

Oxidative Stress and Antioxidant Enzyme Alterations in *Poecilia reticulata* Tissues Tau-Fluvalinate Exposure

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Research Article

History

Received: 23.06.2025

Accepted: 29.03.2026



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ABSTRACT

Tau-fluvalinate is a synthetic pyrethroid insecticide and acaricide that is extensively utilised for the management of numerous agricultural pests. However, mounting evidence suggests that tau-fluvalinate exerts a deleterious effect on the health of aquatic organisms, resulting in tissue damage and mortality. The objective of the present study was to evaluate the effects of sublethal tau-fluvalinate concentrations on oxidative stress and antioxidant enzyme activities in the liver, gills, and muscles of *Poecilia reticulata*. The fish were exposed to tau-fluvalinate concentrations of 0.0033, 0.0065, and 0.013 µg/L for a period of 96 hours. The activities of superoxide dismutase, catalase and glutathione peroxidase, in addition to malondialdehyde levels, were analysed in the liver, gills and muscles. A statistical analysis was conducted on these data (P<0.05). In all tissues, exposure to tau-fluvalinate resulted in significant decreases in antioxidant enzyme activities and marked increases in malondialdehyde levels, indicating increased oxidative stress. The findings reveal that sublethal tau-fluvalinate exposure weakens the antioxidant defence system and causes tissue damage in the liver, gills, and muscles of *Poecilia reticulata*.

Keywords: Aquatic ecotoxicology, Antioxidant defense, *Poecilia reticulata*, Pyrethroid toxicity, Tau-fluvalinate

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1. Introduction

The rapid growth of the world's population, intense urbanisation, and declining arable farmland have led to a significant increase in the use of plant growth promoters to increase agricultural production, quality, and diversity per unit area. Pesticides can be classified by their mode of action against target pests and by their chemical structure. This diversity distinguishes them by their functions (insects, weeds, or fungi) and by their molecular structure and interaction with biological systems [1]. However, given that pesticides are not formulated to target specific organisms, they also pose a risk to other living things, including humans. Moreover, once applied, these substances have been shown to remain intact for extended periods within the habitats of both target and non-target organisms [2]. These substances have the capacity to accumulate over time in the sediments and suspended particles of aquatic environments [3]. Water, which is essential for life and a balanced ecosystem, is being polluted by the excessive use of metals, pesticides and other pollutants [4]. This has the potential to result in the movement of pesticides from their intended locations to bodies of water such as lakes and rivers, due to the natural occurrence of precipitation and floods. The pesticides are then able to accumulate within the water systems over time [5]. The transport of pesticides into water systems is primarily influenced by environmental parameters and application strategies. A range of factors have been identified as playing a critical role in

determining contamination, including solubility, soil structure, climatic conditions, rainfall patterns, proximity to water sources, and application techniques [6]. The contamination of surface waters by pesticides used in agriculture has become a global problem. A substantial proportion of studies conducted on rivers and streams pertaining to fish have indicated the presence of one or more pesticides, with this occurrence being observed in 96% of the relevant studies. This prevalence of pesticides has also been documented in all studies involving surface water samples, as well as in 33% of those focused on groundwater [7]. Pyrethroids are lipid-soluble compounds that rapidly diffuse in nerve tissue, thereby specifically disrupting the function of sodium ion channels. Disruption of the normal functioning of voltage-gated sodium channels leads to excessive sodium ion influx into the cell through these channels, increasing the excitability of the neuronal membrane and decreasing its polarization [8]. The disruption of the function of voltage-gated sodium channels has been demonstrated to cause serious functional losses in the nervous system, muscles, and salivary glands due to impaired neural transmission, resulting in sudden paralysis, seizures, and death in insects [9]. Furthermore, it has been documented that pyrethroids disrupt neurophysiological equilibrium by affecting voltage-gated chloride and calcium channels [8]. Despite the prevalent perception that pyrethroids are innocuous for vertebrates due to their conversion into

non-toxic metabolites [10], research findings indicate that these compounds pose a considerable threat to aquatic organisms, particularly fish. In contrast, the effects of these substances on mammals, birds, and amphibians are more limited, but they have been reported to affect many organs, including the liver, kidneys, brain, heart, spleen, and thymus, in various vertebrate species such as fish, rats, frogs, rabbits, and chickens [11].

Tau-fluvalinate (T-FLV) ($C_{26}H_{22}ClF_3N_2O_3$) is a synthetic pyrethroid insecticide and acaricide used to control various pests. Within the domain of agricultural applications, this compound finds utilisation in the management of various pests, including aphids, red spider mites, thrips, and whiteflies. Additionally, reports have emerged of its employment in beehives, across diverse plant species, in the context of ornamental plant cultivation, and within ant nests [12]. In addition, it is extensively employed in the management of Varroa mites in honeybee colonies [13]. Research has demonstrated that T-FLV induces uncontrolled nerve transmission by maintaining voltage-gated sodium channels in the nerve cells of harmful insects and mites in a perpetually open state, leading to convulsions, tremors, paralysis, and ultimately death [14]. However, it has also been reported that T-FLV may have potential toxic effects in aquatic vertebrates such as fish [15].

The species utilised in this study is *Poecilia reticulata*, a vertebrate model organism that is economically and widely favoured in chemical research [16]. Although the toxic effects of pyrethroids on aquatic organisms have been extensively studied, there is limited information available on the sublethal effects of tau-fluvalinate on oxidative stress parameters and antioxidant defence systems in fish.

The present study hypothesises that sublethal exposure to T-FLV instigates oxidative stress and significantly alters antioxidant enzyme activities in a tissue-specific manner. The present study aims to evaluate the effects of sublethal T-FLV exposure on oxidative stress biomarkers and key antioxidant enzyme activities in the liver, gill, and muscle tissues of *Poecilia reticulata*.

2. Materials and Methods

2.1. Test Samples and Chemicals

Female adult specimens of *Poecilia reticulata* (average length: 3.53 ± 0.23 cm; mean weight: 2.49 ± 0.31 g), supplied by Malawi Aquarium and Pet Products. İth. İhr. Ltd. Co. , İzmir, Türkiye. The experimental animals were acclimatised for 15 days in aquariums containing dechlorinated tap water in order to adapt to the environmental conditions. During this period and throughout the experiment, the fish were fed a standard commercial diet containing 46.0% crude protein, once daily at 8 a.m. The toxic substance used in this study is tau-fluvalinate, a synthetic pyrethroid-type insecticide [(RS)- α -cyano-3-phenoxybenzyl N-(2-chloro- α,α,α -trifluoro-p-

tolyl)-D-valinate], which was purchased from ADAMA Agricultural Solutions Ltd. (İzmir, Türkiye).

2.2. Experimental Design

This study used eight glass aquariums, each measuring 45 cm in depth, 50 cm in length, and 20 cm in width. The aquariums were filled with tap water that had been left to stand for 48 hours and was then aerated and dechlorinated. Eighty healthy female *Poecilia reticulata* were used as the experimental subjects. Before the start of the study, the fish underwent a 15-day acclimatisation period in aquariums containing aerated, rested, and dechlorinated tap water. During this period, the fish were fed standard commercial feed at least once daily at the same time each day. Four days before the experiment, a critical health check was conducted to ensure that the disease or mortality rate did not exceed 5% [17]. The 96-hour LC_{50} value of fluvalinate in *Poecilia reticulata* was determined to be $0.026 \mu\text{g/L}$ [18]. The eighty healthy female guppies were then randomly assigned to four groups of twenty fish each. Each group was divided into two replicate experimental units, each containing ten fish. The experimental groups are as follows:

Control Group: No chemical treatment was applied, and the fish were fed a standard diet.

1. Experimental group: The fish were given $0.0033 \mu\text{g/L}$ T-FLV-1 and fed a standard diet.

2. Experimental group: The fish were given $0.0065 \mu\text{g/L}$ of T-FLV-2 and fed a standard diet.

3. Experimental group: The fish were given $0.013 \mu\text{g/L}$ of T-FLV-3 and fed a standard diet.

The aquarium water was completely changed every 24 hours, and freshly prepared T-FLV solutions (at concentrations of $0.0033 \mu\text{g/L}$, $0.0065 \mu\text{g/L}$, and $0.013 \mu\text{g/L}$) were added. Fish feeding continued regularly throughout the experiment. The aquarium water temperature, pH, and dissolved oxygen levels were measured every 24 hours. Optimal conditions for the fish were maintained throughout the experiment. The water temperature was kept at $24 \pm 1^\circ\text{C}$ using thermostatically controlled heaters, and the oxygen level was kept at 7–8 mg/L using an air pump. After treatment, the fish were anaesthetised using clove oil. The liver, muscle, and gill tissues of the control and treatment groups were stored in a deep freezer at -80°C [19]. The activity of the antioxidant defence system enzymes and the content of lipid peroxidation and malondialdehyde were obtained from tissue supernatants.

2.3. Oxidative Stress Biomarkers

Superoxide dismutase enzyme (SOD, EC 1.15.1.1) activity was measured using the Randox-Ransod enzyme kit (Randox Laboratories Ltd., GB). This method involves producing superoxide radicals and storing certain portions of them. According to this method, xanthine oxidase produces superoxide radicals ($O_2^{\cdot-}$). These radicals then react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium (INT) to form a red coloured formazan dye. SOD enzymes present in the environment dismutate

superoxide radicals, splitting them into less reactive species such as oxygen and hydrogen peroxide [20]. SOD activity was expressed as U/mg of protein.

The catalase (CAT, EC 1.11.1.6) enzyme activity was determined using a spectrophotometric method [21]. This method involves monitoring the consumption of hydrogen peroxide (H₂O₂) at a temperature of 37°C and a wavelength of 240 nm. The CAT activity was expressed as U/mg protein.

The activity of glutathione peroxidase (GSH-Px, EC 1.11.1.9) was determined using a spectrophotometric method adapted from the Ransel commercial kit (Randox Laboratories, UK). In this assay, GSH-Px catalyses the reduction of cumene hydroperoxide in the presence of reduced glutathione (GSH). The resulting oxidised glutathione (GSSG) is then converted back to its reduced form (GSH) by the enzyme glutathione reductase (GR), with NADPH being oxidised in the reaction medium simultaneously [22]. Concurrently, NADPH is oxidised to NADP⁺. As NADPH exhibits a maximum absorption band at 340 nm, the activity of the GSH-Px enzyme was determined by monitoring the rate of decrease in the optical density at this wavelength. The GSH-Px activity was expressed as U/mg protein.

Malondialdehyde (MDA) is a significant product of lipid peroxidation, which is defined as the reaction of free radicals with fatty acids. In this method, the malondialdehyde (MDA) concentration was measured at

a wavelength of 532 nm using a spectrophotometer. This measurement was based on the principle of forming a coloured complex with thiobarbituric acid (TBA) in an acidic environment and at high temperature. MDA levels were expressed as nmol/mg [23].

2.4. Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA). All data are presented as the mean \pm standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to determine differences between the experimental groups. The Tukey post-hoc test was used to identify differences between groups. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Superoxide Dismutase

Biochemical analyses revealed a significant impairment of the primary enzymatic antioxidant defense system in *Poecilia reticulata* following 96 hours of T-FLV exposure. Statistical comparisons between the control and T-FLV treatment groups (T-FLV-1, T-FLV-2, and T-FLV-3) showed a significant decrease in SOD activity in the liver, gill, and muscle tissues compared to control values (Figure 1).

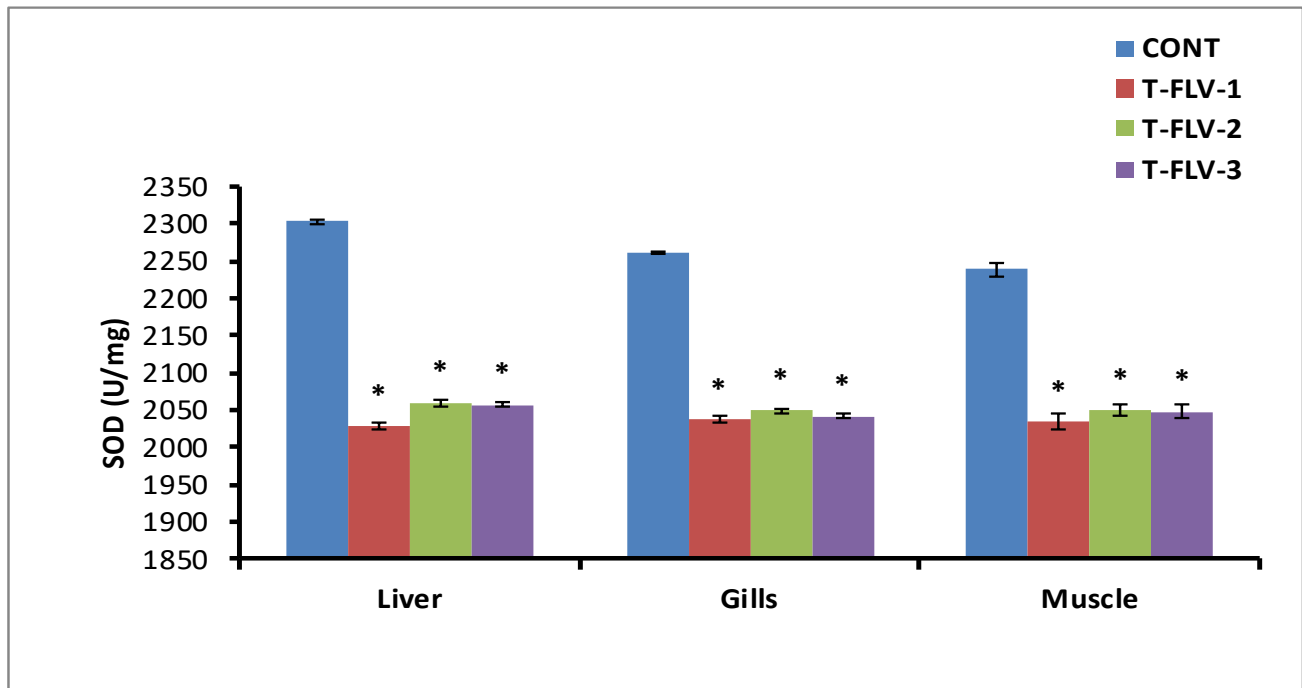


Figure 1. SOD Activities in the liver, gills, and muscles of the *Poecilia reticulata* exposed to Tau-Fluvalinate. All values are means \pm SEM. *Differences from the control group are statistically significant ($P < 0.05$).

3.2. Catalase

As shown in Figure 2, 96-hour exposure to T-FLV at varying concentrations caused a significant decline in CAT activities in the liver, gills, and muscle tissues of *Poecilia reticulata* compared to the control group.

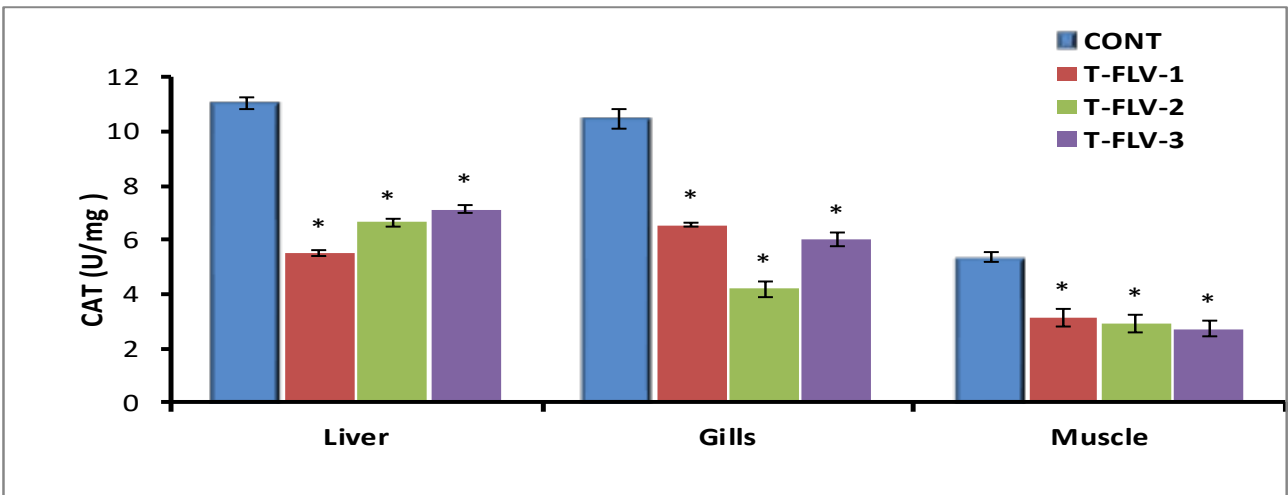


Figure 2. CAT activities in the liver, gills, and muscles of the *Poecilia reticulata* exposed to Tau-Fluvalinate. All values are means \pm SEM. *Differences from the control group are statistically significant ($P < 0.05$).

3.3. Glutathione Peroxidase

The study determined that GSH-Px enzyme activity was significantly reduced in the liver, gill, and muscle tissues of *Poecilia reticulata* treated with different concentrations of T-FLV for 96 hours compared to the control group (Figure 3).

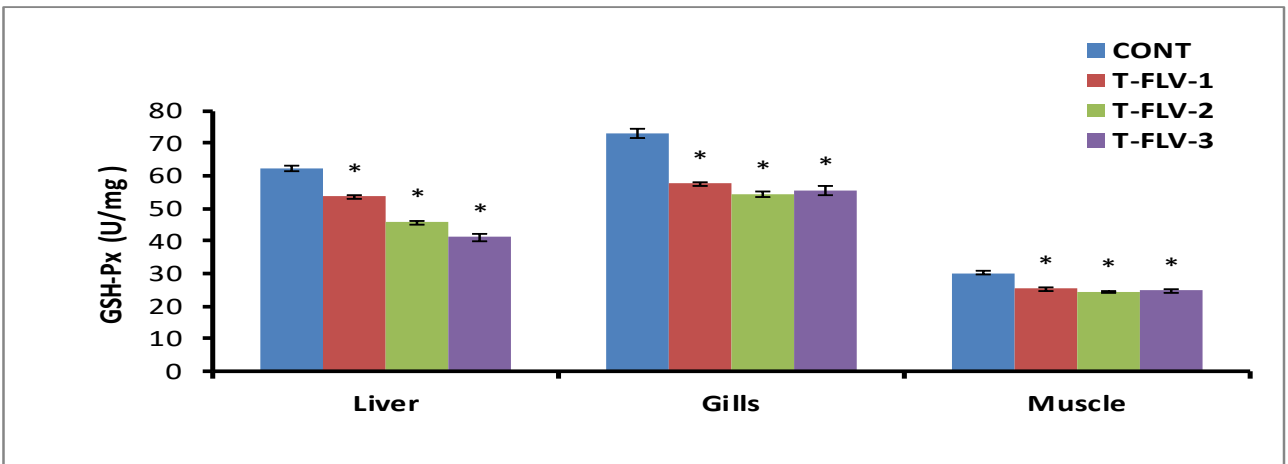


Figure 3. GSH-Px Activities in the liver, gills, and muscles of the *Poecilia reticulata* exposed to Tau-Fluvalinate. All values are means \pm SEM. *Differences from the control group are statistically significant ($P < 0.05$).

3.4. Malondialdehyde

In *Poecilia reticulata* exposed to different concentrations of T-FLV for 96 hours, malondialdehyde (MDA) levels were significantly increased in the liver, gill, and muscle tissues compared to the control group (Figure 4).

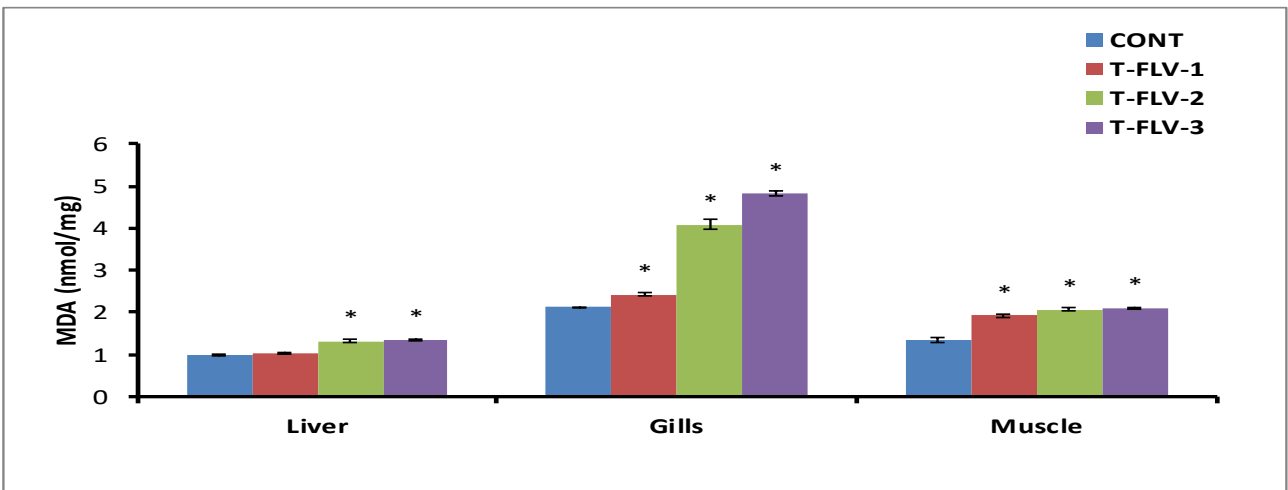


Figure 4. MDA levels in the liver, gills, and muscles of the *Poecilia reticulata* exposed to Tau-Fluvalinate. All values are means \pm SEM. *Differences from the control group are statistically significant ($P < 0.05$).

4. Discussion

The present study demonstrates that exposure to tauflualinate (T-FLV) significantly disrupts the antioxidant defence system of *Poecilia reticulata*. The results obtained indicate that there is a concentration-dependent suppression of key antioxidant enzymes (SOD, CAT, and GSH-Px) in multiple tissues, as well as a marked increase in MDA levels. The disruption of ecosystem balance and the leakage of agricultural pollutants such as T-FLV pose a significant threat to aquatic organisms and the food chain [24]. However, the present study specifically confirms that T-FLV acts as a potent trigger of systemic oxidative stress in *Poecilia reticulata*. In the present study, the decline in SOD, CAT, and GSH-Px activities detected in the liver of *Poecilia reticulata* signifies a direct failure in the detoxification of reactive oxygen species (ROS). As the liver is the primary site for the biological activation of xenobiotics [25], the decrease in these enzymes, which are necessary for ROS neutralisation, explains the simultaneous increase in MDA levels that was recorded. This increase in MDA is indicative of the destructive effects of free radicals on cellular lipids and proteins. The findings in the liver are consistent with broader studies on pyrethroids; for example, in Van fish (*Alburnus tarichi*) exposed to cypermethrin [26], and in *Channa punctata* exposed to deltamethrin [27,28]. Similar decreases in SOD, CAT, and GSH-Px activities and increases in MDA levels were observed. Despite the extensive literature on the subject, which demonstrates that sublethal concentrations of pesticides inhibit various enzymes in different fish species [29], this study emphasises that T-FLV toxicity specifically disrupts the liver detoxification balance by exceeding the antioxidant capacity of *Poecilia reticulata*.

The results of the present study demonstrate that exposure to T-FLV leads to elevated levels of ROS production and diminished antioxidant defence in gill tissues. A significant decrease in the activities of SOD, CAT, and GSH-Px was observed in the gills, which act as the primary entry point for pyrethroids [1,10]. This localized antioxidant depletion is likely due to direct interaction between the pesticide and the respiratory epithelium. This finding is consistent with the decrease in CAT activity reported in *Heteropneustes fossilis* exposed to fluvalinate [30], which has been attributed to either enzyme inhibition or reduced synthesis [31]. Analogous patterns of antioxidant depletion and heightened vulnerability to oxidative stress have been observed in *Clarias batrachus* [32] and *Alburnus tarichi* [33] under pesticide stress. The present study corroborates the hypothesis that the gills of *Poecilia reticulata* are highly sensitive to T-FLV-induced oxidative damage [34,35] and represent a critical site of toxic effect.

It is of particular significance that this study demonstrates the marked reduction in SOD, CAT and GSH-Px activity in the muscle tissue of *Poecilia reticulata* consequent to T-FLV, together with an attendant increase in LPO. Despite the lower metabolic rates exhibited by

muscle tissues and their less frequent study within the domain of ecotoxicology, they play a vital role in the accumulation and transport of pesticides [36]. Although many toxic substances are known to trigger ROS formation by crossing the intestinal barrier [37], the oxidative stress observed in muscle tissue in this study clearly demonstrates that the effect of T-FLV is not limited to the digestive system but reaches a systemic dimension. The findings, which are specific to muscle tissue, are in accordance with observations made in *Brycon cephalus* exposed to methyl parathion [38], and *Cyprinus carpio* exposed to diazinon [39], where the activities of CAT and GSH-Px were inhibited by pesticide-induced stress. Furthermore, the results of the present study demonstrate that the effects of the suppression of the activity of the enzymes observed in the present study are analogous to the effects observed in the spleen and heart tissues of *Oncorhynchus mykiss* under cypermethrin exposure [40]. In conclusion, the present findings indicate that the toxic effect of T-FLV on muscle tissue stems from its capacity to increase ROS production, thereby suppressing the antioxidant defence system and negatively affecting protein synthesis in *Poecilia reticulata*.

5. Conclusion

The results of our study suggest that *Poecilia reticulata* is highly sensitive to the effects of the commercial formulation of T-FLV. Despite the low concentrations of T-FLV and the short exposure duration, the antioxidant defence system in *Poecilia reticulata* was overwhelmed by this pyrethrin, leaving it insufficient to protect against the damaging effects of oxidative stress. The increasing, uncontrolled use of pyrethroids in agricultural pest control could have serious ecological consequences, particularly given the chronic exposure of non-target aquatic organisms to these xenobiotics. Our results suggest that monitoring the presence of chemicals such as T-FLV, which are used in pest control in agricultural areas, within aquatic ecosystems could significantly contribute to assessing ecological and environmental health risks, as well as developing measures to protect water basins.

Conflict of Interest

There are no conflicts of interest in this work.

Acknowledgments

This study was supported by the Van Yüzüncü Yıl University Scientific Research Projects Coordination Office under project number FYL-2018-7267.

Ethical Approval Statement

In this study, the ARRIVE guidelines (Directive 2010/63/EU) were fully followed to ensure animal welfare and experimental transparency. All routine procedures

and protocols were meticulously implemented to minimize animal suffering and to safeguard their welfare. The study was approved by the Local Ethics Committee for Animal Experiments at Van Yüzüncü Yıl University (Ethics approval reference number: YÜHADYEK-2018/03).

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