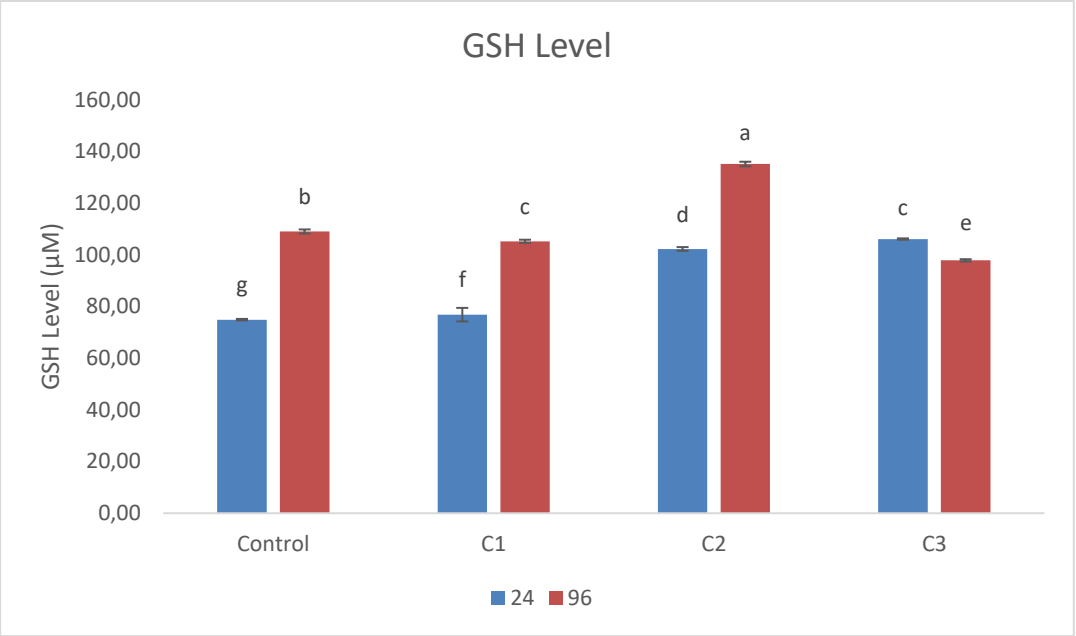



**Figure 2**. Changes in GSH (pg/mL) enzyme activity in *D. polymorpha* treated with different doses of GCH. Different letters on the bars indicate a statistically significant difference between groups in the same treatment period, $^{abc}p<0.05$ (according to Duncan's multiple comparison test) shows statistical difference between 24$^{th}$ and 96$^{th}$ hours in the same treatment group (Independent T-test).

**CAT activity**

It was determined that the CAT activity in the test organism exposed to GCH at different concentrations and durations decreased statistically significantly ($p<0.05$) compared to the control group (Figure 3).

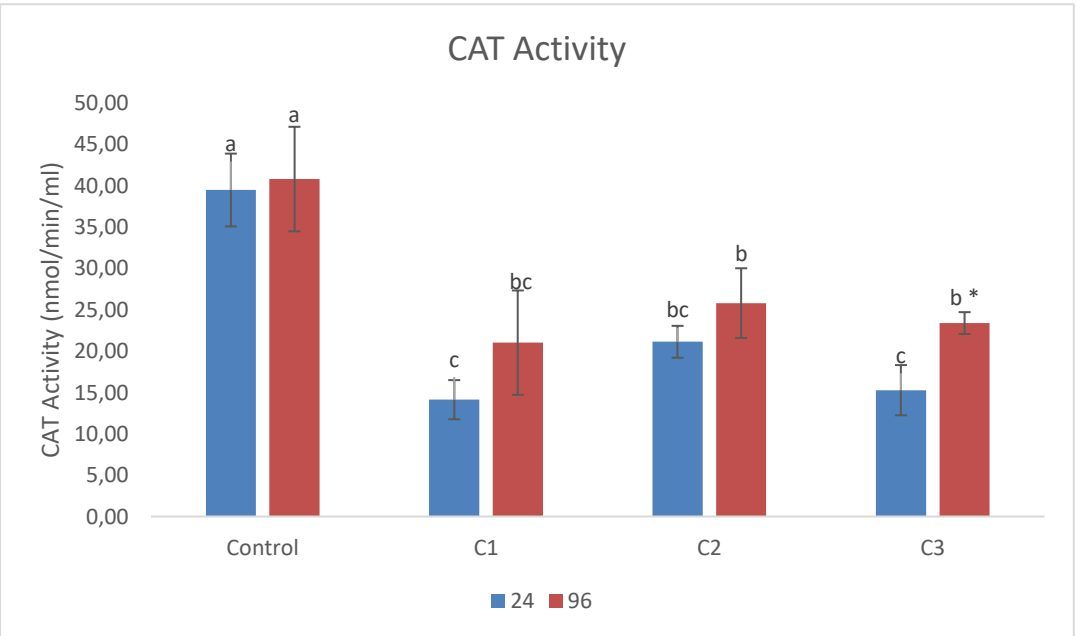



**Figure 3.** Changes in CAT (pg/mL) enzyme activity in *D. polymorpha* treated with different doses of GCH. Different letters on the bars indicate a statistically significant difference between groups in the same treatment period, $^{abc}p<0.05$ (according to Duncan's multiple comparison test) shows the statistical difference between 24$^{th}$ and 96$^{th}$ hours in the same treatment group (Independent T-test).

**SOD activity**

It was determined that the SOD activity in the test organism exposed to GCH at different concentrations and durations decreased statistically significantly ($p<0.05$) compared to the control group (Figure 4).

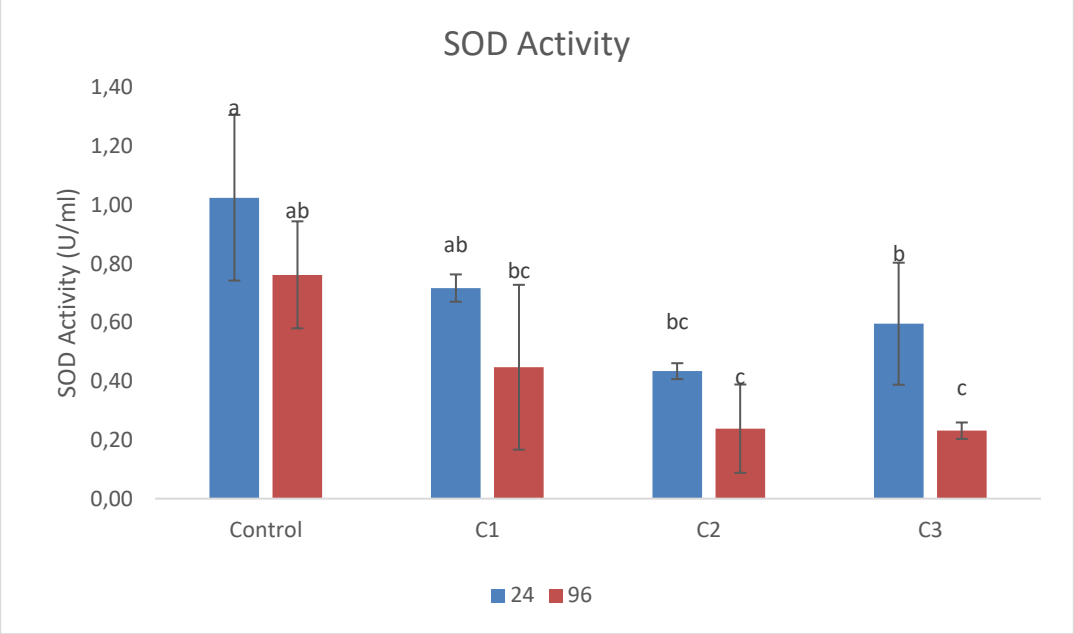



**Figure 4.** Changes in SOD (pg/mL) enzyme activity in *D. polymorpha* treated with different doses of GCH. Different letters on the bars indicate a statistically significant difference

**GPx activity**

It was determined that GPx activity in the test organism exposed to GCH at different concentrations and durations increased statistically significantly ($p<0.05$) at the 24$^{th}$ hour compared to the control group (Figure 5).

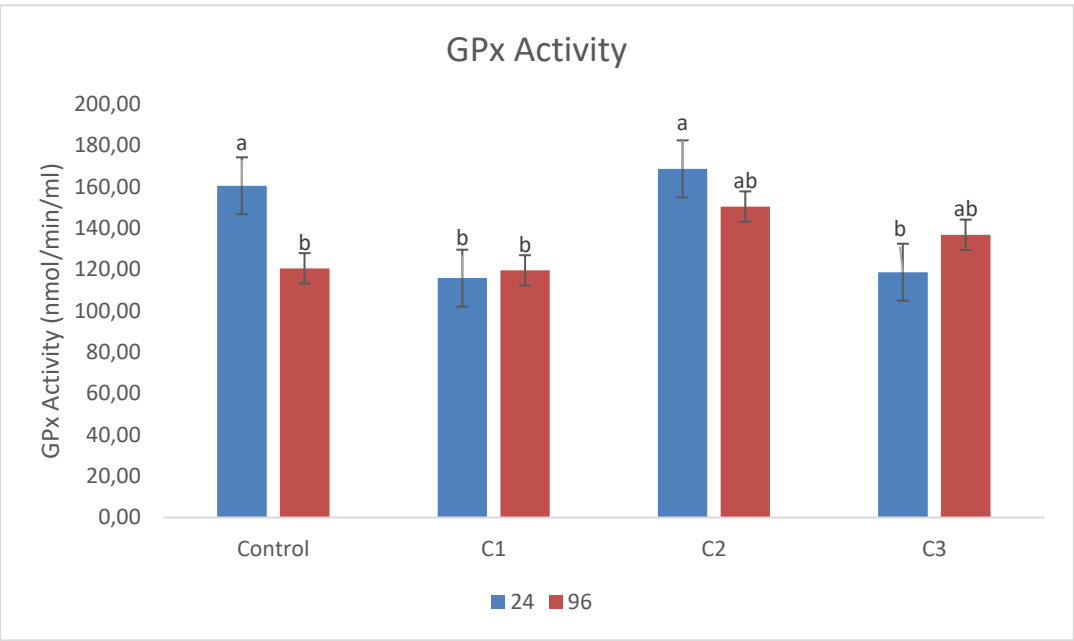



**Figure 5.** Changes in GPx (pg/mL) enzyme activity in *D. polymorpha* treated with different doses of GCH. Different letters on the bars indicate a statistically significant difference between groups in the same treatment period, $^{abc}p<0.05$ (according to Duncan's multiple comparison test) shows statistical difference between 24$^{th}$ and 96$^{th}$ hours in the same treatment group (Independent T-test).

**Histopathological data**

Normal histological appearance was observed in the gill tissues of the Control-24 group (Figure 6a). In addition, mild histopathological changes were observed in the gill tissues of the C1-24 group (Figure 6b), moderate level (Figure 6c) in the C2-24 group, and severe histopathological changes in the C3-24 group (Figure 6d).

Normal morphological appearance was observed in the gill tissues of the Control-96 group (Figure 7a). In addition, mild histopathological changes were observed in the tissues of the C1-96 group (Figure 7b), moderate level (Figure 7c) in the C2-96 group, and severe histopathological changes in the C3-96 group (Figure 7d).

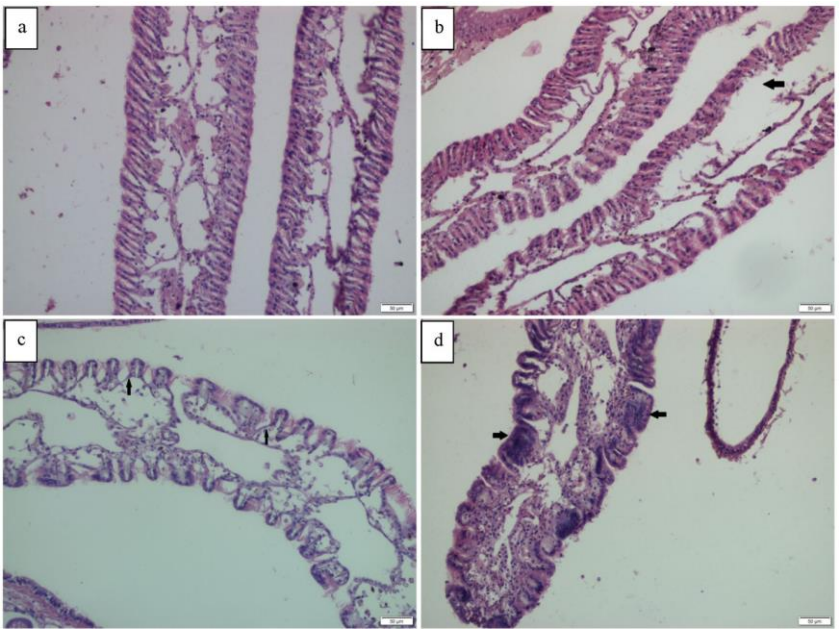



**Figure 6.** Normal histological structure in the gill tissue of the Control-24 group (a), separation (arrow) in the gill tissue of the C1-24 group (b), epithelial lift (arrows) in the gill tissue of the C2-24 group (c), epithelial hyperplasia in gill tissue of group C3-24 (arrows) (d), H&E.

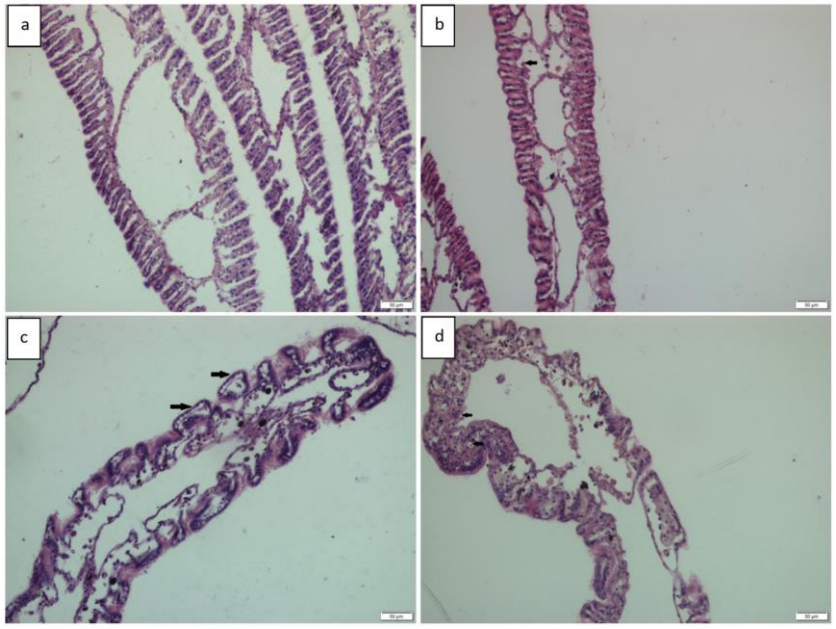



**Figure 7.** Normal histological structure in the gill tissue of the Control-96 group (a), cellular desquamation (arrow) in the gill tissue of the C1-96 group (b), enlargement of the epithelial layer in the gill tissue of the C2-96 group and missing (arrows) (c), Necrosis of gill tissue from group C3-96 (arrows) (d), H&E.

## Discussion

According to various research data, it is stated that oxidative stress is the underlying mechanism of various pollutant-induced cytotoxicity (Yang et al. 2004; Milatovic et al. 2007).

Oxidative stress is defined as the potential for damage to tissues and cellular components with an imbalance between ROS production and removal and is generally accepted in toxicology studies. Pollutants including pesticide pollution, oxidative damage and antioxidant damage caused by the antioxidant defense developed against the formation of oxidative stress in organisms. The sensitivity of the defense to these effects is used to evaluate the toxic effects of these compounds (Valavanidis et al. 2006).

The biological role of superoxide dismutase (SOD) antioxidant defense is to defend cells against the toxic effects of $O_2$ by catalyzing dismutation reactions (Prasad 2004). Since the SOD-CAT system represents the first line of defense against oxidative stress, an increase in catalase (CAT) and SOD activity is usually observed in response to environmental pollutants (McCord 1996). While the SOD enzyme converts the superoxide anion radical ($O_2^-$) to hydrogen peroxide ($H_2O_2$), the CAT enzyme converts the released $H_2O_2$ to water and molecular oxygen (Pandey et al. 2003). It is also known that CAT and GPX enzymes compete with each other in the detoxification of $H_2O_2$ (Cheung et al. 2004). Serdar (2021) investigated some biochemical responses of commercial insecticide Cyfluthrin (CFT) in *D. polymorpha* and reported that SOD activity was increased in *D. polymorpha* individuals compared to the control, while CAT activity was inhibited compared to the control. Greco et al. (2011), bivalve *Mya arenaria* living organism in their study, dichlorophenoxyacetic acid (2,4-D), 2-(2-methyl-4-chlorophenoxy) propionic acid (mecoprop), and a formulation containing 3,6-dichloro-2-methoxy benzoic acid. They observed enzyme activities by exposing them to acid (dicamba) herbicides. As a result of the study, they found a decrease in SOD enzyme activities. Tutuş (2016) investigated the effects of organophosphorus insecticide chlorpyrifos (CPF) and avermectin insecticides abamectin (ABM) and emamectin benzoate (EB) on antioxidant parameters and lipid peroxidation in the liver selected as the target organ in *Oreochromis niloticus*. As a result of the study, SOD activity showed a decrease in the effect of all pesticides tested in durations. Xiong et al. (2011), investigated the acute toxicity, oxidative stress, and damage of NPs in *Danio rerio*, the fish were exposed to the effects of $TiO_2$ and ZnO NPs, and they determined decreases and increases in SOD activity. Yuksel et al. (2020), found reductions in SOD activity in *Gammarus pulex*, where they applied malathion. Florescu et al. (2021), found decreases and increases in SOD activity in the intestines of Sturgeon Fish (*Acipenser stellatus*) fry. Magara et al. (2021), found a decrease in SOD activity in the oxidative stress determination studies of Bronopol and Detarox AP on *Sinanodonta woodiana*. It could be said that the changes in SOD enzyme activities in *D. polymorpha* individuals exposed to GCH application are in agreement with the studies in the literature.

Korkmaz et al. (2018) evaluated the toxic effects of adult zebrafish (*D. rerio*) exposure to organophosphate group pesticides for alone and pyrethroid group pesticides cypermethrin and their mixtures at sublethal doses, and the reversibility of these effects using various biochemical markers. CAT and GPx enzyme parameters were decreased. Hao et al. 2009 investigated the subacute effect of $TiO_2$-NP on oxidative stress and histopathological variables in *Cyprinus carpio*. Fish were exposed to 10, 50, 100, and 200 mg/L $TiO_2$-NP for 8 days, and CAT and peroxidase activities and lipid peroxidation levels in the liver, gill, and brain tissues were investigated. As a result of the study, significant decreases were determined in CAT and peroxidase activities in the examined tissues, especially at high ambient concentrations (100 and 200 mg/L) of $TiO_2$-NP. Tonn et al. (2016), found decreases in CAT values in their study with *Holothuria forskali*. Xiong et al. (2011), observed increases and decreases in CAT levels in their study. Yuksel et al. (2020), found decreases in CAT activity in study. Florescu et al. (2021), detected reductions in CAT activity. Magara et al. (2021), determined reductions in CAT activity. Chen et al. (2020), they investigated the levels of different toxic metals and the extent of oxidative stress responses in *Sinonovacula constricta*. As a result of the research, they observed first increases and then decreases in CAT activity. It could be said that the changes in CAT enzyme activities in *D. polymorpha* individuals exposed to GCH application are in agreement with the studies in the literature.

The decrease in GCLC protein synthesis, which provides the production of SOD, GPX, GST and GSH, leads to a decrease in the antioxidant defense of the cell and thus an increase in the amount of MDA. The CAT enzyme is sensitive to excessive superoxide radical production and its activity decreases under these conditions Kono and Fridovich (1982), this inhibition is also hypothesized to contribute to the increase in MDA levels. Depletion leads to an imbalance in the redox state and ability to cope with organic xenobiotics metabolized by glutathione S-transferase (GST) and glutathione peroxidases (GPx). The effects of various

xenobiotics on GPx activity in aquatic organisms have been investigated. Yuksel et al. (2020), observed decreases in GPx values of malathion substance in *G. pulex* in their study. The study of Magara et al. 2021 examined the acute and non-lethal toxicity of Bronopol and Detarox® AP in the crustacean *S. woodiana* and found decreases in GPx levels as a result. Chen et al. (2020), in their study investigated the levels of different toxic metals and the extent of oxidative stress responses in *S. constricta* and reported decreases in GPx levels. Changes in GPx activities in *D. polyhmorpha* exposed to GCH are similar to the literature.

GSH is recognized as a primary line of defense against free oxygen radicals in the antioxidant system (Cnubben et al. 2001; Dickinson and Forman 2002). Chemicals can alter the reduced amounts of GSH Rama (1998). GPx reduces reactive lipid hydroperoxides to prevent malondialdehyde (MDA) formation. Glutathione (GSH) is an important antioxidant that functions as a direct scavenger of oxidants as well as an antioxidant enzyme substrate (Ferrari et al. 2007; Serdar et al. 2021), in their study on *D. polymorpha* individuals, some biochemical responses of the commercial insecticide Beta-Cyfluthrin (β-CF) were investigated in *D. polymorpha*. It was reported increased levels of MDA and GSH in *D. polymorpha* exposed to β-CF compared to control. Kaya et al. (2013) used heavy metals (Cu, Fe, Cd, Pb, Zn and Mn) to analyze the biomarker (Glutathione), which is an indicator of oxidative stress. As a result of the analyzes made, they observed increases in GSH levels. Xiong et al. (2011), in their studies investigating the acute toxicity, oxidative stress, and damage of NPs in *D. rerio*, fish were exposed to $TiO_2$ and ZnO NPs, radical production and its biochemical effects were investigated. As a result of the research, GSH levels decreased and increased depending on the type of NP and tissues. Yuksel et al. (2020) investigated the oxidative stress and antioxidant levels of malathion pesticides on *G. pulex*. As a result of the study, they observed a decrease in GSH levels. Chen et al. (2020) investigated the levels of different toxic metals and the extent of oxidative stress responses in *S. constricta*. They observed increased GSH levels. In this study, which was conducted to investigate the effect of GCH pesticide on *D. polymorpha* individuals, it is thought that the increase in GSH levels is compatible with the literature.

Florescu et al. (2021) were study on *A. stellatus* also determined increases in MDA levels, Liang et al. (2016), investigated the changes in mRNA expression of genes related to endoplasmic reticulum (ER) stress marker and unfolded protein response (UPR) and redox enzyme and apoptosis in the ammonia of hepatopancreas in pacific white shrimp and observed that MDA increased as a result of their research. Chen et al. (2020) and Magra et al. (2021) observed that the MDA level increased in their study. In this study, it is thought that the changes in MDA levels in *D. polymorpha* individuals are caused by exposure to GCH pesticide.

Histopathology is the examination of changes in organs, tissues, and cells under a microscope using various methods. Information obtained from studies in the field of histopathology sheds light on the microscopic structure of tissues (Vikipedi Özgür Ansiklopedi 2023).

Exposure of organisms to pollutants causes damage to tissues and cells, thus harming the organism. In this study, it is thought that the histological effects in *D. polymorpha* are also dependent on the GCH concentration and time of exposure. Binelli et al. (2004), degenerations were observed with the accumulation of basophilic material in the cell apex of *D. polymorpha*, which they exposed to DDT. Mantecca et al. (2006), examined the histopathological data of *D. polymorpha*, in which they applied the herbicide paraquat (PQ), and as a result, they observed severe lesions such as cellular vacuolation, lysis and germinative epithelial thinness in the digestive gland and testis. Giamberini et al. (1996) investigated histopathological changes in the gills of *D. polymorpha* exposed to a new molluscicide and found serious degenerations in the tissues as a result of the research. Fisher et al. (1991) examined *D. polymorpha*, which they exposed to potassium in their study, and reported that potassium caused deterioration in the gill epithelium. Yancheva et al. (2020), applied cadmium (Cd) and polyaromatic hydrocarbons (PAHs) in *D. polymorpha* in their study and emphasized that Cd and PAHs cause serious damage to the gills of *D. polymorpha*. Shan et al. (2020) exposed *Corbicula fluminea* to imidacloprid and observed significant histopathological changes as a result of degeneration of ciliates, contraction, and adhesion of lymphocytes, and swelling of epithelial cells in the gills and marked degeneration of digestive tubules, hemolytic infiltration of connective tissue, and epithelial cell necrosis. Benjamin et al. (2019) stated in their study that the digestive gland of *C. fluminea*, which they exposed to Bisphenol A (BPA), was the most affected tissue followed by the gill and then the adductor muscles. Baratange et al. (2022), in their study applied carbamazepine and methylmercury to *D. polymorpha* and found that exposure to MeHg caused a high degree of gill fibrosis, deformation, and change as a result of the application: 25-50% of the respiratory surface of the gills changed and 20-40%

of the digestive tubules observed numerous fibrosis such as ongoing and terminal lysis or necrosis and cell changes such as pycnotic nuclei. Hossain et al. 2023 reported that chlorpyrifos caused moderate to severe pathological symptoms in the treatment groups when compared with the control in the gill, muscle and ovary histopathology of *Lamellidens marginalis*. Moreira et al. 2023 reported that mild to severe filament degradation and ciliary deformation occurred in Limnoperna fortunei under the influence of two biocides -MXD-100$^{TM}$ and sodium dichloroisocyanurate (NaDCC). Dethe and Ahire 2023, Toxicity of Imidacloprid, reported histopathological changes in gill, mantle and digestive gland on *L. marginalis*. Histopathological damage to the tissues of *D. polymorpha* individuals is thought to be due to exposure to GCH.

## Conclusion

According to the results of the data obtained from the study, the toxic effect of GCH pesticide on *D. polymorpha* was determined. It was concluded that GSH, MDA, SOD, CAT, and GPx are useful biomarkers in investigating the toxic effects of filtered test organism *D. polymorpha*. The results obtained indicate that changes in biomarker levels are dependent on both the concentration and duration of exposure show that the response of the test organism to the toxic substance varies with the concentration of the toxic substance and the duration of administration.

Changes in oxidant-antioxidant levels in aquatic environments may be directly related to pesticide pollution, as well as other environmental stress factors that can act together with pesticides. Environmental factors such as dissolved oxygen level, temperature, salinity, and the presence of organic pollutants can affect the oxidant-antioxidant status of organisms (Livingstone 2003). In future studies, it is recommended to carry out long-term follow-up studies, taking into account these stress factors. As a result of organisms being exposed to pollutants, deterioration occurs in their cells, tissues and organs and histological damage occurs. The magnitude of this damage varies according to the duration and concentration of exposure, and it is thought that the damage increases as the concentration and duration increase.

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