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Organophosphate Pesticide as an Environmental Stressor and its Impact on the Biochemical Parameters in Liver, Kidney and Gill of the Subtropical Catfish *Heteropneustes fossilis* (Bloch, 1794)

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ABSTRACT: The widespread use of pesticides in agriculture has led to increased contamination of aquatic ecosystems, posing severe risks to non-target organisms. This study investigates the impact of the organophosphate pesticide, monocrotophos, on the freshwater catfish *Heteropneustes fossilis*. Fish were exposed to varying concentrations (3, 6, and 8 ppm) of monocrotophos for 72 hours, and biochemical parameters were assessed. Behavioral alterations, including erratic swimming, excessive mucus secretion, and respiratory distress, were observed in exposed fish. Protein content significantly declined ($p<0.05$) in the liver, gills, and kidney, indicating metabolic disturbances. Lipid peroxidation (LPO) levels increased markedly, suggesting oxidative stress, with the highest LPO observed in gills (83.64% increase at 8 ppm). Catalase activity showed a dose-dependent increase, with a 590.4% rise in gills and 249% in liver at the highest concentration, reflecting a compensatory response to oxidative damage. These findings suggest that monocrotophos exposure induces biochemical and physiological stress in *H. fossilis*, which could have ecological and human health implications. The study underscores the need for stringent regulations on pesticide usage to protect aquatic biodiversity.

Keywords: Organophosphate, Pesticide, Environmental Stressor. Lipid peroxidation, Catfish *Heteropneustes fossilis*, (LPO).

INTRODUCTION

The aquatic ecosystem has been increasingly contaminated due to the continuous discharge of agricultural runoff, which carries pesticide residues. Over the past few decades, the extensive application of pesticides has significantly risen to manage pests and weeds (Ortiz-Ordoñez *et al.,* 2011; Jacquin *et al.,* 2019). In many parts of the Indian subcontinent, agricultural fields are located in proximity to water bodies, increasing the risk of water pollution through runoff. The presence of these chemicals in aquatic environments poses a serious threat to non-target organisms, particularly fish, which serve as bioindicators of water quality (Hedayati & Niazie 2015; Doherty *et al.,* 2016). These pollutants can enter fish through dermal contact, gill absorption, or ingestion, leading to physiological damage, metabolic disruption, and oxidative stress (Blahova *et al.,* 2014; Jacquin *et al.,* 2019). Cellular and enzymatic alterations in fish have been identified as valuable biomarkers for assessing the level of pesticide contamination in aquatic systems (Agrahari *et al*., 2006; Ortiz-Ordoñez *et al.,* 2011; Lakshmaiah, 2016; Özaslan *et al.,* 2018).

Heteropneustes fossilis, a widely distributed freshwater catfish in the Indian subcontinent, holds significant nutritional and economic value. This species, found predominantly in slow-moving and shallow water bodies such as ponds, ditches, and marshlands, is a rich source of protein, iron, and calcium (Rahman *et al*., 2017). Due to its nutritional benefits, it is widely consumed in countries such as India, Pakistan, Sri Lanka, Bangladesh, and Myanmar (Alok *et al.,* 1993). Organophosphate pesticides, known for their biodegradability and short environmental persistence, are commonly employed in agriculture. Among these, monocrotophos is widely used due to its cost-effectiveness and efficiency. However, its adverse effects on aquatic organisms have been documented (Velmurugan *et al*., 2007). Although previous research has evaluated the toxicity of monocrotophos on various freshwater fish species (Tamizhazhagan & Pugazhendy 2016), its impact on *H. fossilis* remains unexplored. The

present study aims to bridge this knowledge gap by assessing the effects of monocrotophos on blood parameters, vital organ histopathology, and stress enzyme activity in *H. fossilis.*

MATERIALS AND METHODS

A. Collection and Acclimatization

Healthy juvenile *H. fossilis* (mean length: 13.9±1.18 cm; mean weight: 49.53±0.71 g) were procured from pesticide-free freshwater ponds in Odisha, India. To mitigate infection risks, fish were treated with a 10 ppm potassium permanganate ($KMnO_4$) bath for 5 minutes before being transferred to 1000 L plastic tank containing dechlorinated water (pH 7.4±0.38, temperature 28.43±0.84°C). Fish were acclimatized for 15 days under laboratory conditions and fed daily with chicken liver and egg white. Water changes were performed 30 minutes after feeding to maintain water quality.

B. Test Chemical and Experimental Design

Monocrotophos (Monokem, 36% SL) was sourced from Sumitomo Chemical Pvt. Ltd., Chennai, India. The median lethal concentration (96 h LC50) was determined to be 20 ppm using probit analysis following EPA guidelines (EPA, 2002). Experimental groups included control (no pesticide) and three monocrotophos treatments: T1 (3 ppm), T2 (6 ppm), and T3 (8 ppm), each conducted in triplicate. Fish (n=23 per tank) were maintained in 400 L borewell water, and pesticide concentrations were carefully prepared and administered. Fish remained unfed during the experiment, and water quality parameters were monitored throughout the study.

C. Sampling and Biochemical Analysis

Blood samples were collected from the caudal vein using EDTA-coated syringes at 24 h and 72 h post-exposure. Fish were anesthetized with clove oil (50 µL L^{-1}) before blood extraction. Organ tissues (gills, liver, kidney) were carefully dissected and homogenized in chilled phosphate buffer (0.05 M, pH 7.4) to prevent enzymatic degradation. Homogenized samples were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was stored at -20°C for further biochemical assays. Lipid peroxidation (LPO) was assessed by measuring malondialdehyde (MDA) levels at 532 nm (Ohkawa *et al.*, 1979), while catalase activity was measured spectrophotometrically at 340 nm using hydrogen peroxide (Cohen *et al.*, 1970).

D. Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 22.0, followed by Duncan's Multiple Range Test (DMRT) to determine significant differences ($p<0.05$).

RESULTS

A. Behavioural Alterations and Mortality

Fish exposed to monocrotophos exhibited increased opercular movements, loss of equilibrium, frequent surfacing, excessive mucus secretion, and erratic swimming. Behavioral abnormalities intensified with increasing pesticide concentrations (Table 1). The 96 h LC50 for *H. fossilis* was determined as 24 ppm (mg L^{-1}).

Table 1: Behavioural changes in *H. fossilis* exposed to sub-lethal concentration of monocrotophos for 24h and 72h.

Behaviour (24 h & 72 h)	Experimental groups			
	Control	T_1	T_2	T_3
Water surfacing	-	+	++	+++
Lethargic	-	+	+	++
Mucus secretion	-	+	+	++
Irregular movement	-	+	++	++
Hypoxic condition	-	+	++	+++
Loss of equilibrium	-	+	++	+++

None (-), Moderate (+), High (++), Very high (+++); All data are recorded on the basis of clear observation on a frequent basis

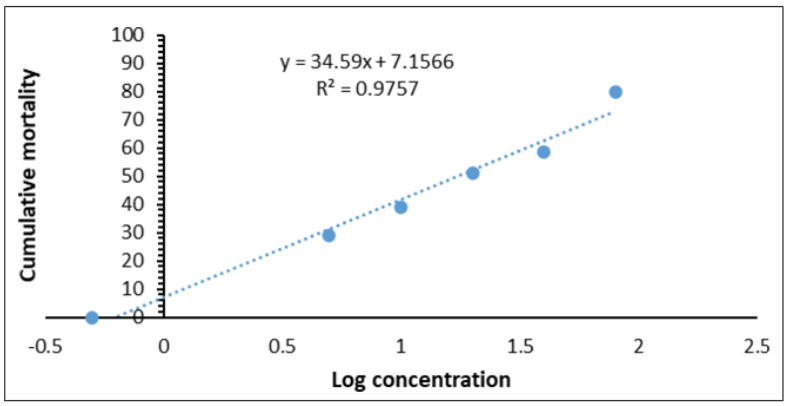

Fig. 1. Probit line for 96h LC50 of monocrotophos for *Heteropneustes fossilis.*

B. Protein Content and Lipid Peroxidation

Treated fish exhibited significant reductions ($p<0.05$) in protein content across all tissues, with the lowest values observed at higher pesticide concentrations (T3). Liver tissue protein levels declined more significantly compared to other organs (Table 2. Fig. 1). LPO activity increased progressively in all tissues with increasing monocrotophos exposure, particularly in the gills (83.64% increase at T3) and liver (56.39% increase at T3) (Table 3).

Table 2: Tissue protein content changes in *H.fossilis* exposed to sub-lethal concentration of monocrotophos for 24h and 72h.

Parameters		Experimental groups				*p value*
		Control	T_1	T_2	T_3	
	Gill 24 h	103.141±0.382^d	94.876±0.344^c	91.460±0.292^b	86.501±0.335^a	<0.001
	Gill 72 h	102.021±0.21^d	91.736±0.477^c	88.485±0.397^b	83.306±0.382^a	0.000
Protein	Liver 24 h	59.614±0.291^d	56.474±0.307^c	52.507±0.240^b	48.209±0.146^a	<0.002
(mgg^{-1}tissue)	Liver 72 h	59.003±0.087^d	53.113±0.146^c	49.256±0.191^b	45.179±0.480^a	<0.001
	Kidney 24 h	92.066±0.344^d	87.603±0.252^c	81.157±0.668^b	77.190±0.165^a	0.000
	Kidney 72 h	91.823±02134^d	85.454±0.252^c	76.529±0.286^b	71.791±0.386^a	<0.001

Values are expressed in mean ± SE(n=3) ; Mean values in the same row with different superscripts differ significantly ($p<0.05$)

Table 3: Tissue lipid peroxidation (LPO) changes in *H. fossilis* exposed to sub-lethal concentration of monocrotophos for 24h and 72h.

Parameters		Experimental groups				*p value*
		Control	T_1	T_2	T_3	
	Gill 24 h	1.764±0.023^a	2.058±0.031^b	2.365±0.073^c	2.636±0.019^d	0.001
	Gill 72 h	1.612±0.098^a	2.280±0.020^b	2.862±0.035^c	3.258±0.031^d	0.000
LPO (nmolmg^{-1}	Liver 24 h	2.609±0.016^a	3.124±0.031^b	3.556±0.016^c	4.080±0.008^d	0.002
protein)	Liver 72 h	2.539±0.097^a	3.395±0.032^b	4.044±0.023^c	4.218±0.012^d	0.001
	Kidney 24 h	4.236±0.019^a	4.711±0.023^b	6.089±0.027^c	6.235±0.012^d	0.000
	Kidney 72 h	4.112±0.099^a	5.871±0.012^b	6.413±0.008^c	6.498±0.016^d	0.002

Values are expressed in mean ± SE(n=3); Mean values in the same row with different superscripts differ significantly ($p<0.05$)

C. Catalase Activity

Catalase activity increased significantly ($p<0.05$) in all tissues in response to monocrotophos exposure. Compared to control, activity levels rose by 590.4% in gills (Fig.3) and 249% in liver (Fig. 2) after 24 h at T3. A similar trend was observed at 72 h, suggesting enhanced production of reactive oxygen species (ROS) and a compensatory activation of antioxidant defense mechanisms.

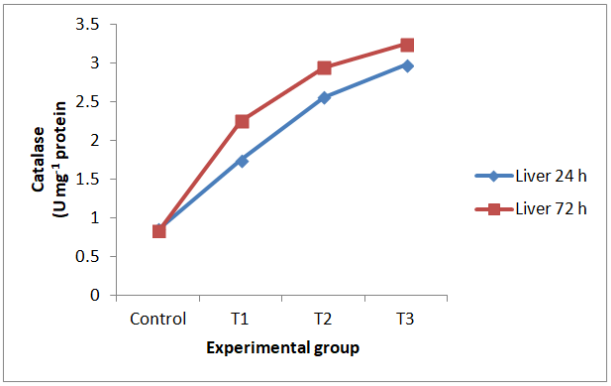

Fig. 2. Catalase activity in liver tissue of *H. Fossilis* to Monocrotopos exposure.

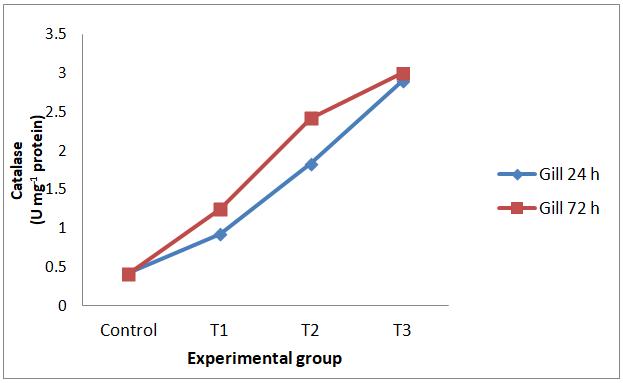

Fig. 3. Catalase activity in gill tissue of *H. Fossilis* to Monocrotopos exposure.

DISCUSSION

The findings of this study align with previous studies on fish exposed to pesticides (Tilak & Yakobu 2002; Nagaraju & Rathnamma 2013; Rohankar *et al.*, 2012). The observed increase in lipid peroxidation suggests heightened oxidative stress, which can impair cellular integrity and metabolic functions. Additionally, catalase activation as a response to oxidative stress in fish has been widely documented (Ortiz-Ordoñez *et al.*, 2011). The reduction in protein content in exposed fish may

indicate increased proteolysis or impaired protein synthesis, both of which are common consequences of pesticide-induced stress. The observed behavioral alterations in *Heteropneustes fossilis* exposed to monocrotophos, including increased opercular movements, loss of equilibrium (Adedeji *et al*., 2009), frequent surfacing, excessive mucus secretion, and erratic swimming, are consistent with previous studies on organophosphate toxicity in fish. For instance, exposure to monocrotophos has been reported to cause restlessness, increased opercular movement, erratic and jerky movements, and excessive mucus secretion in *Clarias batrachus*. Similarly, *Labeo rohita* exhibited significant behavioral changes upon monocrotophos exposure, highlighting the neurotoxic effects of this pesticide (Devi and Mishra 2013).

The significant reductions in protein content observed across all tissues, particularly in the liver, suggest increased proteolysis or impaired protein synthesis. This aligns with findings in *Clarias batrachus*, where monocrotophos exposure led to decreased total protein levels in gill, kidney, liver, and muscle tissues. Such protein depletion may result from increased energy demands due to stress, leading to the breakdown of proteins for energy production.

The elevated lipid peroxidation (LPO) levels, indicated by increased malondialdehyde (MDA) concentrations, point to heightened oxidative stress in exposed fish. Increased LPO activity has been documented in various fish species subjected to pesticide exposure. For example, monocrotophos exposure in zebra fish resulted in elevated MDA levels, indicating oxidative damage to cellular membranes. Similarly, studies on *Clarias batrachus* have shown increased lipid peroxidation upon monocrotophos exposure.

The significant increase in catalase activity across all tissues suggests a compensatory response to neutralize the excess reactive oxygen species (ROS) generated due to pesticide exposure (Tripathi and Singh 2013). Enhanced catalase activity has been observed in other aquatic organisms exposed to monocrotophos. For instance, in freshwater mussels, acute exposure to monocrotophos led to increased antioxidant enzyme activities, including catalase, as a defense mechanism against oxidative stress. Similarly, zebra fish exposed to monocrotophos exhibited increased catalase activity in the brain, indicating an adaptive response to oxidative stress.

These findings underscore the detrimental impact of monocrotophos on the physiological and biochemical health of *H. fossilis*. The observed behavioral disruptions, protein depletion, and heightened oxidative stress markers suggest that prolonged pesticide exposure could compromise fish health and, through bioaccumulation, pose risks to human consumers. Further research is warranted to explore long-term ecological consequences and develop mitigation strategies to reduce pesticide contamination in aquatic environments.

CONCLUSIONS

The study highlights the detrimental impact of monocrotophos on *H. fossilis*, evidenced by behavioural disruptions, protein depletion, and heightened oxidative stress markers. The findings suggest that prolonged pesticide exposure could compromise fish health and, through bioaccumulation, pose risks to human consumers. Further research is warranted to explore long-term ecological consequences and develop mitigation strategies to reduce pesticide contamination in aquatic environments.

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