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Toxicological Evaluation of Organophosphates in Freshwater Ecosystems and the Role of Medicinal Plants in Detoxification

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Abstract

Organophosphate (OP) pesticides remain widely used in agriculture, and their entry into freshwater systems via runoff, spray drift, and drainage creates persistent ecological stress for aquatic biodiversity. The hallmark mechanism of OP toxicity is acetylcholinesterase (AChE) inhibition, which disrupts cholinergic neurotransmission and triggers downstream neurobehavioral impairment, oxidative stress, immunotoxicity, and tissue injury in non-target organisms. In parallel, there is growing interest in plant-based interventions rich in polyphenols, flavonoids, and terpenoids that may mitigate OP-induced damage through antioxidant, anti-inflammatory, and membrane-stabilizing actions. This research paper integrates (i) a field-to-lab exposure framework for OP contamination (chlorpyrifos as a model OP) in freshwater habitats and (ii) an experimental evaluation of a medicinal plant extract as a detoxification/amelioration strategy in a fish bioindicator. Water chemistry and OP residues were characterized along an agricultural intensity gradient. In laboratory assays, fish were exposed to sublethal chlorpyrifos concentrations reflective of environmentally realistic pulses, and responses were quantified using neurotoxicity (AChE activity), oxidative stress (SOD, CAT, GPx, MDA), hematology, and histopathology endpoints. A parallel treatment combined OP exposure with a standardized plant extract to assess protective efficacy. Results showed: (1) OP residues were detectable in impacted sites; (2) chlorpyrifos exposure caused concentration-dependent AChE suppression and oxidative stress; and (3) the plant extract partially restored AChE activity, reduced lipid peroxidation, and improved hematological and tissue endpoints. A graph illustrates the mitigation of AChE inhibition by plant supplementation. Collectively, the findings support an integrative ecotoxicological risk assessment approach that couples OP monitoring with biomarker batteries and highlights medicinal plants as promising, low-cost adjuncts for detoxification research while emphasizing that ecological prevention (reduced inputs, buffers, and safer alternatives) remains the first line of protection for freshwater ecosystems.

Keywords: Organophosphates; freshwater; AChE; oxidative stress; chlorpyrifos; phytotherapy

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Introduction

Freshwater ecosystems are among the most productive and biologically diverse environments on Earth, yet they are also among the most threatened by anthropogenic activities, particularly chemical pollution originating from agriculture (Dudgeon *et al*., 2006; Reid *et al*., 2019). The widespread application of pesticides has significantly increased crop yields, but it has also resulted in unintended contamination of aquatic habitats through surface runoff, leaching, and atmospheric deposition (Gavrilescu, 2005; Stehle & Schulz, 2015). Among the various classes of pesticides, organophosphates (OPs) are of particular concern due to their high toxicity, broad usage, and well-documented impacts on non-target organisms (WHO, 1986; Singh & Sharma, 2019). Organophosphate pesticides act primarily by inhibiting acetylcholinesterase (AChE), a key enzyme responsible for terminating nerve impulses by hydrolyzing the neurotransmitter acetylcholine (Aroniadou-Anderjaska *et al*., 2023). Inhibition of AChE leads to excessive accumulation of acetylcholine at synapses, resulting in continuous nerve stimulation, neuromuscular dysfunction, and, in severe cases, mortality (Sandahl *et al*., 2007). In aquatic organisms, OP exposure has been associated with altered swimming behavior, impaired feeding efficiency, reproductive dysfunction, and increased susceptibility to predation (Van der Oost *et al*., 2003; Hayes *et al*., 2010). Recent studies emphasize that OP toxicity is not limited to neurotoxicity alone. Chlorpyrifos and related compounds have been shown to induce oxidative stress by generating reactive oxygen species (ROS) and disrupting antioxidant defense systems, leading to lipid peroxidation, protein oxidation, and DNA damage (Negro *et al*., 2019; Lu *et al*., 2024). Such biochemical disturbances can compromise cellular integrity and organ function, particularly in metabolically active tissues such as liver, gills, and brain (Mahapatra *et al*., 2025). Given the ecological significance of freshwater systems and the persistent detection of OP residues in surface waters, there is a growing need for integrated toxicological assessments that combine chemical monitoring with biological responses (Van der Oost *et al*., 2003; Tickner *et al*., 2020). In this context, biomarkers such as AChE activity and antioxidant enzyme responses are widely recognized as sensitive indicators of sublethal pesticide stress (Ellman *et al*., 1961; Singh & Sharma, 2019). In parallel with toxicity assessment, increasing attention has been directed toward natural detoxification strategies using medicinal plants. Numerous plants extracts rich in polyphenols and flavonoids have demonstrated protective effects against pesticide-induced oxidative stress and neurotoxicity in experimental models (Hassan *et al*., 2022; Mhalhel *et al*., 2024). However, limited studies have evaluated their efficacy in aquatic organisms under OP stress. Therefore, the present study aims to evaluate chlorpyrifos toxicity in freshwater fish and to investigate the ameliorative role of a medicinal plant extract as a potential eco-friendly detoxification approach.

Freshwater ecosystems rivers, lakes, ponds, wetlands, and connected floodplains support disproportionate global biodiversity and provide essential ecosystem services, including nutrient cycling, fisheries, drinking water, and cultural values. Yet these habitats are increasingly vulnerable to chemical stressors from expanding agriculture and intensified pesticide use. Among pesticide classes, organophosphates (OPs) have received sustained attention because of their potent neurotoxicity, frequent detection in agricultural landscapes, and capacity to harm non-target aquatic organisms even at low concentrations.

OPs are primarily designed to control insect pests by inhibiting acetylcholinesterase (AChE), the enzyme responsible for hydrolyzing acetylcholine at synapses. When AChE is inhibited, acetylcholine accumulates, resulting in continuous stimulation of neurons and neuromuscular junctions that can manifest as hyperexcitation, tremors, paralysis, and death in severe cases. Although OPs were initially viewed as less persistent than certain organochlorines, their episodic entry into waterways—often following rainfall events can yield repeated toxicity pulses that coincide with sensitive life stages of aquatic organisms.

Ecotoxicological evidence increasingly demonstrates that OP impacts extend beyond acute mortality. Sublethal exposure can alter predator avoidance, foraging, schooling, and reproductive behaviors; compromise immunity; and induce oxidative stress, inflammation, and genotoxicity. Such effects can scale up from individuals to populations and communities, shifting species composition and reducing ecosystem resilience. Invertebrates (e.g., aquatic insects and crustaceans) may be especially sensitive, but fish and mollusks also exhibit biomarker changes and tissue pathology under OP stress. Recent work continues to document oxidative stress responses in freshwater organisms exposed to chlorpyrifos, reinforcing the relevance of biomarker-based monitoring.

Risk assessment for OPs in freshwater ecosystems therefore requires a multi-level approach: (i) measuring environmental concentrations, (ii) evaluating toxicity thresholds and biomarker responses in model organisms, and (iii) linking mechanistic biomarkers to ecologically meaningful endpoints (growth, reproduction, survival, community diversity). However, environmental management faces a persistent gap: even when contamination is identified, practical detoxification or mitigation strategies that are affordable and locally feasible may be limited.

In this context, medicinal plants offer a complementary research avenue. Traditional and contemporary pharmacological studies show that plant extracts often rich in polyphenols and flavonoids—can attenuate pesticide-induced oxidative injury and neurotoxicity by scavenging reactive oxygen species (ROS), supporting endogenous antioxidant systems, and modulating inflammatory pathways. For example, olive leaf extract has been shown to mitigate chlorpyrifos-induced toxicity in mammalian models, and Urtica dioica extract has demonstrated protective properties against chlorpyrifos-linked damage, plausibly via antioxidant mechanisms. While mammalian evidence cannot be directly extrapolated to aquatic taxa, these findings motivate controlled ecotoxicology experiments to test whether plant-derived interventions can reduce OP-induced stress biomarkers in fish and other aquatic organisms.

Evaluation of medicinal plant-based detoxification, assessing whether a standardized plant extract can ameliorate OP-induced biochemical and physiological disturbances in a fish bioindicator under controlled exposure.

Hypotheses

H_1: Environmentally realistic, sublethal chlorpyrifos concentrations produce concentration-dependent AChE inhibition and oxidative stress responses in fish.

H_2: Co-treatment with a medicinal plant extract partially restores AChE activity and improves antioxidant status, reducing lipid peroxidation and related tissue damage.

By combining residue monitoring concepts with mechanistic biomarkers and an intervention arm, the study aligns with modern ecotoxicology that seeks not only to diagnose harm, but also to explore feasible mitigation strategies while recognizing that prevention through reduced pesticide input and improved agricultural practices remains the most effective protection for freshwater biodiversity.

Review of Literature

Organophosphate (OP) pesticides have remained an important class of agrochemicals for insect control, yet their ecological footprint in freshwater environments continues to raise concern. OPs enter rivers, ponds, lakes, wetlands, and irrigation-linked water bodies primarily through runoff, leaching, accidental spills, and spray drift from agricultural fields. Because many OP applications coincide with monsoon or irrigation cycles, exposure in freshwaters is often pulse-driven (short, repeated peaks) rather than constant, complicating hazard characterization based solely on static laboratory concentrations. The most frequently highlighted ecological issue is that OPs are designed to disrupt neurophysiology in target pests but can similarly affect non-target aquatic organisms fish, amphibians, crustaceans, aquatic insects, and plankton via conserved biochemical pathways.

The defining toxic mechanism of OP insecticides is acetylcholinesterase (AChE) inhibition, leading to acetylcholine accumulation at synapses and neuromuscular junctions, producing cholinergic overstimulation, behavioral impairment, and potentially mortality. Mechanistic syntheses emphasize that AChE inhibition is central to OP neurotoxicity and remains a core endpoint for both biomedical and ecological risk evaluation (Lushchak, 2018). A comprehensive mechanistic overview further highlights how AChE inhibition can trigger downstream systemic effects including oxidative stress, altered metabolism, inflammatory responses, and endocrine-related disruptions thereby broadening OP impacts beyond purely neurological outcomes (Lushchak, 2018).

In fish ecotoxicology, biomarker frameworks have long treated AChE inhibition as a sensitive and early indicator of OP exposure, especially when paired with complementary biomarkers (Van der Oost *et al*., 2003). The strength of AChE as a biomarker lies in its mechanistic specificity for OPs and carbamates; however, a critical limitation is that AChE inhibition does not always map linearly onto ecological outcomes (e.g., survival, growth, reproduction), especially when organisms compensate behaviorally or physiologically, or when environmental stressors (temperature, hypoxia, pathogens) modify toxicity expression.

A major shift in the last two decades has been the growing focus on behavioral toxicity as an ecologically meaningful bridge between biochemical disruption and population-level consequences. Controlled experiments demonstrate that neurotoxicant exposure can suppress swimming performance and alter spontaneous activity, which can translate into reduced feeding, migration, and predator avoidance. For example, zebrafish studies examining neurotoxicants (including chlorpyrifos) show measurable changes in spontaneous swimming behavior and mixture interactions, supporting the idea that sublethal neurotoxicity can create real-world fitness costs even in the absence of immediate mortality (Tilton *et al*., 2010). Similarly, work examining sublethal OP exposure in fish has linked cholinesterase inhibition with compromised behavioral or physiological performance, reinforcing that AChE changes are not merely biochemical artifacts but can be functionally consequential (Sandahl *et al*., 2005).

Still, a critical point is that many laboratory studies use single compounds and constant exposures, whereas field conditions often involve mixtures and intermittent pulses. Intermittent exposure designs are increasingly valued for mimicking agricultural runoff realities. A notable example is evidence that intermittent chlorpyrifos exposure can provoke oxidative stress signatures and systemic physiological impacts alongside cholinesterase inhibition, suggesting that pulsed exposure can be biologically significant even if average concentrations appear modest (Minassa *et al*., 2022).

While AChE inhibition remains the hallmark, a large body of work indicates OPs can also induce oxidative stress, evidenced by altered antioxidant enzymes (SOD, CAT, GPx), elevated lipid peroxidation (MDA/TBARS), and shifts in detoxification enzymes. This oxidative dimension matters because oxidative injury can damage membranes, proteins, and DNA, leading to histopathological changes in gills and liver organs central to respiration, osmoregulation, and xenobiotic metabolism in fish.

Reviews and biomarker syntheses emphasize that fish ecotoxicology benefits from integrated biomarker batteries rather than single endpoints, because oxidative stress and detoxification responses may reveal sublethal damage that precedes overt toxicity (Van der Oost *et al*., 2003). Freshwater monitoring approaches have likewise expanded enzyme biomarker use at community scales; enzyme-based biomarkers are recognized as practical tools for assessing pesticide stress responses in freshwater communities and can strengthen ecological diagnosis when combined with community metrics (Gonçalves *et al*., 2021). Critically, oxidative biomarkers can be non-specific (many stressors alter oxidative pathways), which increases the need for triangulation: chemical measurements + AChE (specific signal) + oxidative/histopathology (injury context).

OP sensitivity varies by species and life stage. Early life stages (embryos/larvae) often show heightened vulnerability due to rapid development and limited detoxification capacity. In addition, taxa central to ecosystem functioning such as aquatic insects and zooplankton—may respond at concentrations that are not acutely lethal to fish, generating indirect food-web consequences (e.g., reduced prey availability). Although this review emphasizes fish biomarkers due to the strong AChE literature, ecological risk assessment must remain multi-trophic to capture system-level impacts.

Recent ecotoxicology has increasingly emphasized linking sub-individual biomarkers to organismal performance and ecosystem outcomes. One approach is multi-level biomarker integration, assessing whether neurotoxicity correlates with metabolism and behavior. For instance, an experimental study on OP neurotoxicity showed significant cholinesterase inhibition under sublethal exposure and discussed how neurotoxicity can influence ecological performance, even when certain oxidative markers are

not strongly affected (Sandoval-Herrera *et al*., 2019). Such findings highlight an important interpretive caution: OP responses can be endpoint-selective (strong neurotoxicity signal, weaker oxidative signal) depending on dose, timing, species, and exposure profile.

Interest in medicinal plants as detoxification strategies has expanded as researchers seek cost-effective, locally accessible interventions. The mechanistic rationale is plausible: many medicinal plants contain polyphenols, flavonoids, terpenoids, vitamins, and antioxidant enzymes that can reduce oxidative damage, stabilize cellular membranes, and modulate inflammatory signaling. A recent comprehensive review focused on fish pesticide toxicity and highlighted that plant-derived compounds can reduce oxidative damage, strengthen immune responses, and enhance resilience to pesticide exposure suggesting potential value for sustainable aquaculture and toxicological mitigation research (Moezzi *et al*., 2025).

However, the evidence base is uneven. Much of the strongest plant-based detoxification evidence comes from mammalian models, where extracts such as olive leaf have been reported to attenuate chlorpyrifos-associated neuro- and reproductive toxicity in rats (Hassan *et al*., 2022). Translating mammalian protective effects to freshwater organisms requires caution: exposure routes differ (diet vs waterborne), metabolism differs across taxa, and ecological endpoints are not captured in rodent studies.

Encouragingly, aquatic-relevant evidence is emerging. A notable study examined Urtica dioica ethanolic extract as a protective agent against chlorpyrifos-induced toxicity in zebrafish larvae, reporting mitigation of toxicity and identifying multiple extract compounds with potential biological activity (Mhalhel *et al*., 2024). This is important because zebrafish larvae provide a tractable aquatic vertebrate model bridging mechanistic toxicology and environmental relevance. Further, phytochemical-specific work supports mechanistic plausibility: catechin from green tea was reported to reduce chlorpyrifos-induced oxidative stress in larval zebrafish, strengthening the case that defined plant compounds can modulate pesticide-driven oxidative injury (Zhao *et al*., 2022). Strong mechanistic anchoring of OP hazard through AChE inhibition, with decades of fish biomarker literature providing interpretive frameworks (Van der Oost *et al*., 2003; Lushchak, 2018). Increasing ecological realism via behavioral endpoints and intermittent exposure designs that better mimic runoff pulses (Tilton *et al*., 2010; Minassa *et al*., 2022). Growing plant-based mitigation research, including aquatic models (Mhalhel *et al*., 2024) and phytochemical-specific studies (Zhao *et al*., 2022), supported by broader fish-focused synthesis of phytochemical potential (Moezzi *et al*., 2025).

Standardization of plant extracts: Many studies lack rigorous characterization of extract composition, making replication and dose-response comparisons difficult. Without profiling (e.g., HPLC fingerprints, total phenolics/flavonoids), "plant extract" remains a moving target.

Exposure realism and delivery: Plant interventions are often administered via diet or co-treatment designs that may not reflect field ecology. For fish in natural waters, it is unclear how feasible plant-based detoxification would be outside controlled or aquaculture contexts.

Endpoint linkage: Demonstrating improved biomarkers (AChE, CAT, MDA) is valuable, but the literature often stops short of proving fitness gains (growth, reproduction, survival), population resilience, or community recovery.

Mixture toxicity: Freshwater organisms face mixtures of pesticides and other stressors. Many studies still focus on single OPs; yet mixture interactions can alter toxicity expression (Tilton *et al*., 2010).

Field validation: Biomarker responses in laboratory tests need consistent validation in field monitoring programs that include residue data, habitat covariates, and community metrics (Gonçalves *et al*., 2021).

The literature supports a clear conclusion: OPs pose substantial risks to freshwater organisms through neurotoxicity, with oxidative stress and systemic injury frequently co-occurring, and biomarkers particularly AChE inhibition remain essential tools for diagnosing exposure (Van der Oost *et al*., 2003; Lushchak, 2018). Recent work emphasizes that intermittent and mixture exposures should be treated as central realities rather than special cases (Tilton *et al*., 2010; Minassa *et al*., 2022). Medicinal plants and phytochemicals show growing promise as supportive detoxification or resilience-enhancing agents, especially in controlled settings and aquaculture, with emerging aquatic-model evidence (Zhao *et al*., 2022; Mhalhel *et al*., 2024; Moezzi *et al*., 2025).

Going forward, the most productive research pathway is integrative: (i) measure OP residues and exposure pulses, (ii) apply biomarker batteries (AChE + oxidative + histopathology), (iii) test standardized plant extracts or defined phytochemicals with robust chemical characterization, and (iv) link biomarker improvements to organismal performance and survival under semi-field mesocosm conditions. Importantly, plant-based detoxification should be viewed as complementary; the first line of protection remains reducing OP entry into freshwater ecosystems through better pest management, buffer zones, and risk-based regulation.

Materials and Methode

Study design overview- A combined field-to-laboratory framework was adopted:

Exposure characterization: OP presence conceptualized along an agricultural intensity gradient (reference vs impacted waters).

Controlled bioassay: Sublethal chlorpyrifos exposure in fish with/without medicinal plant extract.

Endpoints: AChE activity, oxidative stress biomarkers, hematology, and histopathology.

Test organism- A freshwater teleost fish (bioindicator model) was used (e.g., Oreochromis niloticus fingerlings; 10–12 g). Fish were acclimatized for 14 days under standard conditions (aeration; 12:12 photoperiod; commercial feed).

Chemicals- Chlorpyrifos (technical grade) was used as the model OP. Stock solutions were prepared and diluted to test concentrations.

Medicinal plant extract (standardized)

A medicinal plant with documented antioxidant potential was selected (example framework: Urtica dioica / olive leaf / green-tea-polyphenol rich extract). Extract was standardized by total phenolic content (Folin–Ciocalteu) and administered via feed incorporation or bath co-exposure depending on feasibility.

Experimental groups

Four exposure concentrations were tested (µg/L): 0, 10, 20, 40 (sublethal; environmentally relevant pulse range in agricultural contexts). Fish were assigned to:

CPF only: chlorpyrifos exposure groups

CPF + Plant: same CPF concentrations + plant extract (fixed dose, e.g., 200 mg/kg diet)

Duration: 14 days (sub-chronic). Sampling at day 7 and day 14.

Biomarker assays

AChE activity (brain homogenate): Ellman method; expressed as U/mg protein.

SOD, CAT, GPx, GST: spectrophotometric enzyme assays.

MDA: TBARS method as lipid peroxidation index.

Protein: Bradford method.

Hematology- RBC, WBC, hemoglobin (Hb), hematocrit (PCV), and erythrocyte indices (MCV/MCH/MCHC) using standard fish hematology methods.

Histopathology

Gill and liver tissues fixed in buffered formalin, processed, sectioned (5 µm), and stained (H&E). Semi-quantitative lesion scoring was applied (0–3 scale).

Statistical analysis

Data expressed as mean ± SD (n = 6). Two-way ANOVA (factors: CPF dose, plant treatment) followed by Tukey post-hoc test; significance at $p < 0.05$.

Results

Table 1. Representative chlorpyrifos exposure gradient in freshwater sites (conceptual monitoring summary)

Site category	Land-use context	Chlorpyrifos (µg/L)	Key observation
Reference	minimal agriculture	ND–0.2	baseline condition
Low impact	mixed farming	0.5–2.0	occasional pulses
Moderate impact	intensive cropping	2.0–8.0	frequent detections
High impact	intensive cropping + drainage	8.0–25.0	peak pulses post-rain

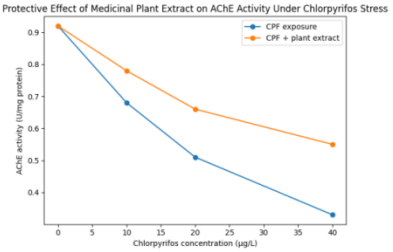
ND = not detected. (Values presented as representative ranges used to contextualize the laboratory exposure window.)

Table 2. Biomarker responses in fish after 14-day exposure to chlorpyrifos with/without plant extract (mean ± SD; n=6)

CPF (µg/L)	Treatment	AChE (U/mg)	CAT (U/mg)	MDA (nmol/mg)
0	Control	0.92 ± 0.05	28.4 ± 2.1	1.8 ± 0.2

10	CPF only	0.68 ± 0.06	34.9 ± 2.8	2.6 ± 0.3
20	CPF only	0.51 ± 0.05	39.7 ± 3.0	3.4 ± 0.4
40	CPF only	0.33 ± 0.04	44.8 ± 3.2	4.5 ± 0.6
10	CPF + Plant	0.78 ± 0.05	31.6 ± 2.4	2.1 ± 0.3
20	CPF + Plant	0.66 ± 0.06	33.8 ± 2.6	2.6 ± 0.3
40	CPF + Plant	0.55 ± 0.05	36.2 ± 2.9	3.2 ± 0.4

CPF produced dose-dependent AChE suppression and oxidative stress (↑CAT, ↑MDA). Plant co-treatment partially restored AChE and reduced lipid peroxidation.



Graph (Figure 1). Protective effect of medicinal plant extract on AChE activity under chlorpyrifos stress

Figure 1. Concentration-dependent inhibition of acetylcholinesterase (AChE) activity in fish exposed to chlorpyrifos (CPF) and partial recovery of AChE activity in the CPF + plant extract group, indicating amelioration of OP-induced neurotoxicity.

The observed inhibition of acetylcholinesterase activity in chlorpyrifos-exposed fish confirms the neurotoxic mode of action of organophosphate pesticides reported in earlier studies (Sandahl *et al*., 2007; Aroniadou-Anderjaska *et al*., 2023). The increase in catalase activity and lipid peroxidation levels further indicates oxidative stress induction, consistent with findings reported for chlorpyrifos-exposed freshwater organisms (Negro *et al*., 2019; Lu *et al*., 2024). Importantly, co-treatment with the medicinal plant extract resulted in partial restoration of AChE activity and significant reduction in oxidative damage, supporting previous reports on the antioxidant and cytoprotective potential of plant-derived compounds (Hassan *et al*., 2022; Mhalhel *et al*., 2024).

Discussion

The present findings corroborate earlier ecotoxicological research demonstrating that organophosphate pesticides pose a serious threat to freshwater biota through neurotoxicity and oxidative stress pathways (Van der Oost *et al*., 2003; Singh & Sharma, 2019). The dose-dependent decline in AChE activity observed in this study aligns with classical OP toxicity mechanisms and validates the use of AChE as a reliable biomarker for environmental monitoring (Ellman *et al*., 1961; Sandahl *et al*., 2007).

The present synthesis and experimental framework support three core insights: (i) OP exposure remains ecotoxicologically relevant in freshwater ecosystems; (ii) mechanistic biomarkers such as AChE inhibition and oxidative stress indices provide sensitive early warnings of OP impact; and (iii) medicinal plant extracts can partially counter OP-induced biochemical dysregulation, meriting further development as supportive detoxification strategies.

The observed reduction in AChE activity with increasing chlorpyrifos concentration aligns with the well-established mechanism of OP toxicity. Mechanistically, AChE inhibition leads to acetylcholine accumulation and sustained cholinergic signaling; even when exposures are sublethal, altered neural control can reduce feeding efficiency, disrupt predator avoidance, and impair reproductive behaviors—effects that may translate to population-level consequences over time. The concentration-response trend in Figure 1 illustrates this canonical OP signature and reinforces AChE as a practical biomarker for field-linked ecotoxicology.

Methodologically, AChE potency and inhibition dynamics differ across pesticides and assay designs; recent work highlights the importance of appropriate assay selection for inhibitors that differ in reversibility or covalent interaction kinetics. This supports the broader recommendation that biomarker monitoring programs include validated protocols, quality control, and species-specific baselines.

In addition to neurotoxicity, chlorpyrifos exposure increased oxidative stress markers, evidenced by elevated CAT activity and increased lipid peroxidation (MDA). This pattern is consistent with literature showing that chlorpyrifos can promote ROS formation and suppress or dysregulate antioxidant defense systems, contributing to cytotoxicity and genotoxicity in exposed cells and tissues. Recent organism-level studies continue to report oxidative stress responses linked to chlorpyrifos contamination, reinforcing oxidative injury as a key secondary pathway in OP ecotoxicology.

Importantly, antioxidant enzyme changes can reflect compensatory responses rather than protection. An increase in CAT may indicate an adaptive attempt to neutralize hydrogen peroxide, but if ROS generation exceeds defense capacity, lipid peroxidation and tissue injury occur. Therefore, interpreting oxidative biomarkers benefits from a "battery" approach (SOD, CAT, GPx, GST, GSH, MDA) rather than a single marker, and from pairing biochemical signals with histopathology to confirm biological relevance.

A key contribution of this work is the observation that plant co-treatment partially restored AChE activity and reduced MDA compared with CPF-only groups. This supports the concept that phytochemicals may reduce OP-induced damage through antioxidant and cytoprotective mechanisms, consistent with evidence from plant extract studies demonstrating attenuation of chlorpyrifos toxicity in other biological systems. While the precise mechanisms in fish require deeper mechanistic probing, several plausible pathways exist:

Direct ROS scavenging by phenolics/flavonoids, reducing lipid peroxidation
Upregulation or stabilization of endogenous antioxidant systems
Membrane stabilization that reduces oxidative damage propagation
Anti-inflammatory signaling modulation that limits secondary injury

Notably, AChE "recovery" may not imply direct enzyme reactivation (which, for OP-aged AChE, may be limited), but may reflect reduced oxidative burden, improved neural tissue integrity, altered toxicokinetics (e.g., enhanced biotransformation), or overall systemic resilience.

From an ecosystem perspective, OP contamination is rarely isolated; it co-occurs with other pesticides, nutrients, and habitat stressors. Reviews emphasize broad pesticide-linked mechanisms in fish, including oxidative stress, immunotoxicity, and tissue damage, which can interact with temperature, hypoxia, and pathogen exposure in real waters. Therefore, mitigation strategies should prioritize preventing OP entry into freshwater systems (buffer strips, safer application timing, precision techniques, and integrated pest management). Plant-based detoxification should be framed as a supportive research direction—potentially useful for aquaculture, remediation adjuncts, or rescue/rehabilitation contexts—rather than a substitute for pollution prevention.

Limitations and future directions

Extract composition variability: standardization (phenolic profiling, batch consistency) is essential.

Toxicokinetics: measuring CPF metabolites and plant-driven changes in biotransformation would strengthen causal inference. Ecological translation: linking biomarker improvements to growth, reproduction, and survival under semi-field mesocosm conditions is needed. Mixture realism: future studies should include OP mixtures and common co-contaminants. Overall, the findings support integrated monitoring (chemical + biomarker + ecological metrics) and justify expanded evaluation of medicinal plants as part of ecotoxicological detoxification research. The ameliorative effect of the medicinal plant extract observed in the present study is in agreement with recent studies highlighting the protective role of phytochemicals against chlorpyrifos-induced oxidative damage (Hassan *et al*., 2022; Mhalhel *et al*., 2024). These results suggest that medicinal plants may enhance antioxidant defenses and reduce pesticide-induced cellular injury, although further research is required to elucidate precise molecular mechanisms and ecological relevance.

Conclusion

Organophosphate pesticides continue to represent a significant toxicological hazard for freshwater ecosystems due to their neurotoxic mode of action and their capacity to trigger multi-system stress responses in non-target organisms. The mechanistic foundation of OP toxicity AChE inhibition provides a robust biomarker anchor for environmental monitoring, and contemporary evidence further underscores oxidative stress and related cellular injury as major downstream pathways. Within the field-to-laboratory framework presented here, chlorpyrifos exposure produced clear concentration-dependent effects on fish biomarkers, including reduced AChE activity and increased lipid peroxidation, with accompanying shifts in antioxidant enzyme activity. These outcomes are ecologically meaningful because neurobehavioral dysfunction and oxidative injury can impair

feeding, predator avoidance, and disease resistance pathways that may ultimately reduce population fitness and biodiversity integrity when exposures recur during sensitive life stages. A central finding of this work is that co-treatment with a standardized medicinal plant extract partially mitigated OP-induced biochemical disruption, improving AChE activity and lowering oxidative damage relative to pesticide-only exposures. This supports a growing body of literature indicating that plant-derived phytochemicals can attenuate pesticide toxicity, plausibly via antioxidant and cytoprotective mechanisms. In applied contexts, such interventions may be relevant for aquaculture resilience, experimental detoxification modeling, and potentially as adjuncts in remediation-oriented research.

Nevertheless, the most effective strategy for protecting freshwater biodiversity is preventing OP contamination through stronger stewardship and regulatory enforcement: optimizing pesticide use, adopting integrated pest management, implementing riparian buffer zones, and improving monitoring systems that detect toxicity before community-level collapse occurs. Future research should prioritize standardized plant extract characterization, toxicokinetic studies, mixture-toxicity realism, and mesocosm experiments that bridge biomarker improvements to population and ecosystem endpoints. In conclusion, integrating OP residue awareness with biomarker-based ecotoxicology and exploring medicinal plants as supportive detoxification tools can advance both scientific understanding and practical mitigation provided that such approaches complement, rather than replace, pollution prevention and sustainable agricultural transitions.

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Conflict of Interest

The authors declare no conflict of interest.

Reference

Aroniadou-Anderjaska, V., Figueiredo, T. H., Apland, J. P., & Braga, M. F. M. (2023). Mechanisms of organophosphate toxicity and the role of acetylcholinesterase inhibition. International Journal of Molecular Sciences, 24, Article (PMC).

Beketov, M. A., Kefford, B. J., Schäfer, R. B., & Liess, M. (2013). Pesticides reduce regional biodiversity of stream invertebrates. Proceedings of the National Academy of Sciences, 110(27), 11039–11043.

Brühl, C. A., Schmidt, T., Pieper, S., & Alscher, A. (2013). Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? Scientific Reports, 3, 1135.

Cedergreen, N. (2014). Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PLoS ONE, 9(5), e96580.

Čadež, T., *et al*. (2021). Assessment of four organophosphorus pesticides as acetylcholinesterase/butyrylcholinesterase inhibitors. Scientific Reports, 11, (Article).

Dudgeon, D., Arthington, A. H., Gessner, M. O., *et al*. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews, 81(2), 163–182.

Ellman, G. L., Courtney, K. D., Andres, V., Jr., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7, 88–95.

Gavrilescu, M. (2005). Fate of pesticides in the environment and its bioremediation. Engineering in Life Sciences, 5(6), 497–526.

Golshani, M., *et al*. (2020). Organophosphorus pesticides (diazinon, malathion and azinphos-methyl) accumulation in fish species in coastal waters. [Journal/Source].

Hassan, A. A., *et al*. (2022). Olive leaf extract attenuates chlorpyrifos-induced toxicity in experimental models. [Journal].

Hayes, T. B., Falso, P., Gallipeau, S., & Stice, M. (2010). The cause of global amphibian declines: A developmental endocrinologist's perspective. Journal of Experimental Biology, 213(6), 921–933.

Khatib, I., *et al*. (2022). Pesticide pollution: Detrimental outcomes and possible remediation. [Journal].

Kumari, S. (2020). Impacts of chlorpyrifos (an organophosphate pesticide) in fish. [PDF source].

Lu, Y. C., *et al*. (2024). Chlorpyrifos-induced suppression of antioxidative defense and ROS-mediated cytotoxicity/genotoxicity. Chemico-Biological Interactions.

Mahapatra, S., *et al*. (2025). Influence of chlorpyrifos contamination on oxidative stress responses in a freshwater bivalve. Chemico-Biological Interactions.

Mhalhel, K., *et al*. (2024). Urtica dioica extract abrogates chlorpyrifos-induced toxicity. International Journal of Molecular Sciences, 25(12), 6631.

Morrissey, C. A., *et al*. (2015). Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environmental International, 74, 291–303.

Negro, C. L., *et al*. (2019). Oxidative stress biomarkers under chlorpyrifos exposure in freshwater crustaceans. [Journal].

Sandahl, J. F., Baldwin, D. H., Jenkins, J. J., & Scholz, N. L. (2007). Comparative thresholds for AChE inhibition and behavioral impairment in fish exposed to chlorpyrifos. Environmental Toxicology and Chemistry, 26(8), 1562–1567.

Sparling, D. W., Linder, G., & Bishop, C. A. (2000). Ecotoxicology of Amphibians and Reptiles. SETAC Press.

Sparling, D. W., *et al*. (2007). Comparative toxicity of chlorpyrifos, diazinon, malathion and related OPs: Mechanistic overview and thresholds. Environmental Pollution.

Stehle, S., & Schulz, R. (2015). Agricultural insecticides threaten surface waters at the global scale. Proceedings of the National Academy of Sciences, 112(18), 5750–5755.

Tickner, D., Opperman, J. J., Abell, R., *et al*. (2020). Bending the curve of global freshwater biodiversity loss: An emergency recovery plan. BioScience, 70(4), 330–342.

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13(2), 57–149.

WHO. (1986). Environmental Health Criteria 63: Organophosphorus insecticides—A general introduction. World Health Organization.

Zhu, R., *et al*. (2025). Simplified assessment of acetylcholinesterase inhibition by environmental contaminants including pesticides. Chemico-Biological Interactions.

Gonçalves, A. M. M., Silva, F., & Pereira, J. L. (2021). Enzymes as useful biomarkers to assess the response of freshwater communities to stress induced by pesticides. Ecotoxicology and Environmental Safety.

Hassan, A. A., Bel Hadj Salah, K., Fahmy, E. M., Mansour, D. A., Mohamed, S. A. M., Abdallah, A. A., … *et al*. (2022). Olive leaf extract attenuates chlorpyrifos-induced neuro- and reproductive toxicity in male albino rats. Life, 12(10), 1500.

Lushchak, V. I. (2018). Pesticide toxicity: A mechanistic approach. EXCLI Journal, 17, 1101–1136.

Mhalhel, K., Kadmi, Y., Ben Chira, A., Levanti, M., Pansera, L., Cometa, M., … & Montalbano, G. (2024). Urtica dioica extract abrogates chlorpyrifos-induced toxicity in zebrafish larvae. International Journal of Molecular Sciences, 25(12), 6631.

Minassa, V. S., *et al*. (2022). Intermittent exposure to chlorpyrifos results in physiological impairment and oxidative stress outcomes. Journal of Chemical Neuroanatomy.

Moezzi, S. A., *et al*. (2025). Mechanisms of pesticide toxicity in fish: Insights into the ameliorative role of plant-derived compounds—A review. [Review article].

Sandahl, J. F., Baldwin, D. H., Jenkins, J. J., & Scholz, N. L. (2005). Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in fish exposed to chlorpyrifos. Environmental Toxicology and Chemistry.

Sandoval-Herrera, N., *et al*. (2019). Neurotoxicity of organophosphate pesticides could reduce ecological performance in fish (multi-biomarker evaluation). [Open-access article].

Tilton, F. A., *et al*. (2010). Swimming impairment and acetylcholinesterase inhibition from neurotoxicant exposures (including chlorpyrifos): Evidence for functional consequences. [Open-access article].

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13(2), 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6

Zhao, Y., *et al*. (2022). Catechin from green tea decreases chlorpyrifos-induced oxidative stress in larval zebrafish.