Continental Veterinary Journal
Journal homepage: www.cvetj.com

Research Article

Studies on the changes in antioxidant enzyme activity induced by parathion in *Hypophthalmichthys molitrix*

Nayab Kanwal[1], Sana Aziz[2*], Sajid Abdullah[1], Muhammad Sarfraz Ali[3], Nisar Ahmad[2]

[1]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan.
[2]Department of Zoology, University of Jhang, Faisalabad, Pakistan
[3]Department of Biosciences, COMSATS University Islamabad (CUI), Pakistan

Correspondence: sana.aziz1994@gmail.com

ARTICLE INFO

ARTICLE HISTORY: CVJ-24-0503

Received	19 May 2024
Revised	21 June 2024
Accepted	23 June 2024
Published online	30 June 2024

Keywords:
Antioxidant enzyme activity
Superoxide dismutase
Catalase
Oxidative stress
Pesticide
Parathion
Hypophthalmichthys molitrix

ABSTRACT

Parathion is a pesticide that has negative effects on the environment and non-targeted species. Fish are commonly used as a model organism to monitor environmental toxicity. This study aimed to evaluate the toxicity of parathion in *Hypophthalmichthys molitrix*. Firstly, a 96-hour LC_{50} was determined by probit software. The 96-hour LC_{50} was found to be 0.98 mg/L. Then, after exposing the fish to different sub-lethal concentrations ($1/3^{rd}$, $1/5^{th}$, and $1/7^{th}$ of the 96-hour LC_{50}), antioxidant enzyme activity was assessed following chronic exposure. After the chronic exposure, fish were sampled and targeted organs were isolated for the analysis of antioxidant enzyme activity in terms of superoxide dismutase (SOD) and catalase (CAT The activity of both enzymes fluctuated significantly in the group exposed to different sub-lethal concentrations and patterns in different organs was Liver > gills > kidney > muscles > heart. Physio-chemical parameters were maintained at optimum levels. The changes in the enzymatic activity can be effectively used as biomarkers for monitoring the level of pesticides in aquatic systems.

To Cite This Article: Kanwal N, S Aziz, S Abdullah, MS Ali and N Ahmad, 2024. Studies on the changes in antioxidant enzyme activity induced by parathion in *Hypophthalmichthys molitrix*. Continental Vet J, 4(1): 40-45.

Introduction

Many freshwater and land-based ecosystems are suffering from high levels of pollution caused by toxic substances, with agricultural pesticides and fertilizers being major contributors to the problem on a global scale. These pesticides can harm non-target organisms, including fish, which are important components of aquatic biota. Methyl parathion (MP) is one of several organophosphate pesticides (OPs) commonly used in agriculture and pest control programs due to their effectiveness against a wide variety of insect pests. The use of pesticides is a major contributor to chemical pollution in aquatic ecosystems. Parathion-methyl is an organo-phosphorous and toxicity exhibit pesticide frequently employed in river agriculture-producing premises. The frequent application of these pesticides might jeopardize the toxicity and influence the number of fishes and invertebrate species that live in aquatic environments near agricultural areas. Because there is an excess of toxicity data due to pesticide accumulation in freshwater Amazonian River fishes, some concentrations of pesticides increased the sustainable risks of parathion-methyl in the region (Rico et al. 2010). However, this chemical is highly toxic to aquatic organisms and has been classified as "extremely hazardous" for the environment by

WHO (World Health Organization 2020). Fish can serve as reliable indicators of environmental pollution, offering insights into the quality of aquatic systems (Dautremepuits et al. 2004). The primary reason for the toxicity of organophosphates is the inhibition of acetylcholine esterase. The toxicity of these pesticides is also associated with an increase in lipid peroxidation and changes in ATPase activity (Hazarika and Sarkar 2001). Acetylcholinesterase (ACHE) is responsible for inactivating the neurotransmitter acetylcholine. When ACHE is blocked, acetylcholine builds up in cholinergic synapses, leading to synaptic blockage and disrupting signal transmission (Ferrari et al. 2007).

Fish raised in areas heavily polluted with heavy metals, and pesticides inhibited brain and muscle function (Lionetto et al. 2003). The most important enzymes for the detoxification of reactive oxygen species in all organisms are superoxide dismutase (SOD), catalase, glutathione peroxidases (GPXs), and transferases (Slaninova et al. 2009). Prolonged exposure to organophosphates can lead to reduced swimming performance and peroxidative damage in the brain and gills (Ahmad et al. 2000). The long-time administration of organophosphates caused gradual exhaustion of SOD, GR, GPX, and GST or the increase of antioxidative defense systems (Gultekin et al. 2000). The ability of organophosphates to induce oxidative stress is not fully understood (Oruc and Usta 2007). So, that's why silver carp was used to assess parathion toxicity. Firstly, 96-hr LC_{50} was checked then after exposure to different sub-lethal concentrations, antioxidant enzyme activity was checked.

Materials and Methods

Study area

The research work entitled "Studies on the changes in antioxidant enzyme activity induced by parathion in *Hypophthylmichthy molitrix"* was organized in the Aquatic Toxicology Laboratory at Research Farm of Fisheries, Department of Zoology, Wildlife and Fisheries, UAF. The fish *H. molitrix* was obtained from the Satyana Road Fish Hatchery, Faisalabad.

Test chemicals

Parathion and all other used chemicals in the study were of analytical quality and purchased from Sigma Aldrich.

Preparation of stock solution

The stock solution was made to prepare the working solution for the experiment by the successive dilution of the stock solution. By dissolving methyl parathion (0.2-2.0mgL^{-1}) in liquid ethanol (50ml), a stock solution of parathion was generated, and the desired concentrations of test media in tap water were prepared by adding appropriate quantities of this stock solution to the tank. The water was continuously aerated.

Acclimatization of fish

The experiment was performed in glass aquaria, and fish were used to acclimatize in an oxygenated tank and also with dechlorinated tap water for 14 days before the experiment. At the time of acclimatization, fish were provided with commercial feed pellets equal to twice the initial body weight. Before the trial, it was assured that all aquariums were properly cleaned with distilled water to eradicate any pollutants and dust contaminants. To avoid the accumulation of metabolic wastes, $1/4^{th}$ of the water was renovated daily basis and any fish that exhibited strange activity or behavior were eliminated from tanks. During this period, fish were maintained in glass aquaria with a constant temperature (26±1°C), a 12-hour light and dark cycle, and fed a standard diet.

Acute toxicity testing

An experiment was conducted to determine 96-hr LC_{50} and LC_{99}. For toxicity testing, different concentrations of parathion (0.2-2.0mgL^{-1}) were tested, separately. The experiment was consisted of treatment groups (with parathion) and of the control group (no pesticide) in three replications. On the base of concentration level, exposure ranges were selected to its natural environment like previous research to pesticide toxicity on fish bodies were conducted. 65% of water and waste were removed at regular intervals.

Oxidative stress analysis

To assess the oxidative stress, three sub-lethal doses ($1/3^{rd}$, $1/5^{th}$, and $1/7^{th}$ concentrations of 96-h LC_{50}) of the parathion were administered to *Hypophthalmichys molitrix* over 15 days. Table 1 mentions the sub-lethal concentrations of Parathion tested to determine oxidative stress. The concentrations are presented in mg/L^{-1}. To test oxidative stress in terms of superoxide dismutase (SOD) and catalase (CAT) in the liver, kidney, gills, muscle, and heart of *Hypophthalmichys molitrix*, the sampling process was done with three replicates. Different samples of different fish organs were obtained. Then, all of the organs were homogenized individually and buffered in cold form with a pH of 6.5. All of the homogenates were centrifuged for 15 minutes at 4^oC and 10,000 rpm. Translucent supernatants were obtained, which were observed by spectrophotometer by using the sophisticated protocol of Weydert and Cullen (2010) with slide alterations.

Determination of physiochemical parameters

During the experiment, the digital meters will be used to calculate the physio-chemical parameters viz. temperature, electrical conductivity, DO, and pH. Total hardness, carbonates, bicarbonates, total alkalinity, magnesium, and calcium will be observed by the procedure of A.P.H.A. (2005).

Statistical analysis

The recorded data was statistically analyzed by ANOVA and correlation. Tukey's test was performed to find out the comparison between treatments by

using the statistical software Statistix 8.1. To calculate the LC_{50} and lethal concentration of parathion for the probit analysis was used.

Results

Acute toxicity of parathion

At various concentrations of parathion, the mortality of fish was seen in three different replicating groups, which are illustrated in Table 2. During 96-hr of acute exposure to parathion, varying concentrations of parathion, which ranged from 0.2 mg L^{-1} to 2.0 mg L^{-1} were tested. 96-h LC50 of parathion was 0.98 mg/L. The Pearson goodness of fit test resulted in a Chi-Square value of 11.0266 with 9 degrees of freedom and a p-value of 0.74.

Table 1: Sub-lethal concentrations of parathion tested during chronic expsoure.

Chemical	**Treatments**	**Exposure doses (Fish Species *Hypophthalmichys molitrix*)**
Parathion (mg/L^{-1})	1/3rd of 96-h LC_{50}	0.605
	1/5th of 96-h LC_{50}	0.363
	1/7th of 96-h LC_{50}	0.259

Table 2: 96h acute toxicity of *Parathion* (mg/L) for *Hypophthalmichthys molitrix*
CI, confidence interval (mg/L); LCL, lower confidence limit (mg/L); UCL, upper confidence interval (mg/L); Lethal Conc., lethal concentrations (mg/L); DF, degree of freedom

Fish species	**Chemical**	**LC_{50}**	**95%CI**	**Lethal conc.**	**95%CI**	**Pearson goodness of fit tests**		
			(LCL-UCL)		**(LCL-UPL)**	**Chi-Square**	**DF**	**P-value**
H. molitrix	Parathion	0.98	0.73-1.18	2.23	1.91-2.81	11.02	9	0.27

Table 3. Means of Physio-chemical alterations in water studied during exposure of acute toxicity of parathion for *Hypophthalmichys molitrix.*

Parameters	**Unit**	**Means**	**Analysis method**
Temp.	^{0}C	30.19	Temperature-meter
pH	mg/L^{-1}	7.25	-
DO	mg/L^{-1}	4.93	Oxygen-meter
CO_2	mg/L^{-1}	0.81	Carbon-dioxide meter
T.H	mg/L^{-1}	223	Titration-method
Ca	mg/L^{-1}	18.972	Spectro-photometry
Mg	mg/L^{-1}	44.381	Spectro-photometry

CO_2 = Carbon dioxide DO = Dissolved oxygen Mg = Magnesium Ca = Calcium T.H = Total Hardness T=Temperature

Physio-chemical parameters of the test media

All the physio-chemical parameters (temperature, pH, dissolved oxygen, carbon dioxide, total hardness, calcium, and magnesium) were monitored on a daily basis in a glass aquarium because they also impact the activity of antioxidant enzymes of fish (Table 3).

Oxidative stress analysis

During acute exposure of pesticide fish were subjected to parathion sub-lethal concentrations for almost (15 days) and sampling was done after trial. The antioxidant activity of SOD and CAT was analyzed from different organs of fish. Organ-specific response of mean Superoxide dismutase (SOD) and catalase (CAT) activity in fish organs subjected to different concentrations of parathion were noticed in the following order Liver>gills>kidney>muscles>heart (Table 4).

Discussion

Pesticides are widely used in agriculture and have a negative impact on non-target organisms, including fish, which are crucial to the aquatic environment. Methyl parathion (MP) is an organophosphate pesticide (OP) commonly used in insect control programs due to its effectiveness against a wide range of insect pests (Monteiro, 2009). Water pollution can particularly affect fish because their tissues absorb contaminants, disrupting various morphological and metabolic functions (Kirici et al. 2017). For aquatic animals living in habitats exposed to water-borne pollutants, the generation of reactive oxygen species (ROS) can cause oxidative damage, potentially serving as an antioxidant defense mechanism (Livingstone 2001).

Parathion had very toxic effects on *Hypophthalmichthys molitrix*. The fish were kept in glass aquaria to analyze the lethal concentrations. Various concentrations of parathion were found to be lethal to the fish. Research showed that parathion, at different concentrations, proved to be toxic to fish organs and caused mortality. Fish mortality was observed in three replicates at various concentrations of parathion, ranging from 0.2-2.0mg/L. The LC50 value was estimated to be 0.98mg/L, representing the lethal concentration at which 50% of the fish population died during the 96-hour experiment. Another study examined the effects of the pesticides methyl parathion, dichlorvos, and chlorpyrifos on adult zebra fish, which were exposed to various concentrations (5, 10, and 25mg/L) for 24 and 48 hours in an acute toxic study. Chlorpyrifos showed mortality in all concentrations, while Methyl parathion and Dichlorvos showed the same effect at 25mg/L. The LC50 value for Methyl parathion and Dichlorvos was 5 and 10mg/L, respectively, which is considered lethal (Sukirtha and Usharani 2013). In another study, different treatments (2-4) were given to Rhamdia quelen jundia fish, which were exposed to sub-lethal concentrations (16.6, 33.3, and 50% of the LC50) of each agrichemical for 96 hours. At the end of this period, the fish were subjected to an acute stress-handling stimulus by being chased with a pen net. The findings of this research clearly show that severe contact (acute exposure) with sub-lethal levels of methyl-parathion has an adverse effect on another acute stressful event in jundia fingerlings as well (Cericato et al. 2008).

Table 4. Alterations in SOD and CAT activity (UmL^{-1}) during $1/3^{rd}$, $1/5^{th}$ and $1/7^{th}$ of 96-h LC_{50} conc. of parathion exposure in different organs of *Hypophthalmichys molitrix*.

Different organs of Fish	Parathion Concentration (mg/L^{-1})	SOD activity (UmL^{-1})	CAT activity UmL^{-1}
Liver	Control	69.47±0.04	576.63±0.03
	1/3rd of LC_{50} conc.	46.17±6.63	489.79±7.38
	1/5th of LC_{50} conc.	49.91±8.61	500.21±5.25
	1/7th of LC_{50} conc.	50.16±0.42	561.63±3.22
Gills	Control	58.65±0.01	530.40±0.022
	1/3rd of LC_{50} conc.	29.00±6.33	496.79±4.81
	1/5th of LC_{50} conc.	35.81±0.07	500.24±2.68
	1/7th of LC_{50} conc.	40.76±8.44	536.89±1.22
Kidney	Control	54.50±0.02	545.20±0.03
	1/3rd of LC_{50} conc.	31.18±4.14	530.88±3.68
	1/5th of LC_{50} conc.	47.58±1.78	520.47±3.12
	1/7th of LC_{50} conc.	52.21±3.23	540.02±2.51
Muscles	Control	20.73±0.02	478.34±0.03
	1/3rd of LC_{50} conc.	17.30±4.97	466.94±2.41
	1/5th of LC_{50} conc.	19.91±4.42	458.86± 1.66
	1/7th of LC_{50} conc.	23.59±1.75	469.81±1.09
Heart	Control	18.11±0.03	357.94±0.02
	1/3rd of LC_{50} conc.	16.27±0.71	346.98±0.38
	1/5th of LC_{50} conc.	15.25±0.61	340.86± 1.66
	1/7th of LC_{50} conc.	19.32±4.22	351.96±0.27

The present study also investigated the changes in antioxidant enzyme activity induced by parathion exposure in *Hypophthalmichthys molitrix*. The results showed significant alterations in the activity levels of Superoxide Dismutase (SOD) and Catalase (CAT) in the fish's liver, gills, kidney, muscles, and heart following parathion exposure. Superoxide dismutase activity in fish exposed to different concentrations of parathion fluctuated in the following order: liver > gills > kidney > muscles > heart. SOD activity was significantly decreased and led to oxidative stress in the fish. The present research observed changes in SOD activity in different tissues of *Hypophthalmichys molitrix* due to parathion exposure. It suggests an upregulation of the SOD enzyme as a defense mechanism against the accumulation of superoxide radicals. However, when severe disruptions occurred, the level of antioxidant enzymes decreased. Another experimental result highlighted that the fish were exposed for 96 hours to different sublethal concentrations of MP and CPF (1/4 LC50, 1/8 LC50, and 1/10 LC50), and their oxidative stress-induction potential was estimated in the brain, liver, and gills of the fish. MDA content was induced in all tissues, but the maximum rise was observed in the gills (161 and 153% for MP and CPF, respectively). SOD levels fluctuated in all treatment groups relative to the control (Sharbidre et al. 2011). Other studies have found that poor SOD

activity under high metal stress might lead to a buildup of more reactive oxygen species in animals, resulting in cell damage (Phull et al. 2018).

The recent research analyzed the catalase (CAT) activity and observed the impact of parathion exposure on CAT activity in various fish organs. The study found that there were significant alterations in CAT activity in the liver, gills, kidney, muscles, and heart when exposed to sub-lethal concentrations of parathion. This indicates impaired antioxidant defense mechanisms and changes in antioxidant activity in fish organs. Another study examined the enzymatic activity of antioxidant enzymes such as SOD, CAT, and GPx in the presence of the pesticide simazine at concentrations of 2mg/L and 4mg/L. The study found that enzyme activity increased at 14 and 28 days but decreased after 60 days of exposure. Similar alterations in enzyme activity were observed in the liver, gills, and brain of the fish. Prolonged exposure to simazine resulted in excessive generation of reactive oxygen species (ROS), leading to oxidative damage to cell lipids and proteins, as well as inhibition of antioxidant capabilities in fish tissues (Stara et al. 2008).

In our recent research study, we found that temperature had a positive association with parathion concentration. The toxicity of parathion increased with rising temperatures, affecting the *Hypophthalmichthys molitrix*. We observed that the mortality rate of common carp exposed to parathion and malathion was higher at higher temperatures (Gupta et al. 2016). Our current study suggested that pH showed a highly significant correlation with concentration and a negative association with temperature. The research indicated that pH played a crucial role in pesticide stability, with different pesticides exhibiting varying sensitivities to pH changes. Although specific research studies on the effects of parathion on antioxidant enzyme activity in *Hypophthalmichthys molitrix* showed severe toxicity, existing research on other organisms suggests that parathion exposure can lead to a decrease in antioxidant enzyme activity. This reduction in enzymatic defense mechanisms can have adverse effects on the silver carp, potentially leading to oxidative stress and various physiological impairments.

The latest research shows that *Hypophthalmichthys molitrix* can be used as biomarkers for detecting severe toxic effects caused by parathion. The findings of this study contribute to our understanding of how *Hypophthalmichthys molitrix* responds to oxidative stress when exposed to parathion. The changes observed in antioxidant enzyme activity demonstrate the fish's adaptive mechanisms to counteract pesticide-induced oxidative stress. These findings are important for evaluating the potential impact of parathion on fish health and for understanding the ecological consequences of pesticide contamination in aquatic environments.

Conclusion

The 96-hour LC_{50} acute toxicity of parathion to H. molitrix was 0.98mg/L. Our study demonstrated that parathion induces toxicity in fish after exposure to different sub-lethal concentrations, most probably due to the production of reactive oxygen. Chronic exposure of parathion to the observed experimental group caused significant variations in antioxidant enzyme activity. The changes in antioxidant enzyme activity (SOD & CAT) at various concentrations of parathion showed fluctuations in the following order: liver > gills > kidney > muscle > heart.

Funding

No funds were provided by any national or international funding agencies.

Ethical Statement

No Ethical permissions were required for this article.

Availability of Data and Material

The data can be obtained from the corresponding author on a reasonable request.

Consent to Participate

All the authors gave their consent for equal participation.

Consent for Publication

All the authors gave their consent for publication.

Competing Interest

The authors declare that they have no relevant financial or non-financial interests to disclose

Author Contribution

NK executed the laboratory research. SA supervised the research work. SA and MSA helped in methodology, formal analysis, data curation, and manuscript review and editing. NA conceived the idea.

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