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"SUBLETHAL EFFECTS OF AGRICULTURAL INSECTICIDES IN PROTEIN METABOLISM OF FISH *Clarias batrachus*: BIOCHEMICAL AND HISTOPATHOLOGICAL PERSPECTIVES"

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Abstract

This study evaluates the impact of organophosphorus insecticides on Protein metabolism in brain, gill, and liver tissues of the freshwater fish Clarias batrachus. Fish were exposed to sublethal concentrations, and biochemical changes were analyzed. Significant alterations in all substrates levels were observed across tissues, indicating metabolic stress and toxicity. Protein metabolism plays a crucial role in maintaining physiological homeostasis in aquatic organisms, particularly in fish where nitrogenous waste regulation is vital for survival. The present study investigates the effects of organophosphorus insecticide exposure on Protein metabolism in the brain, gill, and liver tissues of the freshwater fish *Clarias batrachus*. Specimens were subjected to sublethal concentrations of the insecticide under controlled laboratory conditions for defined exposure periods. Biochemical analyses revealed a significant elevation in substrate levels across all examined tissues, with the liver exhibiting the highest accumulation, followed by gill and brain. These alterations suggest impairment in ammonia detoxification pathways and disruption of normal metabolic functions. The observed tissue-specific variations indicate differential sensitivity and adaptive responses to toxic stress. The findings highlight the potential of protein metabolism as a sensitive biomarker for assessing organophosphorus insecticide toxicity in aquatic ecosystems. This study contributes to understanding the biochemical impacts of pesticide contamination and underscores the ecological risks associated with their widespread use.

Key words: Organophosphorus Insecticides, Protein Metabolism , Ecological Risks, Sublethal Concentrations

Introduction

Organophosphorus insecticides are widely used in agriculture and often enter aquatic ecosystems through runoff, posing a significant threat to non-target organisms, including fish. These compounds are known to interfere with enzymatic activities and metabolic pathways, leading to biochemical and physiological stress. *Clarias batrachus*, a freshwater teleost, is considered an ideal bioindicator species due to its ecological relevance and tolerance to environmental fluctuations.

Ammonia is a primary nitrogenous waste in fish and plays a critical role in maintaining cellular and metabolic balance. In aquatic organisms, the regulation of ammonia involves complex biochemical pathways, primarily occurring in metabolically active tissues such as the liver, gills, and brain. Any disturbance in these processes can lead to toxic accumulation, affecting physiological functions and survival.

Despite extensive studies on pesticide toxicity, limited information is available on tissue-specific protein metabolism under organophosphorus exposure. Therefore, the present study aims to evaluate alterations in all substrates levels in the brain, gill, and liver tissues of *Clarias batrachus* exposed to sublethal concentrations of organophosphorus insecticides. Protein metabolism is critical in fish physiology. Organophosphorus insecticides are known to disrupt biochemical pathways. This study investigates tissue-specific responses.

Methodology

Experimental Animals

Healthy specimens of *Clarias batrachus* of similar size (50 to 100 grams) and weight were collected and acclimatized under laboratory conditions for two weeks prior to experimentation.

Experimental Design

Fish were divided into control and experimental groups. The experimental groups were exposed to sublethal concentrations of an organophosphorus insecticide for two exposure durations of 48, 72, and 96 hours while the control group was maintained in pesticide-free water.

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Sample Collection

At the end of each exposure period, fish were sacrificed, and tissues such as brain, gill, and liver were carefully dissected, rinsed, and processed for biochemical analysis.

Biochemical Analysis

Ammonia levels in the tissues were estimated using standard colorimetric methods. The results were expressed as µg of ammonia per gram of tissue.

Statistical Analysis

Data were analyzed using appropriate statistical tools, and results were expressed as mean ± standard deviation (SD). Significance was assessed at $p < 0.05$.

Table 1: Ammonia Levels (µg/g tissue)

Tissue	Control	48 hrs	72 hrs	98 hrs
Brain	12	18	25	29
Gill	15	22	30	32
Liver	20	28	35	38

Table 2:Total Proteins (mg/100 mg wet wt. tissue)

Tissue	**Control**	**48 hrs**	**72 hrs**	**96 hrs**
Liver	30.12±0.52	18.45±0.26 (-38.75%)	18.79±0.31 (-37.61%)	19.05±0.28 (-36.75%)
Brain	16.80±0.38	10.25±0.22 (-38.99%)	10.48±0.27 (-37.62%)	10.72±0.35 (-36.19%)
Gill	10.05±0.11	7.34±0.18 (-26.97%)	7.46±0.16 (-25.77%)	7.59±0.14 (-24.48%)

Changes in Protein Content

A significant decrease in protein levels was observed in all tissues of treated fish compared to controls. The decline was progressive with increasing exposure duration.

- **Liver:** Showed maximum reduction, indicating its central role in metabolism and detoxification
- **Muscle:** Moderate decrease due to utilization of structural proteins
- **Gill:** Least reduction but still significant due to direct exposure to toxicants

Mechanism of Protein Depletion

The reduction in protein content may be attributed to:

- Increased proteolysis under stress conditions
- Conversion of amino acids into energy via gluconeogenesis

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- Inhibition of protein synthesis due to toxic effects on ribosomal activity

Comparative Toxicity

Profenofos caused greater protein depletion than dimethoate, indicating higher toxicity. This may be due to its stronger inhibitory effects on metabolic enzymes.

Ecotoxicological Significance

The findings suggest that protein metabolism is highly sensitive to insecticide exposure and can serve as an early warning indicator of environmental contamination. Alterations in protein levels may affect growth, reproduction, and survival of fish populations.

Table 3:Free Amino Acids (µmol/g wet wt. tissue)

Tissue	Control	48 hrs	72 hrs	96 hrs
Liver	645±35.0	910±95.0 (+41.08%)	882±78.0 (+36.74%)	870±42.0 (+34.88%)
Brain	398±45.0	578±105.0 (+45.22%)	540±52.0 (+35.68%)	520±63.0 (+30.65%)
Gill	405±40.0	470±60.0 (+16.05%)	438±42.0 (+8.15%)	430±44.0 (+6.17%)

Table 4:Ammonia (µmol/g wet wt. tissue)

Tissue	Control	48 hrs	72 hrs	96 hrs
Liver	22.40±2.10	32.10±2.65 (+43.30%)	31.05±2.70 (+38.61%)	29.80±2.30 (+33.04%)
Brain	15.10±0.55	9.05±0.06 (-40.07%)	9.28±0.05 (-38.54%)	9.80±0.04 (-35.10%)
Gill	9.60±1.05	5.70±0.62 (-40.62%)	5.95±0.75 (-38.02%)	6.30±0.60 (-34.38%)

Table 5:Glutamine (µmol/g wet wt. Tissue)

Tissue	Control	48 hrs	72 hrs	96 hrs
Liver	115.30±13.20	160.20±15.40 (+38.94%)	155.10±12.60 (+34.50%)	151.40±11.20 (+31.30%)
Brain	100.10±5.10	132.80±9.40 (+32.66%)	127.90±8.10 (+27.77%)	124.60±8.30 (+24.48%)
Gill	28.40±3.60	38.70±5.10 (+36.27%)	36.10±4.80 (+27.11%)	34.80±4.10 (+22.54%)

Table 6 :Urea (µmol/g wet wt. tissue)

Tissue	Control	48 hrs	72 hrs	96 hrs
Liver	17.10±0.95	22.30±0.82 (+30.41%)	21.60±0.79 (+26.32%)	20.80±0.76 (+21.64%)

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Brain	12.80±0.88	16.70±0.70 (+30.47%)	16.40±0.92 (+28.12%)	15.90±0.80 (+24.21%)
Gill	7.10±0.75	4.60±0.15 (-35.21%)	4.75±0.30 (-33.10%)	5.00±0.25 (-29.58%)

Table 7 :Experimental Conditions and Substrate Concentrations for Enzyme Assays at 29°C

Enzyme	P^H	Substrate Concentration	Enzyme concentreation	Buffer concentreation
Acidic Protease	4.0	10 mg (Denatured Hb)	35 mg	100 µmoles (Citrate buffer)
Neutral Protease	7.0	10 mg (Denatured Hb)	35 mg	100µmoles (Phosphate buffer)
Alkaline Protease	8.7	10 mg (Denatured Hb)	35 mg	100 µmoles(Bicarbonate buffer)
GDH	7.3	50 µmoles (Sodium Glutamate)	30 mg	100 µmoles (Phosphate buffer)
AMP Deaminase	6.5	10 µmoles (AMP)	30 mg	50 µmoles (Succcinate buffer)
Adenosine Deaminase	6.4	30 µmoles (Adenosine)	30 mg	50 µmoles (Phosphate buffer)
Glutaminase	5.0	30 µmoles (Glutamine)	30 mg	50 µmoles(Sodium citrayte buffer0

For assaying acidic, neutral and alkaline proteases, 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 30-00 rpm for 15 minutes. A clear cell free supernatent was used for the assay of proteases by the method of Davis and Smith (1955). Acid protgease activity was assayed at pH 4.0 using citgrate buffer, neutral protease at pH 7.0 using phosphate buffer and alkaline protease activity at pH 8.9 with carbonate-bicarbonate buffer and 10 mg of denbatured haemoglobin protein was used as substrate.

To assay glutamate dehydrogenase (GDH), 10% tissue homogenates were prepared in ice cold 0.25 M sucrose solution and centrifuged at 5000 rpm for 15 minutes. A clear cell free supernatent was usesd for the assay of GDH by the method of Lee and Lardy (1965). In addition to substrate, buffer and enzyme, 0.1 µ moles of NAD^+ and 2 µ moles of INT were added to the reaction mixture.

For the assay of adenosine monophosphate (AMP) deaminase, 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes.The clear supernatent was used for the assay of enzyme (Weil-Malherbe and Green 1955 modified by Wagelin et al. 1978) The reaction mixture contained buffer, substate, enzyme, 5 µ moles ATP, 2 µ moles $MgCl_2$ and 1 µ moles EDTA. For assaying adenosine deaminase activity by the method of Agarwal and Parks (1978), 10% tissue homogenats were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes to obtain a clear supernatent which was used as enzyme source.

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To assay the activity of glutaminase by the method of Alton Meister (1955),10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes. The supernatent was used as enzyme source.

Table 8: Effect of Dimethoate on Protein Content (mg/g wet weight) in *Clarias batrachus*

Tissues	Control	Dimethoate	% Decrease in Protein Content Compared to Control
Liver	18.7 ± 0.5	13.8 ± 0.4*	26.2%
Muscle	15.0 ± 0.5	11.2 ± 0.4*	25.3%
Gill	10.3 ± 0.4	7.8 ± 0.3*	24.3%

Table 9:Effect of Profenofos on Protein Content (mg/g wet weight) in *Clarias batrachus*

Tissues	Control	Profenofos	% Decrease in Protein Content Compared to Control
Liver	18.7 ± 0.5	13.1 ± 0.3*	29.9%
Muscle	5.0 ± 0.5	10.8 ± 0.3*	28.0%
Gill	10.3 ± 0.4	7.2 ± 0.3*	30.0%

Table10: Effect of Dimethoate on Protease Activity (U/mg protein) in *Clarias batrachus*

Tissues	Control	Dimethoate	% Decrease in Protein Content Compared to Control
Liver	2.88 ± 0.07	4.15 ± 0.09*	44.1%
Muscle	2.12 ± 0.05	3.26 ± 0.08*	53.8%
Gill	1.68 ± 0.06	2.64 ± 0.07*	57.1%

Table11: Effect of Profenofos on Protease Activity (U/mg protein) in *Clarias batrachus*

Tissues	Control	Profenofos	% Decrease in Protein Content Compared to Control
Liver	2.88 ± 0.07	4.38 ± 0.10*	52.1%
Muscle	2.12 ± 0.05	3.48 ± 0.09*	64.1%
Gill	1.68 ± 0.06	2.86 ± 0.07*	70.2%

Table12:Comparative Profile of Organophosphorus Insecticide(Comparison of Dimethoate, and Profenofos)

Parameter	Dimethoate	Profenofos
Chemical Class	Organophosphate	Organophosphate
Mode of Action	Acetylcholinesterase inhibitor	Acetylcholinesterase inhibitor
Type of Action	Systemic & contact	Contact & stomach
Persistence in Water	Moderate	High (more persistent)
Relative Toxicity to Fish	High	Very high

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Bioaccumulation Potential	Moderate	High
Effect on Protein Metabolism	Significant protein depletion	Severe protein depletion
Protease Activity	High increase	Very high increase
Oxidative Stress Induction	High	Very high
Field Use in Paddy	Common	Common
Environmental Risk	High	Very high

Results and Discussion

The present investigation revealed a significant elevation in ammonia levels in the brain, gill, and liver tissues of *Clarias batrachus* following exposure to organophosphorus insecticides. The increase was progressive with exposure duration, indicating a clear time-dependent metabolic disruption. Among the tissues analyzed, the liver exhibited the highest accumulation of ammonia, followed by gill and brain, reflecting their differential roles in protein metabolism and detoxification.

The liver, being the primary site of protein metabolism, showed pronounced alterations, suggesting impairment of enzymatic pathways involved in ammonia detoxification. Recent studies (2024–2025) have demonstrated that organophosphorus insecticides induce oxidative stress and disrupt hepatic metabolic processes, including alterations in key biochemical indices and enzyme systems . Such metabolic disturbances can lead to reduced efficiency of ammonia conversion pathways, resulting in its accumulation.

The gills also exhibited elevated ammonia levels, which may be attributed to their direct exposure to contaminated water and their role in ammonia excretion. Organophosphorus compounds are known to enter fish primarily through the gill surface, causing structural and functional impairments. A recent ecotoxicological study reported that these insecticides penetrate through gills and induce severe biochemical and neurological disturbances in fish . This supports the observed disruption in ammonia regulation in gill tissues.

Although the brain showed comparatively lower ammonia levels than the liver and gills, the increase remains biologically significant. Elevated ammonia in neural tissues is associated with neurotoxicity and altered neurotransmission. Contemporary research indicates that organophosphorus insecticides target nervous tissues, leading to behavioral and physiological abnormalities in fish . This suggests that ammonia accumulation in the brain may contribute to neurotoxic stress.

At the biochemical level, the observed increase in ammonia may be linked to enhanced amino acid catabolism and inhibition of detoxifying enzymes such as glutamine synthetase and glutamate dehydrogenase. Earlier mechanistic studies have shown that pesticide exposure alters nitrogen metabolism by increasing transaminase activity and disrupting ammonia detoxification pathways . These enzymatic disturbances likely contribute to the accumulation of toxic nitrogenous metabolites. The results indicated a marked increase in ammonia levels in all examined tissues following exposure to the organophosphorus insecticide. The increase was both dose- and duration-dependent, with higher values observed after 96 hrs compared to 48 hrs.of exposure.

Among the tissues, the liver exhibited the highest accumulation of ammonia, suggesting its central role in protein metabolism and detoxification. The gills also showed significant elevation, likely due to their direct contact with the external environment and involvement in ammonia excretion. The brain displayed comparatively lower levels; however, the increase was still significant, indicating potential neurotoxic effects. Furthermore, recent environmental studies highlight that organophosphorus insecticides act as major aquatic pollutants, affecting physiological and biochemical processes in fish even at sublethal concentrations . Chronic exposure leads to cumulative toxicity, impacting organ function and metabolic homeostasis.

Overall, the findings of the present study are consistent with recent advances in fish toxicology, which emphasize that pesticide-induced stress results in metabolic imbalance, oxidative damage, and disruption of nitrogen metabolism. The tissue-specific variations observed in this study further indicate that the liver is the most susceptible organ, followed by gills and brain, under organophosphorus insecticide exposure.These findings are consistent with previous reports

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indicating that pesticide exposure induces metabolic stress and alters nitrogen metabolism in fish. The tissue-specific variations highlight differential sensitivity and adaptive responses among organs.

Conclusion

Exposure to organophosphorus insecticides such as dimethoate and profenofos results in significant disruption of protein metabolism in *Clarias batrachus*. The observed decline in protein content across liver, muscle, and gill tissues indicates enhanced proteolysis and metabolic stress under toxic conditions. Prolonged exposure intensifies these effects, highlighting the cumulative impact of sublethal insecticide concentrations. The study establishes protein metabolism as a sensitive biomarker for assessing pesticide toxicity in aquatic organisms. These findings underscore the ecological risks associated with indiscriminate insecticide usage and emphasize the necessity for stringent environmental monitoring and sustainable agricultural practices to protect aquatic biodiversity.

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