# **Evaluation of the Effect of Temperature on the Toxicity of Lambda-Cyhalothrin in Dreissena Polymorpha Using some Biochemical Biomarkers**

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Received: 28 February 2023	<b>Abstract:</b> Due to increasing climate change, it has become important to determine whether the dose-response relationship of organisms to some substances is affected by temperature. For this reason, in this study, it was aimed to reveal the effect of the temperature variable on the toxic response using the <i>Dreissena polymorpha</i> model organism and some of its biomarkers. For this purpose, acetylcholinesterase (AChE), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) levels in <i>Dreissena polymorpha</i> exposed to subletal concentrations of $\lambda$ -cyhalothrin at different temperatures were measured using commercial ELISA kits. According to the results obtained, there was a statistically significant increase in MDA levels in the groups exposed to $\lambda$ -cyhalothrin, while a decrease in GSH levels was found. AChE levels were inhibited especially in the groups exposed high concentration of $\lambda$ cyhalothrin. It was also found that the inhibition levels increased depending on the application times. While SOD enzyme activity decreased, CAT enzyme activity increased depending on the exposure concentration. It has been observed that different temperature have different effects on the toxicity of $\lambda$ -cyhalothrin. It was observed that $\lambda$ -cyhalothrin caused oxidative stress and neurotoxicity, and the toxicity of $\lambda$ -cyhalothrin changed depending on the toxicity of the toxicity of $\lambda$ -cyhalothrin caused oxidative stress and neurotoxicity.

# Introduction

Lambda-cyhalothrin ( $\lambda$ -cyhalothrin) is a synthetic pyrethroid insecticide. It is used in agriculture, household pest control, food preservation and disease vector control (1). There is limited information on the environmental concentrations in surface waters of these pyrethroids whose are widely used around the World (2, 3).  $\lambda$ -cyhalothrin concentrations in surface waters range from 346 ngL<sup>-1</sup> (4) to 797 ngL<sup>-1</sup> (5). It is known that pesticides cause serious toxicological effects and biochemical dysfunctions. Recent studies (6, 7) have shown that  $\lambda$ -cyhalothrin cause neurotoxicity, hepatotoxicity and oxidative damage.

 $\lambda$ -cyhalothrin itself does not directly generate free radicals, but indirectly contributes to the formation of various types of radicals such as superoxide radical, peroxynitrite, nitric

oxide and nitrogen species such as hydroxyl radical, causing oxidative stress (8, 9). These radicals cause lipid peroxidation in the cell membrane, leading to destabilization and fragmentation of the membrane (10). Lipid peroxidation, induced by reactive oxygen species (ROS), is a common oxidative stress biomarker of toxicants. Lipid peroxidation caused by ROS is the most important biomarker of oxidative stress (11). The MDA, the most important indicator of lipid peroxidation, is a secondary product of lipid peroxidation (12). It is known that pyrethroids show their neurotoxicity mainly by interfering with the function of sodium channels and calcium-dependent chloride channels in the central nervous system (13).  $\lambda$ cyhalothrin can cause neurotoxicity in non-target aquatic organisms and aquatic invertebrates through acetylcholine, a neurotransmitter substance (14, 15, 16). It has been shown in studies that changes in AChE activity are used as potent biomarkers for organophosphorus and carbamate pesticides (17).

GSH, which is a part of the secondary defense system, is a non-enzymatic antioxidant and plays a role in scavenging free radicals (18). Reduced GSH protects cell membranes from lipid peroxidation and non-protein thiol and is one of the main reducers found in cells (19).The most important defense mechanisms against the toxic effects of oxygen metabolism are SOD and CAT. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, whereas CAT; converts hydrogen peroxide into water. These enzymes play a very important role in mitigating the toxic effects of ROS (20).

*D. polymorpha*, commonly known as the zebra mussel, is native to the Palearctic region of the world, the freshwater drainage basins of the Caspian and Black Sea, and the Dniester, Volga, Danube, and Ural rivers (21). Climate change is causing rivers and lakes to warm and glaciers to shrink, which in turn changes the quantity and quality of melt water (22). Because it is sensitive to environmental changes, one of the most valuable invertebrate models for freshwater ecotoxicological studies is the bivalve zebra mussel (*Dreissena polymorpha*), which has been extensively used for biomonitoring of organic pollutants and for the evaluation of several biomarkers, both in vitro and in vivo (23).

Temperature, one of the main environmental stressors, can interact with insecticides (24). It has been studied in many aquatic organisms, including amphibians, crustaceans, annelids and arthropods, where chemical pollutants may interact with natural stressors (eg, temperature, predation, larval competition) (25).

In this study, we evaluated the effects of  $\lambda$ -cyhalothrin on *D. polymorpha* at different temperatures to determine whether temperature and pesticides interact synergistically on some biochemical biomarkers in *D. polymorpha*.

# **Material and Methods**

### Chemicals

All chemicals were used directly without further purification.  $\lambda$ -cyhalothrin was purchased from local chemicals market from Turkey. The studied concentrations were prepared by diluting commercially purchased  $\lambda$ -cyhalothrin with distilled water.

### Model organism

The model organism *D. polymorpha* individuals used in the study were obtained from culture collection of Fiseheries Labaratuavary, Munzur University. The cultured *D*.

*polymorpha* individuals were brought alive to the Toxicology Research Laboratory and adapted to these conditions for 30 days.

#### Adaptation of D. polymorpha to laboratory conditions

D. polymorpha were selected from healthy individuals of similar size. Laboratory temperature and lighting was controlled. In the illumination, a photoperiod of 12:12 light:dark was applied. Temperature was set  $19\pm0.5$  °C,  $22\pm0.5$  °C and  $25\pm0.5$  °C with thermostat at all experimental stages. Also external filter was used for water circulation in stock aquariums where the test organism is kept. Nutrition and mobility of living organisms has been observed during adaptation.

#### **Experimental design**

The following 12 test groups at the ratios of 1/20, 1/10 and 1/5 of  $LC_{50}$  values created at different tempatures 19, 22 and 25 °C. Ten *D. polymorpha* individuals were used in all groups.

Control group, organisms not exposed to any substance at 19°C. Group A, organisms were exposed to  $\lambda$ -cyhalothrin at 1/20 of the LC<sub>50</sub> value at 19 °C. Group B, organisms were exposed to  $\lambda$ -cyhalothrin at 1/10 of the LC<sub>50</sub> value at 19 °C. Group C, organisms were exposed to  $\lambda$ -cyhalothrin at 1/5 of the LC<sub>50</sub> value at 19 °C. Control group, organisms not exposed to any substance at 22 °C. Group D, organisms were exposed to  $\lambda$ -cyhalothrin at 1/20 of the LC<sub>50</sub> value at 22 °C. Group E, organisms were exposed to λ-cyhalothrin at 1/10 of the LC<sub>50</sub> value at 22 °C. Group F, organisms were exposed to  $\lambda$ -cyhalothrin at 1/5 of the LC<sub>50</sub> value at 22 °C. Control group, organisms not exposed to any substance at 25 °C. Group G, organisms were exposed to  $\lambda$ -cyhalothrin at 1/20 of the LC<sub>50</sub> value at 25 °C. Group H, organisms were exposed to  $\lambda$ -cyhalothrin at 1/10 of the LC<sub>50</sub> value at 25 °C. Group I, organisms were exposed to  $\lambda$ -cyhalothrin at 1/5 of the LC<sub>50</sub> value at 25 °C.

#### Supernatant preparation

For supernatants preparation, the shells of *D. polymorha* organisms were opened by cutting the adductor muscles with the help of scapula, scalpel and spatula and then 0.5 g of *D. polymorpha* individuals were weighed and 1/5 w/v in PBS buffer containing 5  $\mu$ L of protease inhibitor cocktail and homogenize using a homogenizer with ice. These homogenized samples were placed in a cooled centrifuge at 17000 g for 15 minutes. The obtained supernatants were stored in a deep freezer at -80 °C.

#### **Determination of biochemical response**

In our study, AChE, SOD, CAT enzymes and GSH, MDA levels were used to determine the biochemical response. GSH, SOD, CAT and MDA kits were purchased from CAYMAN and

AChE kits used in the study were purchased from CUSABIO company (Catalog numbers GSH: 703002, SOD: 706002, CAT: 706002 AChE: CSB-E17001Fh, MDA: 10009055).

GSH assay kit utilizes a carefully optimized enzymatic recycling method, using glutathione reductase, for the quantification of GSH. The sulfhydryl group of GSH reacts with

DTNB (5,5'-dithio-bis2-(nitrobenzoic acid), Ellman's reagent) and produces a yellow colored 5-thio2-nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 405-414 nm provides an accurate estimation of GSH in the sample.

Superoxide dismutase assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical measured in change in absorbance per minute at 25°C and pH 8.0.

Catalase Assay Kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to a purple color.

TBARS Assay Kit provides a simple, reproducible, and standardized tool for assaying lipid peroxidation. The MDA-TBA adduct formed by the reaction of MDA and TBA under high temperature (90-100°C) and acidic conditions is measured colorimetrically at 530-540 nm or fluorometrically at an excitation wavelength of 530 nm and an emission wavelength of 550 nm.

AChE assay kit employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for AChE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AChE present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for AChE is added to the wells. The color development is stopped and the intensity of the color is measured.

#### Statistical analysis

Statistical analysis were carried out using SPSS 24.0 statistics program. The  $LC_{50}$  value of  $\lambda$ -cyhalothrin Insecticide in *D. polymorpha* was calculated using Probit analysis. The statistical difference between different groups was determined by the Duncan's multiple range test. The difference between the application times (24 and 96 hours) was determined by independent t-test.

### Results

 $LC_{50}$  values of  $\lambda$ -cyhalothrin were determined with range determination tests in *D. polymorpha* individuals for 19, 22 and 25 °C and the results are shown in Table 1.

<b>Table 1:</b> LC <sub>50</sub> values of $\lambda$ -cyhalothrin for <i>D. polymorpha</i> individuals at
different temperatures1

Temperature (°C)	$LC_{_{50}}$ value (mg/L $\lambda$ -cyhalothrin)
19	2.23 ± 0.27 <sup>b</sup>
22	2.61± 0.1 <sup>0ab</sup>
25	2.71±0.21ª

<sup>1</sup>All data are presented as the mean ± standard error of the mean (SEM). Different letters on the means indicate a statistically significant difference between different temperatures

AChE activity increased at 19 °C at the end of the 24th hour in groups A and B when decreased as statistically significant in the group C compared the control group. AChE activity decreased in group D and increased in groups E and F at the end of 24th hour at 22 °C. An increase was detected at the end of the 96th hour at 22 °C. A statistically significant decrease was detected in group I at the end of the 24th hour at 25 °C in AChE activity but no statistically significant changes were observed at the end of 96th hour. Statistical differences were found in the groups B, D, E, H and I when application times compared (Figure 1).

A statistically significant increase was observed at 19 °C and 22°C in CAT activities in all application groups when compared to the control group at 24 and 96 h (p<0.05). A statistically significant increase was observed at 25 °C in CAT activities in groups G, H and I when compared to the control group during 24 h (p<0.05) but decreased during 96 hours. Statistical differences were found in the groups A, B, C, E, F, G, H, I when application times compared (p<0.05) (Figure 2).

A statistically significant decrease was observed in SOD activities in all application groups at 19, 22 and 25 °C when compared to the control group during 24 h (p<0.05). A statistically significant decrease was observed in the groups A, B, D, E, F, G and H at 19 °C, 22 °C and 25 °C when compared to the control group during 96 h (p<0.05). A statistical difference was found in the groups H and I when application times compared (p<0.05) (Figure 3).

GSH levels were decreased in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin at 19, 22 and 25 °C for 24 and 96 hours (p<0.05). A statistical difference was found in the groups H and I when application times compared (p<0.05) (Figure 4).

No significant changes were found in MDA levels at 19 °C compared to control for 24 hours (p< 0.05) but a statistically





**Figure 1:** Changes in AChE enzyme activities in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin pesticide at 19, 22 and 25 °C for 24 and 96 hours. Different letters on the columns indicate a statistically significant difference between different application doses, and the \* on the columns indicate a statistically significant difference between the application times (p<0.05)

**Figure 2:** Changes in CAT enzyme activities in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin pesticide at 19 °C, 22 °C and 25 °C for 24 and 96 hours. Different letters on the columns indicate a statistically significant difference between different application doses, and the \* on the columns indicate a statistically significant difference between the application times (p<0.05)

significant increase was observed in groups B and D when compared to the control group at 96<sup>th</sup> hours (p<0.05). MDA levels were increased significantly in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin at 22 °C and 25 °C for 24 and 96 hours (p<0.05). A statistical difference was found in the groups B and I when application times compared (p<0.05) (Figure 5).

### Discussion

 $\lambda$ -cyhalothrin is highly toxic to many aquatic organisms including fish, invertebrates and amphibians (26).  $\lambda$ -cyhalothrin is discharged directly water resources through agricultural use and forest spraying procedures and accumulates in sediment (27). Temperature has various effects on a wide range of physiological effects, including bioavailability, adsorption, elimination, and relative toxicity of chemicals in aquatic poikilotherms (28). Temperature can affect the physico-chemical behavior (decomposition, evaporation, transport, transfer and accumulation) of chemicals (29). Temperature can directly affect the mobility of the chemicals and change the uptake rate of these chemicals by aquatic organisms. Due to the rapid spread of chemicals at high temperatures, the rate of uptake of these chemicals into the organism increases. This results in faster reaching the toxicological threshold for the chemical. Temperature can also affect the toxicity of a chemical by affecting its degradation. In a study conducted Tasmin et al. (2014) it was shown that the herbicide diuron has lower toxicity at higher temperatures due to increased chemical degradation/volatility rates at higher temperatures in green algae Pseudokirchneriella ubcapitata (30). Similarly, damselfly Ischnura elegans exposed to the chlorpyrifos had lower toxicity at 24 °C versus 20 °C, as less toxic compounds were formed at a higher biodegradation rate (28). It has





**Figure 3:** Changes in SOD enzyme activities in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin pesticide at 19 °C, 22 °C and 25 °C for 24 and 96 hours. Different letters on the columns indicate a statistically significant difference between different application doses, and the \* on the columns indicate a statistically significant difference between the application times (p<0.05)

**Figure 4:** Changes in GSH levels in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin pesticide at 19°C, 22 °C and 25 °C for 24 and 96 hours. Different letters on the columns indicate a statistically significant difference between different application doses, and the \* sign on the columns indicate a statistically significant difference between the application times (p<0.05)

been observed that pyrethroids are more toxic in winter than in summer, and the 96-hour LC<sub>50</sub> values can change approximately tenfold at 10, 15 and 20°C (31). The zebra mussel is a creature whose body temperature changes according to environmental temperature fluctuations. The metabolic rate of zebra mussels can be affected by various factors, including temperature. The toxicity of pyrethroids was found to increase with decreasing temperature (32). Garcia et al. (2011) evaluated the effects of  $\lambda$ -cyhalothrin insecticide on earthworms using acute and chronic toxicity tests modified for tropical conditions (20 and 28 °C) and on two strains of compost worm (temperate and tropical). It has been observed that the effects of  $\lambda$ -cyhalothrin in soils do not change much at two temperatures. In tropical soils at high temperatures, the effects differ up to ten times. In present study, it has been observed that different temperature treatments have different effects on the toxic effects

of  $\lambda$ -cyhalothrin (33). These findings are consistent with the results of the current study.

In the literature, no study was found that studied the toxic effects of  $\lambda$ -cyhalothrin on *D. polymorpha* at different temperatures. Göksu et al. (2015) investigated the acute toxic effects of  $\lambda$ -cyhalothrin on *Oreochromis niloticus* (L., 1754) offspring in their study.  $\lambda$ cyhalothrin 24-hour LC<sub>50</sub> value was 6.80±0.63 µgL<sup>-1</sup> (34). The LC<sub>50</sub> value for *Channa punctatus* was 6.88 µgL<sup>-1</sup> (35). Chatterjee et al., (2021a) showed that 96 h LC<sub>50</sub> value of  $\lambda$  cyhalothrin to *Tubifex tubifex* are 0.13 mg L<sup>-1</sup>(36). Chatterjee et al., (2021b) evaluate the toxic effects of  $\lambda$  cyhalothrin on the common carp, *Cyprinus carpio L*. The results depicted that 96 h LC<sub>50</sub> value of  $\lambda$  cyhalothrin to the fish was 1.48 µg L<sup>-1</sup> (37). In a study conducted by Bibi et al. (2014), the 96 h LC<sub>50</sub> value of Karate ( $\lambda$ -Cyhalothrin as an active ingredient) was found to be 0.160 µL L<sup>-1</sup> (38). The LC<sub>50</sub> values of  $\lambda$ -Cyhalothrin were



**Figure 5:** Changes in MDA levels in *D. polymorpha* exposed to  $\lambda$ -cyhalothrin pesticide at 19°C, 22°C and 25°C for 24 and 96 hours. Different letters on the columns indicate a statistically significant difference between different application doses, and the \* sign on the columns indicate a statistically significant difference between the application times (p<0.05)

0.571, 0.380, 0.337 and 0.325 ppm at the exposure time of 24, 48, 72 and 96 h, respectively for African catfish *Clarias gariepinus* (39). In present study,  $LC_{50}$  values of  $\lambda$ -cyhalothrin at 19 °C, 22 °C, 25 °C is 2.23 ± 0.27, 2.61± 0.10, 2.71±0.21 respectively. In present study,  $LC_{50}$  values increased as statistically significant with increasing temperature.

The SOD-CAT antioxidant system scavenges free radicals and thus fights against oxygen damage (40). Chatterjee et al. (2021b) evaluated the toxic effects of  $\lambda$ -cyhalothrin on *Cyprinus carpio L*. It was observed that GST exhibited a significant initial increase followed by a decrease, a decrease in CAT, SOD levels, and a significant increase in MDA levels in liver and gill due to increased  $\lambda$ -cyhalothrin concentrations (37). In another study conducted by Chatterjee et al. (2021a), initial induction followed by a subsequent reduction in SOD, GSH, and GST were found in *T. tubifex* exposed to non-lethal concentrations of  $\lambda$ -cyhalothrin (0.013 and 0.026 mg L<sup>-1</sup>) for 14 days. It has been also shown to cause an induction in MDA and CAT over the exposure period (36). Okechukwu and Auta, (2007) investigated the impact of long-term exposure to waterborne  $\lambda$ --cyhalothrin on *Clarias gariepinus* through changes of selected biochemical parameters. C. gariepinus was exposed to 0.0004, 0.0008 and 0.0016 mg  $L^{-1}$  for 8 weeks. The alterations in all parameters were significantly dose and time dependent (41). Ezenwosu et al., (2021) investigated the effect of  $\lambda$ -cyhalothrin on oxidative stress in Clarias gariepinus. On the 7th day, CAT activity increased, on the 14th day; It was determined that SOD activity increased and there was a significant increase in all parameters except SOD on the 21st and 28th days (42). Koc and Akcay (2018) investigated the effects of  $\lambda$ -cyhalothrin on Capoeta capoeta (Guldenstaedt 1773) caught from Kars Brook by biochemical and molecular methods. When the expression levels of CAT and SOD enzymes were investigated by RT-PCR method, it was determined that there was an increase in SOD and CAT enzyme expression levels compared to the control group (43). In present study, it was also shown that  $\lambda$ -cyhalothrin application caused a decrease in SOD enzyme activity in D. polymorpha, and the application time and temperature changed the enzyme activity. CAT enzyme activity increased depending on the application dose. As the dose increased, an increase in enzyme activity was also observed. It was observed that the temperature at which the highest increase occurred at 25 °C increased the toxic effect. At 25 °C, which is the highest application temperature, an inhibition of CAT enzyme activity occurred again. These changes in SOD and CAT enzyme activities are indicative of a defense mechanism developed by the model organism against oxidative stress induced by a polluting,  $\lambda$ -cyhalothrin. The toxic effect gradually increased with increasing dose and temperature.

It has been suggested that MDA, a highly reactive bifunctional molecule, is an end product of membrane lipid peroxidation, one of the pesticide-induced toxicity mechanisms (20). Kumar et al. (2012) showed that Channa punctatus exposed to pyrethroid insecticides  $\lambda$  cyhalothrine for 96 hours significantly increased LPO levels in different organs such as brain, liver, kidney, gill and muscle. The remarkable increase in the LPO indicates strong stress inducing potential of  $\lambda$ -cyhalothrin in fishes (12). In present study, MDA levels were increased significantly in D. polymorpha exposed to ,  $\lambda$ -cyhalothrin at 22 °C and 25 °C for 24 and 96 hours (p<0.05). Serdar et al. (2021) investigated the effect of temperature on the freshwater amphipod Gammarus pulex (I., 1758). It was determined that the MDA level increased with the increase in temperature and Cd concentration. The increased MDA levels reflect the increase LPO found in the present investigation may have resulted from an increase of free radicals as a result of stress condition generated by pesticide exposure (44).

Literature search showed that the toxicity of pyrethroids increases as the temperature decreases (45). The use of

high GSH for conjugation and/or the use of GSH as an antioxidant to neutralize free radicals may cause GSH content depletion (46). In this study, a general decrease was found in GSH levels due to the administration of  $\lambda$ -cyhalothrin. Especially at the end of the 96th hour, the maximum decrease was found at 19 °C. A steady decrease in concentration was observed. It has been observed that this decrease in GSH levels is an adaptive response developed by zebra mussel *D. polymorpha* to cope with the oxidative stress that occurs due to  $\lambda$ -cyhalothrin application, and temperature affects the oxidative response. Decreased temperature increased the severity of the response to oxidative stress.

Inhibition of AChE activity causes decreased cellular metabolism, induce deformities of the cell membrane, and disturbs of metabolic and neural activity, ionic refluxes and differential membrane permeability (47, 48). Razik and El-Raheem (2019) the activity of AChE activity decreased after treated with  $LC_{30}$  and  $LC_{50}$  of the indoxacarb (49). Vieira and Martinez (2018) evaluated the acute effects of  $\lambda$ -cyhalothrin in juveniles of the teleost Prochilodus lineatus exposed for 96 h to four concentrations (5, 50, 250 and 500 ng  $L^{-1}$ ). They observed that AChE activity decrease in the muscles of fish at all concentrations (50). Decremented AChE level exposed to  $\lambda$ -cyhalothrin probably leads to excessive acetylcholine accumulation at the synapses and neuromuscular junctions, resulting in hyperstimulation of the nervous system that causes behavioral changes and eventually death of the organism (51). In the study conducted by Bibi et al. (2014) fry of Cyprinus carpio were exposed to 10% (0.16 µL L<sup>-1</sup>) and 20% (0.032 µL L<sup>-1</sup>) lethal concentration of Karate  $(\lambda$ -Cyhalothrin as an active ingredient) and observed the effects on total protein content and AChE activity in brain, liver and muscle tissues. AChE activity in different tissues of C. carpio decreased in concentration dependent manner and showed tissue specific pattern (38). In this present study, while a general inhibition was observed in AChE levels, this inhibition was observed to be more especially in the groups administered high-dose  $\lambda$ cyhalothrin. Also, as the application times increase, it was seen that the inhibition levels increase. It has been observed that different temperatures cause changes in enzyme levels. It has been suggested that changes in exposure temperature may alter the binding affinities of lipophilic toxins within the lipid-rich neural fat body sheath associated with insect nervous systems, thereby interfering with ion channel activation and membrane. Neurons associated with the neural membrane may be affected by temperature, which may lead to disruption of the permeability mechanism (52). In present study, AChE levels were inhibited especially in the groups administered high-dose  $\lambda$ -cyhalothrin. It was also found that the inhibition levels increased depending on the application times.

### Conclusions

It was determined that  $\lambda$ -cyhalothrin has a toxic effect on *D. polymorpha* and this toxic effect increases depending on

the temperature. In our study, it was concluded that the D. polymorpha model organism and some of its biochemical parameters (AChE, CAT, SOD, GSH and MDA) are suitable biomarkers for revealing the effect of temperature variable on toxic response. It has also been shown that biochemical parameters such as SOD, CAT, AChE activities and GSH, TBARS levels used are suitable biomarkers for the evaluation of the toxic effects of  $\lambda$ -cyhalothrin.

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# Vrednotenje vpliva temperature na toksičnost Lambda-cihalotrina v modelnem organizmu Dreissena Polymorpha z uporabo biokemijskih označevalcev

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**Izvleček:** Zaradi naraščajočih sprememb podnebja je pomembno ugotavljati, ali temperatura vpliva na razmerje med odmerkom in odzivom organizmov na nekatere snovi. Zato je bil namen te študije prikazati vpliv spremembe temperature na toksični odziv modelnega organizma Dreissena polymorpha in nekaterih njegovih bioloških oiznačevalcev. V ta namen smo s komercialnimi testi ELISA merili koncentracijo acetilholinesteraze (AchE), katalaze (CAT), superoksid dismutaze (SOD), glutationa (GSH) in malondialdehida (MDA) v organizmu Dreissena polymorpha, izpostavljenim subletalnim odmerkom  $\lambda$ -cihalotrina pri različnih temperaturah. V skupinah, izpostavljeni  $\lambda$ -cihalotrinu, se je statistično značilno povečala vsebnost MDA in zmanjšala vsebnost GSH. Ravni AChE so bile znižane zlasti v skupinah, ki so bile izpostavljene visoki koncentraciji  $\lambda$ -cihalotrina. Ugotovili smo tudi, da je bila rast inhibicije odvisna od časa aplikacije. Medtem ko se je aktivnost encima SOD zmanjšala, se je aktivnost encima CAT povečala glede na koncentracijo izpostavljenosti. Ugotovili smo, da različna temperatura različno vpliva na toksičnost  $\lambda$ -cihalotrina.  $\lambda$ -cihalotrina povzroča oksidativni stres in nevrotoksičnost, toksičnost  $\lambda$ -cihalotrina pa se spreminja glede na temperaturo.

Ključne besede: λ-cihalotrin; D. polymorpha; oksidativni stres; nevrotoksičnost; temperatura