



International Journal of Advance Studies and Growth Evaluation

Biochemical Evaluation of Carbohydrate Metabolism in Fresh Water Fish *Clarias batrachus* exposed to Two Organophosphorus Insecticides

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Article Info.

E-ISSN: 2583-6528

Impact Factor (QJIF): 8.4

Peer Reviewed Journal

Available online:

www.alladvancejournal.com

Received: 09/Feb/2026

Accepted: 13/March/2026

Abstract

Agricultural pesticides frequently enter aquatic ecosystems and may affect non-target organisms such as fish. The present investigation evaluated the biochemical responses of the freshwater catfish *Clarias batrachus* following exposure to two organophosphorus insecticides, Dimethoate and Profenofos. Fish were exposed to sub-lethal concentrations of Dimethoate (1.846 mg L⁻¹) and Profenofos (2.100 mg L⁻¹) for a period of 15 days. After the exposure period, fish were transferred to pesticide-free freshwater for 12 days in order to assess recovery responses. Exposure to Dimethoate resulted in significant reductions in carbohydrate and glycogen levels across tissues, whereas Profenofos exposure produced variable alterations depending on the tissue examined. Metabolic intermediates such as lactate and pyruvate exhibited marked fluctuations under pesticide stress. Enzymatic activities related to carbohydrate metabolism also showed significant changes. Lactate dehydrogenase (LDH) activity decreased in liver, kidney, and muscle tissues but increased in gills. Dimethoate strongly inhibited succinate dehydrogenase (SDH) activity in all tissues examined. Malate dehydrogenase (MDH) activity was stimulated by both insecticides. Glycogen phosphorylase activity showed tissue-specific induction and inhibition patterns. Following transfer to pesticide-free water, partial recovery in biochemical parameters was observed, indicating the capacity of fish to restore metabolic balance after cessation of toxic exposure. However, recovery was slower in Dimethoate-exposed fish compared with those exposed to Profenofos, suggesting greater toxicity of Dimethoate. The results demonstrate that organophosphorus pesticides significantly disrupt carbohydrate metabolism in *C. batrachus*. These biochemical responses may serve as useful biomarkers for assessing pesticide contamination in freshwater ecosystems and highlight the potential ecological risk posed by organophosphorus insecticides to edible fish species.

Keywords: Organophosphorus insecticides, biochemical biomarkers, freshwater fish, pesticide toxicity, metabolic enzymes, recovery response.

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Introduction

The extensive application of pesticides in modern agriculture has contributed significantly to improved crop protection and productivity. However, the indiscriminate use of these chemicals has raised serious concerns regarding their environmental impact, particularly in aquatic ecosystems. Pesticide residues often reach rivers, lakes, and ponds through agricultural runoff, posing a threat to aquatic organisms including fish, plankton, and amphibians.

Among the different classes of pesticides, organophosphorus (OP) insecticides are widely used due to their high effectiveness against pests and relatively rapid degradation compared to persistent organochlorine compounds. Despite their shorter environmental persistence, OP pesticides can

exert considerable toxic effects on non-target organisms by interfering with physiological and biochemical processes. These compounds may affect metabolic pathways, enzyme activities, and energy production in aquatic organisms.

Fish are particularly sensitive to pesticide contamination and are widely used as bioindicators in ecotoxicological studies. Sublethal exposure to pesticides can cause significant biochemical and physiological disturbances that may ultimately affect growth, reproduction, and survival. Such biochemical responses often occur before visible signs of toxicity and therefore serve as valuable early indicators of environmental stress.

The freshwater catfish *Clarias batrachus* is an economically important edible species widely distributed in South and

Southeast Asia. It is frequently cultured in paddy fields under integrated rice-fish farming systems, where it is often exposed to agricultural pesticides used in crop protection. Therefore, understanding the toxic effects of commonly used pesticides on this species is of ecological and economic importance.

The present study was undertaken to evaluate the biochemical effects of two commonly used organophosphorus insecticides, Dimethoate and Profenofos, on carbohydrate metabolism in *Clarias batrachus*. Specific attention was given to changes in metabolites such as glucose, glycogen, lactate, and pyruvate, as well as enzyme activities including lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and glycogen phosphorylase. In addition, the recovery potential of fish following cessation of pesticide exposure was examined by transferring the fish to pesticide-free water.

This study aims to provide insights into the metabolic disturbances caused by organophosphorus insecticides and to assess their ecological implications for freshwater fish populations.

Materials and Methods

Experimental Animals

Healthy adult specimens of the freshwater catfish *Clarias batrachus* were collected from local fish markets and transported to the laboratory. The fish were maintained in cement tanks supplied with continuously aerated tap water and acclimatized to laboratory conditions for 10–15 days prior to experimentation. During acclimatization, fish were fed a diet consisting of wheat flour pellets, groundnut cake, and egg white. Feeding was discontinued 24 hours before the start of the experiment.

Water Quality Parameters

The physicochemical characteristics of the experimental water were analyzed according to standard methods of the American Public Health Association (APHA). Parameters such as temperature, pH, dissolved oxygen, alkalinity, chlorides, and nitrate levels were maintained within acceptable limits for freshwater fish.

Table 1: Physicochemical Parameters of Tap Water

S. No.	Parameters	Values
1.	Temperature	29 ^o to 35 ^o C
2.	p ^H	7.6 to 7.8
3.	Electrical conductivity (milliohms/cm)	0.56
4.	Total alkalinity (mg/L as CaCO ₃)	72.0
5.	Bicarbonates (mg/L)	72.0
6.	Carbonates (mg/L as CaCO ₃)	Nil
7.	Chlorides (mg/L)	37.0
8.	Dissolved Oxygen (mg/L)	8.0
9.	B.D.O. (mg/L)	2.0
10.	C.O.D. (mg/L)	0.009
11.	Free CO ₂	43.0
12.	Calcium (mg/L)	2.1
13.	Sodium (mg/L)	3.2
14.	Nitrates (mg/L)	3.4
15.	Fluoride (mg/L)	0.04

Experimental Design

After acclimatization, fish were divided into three groups:

- 1. Control Group:** Maintained in normal tap water.
- 2. Dimethoate-treated Group:** Exposed to a sublethal concentration of 1.846 mg L⁻¹.
- 3. Profenofos-treated Group:** Exposed to a sublethal concentration of 2.100 mg L⁻¹.

Technical grade insecticides with approximately 90% purity were used. The median lethal concentration (LC₅₀) for both insecticides was determined using the probit analysis method. Fish were exposed to the insecticides for 15 days. Following the exposure period, surviving fish were transferred to pesticide-free freshwater for 12 days to assess recovery responses.

Tissue Sampling

At the end of the experimental period, fish were sacrificed and tissues including gills, liver, muscle, and brain were

carefully dissected. Samples were immediately placed in ice-cold conditions for subsequent biochemical analysis.

Biochemical Analysis

Biochemical parameters analyzed included:

- Total glucose
- Glycogen
- Pyruvate
- Lactate

Enzymatic activities determined were:

- Lactate dehydrogenase (LDH)
- Succinate dehydrogenase (SDH)
- Malate dehydrogenase (MDH)
- Glycogen phosphorylase

Standard biochemical methods described in the literature were used for each assay.

Table 2: Levels of Total Glucose, Glycogen, Pyruvate, Lactate, LDH, SDH, and MDH. In tissues of Control and Dimethoate and Profenofos exposed fish *Clarias batrachus*.

Parameters	Tissues	Control	Dimethoate	Profenofos
Glucose (mg/100mg.wet.wt.of tissue)	Liver	129.84±8.21	172.21±11.8	169.31±12.61
	Brain	32.79±1.92	PC= +33.21	PC= +30.96
	Gill	36.64±2.11	53.37±2.11	49.27±2.52
			PC= +33.48	PC= +50.26
			48.91±2.75	44.71±3027

			PC= +33.48	PC= +22.02
Glycogen ($\mu\text{mol}/100\text{gm.wet.wt.of tissue}$)	Liver Brain Gill	1624 \pm 72 111 \pm 14 86 \pm 21	884 \pm 98 PC= -45.56 78 \pm 12 PC= -29.72 53 \pm 6 PC= -38.37	1263 \pm 128 PC= -22.23 86 \pm 14 PC= -22.52 54 \pm 6 PC= -37.21
Pyruvate ($\mu\text{mol}/100\text{gm.wet.wt.of tissue}$)	Liver Brain Gill	64.18 \pm 0.62 19.82 \pm 2.11 69.21 \pm 5.22	50.21 \pm 2.11 PC= -26.68 13.59 \pm 0.61 PC= -32.44 39.28 \pm 1.96 PC= -43.24	46.61 \pm 1.92 PC= -31.93 10.83 \pm 0.96 PC= -45.36 36.61 \pm 3.27 PC= -47.10
Lactate ($\mu\text{mol}/100\text{gm.wet.wt.of tissue}$)	Liver Brain Gill	0.24 \pm 0.008 0.289 \pm 0.14 0.197 \pm 0.06	0.418 \pm 0.05 PC= +74.16 0.396 \pm 0.15 PC= +37.02 0.247 \pm 0.07 PC= +25.38	0.429 \pm 0.08 PC= +78.75 0.401 \pm 0.18 PC= +38.75 0.259 \pm 0.08 PC= +31.47
LDH ($\mu\text{molof formazan formed/mg protein in tissue}$)	Liver Brain Gill	2.43 \pm 0.04 0.69 \pm 0.07 0.81 \pm 0.02	1.61 \pm 0.15 PC= -33.75 0.32 \pm 0.01 PC= -53.62 0.49 \pm 0.02 PC= -39.50	1.52 \pm 0.22 PC= -37.44 0.29 \pm 0.07 PC= -57.97 0.41 \pm 0.02 PC= -49.38
SDH ($\mu\text{molof formazan formed/mg protein in tissue}$)	Liver Brain Gill	6.79 \pm 0.21 2.62 \pm 0.19 0.98 \pm 0.10	3.12 \pm 0.13 PC= -54.05 1.56 \pm 0.21 PC= -40.46 0.57 \pm 0.08 PC= -41.84	3.29 \pm 0.14 PC= -51.54 1.75 \pm 0.23 PC= -33.21 0.62 \pm 0.07 PC= -36.73
MDH ($\mu\text{molof formazan formed/mg protein in tissue}$)	Liver Brain Gill	2.29 \pm 0.05 1.12 \pm 0.08 0.29 \pm 0.04	0.98 \pm 0.01 PC= -57.20 0.46 \pm 0.02 PC= -58.03 0.09 \pm 0.01 PC= -68.96	1.37 \pm 0.03 PC= -40.17 0.530.03 PC= -52.67 0.12 \pm 0.01 PC= -58.62

Each value is mean \pm SD of (6) individual's observation PC denotes percentage change over control. All values are statistically from control significant at 1% level (P<0.01) * Not Significant.

Results

Exposure to sublethal concentrations of Dimethoate and Profenofos caused significant biochemical alterations in *Clarias batrachus*. Although no external symptoms of toxicity were observed, both pesticides produced marked changes in carbohydrate metabolism.

Carbohydrate and glycogen levels decreased significantly in most tissues of fish exposed to Dimethoate. In contrast, Profenofos exposure resulted in tissue-specific responses, including reductions in some tissues and increases in others.

Lactate levels increased in certain tissues of pesticide-exposed fish, indicating a shift towards anaerobic metabolism under toxic stress. Pyruvate levels also showed variable changes depending on tissue type and pesticide exposure.

Enzymatic activities exhibited significant alterations. LDH activity increased in gill tissues but decreased in liver and muscle. SDH activity was strongly inhibited by Dimethoate in all tissues. MDH activity showed induction in several tissues following exposure to both insecticides.

During the recovery period, fish transferred to pesticide-free water showed partial restoration of biochemical parameters. However, recovery was more pronounced in Profenofos-exposed fish compared with those exposed to Dimethoate.

Discussion

The present study demonstrates that organophosphorus

insecticides significantly affect carbohydrate metabolism in *Clarias batrachus*. Carbohydrates serve as the primary energy source in fish, and their depletion under pesticide stress suggests increased metabolic demand for detoxification and survival.

The observed decline in glycogen levels may be attributed to enhanced glycogenolysis in response to energy requirements under toxic conditions. The liver, being the major site of glycogen storage and detoxification, exhibited the most pronounced changes.

Increased lactate levels in certain tissues indicate a shift from aerobic to anaerobic metabolism during pesticide stress. Such metabolic shifts are commonly associated with hypoxic conditions or impaired mitochondrial function.

Alterations in LDH, SDH, and MDH activities further support the disruption of energy metabolism pathways. The increased LDH activity in gill tissues may reflect tissue damage and the release of intracellular enzymes. Partial recovery of biochemical parameters after cessation of pesticide exposure suggests that fish possess adaptive mechanisms to restore metabolic balance. However, the slower recovery observed in Dimethoate-exposed fish indicates that this pesticide exerts more severe toxic effects compared to Profenofos.

Conclusion

The findings of the present study demonstrate that exposure to organophosphorus insecticides leads to significant disturbances in carbohydrate metabolism and enzyme activity in *Clarias batrachus*. Dimethoate produced stronger toxic effects than Profenofos, and recovery following exposure was incomplete within the observed period.

Biochemical parameters such as glucose, glycogen, lactate, pyruvate, and metabolic enzyme activities can serve as sensitive biomarkers for detecting pesticide stress in aquatic organisms. Monitoring these indicators may provide valuable insights into the ecological impact of pesticide contamination in freshwater environments.

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