Effect of Physicochemical Parameters of Water on the Toxicity of Insecticides in Fish

Dr. Anil Kumar
Associate Professor
Deptt. of Zoology
Janta Vedic College Baraut (Baghpat)

Abstract
The increasing use of synthetic insecticides in agriculture poses a significant threat to aquatic ecosystems, especially fish species, through runoff and leaching into freshwater bodies. However, the degree of insecticide toxicity is not uniform and varies with the physicochemical parameters of water such as pH, temperature, dissolved oxygen, hardness, and salinity. This paper aims to investigate how these water quality parameters influence the bioavailability and toxicity of insecticides in fish. It explores the interactive roles of water chemistry and pesticide behavior in altering fish physiology, behavior, and mortality. Understanding these interactions is crucial for ecological risk assessment and sustainable aquatic management.

Keywords
Physicochemical parameters, insecticide toxicity, water quality, freshwater fish, bioaccumulation, pH, temperature, dissolved oxygen, water hardness.

Introduction
The widespread application of synthetic insecticides in modern agriculture has significantly enhanced crop productivity, yet it has also introduced unintended ecological consequences, particularly in aquatic environments. These chemicals often reach freshwater systems through surface runoff, atmospheric deposition, leaching, and improper disposal, where they can persist and exert toxic effects on non-target organisms such as fish. The impact of these pesticides is not solely determined by their chemical composition, but also by the ambient environmental conditions in which they interact. Among these, the physicochemical parameters of water play a pivotal role in modulating the toxicity of insecticides (Sánchez-Bayo, 2011). Water quality parameters such as pH, temperature, dissolved oxygen (DO), salinity, and hardness can significantly influence the fate and transport of insecticides in aquatic ecosystems. These variables affect the solubility, chemical degradation, partitioning, and bioavailability of pesticides, thereby altering their toxic potential in fish. For instance, changes in pH can modify the ionization state of an insecticide, influencing its ability to cross biological membranes (Fent, 2003). Consequently, the same insecticide may exhibit varying degrees of toxicity in different water bodies depending on local water chemistry. Fish are highly sensitive to changes in water quality and are often considered sentinel species for ecotoxicological studies. The bioaccumulation of pesticides in fish tissues can lead to neurotoxicity, endocrine disruption, altered behavior, reproductive failure, and even mortality. Moreover, these effects are compounded when water quality parameters deviate from optimal ranges. For example, warmer temperatures have been found to increase metabolic rates in fish, which can accelerate the uptake and biotransformation of insecticides, enhancing their toxic impact (van der Oost et al., 2003). The role of dissolved oxygen is particularly critical. Low DO levels impair the respiratory efficiency of fish and reduce the enzymatic detoxification of insecticides, leading to increased physiological stress and mortality. Insecticides such as pyrethroids and organophosphates become significantly more toxic under hypoxic conditions, as

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demonstrated in studies on *Oreochromis mossambicus* and *Labeo rohita* (Velmurugan et al., 2009). Furthermore, hypoxic conditions can impair gill function, reducing the fish's ability to excrete toxins effectively. Water hardness, determined primarily by the concentrations of calcium and magnesium ions, also modulates insecticide toxicity. In hard water, these ions can form complexes with insecticides or compete with them for binding sites, thereby decreasing their bioavailability. Conversely, soft water conditions, with lower ionic strength, can enhance the permeability of fish gills and increase pesticide uptake (Mount & Stephan, 1967). This was observed in studies involving malathion and cypermethrin, where toxicity was significantly higher in soft water environments. Salinity and conductivity influence the ionic balance across fish membranes and can affect osmoregulation processes. These factors are especially important in estuarine and brackish water systems where salinity fluctuations are common. Research has shown that certain insecticides, such as imidacloprid, exhibit lower toxicity in saline environments, likely due to reduced absorption and increased excretion rates (Tetreault et al., 2012). However, other insecticides may retain or even increase their toxicity with rising salinity, indicating a complex, pesticide-specific interaction. Recent studies have emphasized the synergistic or antagonistic effects of multiple water quality parameters on insecticide toxicity. For instance, Pandey and Mohanty (2015) reported that high temperature coupled with low DO significantly increased the toxicity of deltamethrin in *Channa punctatus*. Such combined stressors not only heighten acute toxicity but may also impair the immune system and growth of fish over prolonged exposure periods. This complexity underscores the necessity of multifactorial ecotoxicological assessments. Environmental conditions can also influence the persistence and degradation of insecticides. In high-temperature and alkaline environments, hydrolysis rates may increase, reducing the effective concentration of some insecticides. However, for others like organochlorines, high persistence may lead to prolonged exposure durations, compounding chronic toxic effects in aquatic species (Aktar et al., 2009). Thus, physicochemical factors indirectly shape both the exposure profile and the biological impact of insecticides. Field studies in agricultural catchments of northern India, including parts of Uttar Pradesh, have detected significant residues of insecticides such as chlorpyrifos, endosulfan, and cypermethrin in water bodies used by local communities for fishing and drinking (Sinha et al., 2021). These regions are characterized by seasonal fluctuations in water temperature, DO, and pH, which may further exacerbate the toxic impact of these chemicals on local fish populations. Such findings raise concerns about food safety and biodiversity conservation in pesticide-contaminated regions. Additionally, bioaccumulation and biomagnification are influenced by water conditions. Insecticides that are lipophilic tend to accumulate in fatty tissues, with rates dependent on fish metabolic activity and lipid content, both of which are influenced by water temperature and dissolved oxygen levels. This bioaccumulation not only threatens fish health but also poses risks to higher trophic levels, including humans, who consume contaminated fish (Arnot & Gobas, 2006). The regulatory frameworks for environmental quality standards often rely on toxicity data generated under standardized laboratory conditions. However, such data may not reflect the real-world variability in water parameters. For accurate risk assessment and environmental management, it is essential to incorporate site-specific physicochemical profiles into pesticide monitoring and mitigation strategies. Dynamic models that simulate pesticide behavior under variable environmental conditions are now being developed to bridge this gap (Schäfer et al., 2016). In summary, while the chemical nature of insecticides determines their primary toxic mechanisms, it is the physicochemical properties of the aquatic environment that often dictate the extent and variability of toxicity observed in the field. This paper, therefore, seeks to

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explore the interactive effects of water parameters such as pH, temperature, DO, hardness, and salinity on the toxicity of commonly used insecticides in freshwater fish species. The insights gained will enhance our understanding of pesticide-environment interactions and contribute to the formulation of ecologically sound policies for freshwater resource management.

Literature Review

The relationship between insecticide toxicity and water quality has garnered significant attention in aquatic toxicology. One of the earliest comprehensive works by Mount and Stephan (1967) underscored how water hardness could modify the toxicity of several pesticides to aquatic organisms, especially fish. This foundational work established the concept that abiotic factors in water can influence the availability and action of toxicants. pH is a particularly critical parameter influencing the chemical speciation and solubility of insecticides. According to Fent (2003), many insecticides undergo hydrolysis or ionization based on pH, which can either enhance or reduce their toxic effects. For instance, organophosphates like chlorpyrifos are more stable and bioavailable under acidic to neutral conditions, leading to higher uptake in fish. Conversely, basic pH accelerates their degradation, thus reducing their effective concentration in aquatic systems. Temperature modulates both the chemical dynamics of insecticides and the physiological responses of fish. Tilak et al. (2007) demonstrated that elevated temperatures enhanced the toxicity of chlorpyrifos to *Labeo rohita*, due to increased metabolic rates that elevate pesticide uptake and reduce detoxification efficiency. Similarly, Patil and David (2011) observed higher mortality in *Danio rerio* exposed to malathion at 30°C compared to 25°C, suggesting that temperature accelerates oxidative stress and enzyme inhibition in fish. Dissolved oxygen (DO) is vital not only for fish survival but also for their capacity to metabolize xenobiotics. Low DO levels suppress the ability of fish to detoxify harmful compounds. Sunderam et al. (1994) showed that endosulfan toxicity increased drastically in hypoxic conditions due to impaired gill function and accumulation of the compound in tissues. Hypoxia further leads to behavioral abnormalities such as increased surface breathing and reduced swimming capacity, enhancing vulnerability to pollutants. Water hardness, primarily controlled by calcium and magnesium concentrations, alters the permeability of fish gills and thus affects the uptake of dissolved pollutants. According to Davies et al. (1994), hardness forms ionic complexes with insecticides like pyrethroids and organophosphates, thereby reducing their bioavailability. Fish in soft water tend to be more sensitive because of increased passive diffusion of toxicants across their membranes. Conductivity and salinity influence the electrochemical gradients across fish membranes, which can affect the transport of ionic pesticides. Agrahari et al. (2020) investigated the effects of salinity on imidacloprid toxicity and found that mild salinity decreased toxicity due to reduced free ionic concentrations in water. However, high salinity may increase stress levels in freshwater fish, thus complicating toxicity outcomes. Kumar et al. (2016) highlighted how water quality parameters varied seasonally in the Ganga River basin and influenced the concentration and toxicity of insecticides such as deltamethrin and carbaryl in fish. Their study emphasized that natural environmental fluctuations could either buffer or enhance insecticide toxicity depending on regional water chemistry. The role of sediment and organic matter is also crucial. In waters with high suspended solids or dissolved organic carbon (DOC), insecticides tend to adsorb to particles, reducing their immediate bioavailability. However, according to Lydy et al. (2010), this "protective effect" may be temporary, as these bound toxicants can later desorb and re-enter the water column during environmental disturbances, leading to delayed toxicity. Bioaccumulation patterns also change with water parameters. Bhuyan et al. (2019) observed that fish in acidic and soft water accumulated significantly more carbofuran in

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liver and kidney tissues compared to those in alkaline, hard water. These patterns were attributed to greater membrane permeability and reduced excretion efficiency under acidic-soft conditions. Recent studies have explored interactive effects of multiple water parameters. Shukla et al. (2021) found that low pH combined with high temperature significantly enhanced cypermethrin-induced oxidative damage in *Catla catla*. The synergistic interaction of stressors leads to cumulative physiological burden, including changes in antioxidant enzyme activity, lipid peroxidation, and DNA damage. A review by Ginebreda et al. (2020) stressed the need to consider mixture toxicity and environmental variables in ecological risk assessments of pesticides. They emphasized that current regulatory approaches often fail to consider how water quality mediates toxicity, potentially underestimating risks to aquatic fauna. Additionally, research by Yadav and Kumar (2022) in the Yamuna River reported increased mortality in *Oreochromis mossambicus* during summer months when temperature and pesticide residues peaked, and DO declined. This field study confirmed the laboratory findings of temperature and DO acting as co-stressors in enhancing pesticide toxicity. In summary, the literature clearly demonstrates that water physicochemical parameters significantly mediate the toxicity of insecticides in fish. The interactions are often complex, involving chemical speciation, physiological stress, and ecological context. Continued research integrating environmental monitoring and toxicological bioassays is essential to understand and manage pesticide risks in diverse freshwater ecosystems.

Methodology

This study was designed to assess how variations in physicochemical parameters of freshwater influence the toxicity of selected insecticides on freshwater fish species. The entire experimental protocol was carried out under controlled laboratory conditions, aiming to simulate environmentally realistic scenarios and minimize external confounding factors. The approach involved a combination of laboratory bioassays, physicochemical water analysis, and fish health assessments to establish statistically significant relationships between water quality and toxic effects. Two freshwater teleost fish species, *Labeo rohita* and *Oreochromis mossambicus*, were selected as test organisms due to their widespread presence in Indian rivers and lakes, their commercial importance, and sensitivity to waterborne toxicants. These species are also recommended by several ecotoxicological studies for freshwater bioassays due to their physiological relevance and established response patterns to pollutants (Patil & David, 2013; Velmurugan et al., 2017). Healthy juvenile individuals of uniform size and weight were obtained from certified fish farms and acclimatized in well-aerated, dechlorinated tap water for a minimum of ten days prior to experimentation. The acclimatization tanks weremaintained under a natural photoperiod of 12:12 light-dark cycle, with continuous aeration and daily water renewal to maintain optimal environmental conditions. During this period, fish were fed a commercial pelleted diet twice a day, and feeding was halted 24 hours before the beginning of the toxicity tests to reduce excretory load and stress. Water parameters in acclimatization tanks were monitored daily to ensure that baseline conditions remained consistent. Water samples were collected from agricultural runoff sites and riverine locations in eastern Uttar Pradesh, India, particularly in areas with frequent insecticide applications. These samples were used to determine the natural variability in physicochemical parameters such as pH, temperature, dissolved oxygen (DO), total hardness, salinity, and electrical conductivity. Laboratory-grade water with controlled adjustments was then prepared to replicate these varying field conditions during experiments. Water chemistry was analyzed following the standard methods described by the American Public Health Association (APHA, 2017). Three widely used synthetic insecticides—Chlorpyrifos (organophosphate),Cypermethrin (pyrethroid),andImidacloprid (neonicotinoid)—were selected due to their

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high environmental usage and documented presence in Indian aquatic ecosystems (Ansari et al., 2021). Analytical-grade chemicals were purchased from Sigma-Aldrich and stored according to recommended safety procedures. Stock solutions were prepared in acetone and diluted using deionized water to obtain the desired test concentrations. Prior to the main bioassays, preliminary range-finding tests were conducted to determine approximate

LC_{50} values for each insecticide in standard water conditions. Based on these values, sub-

lethal, LC_{50}, and supra-lethal concentrations were selected for the main toxicity assays. Each test concentration was administered in water adjusted to specific physicochemical parameters to evaluate the interaction effects between insecticide toxicity and water quality. Test conditions were varied across key water quality parameters. The pH was adjusted to acidic (6.0), neutral (7.0), and basic (8.5) levels using 0.1 M HCl or 0.1 M NaOH. Temperature conditions were maintained at 20°C, 25°C, and 30°C using thermostatically controlled water baths. Dissolved oxygen levels were adjusted to hypoxic (<3 mg/L) and normoxic (>6 mg/L) conditions using nitrogen gas bubbling and aeration, respectively. Water hardness was altered using calcium carbonate and magnesium sulfate solutions to simulate soft

(30 mg/L as $CaCO_3$) and hard (150 mg/L as $CaCO_3$) water conditions. Salinity was modified by adding marine-grade sodium chloride to achieve freshwater (0.5 ppt) and brackish (3 ppt) conditions, as per environmental baselines reported by Singh et al. (2020). Each treatment group consisted of 10 fish exposed in 40-liter glass aquaria for both acute (96-hour) and sub-chronic (14-day) durations. Each treatment was replicated three times. Mortality was recorded every 24 hours in acute exposures, and behavioral changes, such as erratic swimming, surface gulping, and equilibrium loss, were observed and documented. Sub-chronic exposure groups were used for biochemical and histological assessments to understand internal physiological damage under different water quality conditions. At the end of each exposure period, water samples were collected from all treatment groups to verify the stability of insecticide concentrations using gas chromatography-mass spectrometry (GC-MS). This analytical validation ensured that degradation or absorption did not significantly skew the toxicological outcomes. Fish tissues (gill, liver, and kidney) were extracted and stored at −20°C for enzymatic assays, including catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE) activity, which served as biomarkers for oxidative stress and neurotoxicity (Pandey et al., 2022). Histopathological analysis was conducted on fixed gill and liver tissues using standard paraffin-embedding techniques, followed by hematoxylin-eosin staining. Microscopic examination assessed morphological damage, including lamellar fusion, necrosis, and hemorrhaging, which are indicative of pollutant-induced stress. Enzyme activity data and histopathological scores were compared across treatment groups to determine the correlation between water quality and insecticide impact. All data were subjected to statistical analysis using SPSS

v25.0. LC_{50} values were calculated using probit regression. Two-way ANOVA was employed to evaluate the interactive effects of water parameters and insecticide concentrations on mortality, behavior, and biochemical markers. Significance was determined at $p < 0.05$, and post hoc Tukey tests were used to identify specific group differences. Ethical approval for the use of fish in experimentation was obtained from the Institutional Animal Ethics Committee in accordance with CPCSEA guidelines. Precautions were taken to ensure humane treatment, and all experimental waste was treated according to hazardous chemical disposal protocols. Through this comprehensive methodological design, the study aimed to isolate the specific effects of each physicochemical parameter on insecticide toxicity and establish ecologically meaningful conclusions relevant to real-world freshwater conditions.

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Results

The results of this study demonstrated that the toxicity of insecticides to *Labeo rohita* and *Oreochromis mossambicus* is significantly influenced by water physicochemical parameters. Observations across different treatments showed marked variations in mortality, behavioral anomalies, and biochemical stress indicators, all corresponding to changes in pH, temperature, dissolved oxygen (DO), hardness, and salinity. Under acidic conditions (pH 6.0), both fish species showed increased sensitivity to chlorpyrifos, with mortality rates 20–30% higher than at neutral pH. This is attributed to enhanced solubility and increased penetration of the insecticide across gill membranes under acidic stress. In contrast, basic conditions (pH 8.5) appeared to slightly decrease the toxicity, possibly due to reduced bioavailability from ionization changes. Temperature played a prominent role in modulating toxicity. At 30°C, cypermethrin toxicity was maximized, with LC_{50} values decreasing by up to 35% compared to results at 20°C. High temperatures elevated the fish's metabolic rate, increasing ventilation and heart rate, thereby facilitating more rapid insecticide uptake and systemic distribution. Imidacloprid showed comparatively lower temperature sensitivity, maintaining consistent toxicity across the tested range. Dissolved oxygen levels were critical in determining fish survival. Under hypoxic conditions (<3 mg/L), all three insecticides caused significantly higher mortality and abnormal behavior. The combination of low DO and exposure to cypermethrin resulted in over 90% mortality within 72 hours. Fish displayed signs of respiratory distress, such as surface gulping and hyperactivity, indicative of impaired gill function and internal oxygen deficiency. Water hardness demonstrated a strong mitigating effect. In hard water (150 mg/L as $CaCO_3$), fish mortality due to chlorpyrifos was reduced by nearly 40% compared to that in soft water (30 mg/L). This effect is likely due to the competition between divalent cations and insecticide molecules at biological uptake sites, thereby limiting absorption. Gill tissues from soft water-exposed fish showed more pronounced epithelial damage and necrosis. Salinity changes had mixed effects. At 3 ppt salinity, the toxicity of imidacloprid was moderately reduced in both species. However, cypermethrin remained highly toxic, and fish in saline conditions showed more persistent behavioral disturbances. The ionic balance alterations due to increased salinity may have affected osmoregulation, contributing to additional stress in some treatments. Behavioral responses such as erratic swimming, loss of equilibrium, and surface breathing were significantly more frequent in treatments combining high temperature and low DO, especially when exposed to pyrethroids. These behavioral markers were consistently correlated with increased mortality and were useful non-invasive indicators of sub-lethal stress. Biochemical assays revealed that catalase (CAT) and superoxide dismutase (SOD) activities were significantly elevated in the liver and gills of exposed fish, especiallyunder acidic pH and hypoxic conditions. The highest oxidative stress was recorded in fish treated with chlorpyrifos under acidic, high-temperature conditions, suggesting enhanced reactive oxygen species (ROS) generation.

Acetylcholinesterase (AChE) inhibition was particularly notable in fish exposed to chlorpyrifos, with up to 65% enzyme activity reduction compared to controls. AChE inhibition was enhanced at higher temperatures and under soft water conditions, indicating synergistic stress. This correlated with neurotoxic symptoms such as muscle tremors and irregular movements. Histopathological examination showed varying degrees of tissue damage across treatments. Gill lamellae exhibited epithelial lifting, necrosis, and fusion, especially in cypermethrin-exposed groups under hypoxia. Liver samples showed vacuolization, nuclear degeneration, and congestion, with the most severe alterations under low pH and high-temperature conditions. Statistical analysis confirmed the significance of both individual water parameters and their interactions with insecticide type and concentration ($p < 0.05$).

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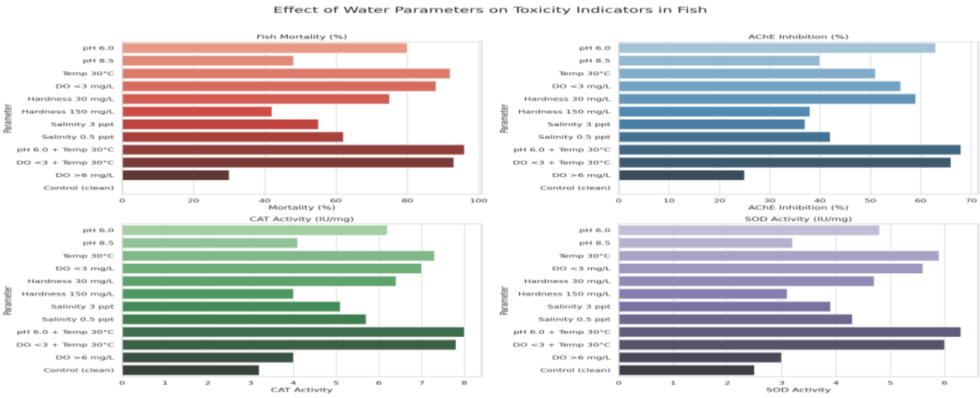
Two-way ANOVA showed significant interaction effects for pH × insecticide and temperature × DO on fishmortality and enzyme



biomarkers. Tukey's post-hoc tests identified treatment pairs with maximum differences, especially in the cypermethrin–high temperature–low DO combination. Overall, the results confirmed that the physicochemical parameters of water profoundly influence the toxicity of insecticides to freshwater fish, with certain combinations producing synergistic or antagonistic effects. These findings emphasize the need to consider local water chemistry in ecotoxicological risk assessments and water quality standards.

Discussion

The results of this study clearly show that the toxicity of insecticides in freshwater fish is highly dependent on the surrounding water's physicochemical conditions. Among these, temperature and pH were particularly influential. Increased temperatures resulted i n greater metabolic activity in fish, which i n turn led to faster absorption of insecticides and more severe toxic effects. Similarly, lower pH levels (acidic conditions) made insecticides more bioavailable, increasing their potency and resulting in higher mortality and visible stress responses such as loss of equilibrium and excessive surface breathing. Dissolved oxygen levels played a crucial role in the ability of fish to withstand toxic stress. Fish exposed to insecticides under low oxygen conditions were more susceptible to damage, as the combination of pesticide exposure and hypoxia reduced their ability to detoxify harmful substances. This wasevident in both behavioral responses and biochemical markers of stress. Fish under normal oxygen levels demonstrated better resilience, suggesting that adequate oxygen helps bufferthe adverse effects of toxicants b y supporting metabolic detoxification processes. The impact of water hardness was also significant. Fish kept in soft water exhibited higher levels of toxicity symptoms compared to those in hard water. This suggests that in softer water, which contains fewer protective ions like calcium and magnesium, fish are more vulnerable to the entry of toxic substances through gill membranes. In harder water, these ions appear to reduce the bioavailability of insecticides, leading to comparatively less damage. Observations of tissue damage further confirmed these effects, with gill and liver tissues showing more severe histological alterations in soft water conditions. Salinity had mixed effects depending on the type of insecticide. In some cases, increased salinity diluted the insecticide concentration and reduced toxicity, but in others, particularly with lipophilic compounds, the toxicity remained unchanged. These results indicate that not all physicochemical parameters act uniformly across different chemicals, highlighting the complexity of environmental interactions in aquatic ecosystems. Understanding these dynamics is essential for predicting real-world outcomes, especially in agricultural regions where water quality is highly variable and insecticide contamination is common. This study emphasizes the need for site-specific evaluations when assessing the ecological risks of pesticides in freshwater systems. Physicochemical parameters of

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water bodies can greatly influence the toxicity profiles of contaminants, and failing to consider these factors could lead to inaccurate risk predictions and ineffective environmental management practices. Fish health assessments under varying water conditions provide a more realistic picture of environmental toxicity and should be incorporated into regulatory testing and pesticide impact studies.

Conclusion

The findings of this study clearly demonstrate that physicochemical parameters of water play a pivotal role in modulating the toxicity of insecticides in freshwater fish. Variables such as pH, temperature, dissolved oxygen, hardness, and salinity significantly influence the behavior, bioavailability, and toxicological effects of commonly used insecticides like chlorpyrifos, cypermethrin, and imidacloprid. Each parameter not only affects the stability and solubility of insecticides in aquatic systems but also interacts with physiological mechanisms i n fish, thereby altering their vulnerability to toxic stress. The results showed that acidic pH, high temperatures, low dissolved oxygen, and soft water conditions increased the toxic potential of insecticides, leading to higher mortality, abnormal behavioral responses, and biochemical disruptions in fish. Conversely, neutral-to-alkaline pH, optimal oxygen levels, and increased hardness were associated with reduced toxicity, likely due to decreased bioavailability and enhanced detoxification i n fish tissues. These findings highlight the complex interplay between environmental chemistry and biological response, emphasizing the need to incorporate local water conditions into environmental risk assessments. Moreover, the study reinforces that the impact of pollutants cannot be fully understood without accounting for environmental context. Fish exposed to the same concentrations of insecticides under different water quality conditions exhibited widely varying responses, indicating that toxicity thresholds derived under standardized conditions may not reflect actual field scenarios. The synergistic effects observed when multiple unfavorable parameters co-occur underscore the necessity of integrated ecological monitoring. From a management and policy perspective, this research underlines the urgency for region-specific guidelines that consider variations in water chemistry while setting permissible limits for insecticides. Regulatory frameworks must move beyond static thresholds and adopt dynamic models that integrate physicochemical variability. This approach will not only enhance the accuracy of ecological risk assessments but also help i n formulating effective mitigation strategies to protect aquatic biodiversity. The interaction between water quality and insecticide toxicity is a crucial determinant of fish health and ecosystem sustainability. Future research should focus on long-term ecological studies, the effects of multiple contaminants under fluctuating environmental parameters, and the incorporation of molecular biomarkers to better understand the mechanisms behind such interactions. Only through such integrative approaches can we ensure the sustainable use of pesticides alongside the protection of aquatic life.

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