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Toxicity and hepatic oxidative stress evaluations in African catfish *Clarias gariepinus* Burchell, 1882 fingerlings exposed to organophosphorus pesticide profenofos

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ABSTRACT

Profenofos is a broad spectrum insecticide used in agriculture for treatment of ectoparasitic disease and pests. The study investigated the toxicity and effects of profenofos (O-4-bromo-2-chlorophenyl-O-ethyl-S-propyl phosphorothioate) on the oxidative stress biomarkers of *Clarias gariepinus* fingerlings. There were concentration and duration – dependent mortalities in *C. gariepinus* exposed to profenofos. The 96 h LC_{50} value estimated by probit analysis was 0.03 mg/L. Based on the 96 h LC_{50}, fish were exposed to two sublethal concentrations of profenofos (1/10th of 96 h LC_{50}=3.00 µg/L) and 1/5th of 96 h LC_{50}=6.00 µg/L). The liver tissue was analyzed on day 1, 7, 14 and 21 for the assessment of oxidative stress biomarkers. The results show that the lipid peroxidation (LPO) values were elevated in *C. gariepinus* exposed to the sublethal concentrations of profenofos throughout the duration of the experiment. The antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase activity increased with increase in concentration and exposure duration when compared with the control. The present study showed that profenofos pesticide is toxic and elevated LPO values and the oxidative stress parameters in *Clarias gariepinus*. Profenofos should be prudently used in terrestrial and aquatic eco-system to avoid eco-toxicological hazards.

Keywords: *Clarias gariepinus, profenofos, toxicity, oxidative stress, fish.*

INTRODUCTION

The growing demand for increased food production to meet the need for the global population has led to sophisticated agricultural technology which pesticides/insecticides play a crucial role. Fish in its natural environment are often exposed to a variety of stressors that can adversely affect their health. The aquatic living resources are very vulnerable to herbicides contamination as run-offs from farms and industries end up in water bodies (Botelho et al., 2009). The washing of packaging materials and application equipment often carried out in the banks of water bodies help in scaling up their contamination potential (Trovo et al., 2005). Furthermore, residues from these pesticides and associated human activities such as urbanization, deforestation, release of domestic, hospital and industrial effluents may contribute to a large build up and discharge into the aquatic environment. Adeogun et al. (2011) highlighted the importance of evaluating growth response and oxidative stress in commercially important fish species.

Profenofos is a broad spectrum, foliar, persistent and toxic organophosphorus insecticide and acaricide widely used in agriculture for the control of pest in agricultural crops such as mango, banana, cotton and pineapple (Kavitha and Rao 2009). It is a potentially ground water contaminating organophosphorous insecticide and has been reported to be highly toxic to aquatic organisms (Shaw, 1995) mainly through the inhibition of the acetylcholinesterase (AChE) activity (Kushwaha et al., 2016). Profenofos has been recognized as a highly persistent pesticide with a half-life of about one week in soil (Bojan et al., 2017). It is lightly soluble in water (20.0 mg/L), readily miscible in organic solvents and thus a better choice for spray compared to persistent organochlorines (Is-

mail et al., 2009). Residues of profenofos have been discovered in fruit samples such as orange, strawberry, okra, mint leaves, curry leaves and pepper (ANVISA 2012).

In the aquatic system, molecules of contaminants may bind to the materials in suspension, accumulate in the sediment or can be absorbed by the aquatic organisms with attendant physiological responses including effect on morphological, biochemical and anti-oxidant responses (Jordan et al., 2013). The assessment of alteration in key enzymatic activities of organisms following exposure to polluted water has been one of the major uses of biomarkers in environmental studies (Almedia et al., 2009). A number of authors have shown that several biomarkers of oxidative stress can provide satisfactory information on the response of fish to environmental stressors (Farombi et al., 2007; Pavloric et al., 2010). Doherty et al. (2010) noted that fish species are suitable candidates for assessment of biomarkers of oxidation stress induced by pollutants because they play a dual role of being on top of the aquatic food chain as vertebrates and respond strongly to stress conditions. Xenobiotics have been described as free radical generators and their biotransformation could lead to increased production of reactive oxygen species (ROS) which are not only highly toxic but cause oxidative and DNA damage in fish (Cavalcante et al., 2008). Under normal condition in the ecosystem animals maintain normal generation and neutralization of ROS (Kumar et al., 2013) but upon exposure to pollutants including pesticides, higher levels of free radicals such as superoxide (O-2) and hydroxyl radicals (OH) are generated. The ROS at excess level react with biological macromolecules to increase the level of lipid peroxidation and alteration in antioxidant enzymes such as catalase (CAT) superoxide dismutase (SOD) (Blahova et al., 2013; Pereira et al., 2013).

African catfish *Clarias gariepinus* is a typical air-breathing catfish with scaleless bony elongated body with long dorsal and anal fins and a helmet like head. According to Skelton (2001), it is probably the most widely distributed fish in Africa. They have ubiquitous distribution in rivers, streams, reservoirs, canals, ponds, dams, and lakes in Africa (Adeyemi, 2014). It is considered to have rapid growth rate both in length and in weight depending on ambient conditions and habitat (Britz and Pienaar 1992). It is an important commercial fish widely consumed in Nigeria due to its cheap source of animal protein for low-income earners. This species acclimatizes easily under laboratory conditions as it has accessory respiratory structures and thus an excellent model for toxicological studies (Nwani et al., 2017). Despite the wide use of profenofos both in agriculture fields and homes in Nigeria, no study to the best of our knowledge has been conducted on the effects of profenofos on non-target indigenous fish species especially *C. gariepinus*. The aim of the present study was thus, to determine the toxicity of profenofos and its effects on the oxidative stress parameters in freshwater African Catfish *C. gariepinus* juveniles.

MATERIALS AND METHODS

Experimental fish specimen and test chemicals

A total of 450 juveniles of *Clarias gariepinus* used for the study were procured from Sacem Fish Farm, Enugu and transported to Fisheries unit of the Applied Biology Special Laboratory Agbani ESUT, Enugu State. They were acclimatized for two weeks under laboratory condition and fed with commercial feed daily at 3% body weight. To maintain hygienic condition and prevent pollution caused by food and feces, fecal matter and other waste materials were siphoned off daily to reduce ammonia content in the water. Also, dead fishes were removed with forceps to avoid possible deterioration of the water quality. During acclimatization, water was changed daily in order for the fishes to adapt to the environment. For the experiment technical grade profenofos (50% EC) with trade name Celcron (Excel Crop Care Industry Ltd, Mubai, India) was purchased from agrochemical retail shop Enugu, Nigeria and used for the study.

Acute Toxicity Test

The test was conducted using a semi-static bioassay in 40 L glass aquaria (60 x 30 x 30 cm). After the range finding test, five concentrations of profenofos (0.01, 0.02, 0.03, 0.04, 0.05 mg/l) were selected for exposures. The fish were divided into six groups (Groups I, II, III, IV, V and VI) of 30 fishes per group. Each group was further replicated into three with each containing 10 fish. The fish in groups I, II, III, IV and V were exposed to 0.01, 0.02, 0.03, 0.4 and 0.05 mg/L of profenofos respectively. The fish in group VI served as the control and contained only tap water without profenofos. On every alternate day, the water in each experimental set up was siphoned out completely using rubber tubing and replaced with fresh preparations of each profenofos test concentrations. The experiment lasted for 96 hours (4 days). After 48 h of exposure, the test solution was changed so as to counter-balance the decreasing pesticide concentration. The survival and mortality after every 24 h was recorded daily and dead fish were removed to avoid the deterioration of the test solution. The median lethal concentration (LC_{50}) value was determined following the probit analysis method described by Finney (1971) using SPSS version 22. The water quality parameters determined using water quality kit (ProLabTM, Florida) indicates that pH varied from 6.40-7.60, temperature ranged from 25.00^{o}C – 25.81^{o}C and dissolved oxygen from 6.0-8.5. Ethical clearance on the use of experimental animal was obtained from the Ministry of Agriculture and Natural Resources, Enugu State, Nigeria, and was strictly followed.

Determination of safe levels

The safe levels of the test pesticide were estimated by multiplying the 96 h LC_{50} with different application factors (AF) and was based on Hart, et al. (1948), Sprague (1971), Committee on Water Quality Criteria (CWQC 1972), National Academy of Science/National Academy of Engineering (NAS/NAE, 1973), Canadian Council of Resources and Environmental Ministry (CCREM 1991) and the International

Joint Commission (IJC 1977).

Determination of sublethal concentrations

The 96 h LC_{50} of profenofos on *Clarias gariepinus* was 0.03 mg/L following the probit analysis method as described by Finney (1971). Based on the 96 h LC_{50} value, the fish was exposed to 3.00 mg/L and 6.00 mg/L sublethal concentrations of profenofos corresponding to 1/10th and 1/5th of 96 h LC_{50} respectively. During the sublethal experiment, a total of 90 fishes were exposed to different sublethal concentrations and a control. Fish were divided into three groups (Groups I, II and III). Groups I and II were exposed to 3.0 mg\L and 6.0 µg/L of profenofos while group III was exposed to only tap water without profenofos and served as the control. Each group was further replicated into three with each containing 10 fish for robust statistical analysis The exposure lasted for 21 days during which the fish were fed with small quantity of food approximately 1% of total body weight about an hour before the test solution was renewed to avoid mortality and cannibalism. Fish were sampled on day 1, 7, 14 and 21. Prior to the sampling, fish were treated with tricainemethanesulfonate (MS 222, 0.1 g/L) to minimize stress. Liver tissues were sampled from two fishes from each triplicate experiment and control on each day and homogenized separately in 0.9% NaCl solution and potassium phosphate buffer (1: 10 W/V, 0.1 M, pH 7.0). The homogenate was centrifuged for 20 min at 10 500 rpm at 4^{o}C to obtain the supernatant which was stored at 4^{o}C for oxidative stress assay

Estimation of oxidative stress parameters

Lipid peroxidation (LPO) was determined by measuring the malondiaehyde (MDA) formation as described by Sharma and Krishna Murti (1986) while catalase (CAT) activity was assayed from the liver homogenate as described by Aebi (1984). Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 at 30^{o}C as described by Misra and Fridovich (1972). The activity of glutathione peroxidase (GPx) was determined by monitoring the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase. The specific activity was determined using the extinction 6.22 mMcm-1 (Lawrence and Burk, 1976). Glutathione reductase (GR) was estimated by measuring the rate of conversion of NADPH using the method of Tayarani et al. (1989). Glutathione S-transferase activity (GST) was measured by the extent of conjugation of GSH with CDNB and the proportionate change in the absorption was determined at 340 nm.

RESULTS

Percentage mortality of *Clarias gariepinus* exposed to profenofos

Juveniles of *Clarias gariepinus* exposed to profenofos examined at different exposure periods (24, 48, 72 and 96 h) depending on different concentrations and mortality rate of fish is presented in Table 1. The pesticide concentration of 0.05 mg\L at 96 h exposure recorded highest fish mortality of 100% representing death of the entire 30 fishes. Also, the least profenofos concentration of 0.01 mg\L at 96 h exposure recorded the lowest mortality of 30% representing the death of 9 fishes out of 30 but no mortality was recorded in the control experiment.

Table 1: Percentage mortality of *Clarias gariepinus* juveniles exposed to different concentrations of profenofos for 24, 48, 72 and 96 h

Profenofos concentrations (mg/L)	Number of fish exposed	Mortalities						
			48 h	72 h	96 h	Total	% Survival	% Mortality
Control	30	0	0	0	0	0	100	0
0.01	30	0	3	3	3	9	70	30
0.02	30	1	3	4	5	11	56	44
0.03	30	2	3	5	5	15	50	50
0.04	30	3	3	6	8	20	33	67
0.05	30	6	6	9	9	30	0	100

Safe levels estimation

The safe levels of profenofos (Table 2) determined by multiplying the various application factors (AF) with the 96 h LC_{50} varied from 3 x 10^{-3} to 3 x 10^{-7} (NAS/NAE 1973). However, following the method of Hart et al. (1948), the safe level was estimated to be 1.86 x 10^{-3}. The safe level determined by the methods of IJC (1977) and CCREM (1991) was 1.5 x 10^{-3}.

Table 2: Estimate of safe levels of Clarias gariepinus juvenile exposed to profenofos pesticide

Pesticide name	96 h LC50 (mg/L)	Methods	Application factor (AF)	Safe levels
Profenofos	0.03	Hart et al. (1948)*		$1.8 X10^{-3}$
		Sprague et al. (1971)	0.1	$3.0X10^{-3}$
		CWQC (1972)	0.01	$3.0X10^{-4}$
		NAS/NAE (1973)	0.1- 0.00001	$3.0 X10^{-3}$-$3.0 X10^{-7}$
		CCREM (1991)	0.05	$1.5 X10^{-3}$
		IJC (1977)	5%96 h LC_{50}	$1.5 X10^{-3}$

Effect of profenofos on oxidative stress parameters in *C. gariepinus*

The changes in LPO values in *C. gariepinus* exposed to the sublethal concentrations of profenofos is presented in Figure 1. The LPO values in the exposed fish were higher than the control throughout the duration of the experiment. The elevated LPO values were significantly different from the control only on day 21 at the 3.00 and 6.00 µg/L profenofos. The activity of the antioxidant enzymes in *C. gariepinus* exposed to the sublethal concentrations of profenofos is presented in Table 3. The CAT activity was elevated throughout the duration of the experiment but was not significantly different from the control except on day 1 at the 6.00 µg/L profenofos. The values of SOD and GR in *C. gariepinus* exposed to 3.00 and 6.00 µg/L profenofos were significantly higher ($p<0.05$) than the control throughout the duration of the experiment. There were concentration and duration-dependent significant increase ($p<0.05$) in GPx except on day 1 at 3.00 µg/L profenofos. The values of GST were significantly higher ($p<0.05$) than the control at 3.00 and 6.00 µg/L profenofos concentrations on day 14 and 21. The GST values were elevated on day 1 and 7 but were not significantly different from the control at 3.00 µg/L profenofos concentration.

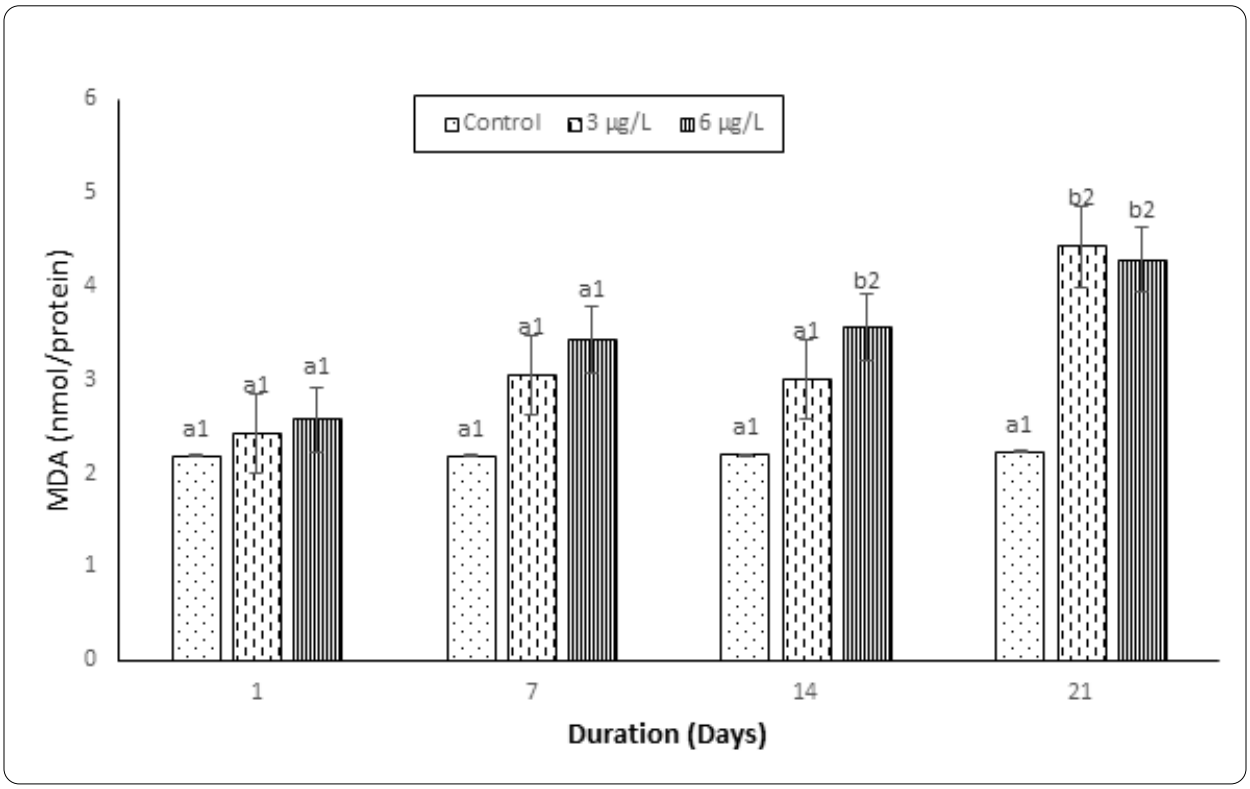

Figure 1: Lipid peroxidation in *Clarias gariepinus* exposed to profenofos. Bars with different alphabet labels were significantly different between durations within the same concentration while bars with different number label were significantly different between concentrations within the same duration ($p<0.05$).

Table 3. Activity of lipid peroxidation, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione transferase in the liver of *C. gariepinus* exposed to sublethal concentrations (3.00 and 6.00 µg/L) of profenofos.

		Duration (Days)			
Parameter	**Concentrations (µg/L)**	**1**	**7**	**14**	**21**
CAT (mg/protein)	Control	1.23 ± 0.07^{a1}	1.22 ± 0.07^{a1}	1.24 ± 0.06^{a1}	1.34 ± 0.15^{a1}
	3	1.35 ± 0.42^{a1}	1.47 ± 0.23^{a1}	1.61 ± 0.08^{a1}	1.55 ± 0.06^{a1}
	6	1.44 ± 0.28^{a1}	1.56 ± 0.35^{a1}	1.60 ± 0.21^{a1}	1.81 ± 0.09^{b2}
SOD (unit/mg protein)	Control	38.50 ± 0.70^{a1}	38.51 ± 0.71^{a1}	38.60 ± 0.79^{a1}	38.50 ± 0.87^{a1}
	3	41.50 ± 0.67^{a2}	44.50 ± 0.87^{b2}	47.50 ± 0.67^{b2}	43.00 ± 1.41^{a2}
	6	44.50 ± 0.91^{a2}	48.56 ± 0.75^{b2}	50.50 ± 0.83^{b2}	46.00 ± 1.42^{a3}
GPx (nmol/mg protein)	Control	19.50 ± 0.71^{a1}	20.50 ± 0.67^{a1}	19.50 ± 0.75^{a1}	23.00 ± 1.41^{a1}
	3	22.50 ± 0.75^{a1}	25.00 ± 1.41^{b2}	26.50 ±0.85^{b2}	27.00 ± 1.40^{b2}
	6	25.50 ± 0.73^{a2}	28.50 ± 0.97^{b3}	29.50 ± 0.95^{b3}	32.00 ± 2.24^{b3}
GR (nmol/mg protein)	Control	13.50 ± 0.71^{a1}	13.50 ± 0.52^{a1}	14.50 ± 0.65^{a1}	15.50 ± 0.47^{a1}
	3	15.50 ± 0.75^{a2}	16.50 ± 0.73^{a2}	18.50 ± 0.73^{b2}	19.50 ± 0.75^{b2}
	6	17.50 ± 0.72^{a2}	19.50 ± 0.75^{b3}	23.50 ± 0.76^{b3}	22.50 ± 0.75^{b3}
GST (mg/protein)	Control	50.50 ± 1.01^{a1}	51.50 ± 1.13^{a1}	50.50 ± 1.11^{a1}	52.50 ± 1.04^{a1}
	3	52.50 ± 1.02^{a1}	53.50 ± 1.03^{a1}	54.50 ± 1.15^{b2}	55.50 ± 1.35^{b2}
	6	54.50 ± 1.05^{a2}	55.50 ± 1.08^{a2}	56.50 ± 1.12^{b2}	57.50 ± 1.09^{b2}

Values with different alphabetic superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure duration

DISCUSSION

The increasing use of pesticides to maximize agricultural production has resulted to environmental pollution of the aquatic systems. Fishes in particular are very sensitive to aquatic environmental contamination (Melefa et al., 2020). Acute toxicity studies give the concentrations and time associated with the death of 50% (LC_{50}) of fish exposed to a given toxicant (Sharafeldin et al., 2015). During such experiment, mortality may be observed in a fish population. Our present results indicate concentration and time-dependent increase in mortality in *C. gariepinus* exposed to profenofos. The 96 h LC_{50} of *C. gariepinus* exposed to profenofos was 0.03 mg/L. The 0.03 mg/L 96 h LC_{50} obtained in the present study was lower than the 62.4 µg/L, 0.27 mg/L and 1.20 mg/L obtained for *Cyprinus carpio* (Ismail et al., 2009), *Oreochromis niloticus* (Kavitha and Rao 2009) and *Labeo rohita* (Nagaraju and Hagos (2016) exposed to profenofos respectively. Our result was also lower than the 2.6 µg/L, 0.10 mg/L and 0.87 mg/l obtained for *Channa punctatus* (Pandey et al., 2011), *Barbonymus goninotus* (Moniruzzaman et al., 2017) and *O. niloticus* (Sharafeldin et al., 2015) exposed to profenofos respectively. Banaee et al. (2016) noted that the toxicity of various pesticides even on the same species may be affected by age, sex, genetic properties, fish size, water quality and the purity of pesticides used. The safe level of profenofos in *C. gariepinus* obtained in the present study was 3×10^{-3} to 3×10^{-7} ((NAS/NAE 1973). However, due to the difficulty in relating laboratory data to field data there have been controversies in deciding the acceptable concentrations that may be considered safe (Pandey et al., 2005).

Environmental pollutants may alter the haematological, morphological, and physiological and biochemical processes in fish organs (Oluah et al., 2020). In attempts to metabolize and detoxify such pollutants, reactive oxygen species (ROS) may be generated. The ROS generated may induce oxidative damage, attack vital biomolecules, lipids, proteins and DNA in the living cells (Odo et al., 2020). The present study indicated the elevation of LPO in *C. gariepinus* exposed to profenofos throughout the duration of the experiment. Similar to our reports, Kavitha and Rao (2009) reported the elevation of LPO in *Oreochromis mossambicus* exposed to profenofos. Bojan et al. (2017) also reported the elevation of LPO in *Labeo rohita* after 7 days exposure to profenofos. Bakry et al. (2013) also reported the elevation of LPO in *Biophalaria alexandrina* snails exposed to profenofos. LPO values have also been elevated in other animals administered various concentrations of profenofos (Rahman et al., 2006; Mansour et al., 2009; Singh et al., 2016).

The fish cells are equipped with antioxidant enzymes that work to counteract the activities of ROS generated by the toxicants (Nwani et al., 2017, Ebeh et al., 2020). Among these antioxidant enzymes are the CAT and SOD that work cooperatively against the effects of the radicals. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide while CAT converts the hydrogen peroxide to water. The values of CAT and SOD in the present study were elevated and indicate their involvement in countering the activities of the free radicals. Similar to our report, Bojan et al. (2017) reported the elevation of SOD and CAT in *Labeo rohita* exposed to profenofos. Elevation of SOD activity has also been reported in *O. niloticus* exposed to profenofos. Elevation of CAT values has also been reported in rats administered profenofos. Contrary to our reports however, Bakry et al. (2013) reported the inhibition of CAT and SOD values in *Biophalaria alexandrina* exposed to profenofos. Kaur and Jindal (2017) also reported the inhibition of CAT and SOD levels in rat exposed to similar insecticide chlorpyrifos. The values of GST were elevated in fish exposed to profenofos. Similar to our findings, elevation of GST values in *O. niloticus* (Kavitha and Rao 2009) and *Labeo rohita* (Bojan et al., 2017) exposed to profenofos have been reported. Basopo and Ngabaza (2015) also reported the elevation of GST in *Helisoma duryi* exposed to a related organophosphorus insecticide chlorpyrifos. The increase in GR and GPx obtained in the present study may suggest defensive mechanism of the fish to counter the oxidative stress due to the insecticide. GPx activity was elevated in *Helisoma duryi* exposed to chlorpyrifos (Basopo and Ngabaza 2015). Contrary to our report, GR activity was inhibited in *O. mossambicus* (Kavitha and Rao) and *B. alexandrina* snails (Bakry et al., 2013) exposed to profenofos. The GPx values were also inhibited in rats administered profenofos (Rahman et al., 2006).

CONCLUSION

The present study indicates that profenofos is highly toxic to *C. gariepinus* even at very low concentrations. Exposure of the fish to sublethal concentrations of profenofos elicited elevation of lipid peroxidation and antioxidant enzymes. The continuous use of profenofos in our household and agricultural fields may pose risk to human health and non-target organisms. The use of profenofos in the environment thus requires stringent precautions to guard against the harmful effects on aquatic life.

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