Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Zoology

Quinalphos Induced Toxicity and Potential Attenuation of Growth, Hematological, Biochemical, Enzymological and Oxidative Stress Biomarkers in *Tubifex tubifex* and *Oreochromis mossambicus:* A Multiple Biomarker Approach

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DOI: 10.36347/sajb.2022.v10i12.002 | **Received:** 07.11.2022 | **Accepted:** 19.12.2022 | **Published:** 22.12.2022

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Abstract **Original Research Article**

The present study was assessed to determine the acute toxicity and changes in oxidative stress enzymes of *Tubifex tubifex* and to evaluate the acute toxicity and changes in oxidative stress enzymes, hematological and biochemical parameters, growth parameters of freshwater fish *Oreochromis mossambicus* during chronic exposure to Quinalphos. The study reveals that the 96h LC_{50} value of Quinalphos to *T. tubifex* and *O. mossumbicus* were 6.28 µg/l and 5.35 µg/l respectively. Besides, the exposed worms and fish also exhibited erratic behavioral responses at the acute level. Acute exposure of Quinalphos to *T. tubifex* induces alterations in oxidative stress enzymes including catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), malondialdehyde (MDA), reduced glutathione (GSH), and glutathione peroxidase (GPx). Additionally, chronic exposure of Quinalphos to *O. mossumbicus* showed increasing the levels of liver CAT, SOD, GST, MDA, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Sublethal levels of Quinalphos elicited substantial changes in protein, glucose, albumin and creatinine of fish in compare to control. Quinalphos also effects different growth (GSI, SGR, FCR) and hematological parameters (the red blood cell, haemoglobin and haematocrit) of treated fish. Moreover, by using integrated biomarker response (IBR) and biomarker response index (BRI) the change in the health status of pesticide exposed fish was determined. These results indicate that Quinalphos alters the survivability and behavioral responses of *T. tubifex* and *O. mossambicus* at the acute level and changes the oxidative stress biomarkers, biochemical and hematological parameters of the fish during chronic exposure.

Keywords: Quinalphos, *Tubifex tubifex*, *Oreochromis mossambicus*, oxidative stress enzymes, biochemical & hematological parameters, growth parameters.

INTRODUCTION

Plant protection chemicals, also known as pesticides, are mainly used for many years in agriculture or in public health protection programs to protect plants from pests, weeds or diseases (Gaona *et al.*, 2019). With the incrementing density of the human population over the past few decenniums, expeditious urbanization and rapid industrial development has been most vulnerably susceptible to freshwater pollution (Ogunnupebi *et al.*, 2020). Every year 80 percent of residential wastewater and commercial wastewater is dumped in water bodies worldwide which disrupt the aquatic ecosystems (Carazo-Rojas *et al.*, 2018). Currently, around four million tonnes are used per year on a global basis, most of which are herbicides (56%), followed by insecticides (19%), fungicides (25%) and other types such as rodenticides and nematicides (Nayak *et al.*, 2021). The toxic effect of pesticides and their substances are mainly inducing chemical damages and also the death of the organism (Sharma *et al.*, 2019; Goi., 2021). A Pesticides capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, persistence in the environment species, its individual sensitivity and also the method by which the poison enters the organism, the time of exposure and on other factors (Tudi *et al.*, 2021).

Amongst the pesticides, organophosphates are water-soluble and actively utilized for both residential and agricultural applications (Sadiqul *et al.,* 2016). Quinalphos is an organophosphate pesticide and is widely utilized in agriculture in India (Sadiqul *et al.,* 2017). It has been routinely used against pests for fruit trees, cotton, vegetables and peanuts scale insect on fruit trees and pest complex on rice (Hemalatha *et al.,* 2021).

Tubifex tubifex is a freshwater benthic Oligochaete worm, which is widely used in aquatic toxicology and environmental risk assessment (Chatterjee *et al.,* 2021; Di *et al.,* 2016a; 2016b). Meanwhile, *T. tubifex* is also a bottom creature of food chain which could accumulate contaminants, and then may have a certain influence on species of high trophic levels (Yuli *et al.,* 2020). This organism is known to be very resistant to pollution and to frequently dominate the macrobenthic community in freshwater habitats (Haque *et al.,* 2020).

The fish are particularly sensitive to pollution (Ghelichpour *et al.,* 2019). They can be exposed to a wide range of toxic substances, including pesticides which can diffuse into their organs and fat tissues and significantly affect their fundamental physiological and biochemical processes (Juan *et al.,* 2020). In our investigation, *Oreochromis mossambicus* is selected as a model fish species due to its high growth rate, prosperous adaptation to different diets, susceptibility to diseases, and effective tolerance to a wide range of environmental conditions (Mutshekwa *et al.,* 2022). Several studies have been carried out on alterations of stress enzymes in fish exposed to pesticides (Singh *et al.,* 2018). However, evidence regarding the toxic effect of this pesticide on alterations of biochemical stress enzymes in *O. mossambicus* is limited.

LC50, a preliminary toxicity study at acute exposure evaluates a lethal endpoint. It is more useful to perform sublethal toxicity studies for a longer period to observe the diminishing at the physiological and biochemical level of the organisms (Bhattacharya *et al.,* 2021; Zhang *et al.,* 2017). Behavioral studies provide an early warning signal about the stress condition of organism for ecotoxicological studies (Chatterjee *et al.,* 2021). As blood is an important regulator of an organism's health, so hematological and biochemical biomarkers alteration of an organism upon exposure to a toxicant is very important to evaluate the adverse conditions (Tabassum *et al.,* 2020). The liver of fish plays an important role in several vital functions if basic metabolism and it is also the major organ of accumulation, biotransformation and excretion of contaminants in fish, including degradation and bio-inactivation of pesticides. Thus, the biochemical parameters in fish liver are sensitive for detecting potential adverse effects and relatively early events of pollutant damage (Ghelichpour *et al.,* 2020). They are indicators for normal liver function and hence, they can be also used as biomarkers for tissue damage (Chang *et al.,* 2020). Cellular levels damage of an organism with toxic substances activates the level of reactive oxygen species (ROS) (Bhattacharya *et al.,* 2021; Chang *et al.,* 2020). This high amount of ROS triggers lipid peroxidation (LPO) that produces malondialdehyde (MDA) and causes severe damage by oxidative stress (Chatterjee *et al.,* 2021; Kumari *et al.,* 2014). Oxidative stress alters the normal conditions which is neutralized by antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione S-transferase (GST) (Bhattacharya *et al.,* 2021; Chang *et al.,* 2020; Kumari *et al.,* 2014). In addition to the stress enzymes, suppression of the AchE leads towards the excessive accumulation of acetylcholine in the synaptic cleft and blocks nerve transmission and subsequently leading towards paralysis and mortality of organisms (Tabassum *et al.,* 2020). Evaluation of the AchE level provides important insight into the neurotoxic potential of the toxicant (Chatterjee *et al.,* 2021). Therefore, assessment of these blood and stress biomarkers can be utilized as a potential tool for aquatic toxicological investigations (Banaee *et al.,* 2011; Tabassum *et al.,* 2020).

Integrated biomarker response (IBR) provides a methodology that combines all the biomarker responses and plays a vital role in determining the toxicity of contaminants (Beliaeff and Burgeot, 2002; Serafim *et al.,* 2012). Moreover, Biomarker Response Index (BRI) has been widely utilized in recent years to integrate multiple biomarker responses (Javed *et al.,* 2017). General unified threshold models of survival (GUTS) were suggested as a potential tool for the precise evaluation of the environmental risk of different toxicants (Jager *et al.,* 2011). Two causations of the process affecting survival were formalized namely the stochastic death (SD) and individual tolerance (IT) approach and were used to describe the death mechanism resulting from the damage of the toxicant. Both approaches can lead to different data interpretations and indicate the time course of effect (Jager *et al.,* 2011).

The objective of the present study is to evaluate the acute toxicity, behavioural changes and oxidative stress enzymes in benthic oligochaete worms (*T. tubifex)* exposed to quinalphos. It also assessed the acute toxicity, ethological changes, growth rate effect and alterations in oxidative stress enzymes, biochemical and hematological parameters parameters in Tilapia (*O. mossambicus*) exposed to Quinalphos for a period of 45 days. In this study, GUTS-SD and IT models were also used to evaluate responses of *T. tubifex* and *O. mossumbicus* to Quinalphos and ultimately to provide prior predictions of toxicity and to find out which model between SD and IT better describes the toxicity data. The entire biomarker dataset was evaluated using integrated biomarker response (IBR) and biomarker response index

(BRI) to assess and compare the health status of pesticide exposed fish.

MATERIALS AND METHODS

Ethical Approval

The fish (*Oreochromis mossumbicus*) experimental bioassay was conducted as per the guidelines approved by Institutional Biosafety Committee (IBSC Approval No. BU/IBSC/22/Zo/36). However, no ethical approval is needed for invertebrates such as *T. tubifex* according to current regulatory studies.

Test Chemical

The test chemical Quinalphos, used in the study was collected from the local market (India MART). Its stock solutions (1% w/v) and subsequent dilutions were made following a standard protocol (Saha *et al.,* 2008).

Test Organism

T. tubifex (Class: Clitellata, Family: Naididae) mean length of 12.9 ± 0.7 mm were procured from a local aquarium store in Burdwan, West Bengal, India. Adult *O. mossambicus* (Class: Actinopterygii, Family: Cichlidae) of both sexes having mean length 7.2±0.49 cm and mean weight 17.4±0.68 g was used for acute and chronic toxicity bioassay. The specimens were given prophylactic treatment by bathing them in 0.05% potassium permanganate ($KMnO_4$) solution for 2 min to prevent any dermal infections.

Maintenance Condition

Worms were adapted in a stock tank containing unchlorinated water for 24 h. Then organisms were transferred to the experimental system. Fish of different sizes were placed in outdoor cement vats for acclimatization for 7 days and were provided with commercial feed. During this acclimation period, continuous aeration and daily water exchange were conducted for all the tanks (Saha *et al.,* 2008).

The values of the different physicochemical parameters of water used in the study were as follows: temperature 29.5 ± 0.5°C, pH 7.1 ± 0.5, free CO_2 18.3 ± 2.0 mg/l, dissolved oxygen 6.2 ± 1.5 mg/l, total alkalinity 164 ± 7.6 mg/l, and hardness 120 ± 4.5 mg/l as $CaCO_3$.

Acute Toxicity Bioassay and Survival Rate Prediction

A static renewal acute toxicity bioassay was conducted in glass beakers of 250 ml each holding 200 ml of water and 10 number of *T. tubifex*. Each experiment was conducted in triplicate. Initially, a range detection test was used to find out the range of levels at which mortality occurs. Following that, a definitive test was performed by subjecting the worms to various nominal concentrations of Quinalphos (00, 0.5, 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 30, 35 µg/l) each associated with a control containing water without any toxicant for 96 h exposure duration. Mortality of the worms was measured at 24, 48, 72 and 96 h. The LC_{50} values at 24, 48, 72 and 96 h were computed utilizing Finney's probit analysis, using log concentration as the dependent variable and probit as the independent variable (Finney, 1971).

For fish the static replacement bioassays were conducted in 15l glass aquaria with 10l of non-chlorinated tap water each containing 10 fish. Each test was accompanied by three replicates with a control consisting of tap water without any toxicant. The fish were not fed for 24h before the commencement of the test. Initial range-finding tests were conducted to estimate the spectrum of concentrations of the test chemical. Then the selected concentrations of Quinalphos (00, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10 µg/l) were used to estimate the 24, 48, 72, and 96 LC_{50} to *O. mossambicus*. The number of dead organisms was counted at every 24h of exposure during the experiment and was removed immediately to avoid any organic decomposition. From each aquarium, 10% of water was removed every 24h and replaced with the desired quantity of Quinalphos to assure a fixed concentration of the toxicant in the solution. The safe level of Quinalphos was calculated based on application factors (AF) using standard protocols (Edwards & Brown, 1967; Bhattacharya *et al.,* 2021).

The survival rate pattern of *T. tubifex* and *O. mossambicus* to pesticides at the acute level was validated using the GUTS modelling that were performed utilizing OpenGUTS standalone software. The model parameters used are k_d (the dominant rate constant), m_w (median of the threshold distribution, h_b (the background hazard rate) and b_w (the killing rate which is only utilized for SD) (Jager *et al.,* 2011; Jager & Ashauer, 2018).

Tissue Homogenization and Centrifugation

At each exposure duration (1, 7 and 14 days), 1 g of the worm was collected and homogenized in 0.1 M phosphate buffer (pH 7.6). The sample was centrifuged at 10,000 *g* for 10 min, and the supernatant was refrigerated at −20°C for subsequent study.

The liver tissue of the fish was homogenized in 2ml of phosphate buffer saline (PBS). The homogenized tissues were spun in a refrigerated centrifuge-(REMI C Model, India) at 5000rpm for 15 minutes at 4^o C. After centrifugation the supernatants were used directly as aliquots and were stored at -20^o C for enzymatic analysis.

Protein Estimation

Protein content of worms and protein content in liver tissue was evaluated utilizing BSA as standard by the method of Lowry *et al.,* (1951). Bovine serum albumin (BSA, Sigma) was used as a standard.

Sublethal and Chronic Toxicity Bioassay

A quantity of 1.5 g of *T. tubifex* was transferred from a stock aquarium in glass beakers each holding 1 L

of unchlorinated tap water to determine the oxidative stress parameters at the sublethal levels. During the period of 1 day, 7 days and 14 days, two sublethal concentrations of Quinalphos (10% of 96h LC_{50}, i.e., 0.63 µg/l and 20% of 96h LC_{50}, i.e., 1.26 µg/l) were administered. These sublethal concentrations of 10% and 20% of 96 h LC_{50} of pesticides were selected in our investigation based on the fact that the values are within the range of environmentally relevant concentrations as reported by several researchers and also the 10% and 20% of 96 h LC_{50} concentrations of toxicants are used by several researchers for the determination of sublethal toxicity (Abhijith *et al.*, 2012; Adel *et al.*, 2017; Rivera-Utrilla *et al.*, 2012; Suryavanshi *et al.*, 2009; Ying, 2006). Control worms were put in another glass beaker with 1 L of water without any toxicant. On day 1 of the experiment, the pesticides were administered (initial treatment). Then, at 2 day intervals, 10% of each test medium was substituted with pesticides at 10% of the original concentration to maintain a constant concentration of pesticides in the test medium. The experiment was carried out in triplicate.

In case of fish specimen bioassays on oxidative enzymes were also conducted in 20l glass aquaria, each containing 10 l of water and five fish. During the period of 15 days, 30 days and 45 days, two sublethal concentrations of Quinalphos (10% of 96h LC_{50}, i.e., 0.5 µg/l and 20% of 96h LC_{50}, i.e., 1 µg/l) were administered same as following procedures of *T. tubifex*. Fish was fed at 2-5% of the bodyweight daily. Amid the experiment, 20% of the test medium was renewed and replaced with the required amount of pesticide. After 15, 30 and 45 days respectively, fish was anesthetized by immersing them in 0.1% 2-phenoxyethanol. Then the fish were decapitated, and the liver was immediately collected for oxidative enzymes examination.

Oxidative Stress Enzymes Analysis

Catalase (CAT) activity was measured following the reduction of hydrogen peroxide to water and molecular oxygen using a standard protocol (Aebi *et al.*, 1984). The estimation of the superoxide dismutase enzyme (SOD) was carried out by the protocol of Kakkar *et al.*, (1984). Glutathione S-transferase (GST) activity was measured through the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (Habig *et al.*, 1974). Reduced glutathione (GSH) level was studied using a standard protocol (Beutler *et al.*, 1963). The activity of glutathione peroxidase (GPx) was determined using the protocol of Lawrence and Burk (1976). Standard protocol was employed for the analysis of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Bergmeyer, 1965) was followed with some minor modifications. The colorimetric assay of lipid peroxidation (LPO) was performed following the standard protocol (Buege & Aust, 1978). The units of CAT, SOD, GST AST, and ALT were expressed as units of activity per milligram of protein (U/mg protein). MDA level was expressed as nmol TBARS per min per milligram of protein (nmol TBARS/min/mg protein). All the parameters were measured using a UV-Vis spectrophotometer (Cecil Aquarius CE 7400) at room temperature (28^{o}C). All assays were run in triplicate.

Sublethal Bioassay on Hematological Parameters

Bioassays on hematological parameters were also conducted in 15 l glass aquaria, each containing 10 L of water and five fish (Majumder and Kaviraj, 2017). Two sublethal concentrations of Quinalphos (0.5 µg/l and 1 µg/l) were employed for the experiment along with a control. There were three replicates for each concentration. Fish specimens were sampled after 15, 30, and 45 days of exposure. By the utilization of a disposable sterile syringe and a needle, blood was obtained by transfixing the heart. The collected blood sample was transferred immediately to vials containing an anticoagulant, EDTA, and was softly shaken to eschew blood hemolysis. Red blood cell (RBC) counts were calculated using a hemocytometer (Mishra *et al.*, 1977). Hemoglobin (Hb) content was estimated by the cyanmethemoglobin method (Blaxhall and Daisley, 1973). The hematocrit value (hct%) was determined with the standard microhematocrit method (Blaxhall and Daisley, 1973).

Sublethal Bioassay on Plasma Biochemical and Enzymological Parameters

Experiments on plasma biochemical parameters were performed in a homogeneous type of experimental setup as depicted above in bioassay on hematological parameters. After the collection of blood, the blood samples were centrifuged at 3000 rpm for 20 min at 4 ◦C for the separation of plasma from the blood sample. Total glucose was quantified by the GOD-POD method utilizing a commercial kit. Total protein was quantified by Lowry *et al.*, (1951). Albumin was estimated utilizing the BCG (bromocresol green) dye-binding method using a commercial kit. Creatinine level was resolute according to modified Jaffe's method utilizing commercial kit.

Growth Parameters Analysis

Bioassays on oxidative enzymes were also conducted in 20l glass aquaria, each containing 10 l of water. During the period of 15 days and 30 days and 45 days two sublethal concentrations of Quinalphos (10% of 96 h LC_{50}, i.e., 0.5µg/l and 20% of 96 h LC_{50}, i.e., 1µg/l) were administered. Fish was fed at 2-5% of the bodyweight daily. The feeding rate of fish was evaluated in a separate 96 h laboratory bioassay. Ten adult fish (two per aquarium) were exposed to each concentration of Quinalphos. Fish were given feed (Osaka Green) daily at 08.00 h and were allowed to feed for 4 h. Unconsumed feed were removed to avoid any decomposition. The amount of food consumed by control fish was considered as 100%. Survival, growth was measured after 45 days replacement bioassays. Length, weight and visceral weight were recorded and 20% of the test medium was renewed and replaced with the required amount of

pesticide weekly. Final biomass was used to estimate yield of fish in each treatment. Formulae used to estimate morphometric indices (CF, SGR, GSI and FCR) were adopted from LeCren (1951) and Bagenal (1978).

Determination of IBR and BRI

IBR was determined by utilizing standard protocol with minor modifications (Broeg and Lehtonen, 2006). Moreover, the biomarker response index (BRI) for determining the health status of the organism was performed using a standard protocol (Hagger *et al.*, 2008).

Statistical Methods

Finney's probit analysis method was employed for estimating LC_{50} values. The Shapiro-Wilk test was used to assess normal distributions and Levene's test was employed to evaluate homogeneity. All data obtained from our study fulfilled the parametric criteria and were analyzed using One-way ANOVA followed by Tukey multiple comparisons test to compare the means among the different treatment groups within each exposure period. The correlation matrix and principal component analysis were performed using software Graphpad prism 9 and JMP Pro 14. $p< 0.05$, $p < 0.01$ and $p < 0.001$ and $p<0.0001$ were accepted as levels of statistical significance. Data are presented as mean ± SEM. GUTS-SD/IT modelling was conducted in open GUTS software to predict the LC50 values of the selected pesticides during long-term exposure (100 days) and to predict the mode of action of the pesticide (Jager *et al.*, 2011).

RESULT

Acute Toxicity and Behavioural Responses

The 24, 48, 72, and 96 h LC_{50} values of Quinalphos to *T. tubifex* and *O. mossambicus* with 95% confidence limits are depicted in Table 1 and Table 2. Based on the 96h LC_{50} value, the safe permissible limit of Quinalphos was determined which is depicted in Table 3 and Table 4 and is reported to be within the range of 0.628– 2.512µg/l to *T. tubifex* and 0.535-2.14µg/l to *O. mossumbicus*. Fig 1 shows the LC_{50} comparison of Quinalphos at 24, 48, 72, 96h and the mortality rate on *T. tubifex.* Fig 2 shows the LC_{50} comparison of Quinalphos at 24, 48, 72, 96h and the mortality rate on *O. mossumbicus*.

Table 1: Lethal concentration values and 95% confidence limits of Quinalphos to *T. tubifex*

Point	Exposure concentration (µg/l)			
	24h	48h	72h	96h
LC_{50}	13.69 (10.872-17.231)	11.37 (8.853- 14.592)	8.31 (6.407-10.771)	6.28 (4.840- 8.150)

Table 2: Lethal concentration values and 95% confidence limits of Quinalphos to *O. mossambicus*

Point	Exposure concentration (µg/l)			
	24h	48h	72h	96h
LC_{50}	7.13 (6.612-7.683)	6.51(6.034-7.032)	5.86 (5.395-6.368)	5.35(4.882-5.858)

Table 3: Safe concentrations of Quinalphos to *T. tubifex* at 96h exposure period

Pesticide	96h LC_{50} (µg/l)	Method	Application Factor (AF)	Safe Level (µg/l)
Quinalphos	6.28	Edwards & Brown, 1967	0.4	2.512
		Burdick, 1967	0.1	0.628

Table 4: Safe concentrations of Quinalphos to *O. mossambicus* at 96h exposure period

Pesticide	96h LC_{50} (µg/l)	Method	Application Factor (AF)	Safe Level (µg/l)
Quinalphos	5.35	Edwards & Brown, 1967	0.4	2.14
		Burdick, 1967	0.1	0.535

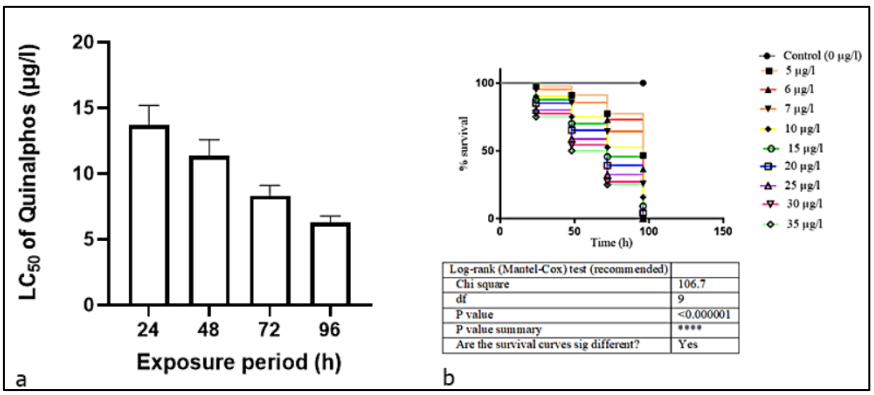
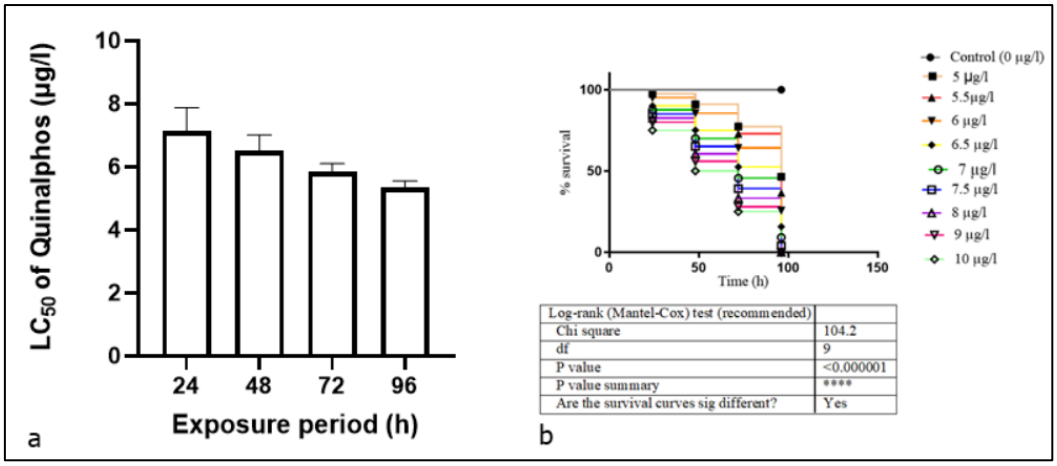


Figure 1: (a) 24, 48, 72 and 96 h LC_{50} values of Quinalphos and (b) Kaplan– Meier survival curves of *T. tubifex* exposed to Quinalphos



Log-rank (Mantel-Cox) test (recommended)	
Chi square	104.2
df	9
P value	<0.000001
P value summary	****
Are the survival curves sig different?	Yes

Figure 2: **(a) 24, 48, 72 and 96 h LC_{50} values of Quinalphos and (b) Kaplan– Meier survival curves of *O. mossumbicus* exposed to Quinalphos**

Moreover, the Kaplan– Meier curve demonstrates that Quinalphos had a concentration and duration- dependent adverse impact on the overall survival rates of *T. tubifex* and *O. mossumbicus* compared with the control group (Mantel log- rank test; $p < 0.05$) (Figure 1a-b) and (Figure 2a-b) respectively. Worms and fish both are found to be 100% viable in all exposure times in control. Conversely, when Quinalphos concentrations increased, as did exposure times (24, 48, 72, and 96 h), the survival rate of *T. tubifex* and *O. mosumbicus* decreased significantly (Mantel log- rank test; $p < 0.05$).

Behavioral Changes

With the increasing concentration of toxicant and the progress of exposure time, *T. tubifex* exhibited various abnormal behaviours (Table 5). The control worms exhibited normal movement and clumping tendency throughout the exposure period. But upon addition of quinalphos, the exposed worms exhibited several erratic behaviours like hyperactivity, increased mucous secretion, enhanced wrinkling effect and decreased clumping tendency.

Table 5: Impact of quinalphos on behavioural responses of *T. tubifex*

Behavioral Responses of *T. tubifex*															
Time (hrs.)	**Behavioral Response**	**Dose (µg/l)**													
		0	**0.5**	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**15**	**20**	**25**	**30**	**35**
24	**HM**	-	-	-	+	+	++	++	++	++	++	+++	+++	+++	+++
	CT	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+	+	-
	MS	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++
	WE	-	-	-	-	-	-	-	+	+	++	++	++	+++	+++
48	**HM**	-	-	-	+	+	++	++	++	++	++	++	++	++	+++
	CT	+++	+++	+++	++	++	++	++	++	+	+	+	+	-	-
	MS	+	+	+	+	+	+	+	++	++	+++	+++	+++	+++	+++
	WE	-	-	-	-	-	+	+	++	++	++	++	+++	+++	+++
72	**HM**	-	-	-	-	+	+	+	+	+	+	+	++	++	++
	CT	+++	++	++	++	++	+	+	+	+	-	-	-	-	-
	MS	+	+	+	+	+	+	+	++	++	++	+++	+++	+++	+++
	WE	-	-	-	-	-	+	+	+	++	+++	+++	+++	+++	+++
96	**HM**	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	CT	+++	++	++	++	++	+	+	+	+	-	-	-	-	-
	MS	+	+	+	+	+	+	+	+	+	++	++	++	++	++
	WE	-	-	-	-	+	+	+	++	++	++	+++	+++	+++	+++

(HM: Hyperactive movement; CT: clumping tendency; MS: mucus secretion; WE: wrinkling effect; –: none; +: mild; ++: moderate; +++: strong) exposed to different concentrations in different exposure periods ($p < 0.05$).

Behavioral changes were observed in control and Quinalphos treated fish throughout the experiment. The control fish were active with a proper body balance throughout the experimental period. They responded towards the slightest disturbances and showed a normal opercular movement. Whereas the treated fish showed several types of abnormal behaviors including increased erratic swimming, jerky movement, mucous secretion, loss of equilibrium and opercular movement.

Table 6: **Impact of quinalphos on behavioural responses of *O. mossumbicus***

Concentration (µg/l)	Erratic swimming	Jerky movement	Mucous secretion	Loss of equilibrium	Opercular movement
0	-	-	-	+++	-
3.5	+	-	-	++	+
4.5	++	+	+	+	+
5.5	++	+	++	+	++
6.5	++	++	++	-	++
7.5	+++	+++	+++	-	+++

(–: none; +: mild; ++: moderate; +++: strong) exposed to different concentrations in different exposure periods ($p < 0.05$).

Survivability Prediction

The model parameters, as well as the fitted performance of GUTS (SD or IT), are given in Table 7. K_d estimated by GUTS– SD was higher than estimated by GUTS– IT and the background rate (h_b) simulated by GUTS– SD was approximately the same as estimated by GUTS– IT in the case of Quinalphos. Moreover, the fitted performance of GUTS– IT was better than that of GUTS– SD in the case of the pesticides based on AIC values (Smaller AIC value indicate the best fit). Thus, the model simulation illustrated that the GUTS- IT model can better predict the survival rate observed in *Tubifex tubifex* for pesticide exposure than the GUTS-SD model at an acute level.

Table 7: Model parameters in case of quinalphos

Symbol	GUTS-RED		Unit	AIC Value	
	SD	IT		SD	IT
k_d	143.8 (6.042 - 143.8)	0.3072 (0.069 - 0.5445)	d^{-1}	257.51	254.92
m_w	1.642 (0.5032 - 1.968)	4.117 (1.206 - 6.127)	µg/l		
b_w	0.04394 (0.03318 - 0.0561)	-	l/µg/d		
h_b	1E-6	1E-6	d^{-1}		
F_s	-	5.711 (3.857 - 10.45)			

[Kd indicates Dominant rate constant; m_w indicates Threshold for mortality; b_w indicates Killing rate; h_b indicates Background hazard rate & F_s indicates Spread factor of the threshold distribution]

Moreover, the forecasted LC_{50} values from GUTS-IT models are given

Table 8: The forecasted LC_{50} values of quinalphos to *T. tubifex*

Time [d]	LC_{50} GUTS-IT (µg/l)
1	15.57 (12.37 - 20.12)
2	8.969 (7.383 - 10.96)
3	6.838 (5.579 - 8.32)
4	5.82 (4.529 - 7.278)
7	4.66 (2.97 - 6.395)
14	4.174 (1.835 - 6.198)
100	4.117 (1.058 - 6.192)

The GUTS model fit is shown in Table 9. Moreover, from the data that the fitted performance of GUTS–SD was better than that of GUTS–IT in case of Quinalphos predicated on Akaike Information Criteria (AIC) values (Smaller AIC value indicate the best fit) (Table 9). Thus, the model simulation illustrated that the GUTS- SD model can better predict the survival rate observed in *Oreochromis mossumbicus* for pesticide exposure than the GUTS- IT model at an acute level.

Table 9: Model parameters in case of quinalphos

Symbol	GUTS-RED		unit	AIC Value	
	SD	IT		SD	IT
k_d	143.8 (8.539 - 143.8*)	1.064 (0.8286 - 1.297)	d^{-1}	277.48	287.36
m_w	3.418 (3.114 - 3.492)	5.117 (4.611 - 5.565)	µg/l		
b_w	0.137 (0.1055 - 0.1728)	-	l/µg/d		
h_b	1E-6	1E-6	d^{-1}		
F_s	-	1.952(1.659 - 2.544)			

[Kd indicates Dominant rate constant; m_w indicates Threshold for mortality; b_w indicates Killing rate; h_b indicates Background hazard rate & F_s indicates Spread factor of the threshold distribution]

Moreover, the forecasted LC_{50} values from GUTS-IT models are given in Table 4.4.

Table 10: The forecasted LC_{50} values of quinalphos to *O. mossumbicus*

Time [d]	LC_{50} GUTS-SD (µg/l)
1	7.816 (7.186 - 8.694)
2	5.81 (5.423 - 6.248)
3	5.337 (4.925 - 5.745)
4	5.191 (4.732 - 5.631)
7	5.12 (4.605 - 5.584)
14	5.117 (4.594 - 5.583)
100	5.117 (4.594 - 5.583)

The survival model output demonstrates that the model deducing SD or IT should be chosen wisely to determine the toxic effects with various toxicant exposure patterns. It is clear that such mechanistic modelling has significant potential for enhancing the accuracy of environmental risk management in the future and can significantly help in effective decision- making.

Effects on Growth Parameters

The indicators of the growth performance of *O. mossumbicus* upon exposure (45 days) to sub-lethal concentrations of Quinalphos (0.5 and 1µg/l) are demonstrated in Figure 3. The sublethal exposure of fish to 10% and 30% of 96 h LC50 of Quinalphos (0.5 and 1mg/L) showed a significant reduction ($p<0.05$) in SGR and a significant increase ($p<0.05$) in GSI and FCR in compare to control.

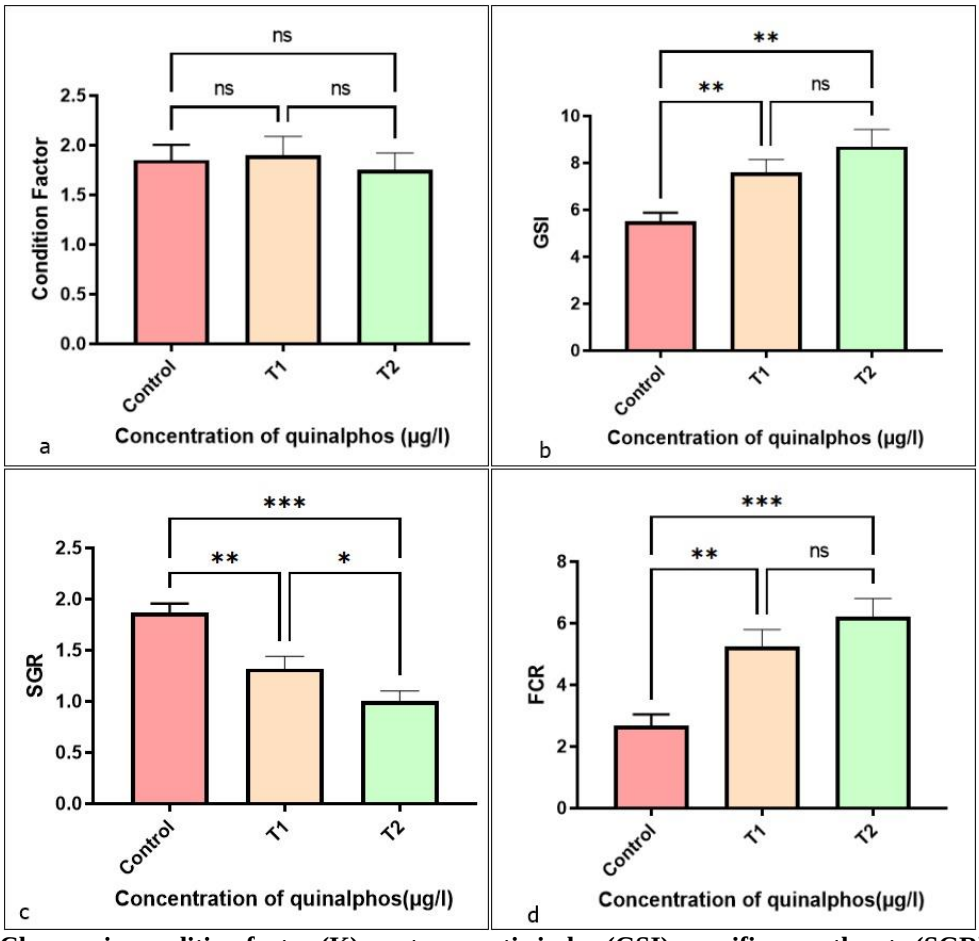


Figure 3: Changes in condition factor (K), gastrosomatic index (GSI), specific growth rate (SGR) and feed conversion ratio (FCR) of *O. mossumbicus* upon addition of Quinalphos (a–d).

The values are represented as mean ± SE. ns indicate non-significant difference and * indicates level of significance (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ and **** = $p<0.0001$). Control indicates 0 µg/l of pesticide (Quinalphos), T1 indicates concentration of 10% of 96 h LC_{50} values of pesticide (Quinalphos), T2 indicates concentration of 20% of 96 h LC_{50} values of pesticide (Quinalphos).

Oxidative Stress Parameters

In case of *T. tubifex* 1d, 7d and 14d exposure period, catalase activity increased significantly (Figure 4) ($p< 0.05$) in two sublethal concentrations of quinalphos (10% of 96h LC_{50}, i.e., 0.63 µg/l and 20% of 96h LC_{50}, i.e., 1.26 µg/l) exposed worms with a control diet with respect to control. SOD activity significantly increased ($p< 0.05$) (Figure 4) in worms exposed to 0.63 µg/l and 1.26 µg/l of pesticide and provided with control diet after 1d, 7d and 14d exposure with respect to control. In 1d, 7d exposure period, the activity of GST increased significantly ($p<0.05$) in worms exposed to 0.63µg/l and 1.26µg/l of Quinalphos with respect to control but 14d exposure period GST activity decreased significantly ($p<0.05$) (Figure 4) with both concentrations of pesticide at 0.63µg/l and 1.26µg/l exposure. MDA activity increased significantly ($p<0.05$) in worms exposed to 0.63 µg/l and 1.26 µg/l of pesticide with a control in 1d, 7d and 14d exposure periods (Figure 4). In 1d, 7d exposure period, GPx activity increased significantly ($p< 0.05$) in 0.63µg/l and 1.26µg/l concentrations with respect to control but decreased significantly after 14d exposure period in higher concentration (1.26µg/l) Figure 4. GSH activity also increased significantly ($p<0.05$) at 1d and 7d exposure period in 0.63µg/l and 1.26µg/l both concentrations but show decreasing activity at 14d exposure period of 1.26µg/l concentration Quinalphos Figure 4.

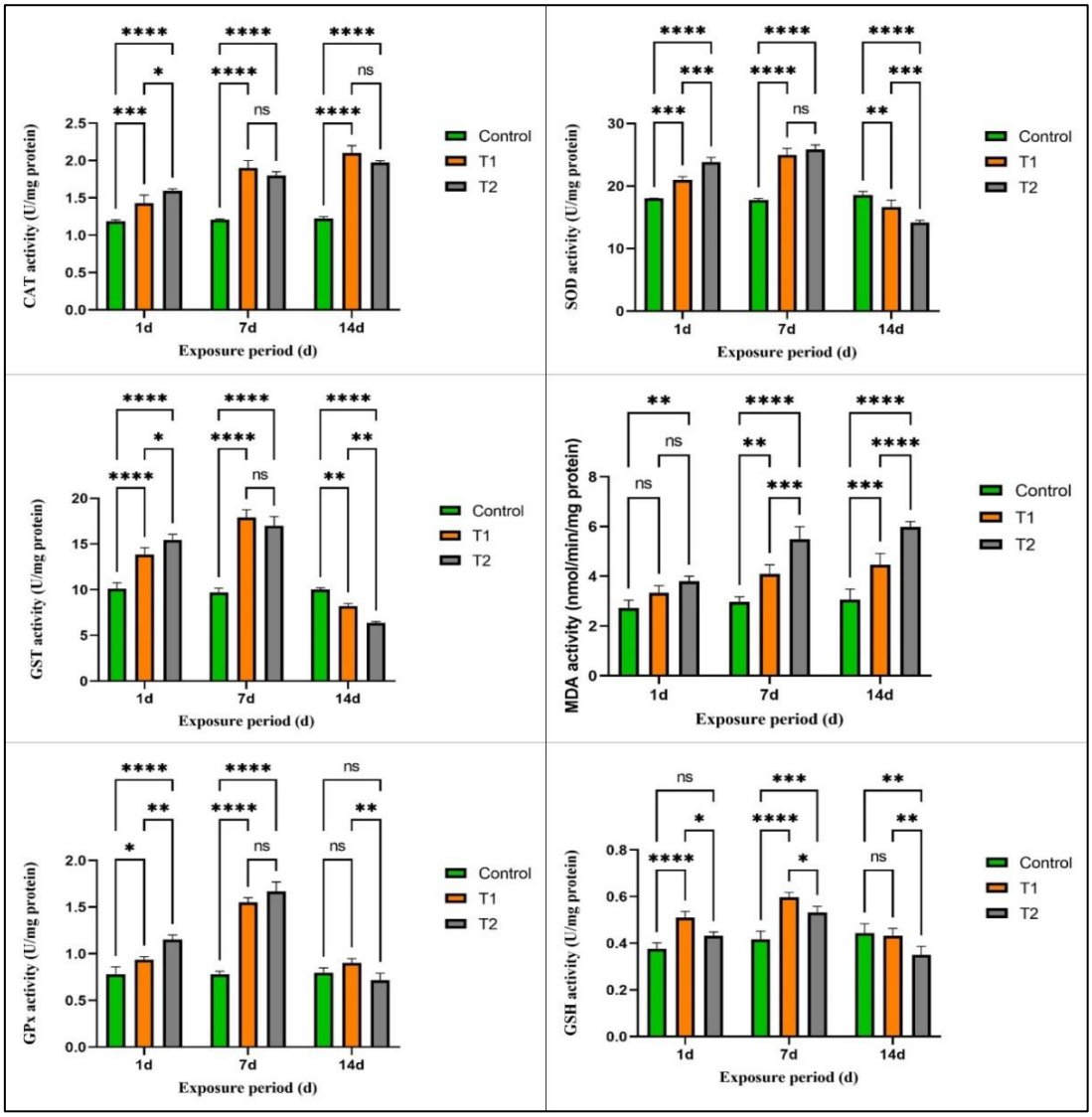


Figure 4: Effects of Quinalphos on CAT, SOD, GST, MDA, GPx and GSH levels in *T. tubifex* at different exposure periods.
The values are represented as mean ±SE, ns indicates non-significant and * indicates level of significance (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ and **** = $p<0.0001$).

In case of *O. mossumbicus* 15d, 30d and 45d exposure period, catalase activity increased significantly ($p< 0.05$) (Figure 5) in with two sublethal concentrations of quinalphos (10% of 96h LC_{50}, i.e., 0.5 µg/l and 20% of 96h LC_{50}, i.e., 1 µg/l) exposed fish supplemented with a control diet with respect to control. SOD activity significantly increased ($p< 0.05$) in fish exposed to 0.5µg/l and 1µg/l of pesticide and provided with control diet after 15d, 30d and 45d with respect to control (Figure 5). In 15d, 30d exposure period, the activity of

GST increased significantly ($p<0.05$) in fish exposed to 0.5µg/l and 1µg/l of Quinalphos provided with a control diet with respect to the control. But 45d exposure period GST activity decreased significantly ($p<0.05$) with 1µg/l exposure (Figure 5). MDA activity increased significantly ($p<0.05$) in fish exposed to 0.5µg/l and 1µg/l of pesticide provided with a control diet in 15d, 30d and 45d exposure periods with respect to the control (Figure 5). In 15d, 30d and 45d exposure period, GPx activity decreased significantly ($p< 0.05$) (Figure 5) in 0.5µg/l and 1µg/l pesticide exposed fish supplemented with a control diet with respect to control. AChE activity also decreased significantly ($p<0.05$) at 15d, 30d and 45d exposure period in 0.5µg/l and 1µg/l both concentrations (Figure 5).

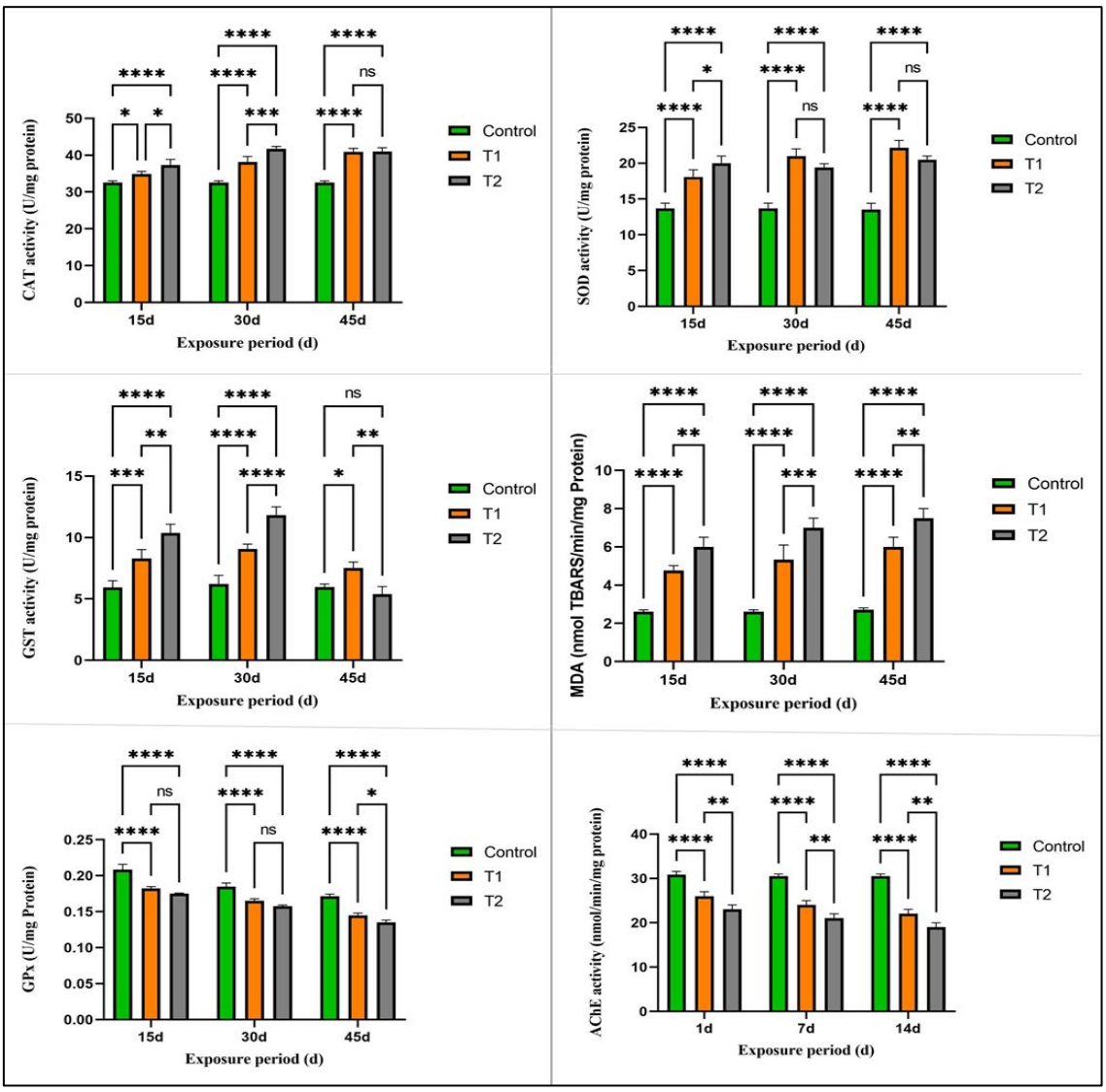


Figure 5: Effects of Quinalphos on CAT, SOD, GST, MDA, GPx and AChE levels in *Oreochromis mossambicus* at different exposure periods.

The values are represented as mean ±SE, ns indicates non-significant and * indicates level of significance (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ and **** = $p<0.0001$).

Biochemical and Haematological Parameters

The 15d, 30d and 45d exposure of *O. mossumbicus* to two sublethal concentrations of quinalphos (10% of 96h LC_{50}, i.e., 0.5 µg/l and 20% of 96h LC_{50}, i.e., 1 µg/l) showed a significant alteration in biochemical ($p<0.05$, Two-way ANOVA, Tukey test) in comparison to control. Total glucose and creatinine increased significantly ($p<0.05$) with two sublethal concentrations of quinalphos 0.5 µg/l and 1 µg/l in 15d, 30d and 45d all exposure periods. Total protein and albumin decreased significantly ($p<0.05$) with time dependent and dose dependent pattern. ALT and AST activities in fish exposed to 0.5 µg/l and 1 µg/l of pesticide combined with a control diet increased significantly ($p<0.05$) in 15d, 30d and 45d exposure periods compared to the control ($p<0.05$) found in Figure 6.

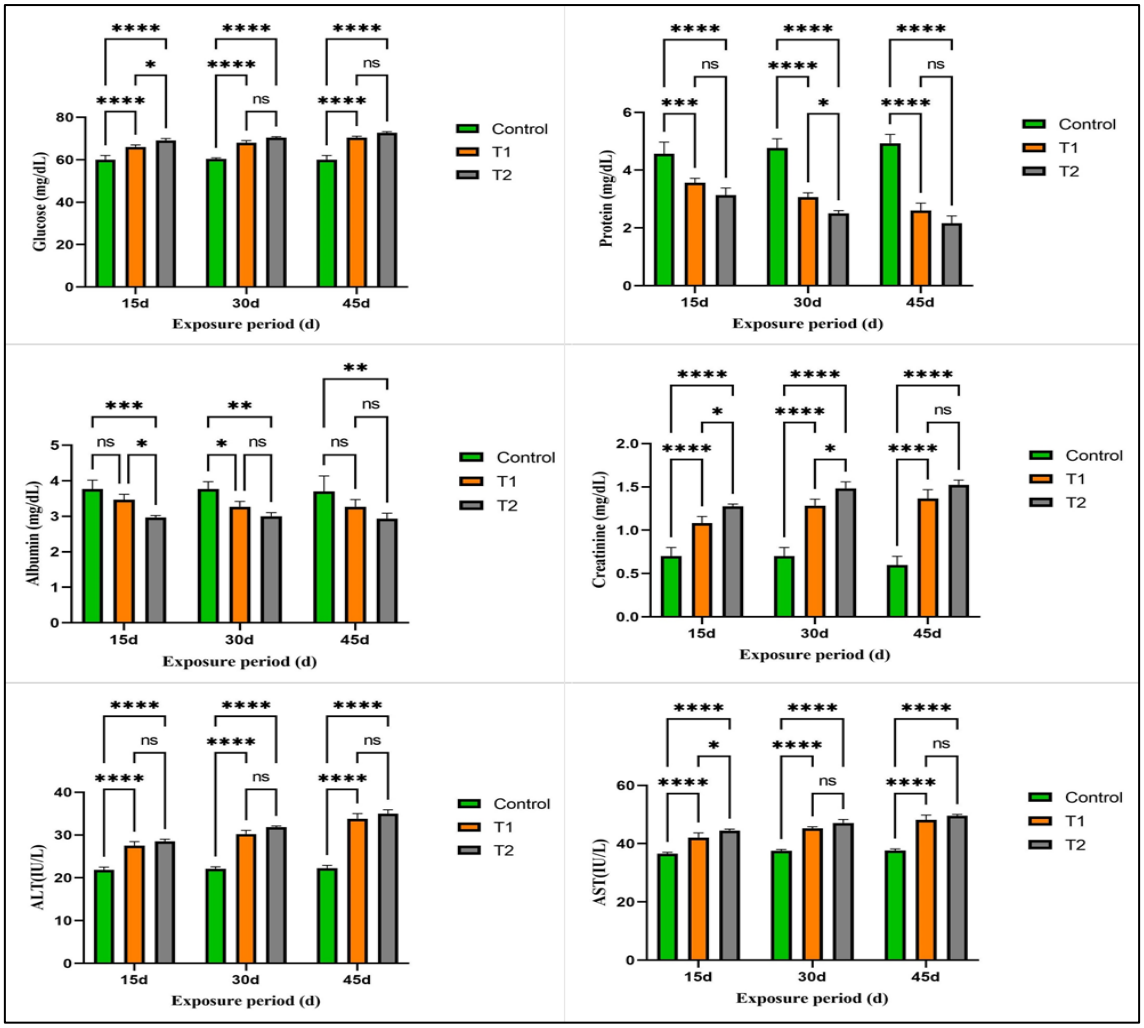


Figure 6: Effects of Quinalphos on Glucose, Protein, Albumin, Creatinine, ALT and AST levels in *O. mossambicus* at different exposure periods.

The values are represented as mean ±SE, ns indicates non-significant and * indicates level of significance (* = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001).

The 15d, 30d and 45d exposure of *O. mossumbicus* to two sublethal concentrations of Quinalphos (10% of 96h LC_{50}, i.e., 0.5 µg/l and 20% of 96h LC_{50}, i.e., 1 µg/l) showed a significant alteration in haematological parameters (p<0.05, Two-way ANOVA, Tukey test) in comparison to control. Red blood cells (RBC), Haemoglobin (Hb) and Haematocrit (Ht) decreased significantly (p<0.05) with 0.5 µg/l and 1 µg/l pesticide concentrations over 15d, 30d and 45d exposure periods (Figure 7).

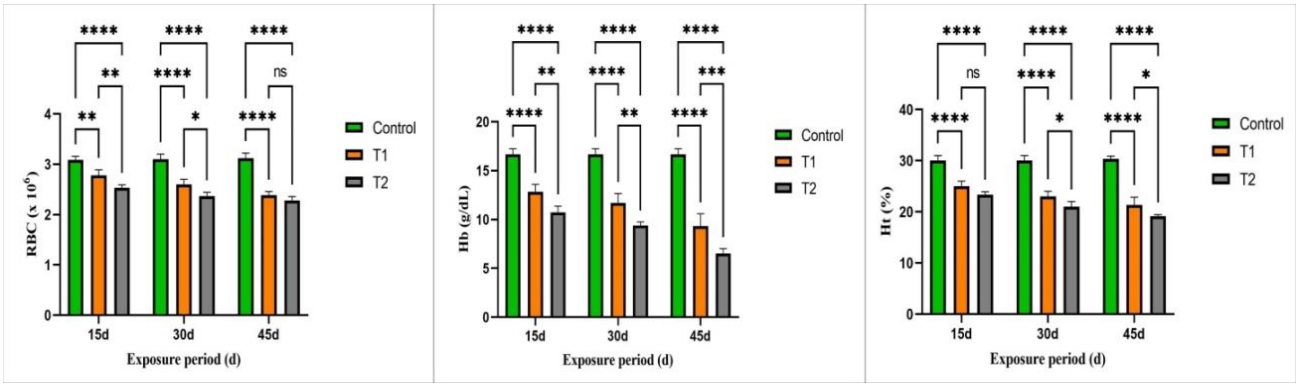


Figure 7: Effects of Quinalphos on RBC, Hb and Ht levels in *O. mossambicus* at different exposure periods.

The values are represented as mean ±SE, ns indicates non-significant and * indicates level of significance (* = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001).

Chemometrics

Two-way ANOVA results revealed significant variations ($p < 0.05$) in each biomarker with various experimental conditions. The Quinalphos concentration-dependent parameters are the stress enzyme parameters (CAT, SOD, GST, GPx, MDA and GSH). The exposure period dependant parameters are CAT, SOD, GST, GPx, MDA and GSH. Moreover, the parameters affected by interactions between Quinalphos concentration and exposure periods are CAT, SOD, GST, GPx, MDA and GSH. Moreover, the results of the PCA are depicted). In Figure 8b which were confirmed and quantified by Pearson correlation plots (Figure 8a). The statistical analysis revealed that the concentrations of Quinalphos are positively correlated with CAT and MDA.

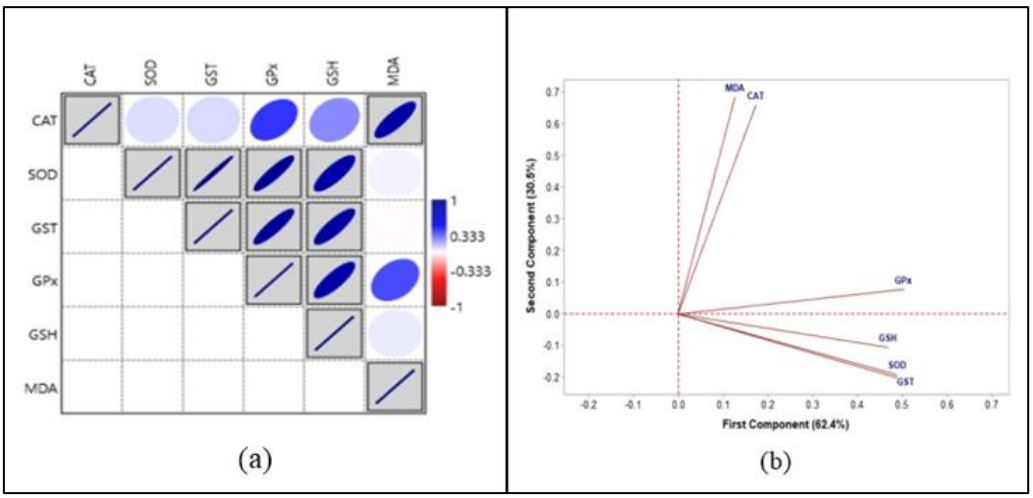


Figure 8: (a) Pearson correlation matrix plot and (b) Ordination diagram of PCA on stress parameters in *T. tubifex* after 14 days exposure to Quinalphos.

Two-way ANOVA results revealed significant variations ($p < 0.05$) in each biomarker with various experimental conditions. The Quinalphos concentration-dependent parameters are RBC, Hb, Ht, glucose, protein, albumin, creatinine, AST, ALT and all the stress enzyme parameters (CAT, SOD, GST, GPx, MDA and AChE) in liver. The exposure period dependant parameters are RBC, Hb, Ht, glucose, protein, creatinine, AST, ALT all the stress enzyme parameters (CAT, SOD, GST, GPx, MDA and AChE) in liver. Moreover, the parameters affected by interactions between Quinalphos concentration and exposure periods are protein, creatinine, AST, ALT, CAT, SOD, GST, GPx, MDA and AChE in liver. Moreover, the results of the PCA are depicted in Figure. 9b which were confirmed and quantified by Pearson correlation plots (Figure. 9a). The statistical analysis revealed that in the case of hematological parameters, the concentrations of Quinalphos are negatively correlated with RBC, Hb, and Ht (%). In the case all parameters' concentrations of Quinalphos are positively correlated with AChE, GPx, Protein, Albumin, RBC, Hb, Ht. Whereas Glucose, Creatinine, SOD, CAT, MDA, AST, ALT negatively correlated with Quinalphos concentrations.

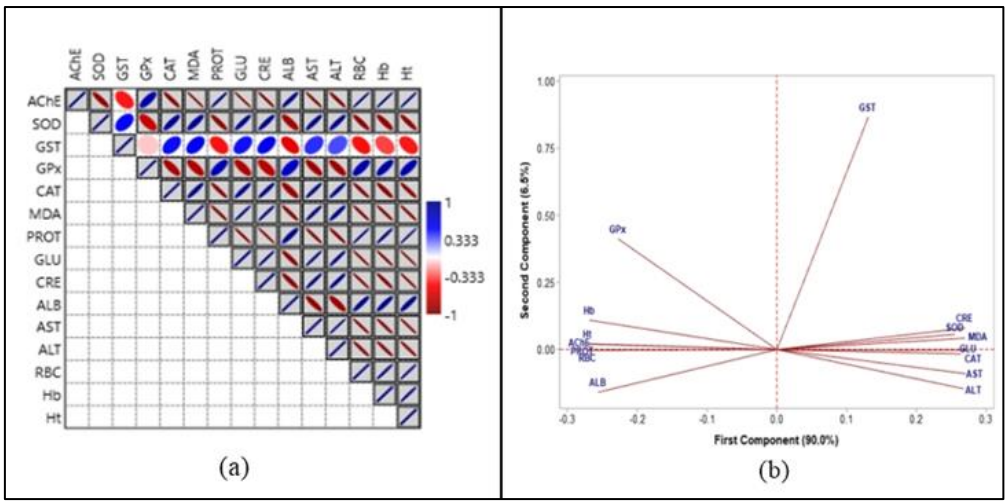


Figure 9: (a) Pearson correlation matrix plot and (b) Ordination diagram of PCA on hematological parameters, biochemical as well as enzymological parameters and stress parameters in liver in *O. mossumbicus* after 45 days exposure to Quinalphos.

IBR and BRI

IBR values in the star plot showed T2-30d were the most affected group under Quinalphos induced toxicity (Figure 10). IBR values ranged from 0.75 to 3.00. According to this index, the rank of the most affected group could be ordered as: T2-7d >T1-7d >T2-14d >T2-1d >T1-1d >T1-14d >C-14d >C-7d >C-1d. The control group (C-1d, C-7d, C-14d) remains unaffected in terms of Quinalphos-induced toxicity.

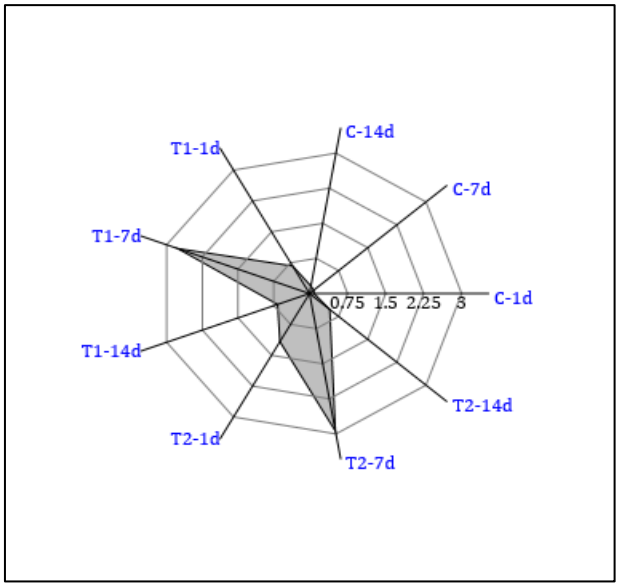


Figure 10: **Concentration and duration-dependent star plots representing the IBR values in Quinalphos of *T. tubifex*. C indicates control (0 µg/1), T1 indicates the concentration of Quinalphos at 10% of 96 h LC50 value and T2 indicates the concentration of Quinalphos at 20% of 96 h LC50.**

IBR values in the star plot showed T2-30d were the most affected group under Quinalphos induced toxicity (Figure 11a). IBR values ranged from 0.75 to 2.25. According to this index, the rank of the most affected group could be ordered as: T2-45d >T2-30d >T1-45d >T1-30d >T2-15d >T1-15d >C-45d >C-30d >C-15d. The control group (C-45d, C-30d, C-15d) remains unaffected in terms of Quinalphos-induced toxicity. Figure 11b Moreover, BRI values representing the overall general health status of the fish.

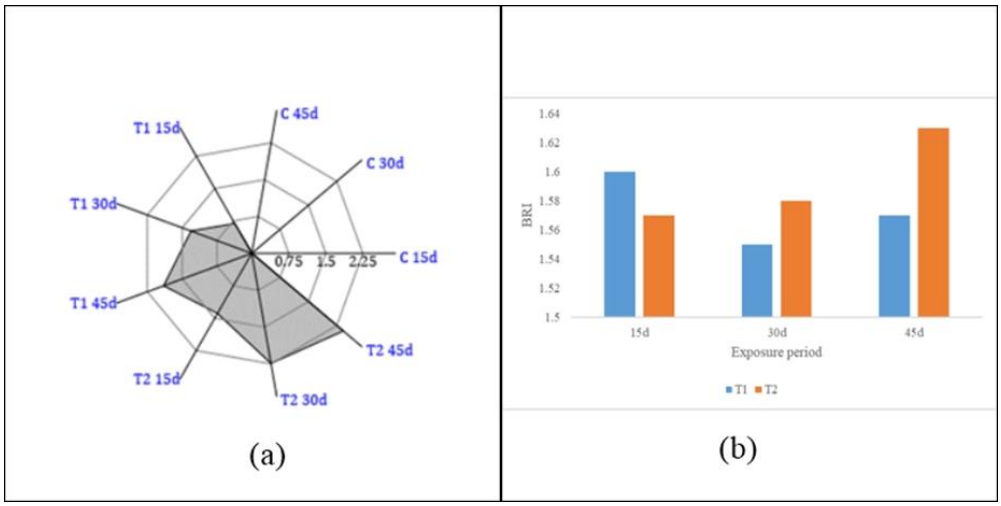


Figure 11: (a) Concentration and duration-dependent star plots representing the IBR values in Quinalphos. C indicates control (0 µg/1), T1 indicates the concentration of Quinalphos at 10% of 96 h LC_{50} value and T2 indicates the concentration of Quinalphos at 20% of 96 h LC_{50} and (b) BRI values representing the health status of *O. mossumbicus* upon exposure to Quinalphos for 45 days.

DISCUSSION

In the present analysis, the 96 h LC_{50} value of Quinalphos to *T. tubifex* is 6.28µg/l and *O. mossambicus* is 5.35µg/l, suggesting that it is extremely toxic and is lower than the LC_{50} value of other fish species such as 1.4 ppm in *Barbonymus gonionotus* (Sadiqul I. M., 2017), 2.75ppm in *Cyprinus carpio* (Padmanabha *et al.*, 2016), 1.09ml/l in *Clarias batrachus* (Vinoy K.

Shrivastava., 2013) and 10.9µl/l in *Cyprinus carpio* (Hemalatha D., 2021). These variations between different test species depend on species, age, size, maturity, fitness, variety, bodyweight water physiochemical parameters and duration of exposure (Wang *et al.,* 2019).

Moreover, the survival model performance provides evidence that the model presuming SD or IT should be selected to constantly predict toxic effects for different toxicant exposure patterns. Such mechanistic effect model mechanisms considered how chemicals affect individuals and ecological systems such as populations and communities. It also included for making decision a standardized way in future regulatory risk assessments (He *et al.,* 2019).

In our present study, Quinalphos exposed worms show various abnormal behaviours i.e., increased mucous secretion, increased hyperactive movement, decreased clumping tendency, fragmentation of body segments. Similar type worms' behavioural changes were found in others pesticide, Cartap hydrochloride (Medda *et al.,* 2019). Treated fish also showed several abnormal behaviors including erratic swimming, jerky movement, mucous secreation, loss of equilibrium and opercular movement. Probably the reduction of RBC and AchE levels of fish were responsible for increased opercular movement and erratic swimming behaviour. Similar AchE level abnormalities were recorded in *Oreochromis sp.* after exposure to Carbofuran pesticide (Hamed *et al.,* 2018). These changes in the behaviour of the organism help to evaluate the relation between the organism and its surrounding environment (Ghelichpour *et al.,* 2019).

Aquatic organisms require optimum conditions for regular growth and wellbeing (Abdel-Wahab *et al.,* 2021). Similar type modification was showed in C*larias batrachus* due to Quinalphos texposure (Shrivastava *et al.,* 2013). The results show the reduced growth rate and feed conversion ratio of tilapia delivered quinalphos compared with control. Nevertheless, quinalphos toxicity induced abnormal and inflammatory features in the intestine of tilapia, explaining the lowered feed efficiency and growth performance (Padmanabha *et al.,* 2016). Exposure to pesticides resulted in impaired intestinal health in tilapia (Neamat-Allah *et al.,* 2020). The damaged intestinal features and inflammation are probably attributed to the oxidative stress induced by Quinalphos exposure. The growth parameter serves as a designator of populations' life conditions that could be utilized to detect stress due to contamination (Abdel-Warith *et al.,* 2021). Decrease in the SGR of fish occurs due to disruption of the metabolic processes in the fish's body (Kim *et al.,* 2018). The increase in the value of the FCR is probably due to the toxic effects of toxicants in the fish's body, which interfere with the function of respiration and inhibit the metabolic activity of the fish's body so that the process of digestion of food is disrupted (Padmanabha *et al.,* 2015; Sunanda *et al.,* 2016). As a result, the decreased growth rate of *O. mossumbicus* enlisted in our current study is most likely due to decreased appetite resulting in reduced feed intake or increased expenditure of energy in the presence of toxicant for continuing normal metabolic process, leaving less energy available for growth (Abdel-Tawwab *et al.,* 2013). Moreover, dose-depend decrease in specific growth rate and increase in the feed conversion ratio in our study might be related to altered stability or downregulation of growth hormone (Guo *et al.,* 2021). Declines in growth parameters were reported in *O. mossambicus* upon exposure to phenol and aniline (Saha *et al.,* 1999; Bhunia *et al.,* 2003). The growth of *O. niloticus* was reported to be reduced when exposed to sub-lethal concentrations of cypermethrin (Majumder and Kaviraj *et al.,* 2017) and abmectin (Mahmoud *et al.,* 2021).

In this study, alterations of different biomarkers in *T. tubifex* have suggested towards effects of Quinalphos have negative effects on the survivability of the worms. Similar oxidative stress enzymes study was assessed (Chatterjee *et al.,* 2021) in *T. tubifex* by the sublethal effects of other pesticides like profenofos, cyhalothrin and biopesticide azadirachtin.

Results of the hematological, biochemical and enzymological parameters, alterations of stress enzymes in liver in this study have suggested towards effects of Quinalphos on interlinked physiological and biochemical processes that have detrimental effects on the survivability of the fish.

In this present study, the gradual decrease in RBC, hemoglobin and Hct% indicates structural abnormalities of RBCs that affect its oxygen-carrying capacity and leads towards anaemic condition (Chen *et al.,* 2017). Similarly, type of reductions in hematological parameters of *O. mossumbicus* after exposure to Chlorpyrifos (Ghayyur *et al.,* 2019) and to Paclobutrazol (Tahir R., 2021) respectively. The alterations in plasma biochemical parameters serve as an excellent biomarker to check the general health status of an organism (Javed *et al.,* 2017). Among these parameters, plasma protein, glucose is important sensitive index denoting the stress state of an organism (Javed *et al.,* 2017). The alteration in glucose level indicates metabolic depression caused by damages to vital organs (Tabassum *et al.,* 2020). In our present investigation, the gradual expansion in the glucose level is probably due to the activation of stress-induced catecholamine- mediated gluconeogenesis in the liver and glycogenolysis in skeletal muscles (Kuo *et al.,* 2015). Similar types of alterations in glucose level were observed in *O. mossumbicus* after exposure to Chlorpyrifos (Ghayyur *et al.,* 2019). The alteration in total protein indicates the weakening of the immune system as well as liver and kidney dysfunction (Tahir *et al.,* 2021). In this study, the gradual reduction of total protein level resulted the

decreased rate of protein synthesis indicates the destruction or necrosis of hepatocytes and consequent impairment in the protein synthesis machinery after addition of the pesticide (Chatterjee *et al.,* 2021). Similar results of reduction in total protein content were reported in *O. mossumbicus* after exposure of Abamectin (Al Ghais *et al.,* 2019). Albumin plays important role maintenance of the osmotic balance between the circulating blood and the tissue membrane (Dogan and Can, 2011). In the present study, the reduction in albumin level indicates liver dysfunction, and malnutrition upon the addition of pesticide due to the inhibition of albumin biosynthesis in the liver, (Ghelichpour *et al.,* 2020). Similar types of reductions in albumin level were documented in *Oreochromis sp*. during exposure to pesticide Atrazine (Abdel-Warith *et al.,* 2021). Creatinine can be used as a sensitive indicator of kidney functions. In our present study, the amplified creatinine levels upon pesticide exposure might be due to kidney dysfunction by structural damage (Mirghaed *et al.,* 2018). A similar result was reported in *Oreochromis* sp. during exposure to pesticide Atrazine (Abdel-Warith *et al.,* 2021). Cholesterol is an important biomolecule that serves several important functions including membrane formation and serving as a precursor for the synthesis of steroid hormones (Nematdoost Haghi and Banaee, 2017). In the present study, the incremented level of cholesterol indicates abnormal lipid metabolism probably caused due to damage in liver tissues (Tahir *et al.,* 2021). A similar type of augmented cholesterol level was observed in *O. mossumbicus* after exposure to Chlorpyrifos (Ghayyur *et al.,* 2019). Accumulative level of blood serum enzymes like AST and ALT indicates as reflection of tissue damage or organ disfunction caused by toxicity-induced stress (Tabassum *et al.,* 2020). In our study, the increase recorded in AST activity suggests that an important reaction of the molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids) that AST catalyzes was affected. Similarly, the increase in ALT indicates the intensive glycogenesis to coop-up the severe energy crisis occurred due to quinalphos toxic stress. (Hamed *et al.,* 2018., Nematdoost Haghi and Banaee *et al.,* 2017). Similar types of incremented levels of AST and ALT were observed in *O. mossumbicus* after exposure to abamectin insecticide (Kushwaha *et al.,* 2020).

Pesticides can disrupt the optimum redox homeostasis in an organism by generating reactive oxygen species (ROS) that lead to physiological and biochemical alterations (Bhattacharya *et al.,* 2021). Among the stress enzymes, SOD and CAT work as first-line defense enzymes where SOD transforms superoxide anion free radical ($O2^-$) into molecular oxygen and hydrogen peroxide (H2O2) thereby damaging the cells (Chang *et al.,* 2020). In the current research, quinalphos induced ascent in SOD activity in the *T. tubifex* and liver of *O. mossumbicus* might be due to the initiation of formation of the breakdown of $O2^-$ into H2O2 to protect the cell from oxidative stress (Abdel-Wahab *et al.,* 2021). Along with SOD, CAT also acts as a first-line defense enzyme by breaking down the H2O2 to molecular oxygen and water (Chang *et al.,* 2020). In the present study, the augmented CAT level is probably due to the upregulation of nuclear Nrf2 expression that leads towards CAT production to neutralize effects of ROS generation caused by the toxicant (Hamed *et al.,* 2018). Similar type CAT and SOD study was reported in *T. tubifex* after exposure of profenofos, λ cyhalothrin and azadirachtin (Chatterjee *et al.,* 2021) and in *O. mossumbicus* after exposure of fumaronitrile (Chinnadurai *et al.,* 2022). GST is a multifunctional bio-processing enzyme of phase II biotransformation system that modulates GSH and facilitates the excretion of toxic compounds through nucleophilic additions (Bhattacharya *et al.,* 2021). In our present investigation, the initial increment in the GST level of was probably due to the higher formation rate of glutathione disulfide (GSSG) (Chang *et al.,* 2020). However, a subsequent decrease in GST activity with the increasing exposure period indicates the compromised detoxification process of the organism under long-term exposure due to the downregulation of gene expression associated with GST enzymes (Chinnadurai *et al.,* 2022). Another important enzyme of the 'glutathione enzyme system', GPx played an important role in the transformation of H_2O_2 to molecular oxygen and water, oxidation of GSH into GGSG (Chang *et al.,* 2020). In the present investigation, the gradual reduction in GPx level in the liver of quinalphos exposed fish indicates a high level of toxicant-induced ROS developed through lipid peroxidation as the liver is the key organ in vertebrates and injury to the hepatic system reflects in physiological and biochemical parameters of the organism (Tabassum *et al.,* 2020). Different abnormalities in cellular and biochemical levels due to the generation of excessive ROS are reported by previous researchers (Klotz and Steinbrenner *et al.,* 2017). The elevation in the ROS level acts as a signal for the induction of stress-responsive survival pathway. The initial induction of GST and GPx levels is may be due to activation of some nuclear transcription factors like FOXO, NRF2 and p53 that promote GSH synthesis (Al Ghais *et al.,* 2019). The initial upregulation of antioxidant enzymes in pesticide exposed fish might also be due to the same reason. However, long-term exposure to quinalphos caused oxidative damage to the worms and fish through suppression of the antioxidant defense system. This is also reflected in the present study as GST and GPx level decreased at the highest exposure period (Chatterjee *et al.,* 2021). LPO is caused by the ROS-mediated degradation of unsaturated fatty acids from the plasma membrane of cells and organelles (Chang *et al.,* 2020). Malondialdehyde (MDA) is the final by- product of lipid peroxidation and can be used as a bioindicator of augmented concentration of ROS and cellular injury (Kumari *et al.,* 2014). In the present study, the incremented MDA level in worms and fish liver of quinalphos exposed *T. tubifex* and *O. mossumbicus*

indicates ROS mediated hepatic cell injury and subsequently leads to DNA damage and conclusively apoptosis (Bhattacharya *et al.,* 2021). A similar type of alterations in oxidative stress biomarkers was reported by several researchers upon exposure of several classes of pesticides and contaminants present in water to *T. tubifex* and *O. mossumbicus* (Abdel-Wahab *et al.,* 2021). AchE acts as an important biomarker in toxicant-induced neurodegenerative studies (Tabassum *et al.,* 2020). Decremented AchE level in liver of quinalphos exposed *O. mossumbicus* in this study probably leads to excessive acetylcholine (Ach) accumulation at the synapses and neuromuscular junctions, resulting in hyperstimulation of the nervous system that causes behavioral changes and eventually death of the organism (Legradi *et al.,* 2018). Similar inhibition in AchE activity in various tissues of the fish has been reported in *Oreochromis sp.* after exposure to Carbofuran pesticide (Hamed *et al.,* 2018). Reduced glutathione (GSH) is a primary nonprotein thiol that acts as an antioxidant against ROS, protecting cells from the detrimental effects of lipid peroxidation (Faheem & Lone *et al.,* 2018). In the current study, increased GSH level might be an adaptive response to oxidative stress and a defensive strategy upon exposure to toxicants (Kushwaha *et al.,* 2020). However, a substantial decrease in GSH activity on day 14 at concentrations might be due to reduced adaptive response towards toxicants or transformation of GSH to oxidized glutathione for contaminant detoxification (Sreejai & Jaya *et al.,* 2010). Mosleh *et al.,* (2007) reported similar patterns in GSH levels upon exposure of a tubificid worm, Tubifex to chitosan.

IBR is commonly utilized as an indicator of environmental stress to determine the adverse toxicological effects of the toxicants on the exposed organisms (Chang *et al.,* 2020). It integrates alterations of all the biomarkers into a single point and helps in evaluating the organism's health status (Li *et al.,* 2011). In this present analysis, the calculated IBR results are in line with the findings of previous researchers (Li *et al.,* 2011). In integration, biomarker weights and scores for exposed worms and fish parameters are used to calculate BRI, which indicates the general health status of the worms and fish (Hagger *et al.,* 2008). The BRI value of quinalphos was 2.7 which indicate major alterations of the health status in comparison to the control (Hagger *et al.,* 2008). The integrated index may aid in reducing the variation of multiple responses and providing an accurate scale of toxicity and risk assessment. Thus based on the present study, it can be concluded that quinalphos impacts worms and fish health adversely by altering its normal physiological and biochemical processes and disrupting the optimum redox homeostasis.

CONCLUSION

In conclusion, Quinalphos exposure changed *T. tubifex* survival time as well as antioxidant responses. All characteristics evaluated in this study were affected by pesticide concentrations and treatment length in different ways, which might be connected to diverse molecular and genetic mechanisms that need to be investigated further in the future. According to the result of this research, oxidative stress measures may be beneficial in measuring the overall health of aquatic species, particularly sediment macroinvertebrates, in a state of stress. According to our findings, Quinalphos induces significant changes in survivability and behavioural abnormalities at the acute level during short-term exposure and attenuation of haematological, biochemical, and stress parameters at the sublethal level during long-term exposure. As a result, the current findings on the pesticide's toxicity to *O. mossumbicus* might be used as a possible tool for raising public awareness about pesticide indiscriminate use. As a result, a strong emphasis should be focused on the development of biopesticides or microbial pesticides that are highly biodegradable and have lower toxicity than chemical pesticides.

Funding

The research did not receive any specific grant from funding agencies in the public, commercial or nonprofit sectors.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Zoology, The University of Burdwan and DST PURSE PHASE II for providing infrastructural facilities to carry out the work.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- Abdel-Warith, A. W. A., Younis, E. M., Al-Asgah, N. A., Gewaily, M. S., El-Tonoby, S. M., & Dawood, M. A. O. (2021). Role of Fucoidan on the Growth Behavior and Blood Metabolites and Toxic Effects of Atrazine in Nile Tilapia Oreochromis niloticus (Linnaeus, 1758). *Animals*, 11, 1448. https://doi.org/ 10.3390/ani11051448.
- Aebi, H. (1984). Catalase in Vitro. *Methods in Enzymology*, 105(C), 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3
- Ajima, M. N. O., Pandey, P. K., Kumar, K., & Poojary, N. (2017). Neurotoxic effects, molecular responses and oxidative stress biomarkers in Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) exposed to verapamil. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, 196, 44–52. https://doi.org/10.1016/j.cbpc.2017.03.009

- Al Ghais, S. M., Varadharajulu, S., & Kumbhar, P. (2019). Effects of Abamectin on Tilapia mossambica peters changes in reduced glutathione (GSH) and protein content. *International Journal of Fisheries and Aquatic Studies*, 7(4), 280–284.http:// www.fisheriesjournal.com/archives/2019/vol7is sue4/PartD/7-4-9-917.pdf.
- Ali, D., Almarzoug, M. H. A., Al Ali, H., Samdani, M. S., Hussain, S. A., & Alarifi, S. (2020). Fish as bio indicators to determine the effects of pollution in river by using the micronucleus and alkaline single cell gel electrophoresis assay. *Journal of King Saud University - Science*, 32(6), 2880–2885. https://doi.org/10.1016/j.jksus.2020.07.012.
- APHA. (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed.; APHA:Washington, DC, USA, 2005. American Water Works Association/American Public Works Association/Water Environment Federation, 552. https://doi.org/10.2105/AJPH.51.6.940-a
- Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. *Landes Bioscience.* https://doi.org/10.1155/2014/360438
- Bafana, A., Dutt, S., Kumar, A., Kumar, S., & Ahuja, P. S. (2011, February). The basic and applied aspects of superoxide dismutase. *Journal of Molecular Catalysis B: Enzymatic.* https://doi.org/10.1016/j.molcatb.2010.11.007
- Balami, S., Sharma, A., & Karn, R. (2020). Significance of Nutritional Value of Fish for Human Health. *Malaysian Journal of Halal Research*, 2(2), 32–34. https://doi.org/10.2478/mjhr-2019-0012
- Beliaeff, B., & Burgeot, T. (2002). Integrated biomarker response: A useful tool for ecological risk assessment. In *Environmental Toxicology and Chemistry* (Vol. 21, pp. 1316–1322). SETAC Press. https://doi.org/10.1002/etc.5620210629
- Bergmeyer, H. U. (1965). Principles of Enzymatic Analysis. In *Methods of Enzymatic Analysis* (pp. 3–13). Elsevier. https://doi.org/10.1016/b978-0-12-395630-9.50008-6
- Bhattacharjee, P., Borah, A., & Das, S. (2020). Quercetin-induced amelioration of deltamethrin stress in freshwater teleost, Channa punctata: Multiple biomarker analysis. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, 227. https://doi.org/10.1016/j.cbpc.2019.108626
- Bhattacharya, R., Chatterjee, A., Chatterjee, S., & Saha, N. C. (2021). Oxidative stress in benthic oligochaete worm, Tubifex tubifex induced by sublethal exposure to a cationic surfactant cetylpyridinium chloride and an anionic surfactant sodium dodecyl sulfate. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, 240. https://doi.org/10.1016/j.cbpc.2020.108906
- Broeg, K., & Lehtonen, K. K. (2006). Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. *Mar. Pollut. Bull.*, 53, 508–522. https://doi.org/10.1016/j.marpolbul.2006.02.004.
- Buege, J. A., & Aust, S. D. (1978). Microsomal Lipid Peroxidation. *Methods in Enzymology*, 52(C), 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6
- Burdick, G. E. (1967). Use of bioassays in determining levels of toxic wastes harmful to aquatic organisms. In *A symposium on water quality criteria to protect aquatic life* (pp. 7–12). Trans. Am. Fish. Soc. Washington.
- Carazo-Rojas, E., Pérez-Rojas, G., Pérez-Villanueva, M., Chinchilla-Soto, C., Chin-Pampillo, J. S., Aguilar-Mora, P., … Vryzas, Z. (2018). Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. *Environmental Pollution*, 241, 800–809. https://doi.org/10.1016/j.envpol.2018.06.02
- Chang, T., Wei, B., Wang, Q., He, Y., & Wang, C. (2020). Toxicity assessment of municipal sewage treatment plant effluent by an integrated biomarker response in the liver of crucian carp (Carassius auratus). *Environ. Sci. Pollut. Res.*, 27, 7280–7288. https:// doi.org/10.1007/s11356-019-07463-2.
- Chatterjee, A., Bhattacharya, R., Chatterjee, S., & Saha, N. C. (2021). λ cyhalothrin induced toxicity and potential attenuation of hematological, biochemical, enzymological and stress biomarkers in Cyprinus carpio L. at environmentally relevant concentrations: A multiple biomarker approach. *Comp Biochem Physiol C Toxicol Pharmacol*, 250, 109164. doi: 10.1016/j.cbpc.2021.109164. Epub 2021 Aug 12. PMID: 34390845.
- Chatterjee, A., Bhattacharya, R., Chatterjee, S., & Saha, N. C. (2021). Acute toxicity of organophosphate pesticide profenofos, pyrethroid pesticide λ cyhalothrin and biopesticide azadirachtin and their sublethal effects on growth and oxidative stress enzymes in benthic oligochaete worm, Tubifex tubifex. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, 242. https://doi.org/10.1016/j.cbpc.2020.108943.
- Chinnadurai, K., Prema, P., Veeramanikandan, V., Kumar, K. R., Nguyen, V.-H., Marraiki, N., Balaji, P. (2022). Toxicity evaluation and oxidative stress response of fumaronitrile, a persistent organic pollutant (POP) of industrial waste water on tilapia fish (Oreochromis mossambicus). *Environmental Research*, 204, 112030. doi:10.1016/j.envres.2021.112030.
- Costa, A. I. G., Queiroz, M. E. L. R., Neves, A. A., De Sousa, F. A., & Zambolim, L. (2015).

Determination of pesticides in lettuce using solid-liquid extraction with low temperature partitioning. *Food Chemistry*, 181, 64–71. https://doi.org/10.1016/j.foodchem.2015.02.070.

- Dar, S. A., Yousuf, A. R., Balkhi, M. ul H., Ganai, F. A., & Bhat, F. A. (2015). Assessment of endosulfan induced genotoxicity and mutagenicity manifested by oxidative stress pathways in freshwater cyprinid fish crucian carp (Carassius carassius L.). *Chemosphere*, 120, 273–283. https://doi.org/10.1016/j.chemosphere.2014.07.031
- Edwards, R. W., & Brown, V. M. (1967). Pollution and fisheries: a progress report. *Wat. Pollut. Control*, 66, 63–78.
- Elia, A. C., Giorda, F., Pacini, N., Dörr, A. J. M., Scanzio, T., & Prearo, M. (2017). Subacute toxicity effects of deltamethrin on oxidative stress markers in rainbow trout. *Journal of Aquatic Animal Health*, 29(3), 165–172. https://doi.org/10.1080/08997659.2017.1349006
- Faheem, M., Khaliq, S., & Lone, K. P. (2019). Effect of bisphenol-A on serum biochemistry and liver function in the freshwater fish, Catla catla. *Pakistan Veterinary Journal*, 39(1), 71–75.
- Fern´andez San Juan, M. R., Cortelezzi, A., Albornoz, C. B., Landro, S. M., Arrighetti, F., Najle, R., & Lavarías, S. M. L. (2020). Ecotoxicology and environmental safety toxicity of pyrethroid cypermethrin on the freshwater snail Chilina parchappii: lethal and sublethal effects. *Ecotoxicol. Environ. Saf.*, 196, 110565 https://doi.org/10.1016/j. ecoenv.2020.110565.
- Ghane, M. N., Dhamagaye, H. B., Meshram, S. J., & Salunkhe, A. (2018). Toxic Effects of Paclobutrazol in Oreochromis mossambicus Fingerlings. *Pesticide Research Journal*, 30(1), 112. https://doi.org/10.5958/2249-524x.2018.00019.5
- Ghelichpour, M., Mirghaed, A. T., & Jimenez, A. P. (2019). LC50 determination and intoxication symptoms of a new pyridine carboxamide pesticide flonicamid on common carp cyprinus Carpio. *Rudn J. Agron. Anim. Ind.*, 14, 279–288. https://doi. org/10.22363/2312-797x-2019-14-3-279-288.
- Ghelichpour, M., Taheri Mirghaed, A., Hoseini, S. M., & Perez Jimenez, A. (2020). Plasma antioxidant and hepatic enzymes activity, thyroid hormones alterations and health status of liver tissue in common carp (Cyprinus carpio) exposed to lufenuron. Aquaculture 516, 734634. https://doi.org/10.1016/j.aquaculture.2019.734634.
- Gilden, R., Plisko, M., Hiteshew, K., Friedmann, E., & Milton, D. (2016). Pesticide monitoring on soccer fields via shoe wipes and urine samples. *Environmental Research*, 147, 294–296. https://doi.org/10.1016/j.envres.2016.02.027.
- Goi. (2021). Insecticides / Pesticides Registered under section 9(3) of the Insecticides Act, 1968 for use in the Country:(As on 01.03.2021).
- Grotto, D., Santa Maria, L., Valentini, J., Paniz, C., Schmitt, G., Garcia, S. C., … & Farina, M. (2009). Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. *Quimica Nova. Sociedade Brasileira de Quimica*. https://doi.org/10.1590/S0100-40422009000100032
- Gupta, M., Handa, D., Chaturvedi, A., Singh, R., & Lehl, S. S. (2015). Cartap poisoning: an unusual poisoning in North India. *International Journal of Scientific Reports*, 1(1), 99. https://doi.org/10.18203/issn.2454-2156.intjscirep20150214
- Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249(22), 7130–7139.
- Hagger, J. A., Jones, M. B., Lowe, D., Leonard, D. R. P., Owen, R., & Galloway, T. S. (2008). Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: A case study. *Marine Pollution Bulletin*, 56(6), 1111–1118. https://doi.org/10.1016/j.marpolbul.2008.03.040
- Hagger, J. A., Jones, M. B., Lowe, D., Leonard, D. R. P., Owen, R., & Galloway, T. S. (2008). Application of biomarkers for improving risk assessments of chemicals under the water framework directive: a case study. *Mar. Pollut. Bull.*, 56, 1111–1118. https:// doi.org/10.1016/j.marpolbul.2008.03.040.
- Hamed, H. S., Ismal, S. M., & Faggio, C. (2021). Effect of allicin on antioxidant defense system, and immune response after carbofuran exposure in Nile tilapia, Oreochromis niloticus. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *240*, 108919. https://doi.org/10.1016/j.cbpc.2020.108919.
- Haque, M. I. M., Alam, A. R. U., Akter, N., Siddique, M. A., Sultana, M., Hossain, M. A., & Hasan, M. (2020). Molecular characterization of 'tubifex worms' based on 16S rRNA and cytochrome c oxidase subunit I. *Aquaculture Reports*, *16*, 100292.
- Hemalatha, D., Amala, A., Rangasamy, B., Nataraj, B., & Ramesh, M. (2016). Sublethal toxicity of quinalphos on oxidative stress and antioxidant responses in a freshwater fish Cyprinus carpio. *Environmental Toxicology*, 31(11), 1399–1406. https://doi.org/10.1002/tox.22145
- Hemalatha, D., Nataraj, B., Rangasamy, B., Maharajan, K., & Ramesh, M. (2021). Exploring the sublethal genotoxic effects of class II organophosphorus insecticide quinalphos on freshwater fish Cyprinus carpio. *Journal of Oceanology and Limnology*, *39*(2), 661-670. https://doi.org/10.1007/s00343-019-9104-y.
- Hemalatha, D., Nataraj, B., Rangasamy, B., Maharajan, K., & Ramesh, M. (2021). Exploring the sublethal genotoxic effects of class II organophosphorus insecticide quinalphos on

freshwater fish Cyprinus carpio. *Journal of Oceanology and Limnology*, *39*(2), 661-670. https://doi.org/10.1007/s00343-019-9104-y.

- Iheanacho, S. C., & Odo, G. E. (2020). Dietary exposure to polyvinyl chloride microparticles induced oxidative stress and hepatic damage in Clarias gariepinus (Burchell, 1822). *Environmental Science and Pollution Research*, 27(17), 21159–21173. https://doi.org/10.1007/s11356-020-08611-9
- Javed, M., Ahmad, M.I., Usmani, N., & Ahmad, M. (2017). Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in Channa punctatus exposed to heavy metal loaded waste water /704/172/4081 /631/601 article. *Sci. Rep.*, 7, 1–11. https://doi.org/10.1038/s41598-017-01749-6.
- Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, 21(2), 130–132.
- Kaur, M. (2017). Oxidative Stress Response in Liver, Kidney and Gills of Ctenopharyngodon Idellus (Cuvier & Valenciennes) Exposed To Chlorpyrifos. *MOJ Biology and Medicine*, 1(4). https://doi.org/10.15406/mojbm.2017.01.00021
- Kushwaha, S., Anerao, I., Rajput, S., Bhagriya, P., Roy, H., & Zang, T. (2020). Evaluation of abamectin induced hepatotoxicity in Oreochromis mossambicus. *Cogent Biology*, 6(1), –. doi:10.1080/23312025.2020.1761277.
- Legradi, J. B., Di Paolo, C., Kraak, M. H. S., Van der Geest, H. G., Schymanski, E. L., Williams, A. J., Dingemans, M. M., Massei, R., Brack, W., Cousin, X., & Begout, M. L. (2018). An ecotoxicological view on neurotoxicity assessment. *Environmental Sciences Europe*, 30(1), 46.
- Li, X. Y., Luo, Y. R., Yun, M. X., Wang, J., & Wang, J. J. (2010). Effects of 1-methyl-3-octylimidazolium bromide on the anti-oxidant system of earthworm. *Chemosphere*, 78(7), 853–858. https://doi.org/10.1016/j.chemosphere.2009.11.047
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of biological chemistry*, 193(1), 265–275. https://doi.org/10.1016/0922-338X(96)89160-4
- Majumder, R., & Kaviraj, A. (2017). Cypermethrin induced stress and changes in growth of freshwater fish Oreochromis niloticus. *International Aquatic Research*, 9(2), 117–128. https://doi.org/10.1007/s40071-017-0161-6
- Marigoudar, S. R., Nagarjuna, A., Karthikeyan, P., Mohan, D., & Sharma, K. V. (2018). Comparative toxicity of chlorpyrifos: Sublethal effects on enzyme activities and histopathology of Mugil cephalus and Chanos chanos. *Chemosphere*, 211, 89–101. https://doi.org/10.1016/j.chemosphere.2018.07.137
- Maurya, P. K., Malik, D. S., & Sharma, A. (2019). Impacts of pesticide application on aquatic environments and fish diversity. In Contaminants in Agriculture and Environment: Health Risks and Remediation (pp. 111–128). *Agro Environ Media - Agriculture and Ennvironmental Science Academy, Haridwar, India.* https://doi.org/10.26832/aesa-2019-cae-0162-09
- Mutshekwa, T., Cuthbert, R. N., Mugwedi, L., Wasserman, R. J., Dondofema, F., & Dalu, T. (2022). Behavioural Responses and Mortality of Mozambique Tilapia Oreochromis mossambicus to Three Commonly Used Macadamia Plantation Pesticides. *Water*, 14, 1257. https://doi.org/10.3390/w14081257.
- Neamat-Allah, A. N. F., Abd El Hakim, Y., & Mahmoud, E. A. (2020). Alleviating effects of β-glucan in Oreochromis niloticus on growth performance, immune reactions, antioxidant, transcriptomics disorders and resistance to Aeromonas sobria caused by atrazine. *Aquac. Res.*, 51, 1801–1812. [CrossRef].
- Nishimoto, R. (2019). Global trends in the crop protection industry. *Journal of Pesticide Science. Pesticide Science Society of Japan*. https://doi.org/10.1584/jpestics.D19-101
- Ogunnupebi, T. A., Oluyori, A. P., Dada, A. O., Oladeji, O. S., Inyinbor, A. A., & Egharevba, G. O. (2020). Promising Natural Products in Crop Protection and Food Preservation: Basis, Advances, and Future Prospects. *International Journal of Agronomy. Hindawi Limited.* https://doi.org/10.1155/2020/8840046
- ON, H., PC, G., & EA, C. (2018). Toxic Effect of Dichlorvos-Pesticide on Lipid Peroxidation, Superoxide Dismutase and Catalase of Clarias gariepinus. *Journal of Fisheries & Livestock Production*, 06(03). https://doi.org/10.4172/2332-2608.1000276
- Özkara, A., Akyil, D., & Konuk, M. (2016). Pesticides, Environmental Pollution, and Health. In Environmental Health Risk - Hazardous Factors to Living Species. *InTech.* https://doi.org/10.5772/63094
- Pontes, F. J. S., Maia, R. T., Lima, M. C. P., Ayres, C. F. J., & Soares, T. A. (2016). The role of the conformational dynamics of glutathione S-transferase epsilon class on insecticide resistance in Anopheles gambiae. *Journal of the Brazilian Chemical Society*, 27(9), 1602–1615. https://doi.org/10.5935/0103-5053.20160040
- R., D., S., M., S.K., B., & R.K., M. (2017). An observational study of 11 cases of Cartap poisoning-a rare poisoning. *Asian Journal of Pharmaceutical and Clinical Research*, 10(11), 366–369. Retrieved from http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L619212341
- Rahimikia, E. (2017). Analysis of antioxidants and serum biochemical responses in goldfish under

nickel exposure by sub-chronic test. *Journal of Applied Animal Research*, 45(1), 320–325. https://doi.org/10.1080/09712119.2016.1190732

- Raibeemol, K. P., & Chitra, K. C. (2018). Effects of Chlorpyrifos as Inducer for Oxidative Stress in Liver, Kidney and Spleen of Freshwater Fish, Pseudetroplus maculatus (Bloch, 1795). *Journal of Toxicology*, 8(1), 20–29. Retrieved from www.stmjournals.com
- Reza, A. H. M. M., Rakhi, S. F., Hossen, M. S., & Hossain, Z. (2017). Organ specific histopathology and brain acetylcholinesterase inhibition in rohu, Labeo rohita and silver barb, Barbonymus gonionotus: Effects of three widely used organophosphate pesticides. *Turkish Journal of Fisheries and Aquatic Sciences*, 17(4), 821–832. https://doi.org/10.4194/1303-2712-v17_4_18
- Sadiqul, I. M., Kabir, S. M., Ferdous, Z., Mansura, K. M., & Khalilur, R. M. (2017). Chronic exposure to quinalphos shows biochemical changes and genotoxicty in erythrocytes of silver barb, Barbonymus gonionotus. *Interdiscip Toxicol*, 10(3), 99-106. doi: 10.1515/intox-2017-0016. Epub 2018 Feb 14. PMID: 30174533; PMCID: PMC6107646.
- Sadiqul, I. M., Kabir, S. M., Ferdous, Z., Mansura, K. M., & Khalilur, R. M. (2017). Chronic exposure to quinalphos shows biochemical changes and genotoxicty in erythrocytes of silver barb, Barbonymus gonionotus. *Interdisciplinary toxicology*, 10(3), 99–106. https://doi.org/10.1515/intox-2017-0016.
- Sadiqul, M. I., Ferdous, Z., Nannu, M. T. A., Mostakim, G. M., & Rahman, M. K. (2016). Acute exposure to a quinalphos containing insecticide (convoy) causes genetic damage and nuclear changes in peripheral erythrocytes of silver barb, Barbonymus gonionotus. *Env Poll.*, 219:949–956. [PubMed] [Google Scholar].
- Saha, S., & Kaviraj, A. (2008). Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. *Bull. Environ. Contam. Toxicol.*, 80, 49-52.
- Saravanan, M., Prabhu Kumar, K., & Ramesh, M. (2011). Haematological and biochemical responses of freshwater teleost fish Cyprinus carpio (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. *Pesticide Biochemistry and Physiology*, 100(3), 206–211. https://doi.org/10.1016/j.pestbp.2011.04.002
- Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P.,Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M.,Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and itsimpacts on ecosystem. *SN Appl. Sci*, 1.
- Sharmistha, M., Ritwick, B., Arnab, C., Sarmila, P., & Nimai Chandra, S. (2019). Acute Toxicity and Behavioural Responses of Oligochaete Worm Tubifex tubifex Exposed to Pesticide Cartap Hydrochloride. *Research & Reviews: A Journal of Life Sciences*, 9(3), 1–5.
- Shehzad, G., Sadia, T., Munawar Saleem, A., Naveed, A., & Muhammad Fiaz, K. (2019). Effect of Chlorpyrifos on Hematological and Seral Biochemical Components of Fish Oreochromis mossambicus, *Pakistan J. Zool.*, 51(3), 1047-1052. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.1047.1052.
- Singh, S., Tiwari, R. K., & Pandey, R. S. (2018). Evaluation of acute toxicity of triazophos and deltamethrin and their inhibitory effect on AChE activity in Channa punctatus. *Toxicol. Rep.*, 5, 85–89. https://doi.org/10.1016/j.toxrep.2017.12.006.
- Stoyanova, S., Georgieva, E., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., … Yancheva, V. (2020). Multi-biomarker assessment in common carp (Cyprinus carpio, Linnaeus 1758) liver after acute chlorpyrifos exposure. *Water (Switzerland)*, 12(6). https://doi.org/10.3390/w12061837
- Tahir, R., Ghaffar, A., Abbas, G., Turabi, T. H., Kausar, S., Xiaoxia, D., Naz, S., Jamil, H., Samra, R. S., & Abdelgayed, S. S. (2021). Pesticide induced hematological, biochemical and genotoxic changes in fish: A Review. *Agrobiological Records*, 3, 41-57. https://doi.org/10.47278/journal.abr/2021.005.
- Tan, B. L., Norhaizan, M. E., Liew, W. P. P., & Rahman, H. S. (2018, October 16). Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Frontiers in Pharmacology*, Frontiers Media S.A. https://doi.org/10.3389/fphar.2018.01162
- Tsagkaris, A. S., Uttl, L., Pulkrabova, J., & Hajslova, J. (2020). Screening of carbamate and organophosphate pesticides in food matrices using an affordable and simple spectrophotometric acetylcholinesterase assay. *Applied Sciences (Switzerland)*, 10(2). https://doi.org/10.3390/app10020565.
- Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2021). Agriculture Development, Pesticide Application and Its Impact on the Envi-ronment. *International Journal of Environmental Research and Public Health*, 18(3), 1112–1112.
- Vani, G., Veeraiah, K., Vijaya Kumar, M., Parveen, S. K., & Prasad Rao, G. D. V. (2020). Biochemical changes induced by Cartap hydrochloride (50% SP), carbamate insecticide in freshwater fish Cirrhinus mrigala (Hamilton, 1822). *Nature Environment and Pollution Technology*, 19(5), 1821–1829. https://doi.org/10.46488/NEPT.2020.v19i05.005
- Vinoy, K., & Shrivastava. (2013). Quinalphos (organophosphate) induced histopathological changes in the liver and behavioural modification in the fish - Clarias batrachus (Linn.). *Indo American Journal of Pharm Research*, 3(7).

- Wang, M., Guckland, A., Murfitt, R., Ebeling, M., Sprenger, D., Foudoulakis, M., & Koutsaftis, A. (2019). Relationship between magnitude of body weight effects and exposure duration in mammalian toxicology studies and implications for ecotoxicological risk assessment. *Environmental Sciences Europe*, *31*(1), 1-7. https://doi.org/10.1186/s12302-019-0221-1.
- WHO. (2019). Global situation of pesticide management in agriculture and public health:report of a 2018 WHO-FAO survey. *World Health Organization.*
- Yang, C., Lim, W., & Song, G. (2020, August 1). Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology. *Elsevier Inc*. https://doi.org/10.1016/j.cbpc.2020.108758
- Yuli, A., Priyadi, A., & Firdaus, S. N. (2020). Effect of Tubifex and carrot meal combination on color quality of Botia Chromobotia macracanthus. In *E3S Web of Conferences, EDP Sciences. Indonesia*, 147, 01007.
- Zhang, Q., Zhang, Y., Du, J., & Zhao, M. (2017). Environmentally relevant levels of Λ-cyhalothrin, fenvalerate, and permethrin cause developmental toxicity and disrupt endocrine system in zebrafish (Danio rerio) embryo. *Chemosphere*, 185, 1173–1180. https://doi.org/10.1016/j.chemosphere.2017.07.091.
- Zhang, Q., Zhu, L., Wang, J., Xie, H., Wang, J., Han, Y., & Yang, J. (2013). Oxidative stress and lipid peroxidation in the earthworm Eisenia fetida induced by low doses of fomesafen. *Environmental Science and Pollution Research*, 20(1), 201–208. https://doi.org/10.1007/s11356-012-0962-5