

EVALUATION OF ALKALINE PHOSPHATASE MODULATION IN *CIRRHINUS MRIGALA* FOLLOWING ACUTE PIRIMICARB EXPOSURE

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ABSTRACT

The quantification of toxicity is a highly complex process as toxic effects may manifest in both acute and chronic forms, and their intensity varies considerably depending on several biological and environmental determinants. These include species differences, genetic makeup, physiological conditions, environmental parameters, age, sex, health status, and nutritional background of the exposed organisms. Unlike experimental model organisms, which are often inbred and genetically uniform, the human population is highly outbred and exhibits extensive genetic variability. This genetic heterogeneity significantly influences susceptibility to toxicants, making toxicity assessment in humans more intricate. The simplest indices of toxicity are expressed in terms of organismal mortality or morbidity. Pirimicarb is a selective carbamate insecticide, widely employed as an aphicide for the control of aphid infestations since the 1990s in India. It has a molecular weight of 238.29 g/mol, appears as a colourless crystalline powder, and exhibits physicochemical characteristics such as solubility in water at 2700 mg/L, density of 1.21 g/cm³, and a vapour pressure of 7.28×10^{-6} mmHg. Owing to its strong aphicidal efficiency, Pirimicarb is favoured over other carbamates and is extensively used in integrated pest management practices worldwide. In the present investigation, the alkaline phosphatase response in the freshwater fish *Cirrhinus mrigala* subjected to acute pirimicarb exposure was evaluated. This study was designed to elucidate the enzymatic alterations triggered by pesticide-induced stress and to provide insights into the biochemical consequences of short-term exposure, thereby contributing to the understanding of pirimicarb toxicity in aquatic organisms.

KEYWORDS: Pirimicarb toxicity, *Cirrhinus mrigala*, acute exposure, alkaline phosphatase, environmental risk assessment, aquatic ecotoxicology.

INTRODUCTION

Aquatic toxicology is a multidisciplinary field that primarily investigates the measurement and assessment of contaminant levels to evaluate the hazards posed to aquatic environments. Beyond merely quantifying pollutants, this discipline extends to understanding the mechanisms by which contaminants influence aquatic organisms, ecosystems, and even human health through bioaccumulation and food web transfer. By definition, aquatic toxicology encompasses the study of the adverse effects of synthetic chemicals, naturally occurring substances, and various materials on aquatic biota. These effects can be observed across multiple levels of biological organization, including cellular, tissue, organ,

organismal, population, community, and ecosystem scales. Historically, the detrimental consequences of anthropogenic pollutants on aquatic environments and human well-being have been well documented, with numerous ecological disasters underscoring the urgency of such studies (Pritchard, 1993). The overarching objectives of aquatic toxicology can be outlined as: understanding stress induction, linking biological levels, comparative response analysis, determining threshold concentrations and advancing methodologies to integrate cutting-edge techniques for toxicant screening, effluent regulation, biomonitoring, and ecological risk assessment. In essence, aquatic toxicology serves as a critical link between environmental chemistry, biology,

and human health, ensuring that both ecological balance and public safety are preserved in the face of increasing chemical stressors.

Pirimicarb is a selective carbamate insecticide that has been extensively employed for the control of aphid infestations in agricultural crops since its introduction in India during the 1990s. Owing to its high specificity against aphids and relatively lower toxicity toward beneficial insects such as pollinators and predators, pirimicarb is considered an important tool in integrated pest management programs. Its selective mode of action makes it preferable over several broad-spectrum carbamates and organophosphates. Chemically, pirimicarb is identified as N, N-dimethylcarbamoyl-4,5-dimethylpyrimidin-2-amine. It belongs to the class of synthetic amino pyrimidines and tertiary amino compounds derived from dimethylcarbamic acid. The compound occurs as a colourless crystalline powder. Pirimicarb acts primarily by inhibiting acetylcholinesterase, a key enzyme responsible for hydrolysing the neurotransmitter acetylcholine at synaptic junctions. Inhibition of acetylcholinesterase results in accumulation of acetylcholine, leading to overstimulation of cholinergic receptors, disrupted nerve transmission, paralysis, and eventual death of the target organism. Compared to other carbamates, pirimicarb demonstrates greater aphicidal potency, higher crop safety and reduced impact on non-target beneficial insects, which explains its worldwide adoption in pest management.

Despite its agricultural importance, widespread use of pirimicarb raises concerns regarding its ecotoxicological implications, particularly for non-target aquatic organisms. Runoff from agricultural fields and leaching processes may introduce pirimicarb into aquatic ecosystems, where it can interact with fish and other aquatic fauna, potentially altering biochemical and physiological processes. Such risks necessitate investigations into biomarkers such as enzymatic responses to assess its toxicity and environmental impact.

Historically, toxicology has been deeply interwoven with the origins of therapeutic and experimental medicine, providing the foundation for understanding the adverse and beneficial effects of chemical substances on biological systems (Gallo, 2008). In its earliest form, toxicology focused primarily on describing the antagonistic effects of exogenous agents. However, over the decades, the discipline has advanced significantly, evolving into a modern science that now integrates molecular biology, biochemistry, and systems-level approaches. Today, toxicants are not only studied for their harmful impacts but are also utilized as molecular tools to probe fundamental biological processes (Calabrese and Calabrese, 2013). During the last century, toxicology has expanded its scope by incorporating principles and methodologies from multiple scientific

domains, including biology, chemistry, physics, and mathematics, thereby transforming into a truly multidisciplinary science (Fortun and Fortun, 2005). A notable milestone in this progression was the shift, toward applying toxicological knowledge to safety evaluation and risk assessment. This transition emphasized the role of toxicology not only in describing hazards but also in predicting, preventing, and regulating adverse effects of chemicals on human and environmental health (Choy, 2001).

Mechanistically, toxicity often be initiated by a single molecular event triggered upon exposure to a toxicant, which then propagates into a cascade of biochemical and physiological disturbances (Groh et al., 2005). Such interactions can disrupt metabolic pathways and ultimately lead to structural and functional alterations within cells. Toxicants may bind or react with critical cellular macromolecules including proteins, carbohydrates, lipids, and nucleic acids (DNA and RNA), resulting in impaired cellular function, oxidative stress, and long-term genomic instability (Srere, 1987). Thus, toxicology has evolved from a descriptive science of poisons to a highly sophisticated discipline capable of elucidating molecular mechanisms of toxicity, predicting health and ecological risks, and supporting the design of safer chemicals and therapeutic interventions. Toxicants represent a highly diverse group of substances, and their classification can be approached from multiple perspectives depending on their chemical nature, function, mode of action or origin (Ashauer and Jager, 2018). Such categorization is essential for understanding their toxic kinetics, predicting ecological and health risks and establishing regulatory guidelines. Another widely applied basis of classification is the route of exposure and intended use. Toxicants are frequently grouped according to the pathway through which organisms encounter them such as inhalation, ingestion, dermal absorption or aquatic exposure. Similarly, their functional uses often dictate their toxicological grouping, including pesticides, pharmaceuticals, food additives, solvents, heavy metals, or radioactive materials. Beyond these categories, toxicants may also be classified according to mode of toxic action e.g., enzyme inhibitors, mutagens, teratogens, endocrine disruptors, or neurotoxins, chemical structure e.g., organophosphates, carbamates, chlorinated hydrocarbons or target organ toxicity e.g., hepatotoxins, nephrotoxins, neurotoxins. Such multidimensional classification systems not only facilitate the systematic study of toxicants but also help in risk assessment, environmental monitoring and public health management. They provide a framework for linking the physicochemical properties of toxicants with their biological impacts across species and ecosystems, enabling scientists and regulators to predict hazards more accurately.

The aquatic environment serves as both a sink and a transport medium for a wide variety of toxicants, many of which enter through diverse anthropogenic activities

such as wastewater discharge, agricultural runoff, land spillage and industrial effluent release (Ritter et al., 2002). Once introduced, these contaminants can persist, bio accumulate, and exert harmful effects on aquatic organisms, ultimately influencing ecosystem balance and posing risks to human health through trophic transfer. The toxic effects induced in freshwater and marine organisms are widely recognized as early indicators of environmental and human health risks (Jaishankar et al., 2014). Aquatic organisms, especially fish and invertebrates, are sensitive biological sentinels that reflect the degree of contamination in their habitats, making them valuable for biomonitoring studies. Traditionally, acute lethal toxicity tests have been the primary method for assessing chemical hazards in aquatic systems. These tests determine lethal concentration thresholds, such as LC_{50} , and provide a standardized means to compare toxicant effects across species and chemicals. However, acute tests often represent short-term, high-dose scenarios that are less likely to occur under realistic environmental conditions (Coelho et al., 2011). Consequently, while they remain important for regulatory purposes and interspecies comparisons, they may not fully capture the long-term ecological implications of contaminant exposure.

To address this limitation, sub-lethal and chronic toxicity assessments have gained prominence. These approaches examine disturbances in behaviour, physiology, reproduction, growth and metabolism, which may occur at concentrations well below lethal thresholds. One advanced methodology is the complete life cycle fish test, designed to evaluate chemically induced alterations across developmental stages, thereby providing a more holistic view of toxicant impacts (Young et al., 2006). Furthermore, multidisciplinary research in aquatic toxicology has expanded our understanding of how anthropogenic activities and chemical contaminants alter aquatic systems. Fields such as molecular biology, environmental chemistry, physiology and ecology now converge to unravel mechanisms of toxicity and predict ecological consequences (Scott & Sloman, 2004). Importantly, aquatic toxicology not only enhances our comprehension of ecological impacts but also plays a crucial role in human health risk assessment. By quantifying exposure routes, bioaccumulation pathways, and toxic responses, this discipline provides valuable insights into the potential hazards faced by human populations reliant on contaminated water sources and aquatic food chains (Fitzgerald et al., 2021).

In essence, aquatic toxicology represents a vital framework for understanding and mitigating the risks posed by chemical contaminants. Through acute and chronic toxicity testing, mechanistic studies, and multidisciplinary approaches, it bridges the gap between environmental monitoring, ecological protection and human health safety. Pesticides are defined as substances or formulations designed to prevent, suppress or eliminate pests, thereby protecting agricultural

productivity, public health and stored products (Zacharia, 2011). While the formulations and application methods may vary widely, the fundamental principle underlying all pesticides remains consistent: they are chemically active substances capable of exerting toxic effects on target organisms while ideally posing minimal risk to non-target species (Ortiz-Hernández et al., 2013). This selective toxicity is central to their effectiveness in pest management and environmental safety.

The target organisms of pesticides encompass a broad spectrum of pests that threaten human well-being, agriculture, and ecological balance. These include insects, weeds, mites, nematodes, molluscs, birds, fish, and microbial pathogens (Costa, 2008). These organisms are considered pests because they compete with humans for food resources, transmit or cause diseases, damage crops and create nuisances in domestic and industrial environments. The diversity of pest species necessitates the development of specialized pesticide classes such as insecticides, herbicides, fungicides, rodenticides and bactericides each designed to act on specific biological pathways or vulnerabilities of the target organism. Although pesticides are formulated to maximize efficacy against pests, their non-target effects cannot be entirely eliminated. Sub-lethal exposure or bioaccumulation may impact aquatic and terrestrial organisms, potentially leading to disruptions in ecological balance and indirect risks to human health. Therefore, understanding the toxicological profiles, modes of action and environmental fate of pesticides is crucial for developing safer and more sustainable pest management strategies. In summary, pesticides are powerful chemical tools designed for pest control, with effectiveness determined by selective toxicity, spectrum of action and safe integration into human and environmental systems. Their study requires a careful balance between maximizing pest suppression and minimizing ecological and human health risks.

Pirimicarb is a selective insecticide that belongs to the chemical class of carbamate pesticides, widely recognized for their acetylcholinesterase-inhibiting properties (Bullock, 1973). Among insecticides, pirimicarb is particularly effective against aphids, making it a preferred choice in integrated pest management programs. It is most commonly applied as a foliar spray on crops infested with aphids (Furk et al., 1980). Aphids are small, sap-sucking insects classified under the superfamily Aphidoidea (Van Emden, 2017). Commonly known by vernacular names such as greenfly, plant louse, ant cow or blackfly, aphids are soft-bodied and generally pinhead-sized, making them inconspicuous yet highly damaging to plants. Many species are characterized by a pair of tubular projections called cornicles on their abdomen, which serve as defensive structures secreting alarm pheromones (Eastop et al., 1976). Additionally, some species secrete waxy filaments from specialized wax glands, giving them a white woolly appearance, which is commonly observed in woolly

aphids. The life cycle of aphids is notably complex, exhibiting both sexual and asexual reproduction. The adult female, often termed the stem mother or wingless female, can produce offspring parthenogenetically, meaning young are generated without fertilization (Kennedy & Stroyan, 1959). These nymphs grow and metamorphose into adults, some of which develop two pairs of membranous wings, enabling dispersal. Adult aphids include both males and females that engage in sexual reproduction, with females laying eggs that give rise to successive generations (Kennedy & Stroyan, 1959). This rapid reproductive cycle, combined with high fecundity and dispersal ability, renders aphids highly adaptive and resilient, making them significant agricultural pests. Pirimicarb's selective toxicity allows it to target these insects efficiently while minimizing collateral effects on non-target organisms, which underscores its importance in crop protection strategies.

Alkaline phosphatase is a membrane-bound metalloenzyme comprising a group of closely related isozymes that catalyse the hydrolysis of organic phosphate esters, releasing inorganic phosphate in the extracellular space (Kaplan, 1972; Sharma et al., 2014). These enzymes require metal ions, specifically zinc and magnesium, as essential cofactors for their catalytic activity. Although all isozymes catalyse the same type of reaction, they exhibit distinct physiological properties that reflect their tissue-specific roles (Moss, 1982). Alkaline phosphatase is widely distributed across various tissues and organs, including the liver, kidney, bones and placenta, among others. In liver tissue, Alkaline phosphatase is predominantly localized in the cytoplasm of hepatocytes and along the canaliculi membrane, where it plays a role in bile formation and secretion (McComb et al., 2013). The enzyme is present in lower concentrations in the placenta, bones, and kidneys, reflecting tissue-specific functional requirements. Interestingly, a major portion of circulating Alkaline phosphatase is found in the serum, while a smaller fraction exists in the intestinal mucosa (Posen, 1967).

Despite its widespread presence, the precise physiological roles of Alkaline phosphatase in many tissues remain only partially understood (Duff et al., 1994). Its activity is implicated in diverse processes, including bone mineralization, liver function, nutrient absorption and cellular metabolism, and variations in Alkaline phosphatase levels can serve as biomarkers of tissue damage or physiological stress. Because of its sensitivity to environmental and chemical stressors, Alkaline phosphatase is widely used in toxicological studies to assess the impact of xenobiotic on organismal health, including in aquatic species exposed to pesticides.

MATERIALS AND METHODS

Aquatic animals serve as valuable models for toxicological assessment, providing a crucial link between laboratory-based experiments and real-world environmental conditions (Couch et al., 1984). Their use

allows researchers to simulate exposure scenarios, monitor physiological and biochemical responses, and evaluate the ecological relevance of contaminants under controlled or semi-controlled conditions. Certain fish and invertebrate species are particularly suitable for both acute and chronic toxicity studies, whether conducted in laboratory aquaria or in field environments (Hill, 1989). These organisms provide important insights into the effects of natural and anthropogenic stressors, including chemical pollutants, on specific organs and physiological systems. The selection of species often takes into account morphological features that mirror the functional aspects of target organs, enabling precise assessment of toxic effects on processes such as metabolism, reproduction, growth, and behaviour (Nebeker et al., 1984). Small-bodied aquatic animals, particularly certain freshwater and estuarine fish, are widely favoured for toxicological studies due to several advantages: compact size, ease of collection and maintenance, short life cycles, complex yet observable behavioural patterns and adaptability to confined laboratory conditions (Chapman & Wang, 2001). These attributes facilitate high-throughput testing, longitudinal observation and controlled experimentation, making them ideal models to evaluate the impact of pesticides, heavy metals, and other environmental toxicants on aquatic health. Overall, the use of aquatic animals as experimental models enables researchers to bridge laboratory findings with ecological relevance, helping to predict the potential consequences of chemical contaminants on both aquatic ecosystems and higher trophic levels, including humans.

The present study was conducted on the freshwater fish *Cirrhinus mrigala*, commonly known as white carp or mrigala, which is recognized as one of the major Indian carps. It is widely distributed in rivers, streams, and freshwater bodies across India and serves as a key species in aquaculture throughout South Asia (Borah & Bordoloi, 2020). *Cirrhinus mrigala* is a benthopelagic and potamodromous species that primarily feeds on plankton, reflecting its important ecological role in freshwater ecosystems. Fish are considered highly sensitive bio indicators due to their susceptibility to a wide range of environmental changes, including chemical, physical, and biological stressors (Huntingford et al., 2006). Among aquatic organisms, fish are the most significant non-target species affected by aquatic pollution, serving as early indicators of ecosystem health (Świacka et al., 2021). In this context, the present study aimed to investigate the lethal and sub-lethal effects of the insecticide pirimicarb on *Cirrhinus mrigala* an economically and ecologically important freshwater fish. Several attributes make this species particularly suitable for laboratory-based toxicological investigations: it is widely cultured, highly demanded for polyculture systems, easily available, highly fecund capable of laying millions of eggs and susceptible to aquatic toxicants. Furthermore, *Cirrhinus mrigala* is easily acclimatized to laboratory conditions, facilitating controlled experimental studies to assess biochemical, physiological

and behavioural responses to toxic exposure. The species' sensitivity to environmental contaminants, coupled with its ecological and economic importance, makes it an ideal model organism to evaluate the potential hazards of pesticides like pirimicarb, thereby providing insights into both aquatic ecosystem health and implications for human food safety.

Estimation of Alkaline Phosphatase Activity

For the assessment of alkaline phosphatase activity, vital tissues including liver, intestine and muscle were collected from all experimental groups of *Cirrhinus mrigala*, which included the control group, LC₀, and LC₅₀. Tissue homogenates were prepared in 0.9% chilled saline solution to maintain enzyme stability and were subsequently centrifuged at 3000 rpm to obtain a clear supernatant. For the enzyme assay, 0.2 mL of the supernatant was added to each test tube. To this, 0.8 mL of 0.05 M sodium citrate buffer containing 5×10^{-3} M p-nitro phenyl phosphate was added. The buffer was adjusted to pH 7.6 for alkaline phosphatase, following the protocol described by Linhardt and Walter (1965). The reaction mixtures were incubated at 37°C for 30 minutes, allowing the hydrolysis of p-nitro phenyl phosphate to p-nitro phenol. The enzymatic reaction was terminated by the addition of 4 mL of 0.1 N NaOH, which also stabilized the chromogenic product. A blank solution was prepared by replacing the tissue homogenate with 0.2 mL of distilled water, followed by addition of 1 mL of substrate buffer and 4 mL of 0.1 N NaOH, to correct for any background absorbance. The absorbance of the resulting solutions was measured spectrophotometrically at 400 nm against the blank. The activity of alkaline phosphatase was calculated and expressed in μmol of p-nitrophenol formed per mg of protein. The enzyme activity was determined using the following formula:

Phosphatase activity = Optical density X 2.76 X1000 / amount of protein/mg tissue.

RESULTS

The changes in alkaline phosphatase activity in the liver, intestine and muscle tissues of freshwater fish *Cirrhinus mrigala* following 96 hours of exposure to the insecticide pirimicarb are summarized in Table 01 and illustrated in Graph 01.

In the liver tissue, the alkaline phosphatase activity of the control group was measured as 7.82 ± 0.17 μmol of p-nitro phenol/mg protein. Exposure to LC₀ concentration resulted in a significant increase to 9.89 ± 0.19 μmol /mg

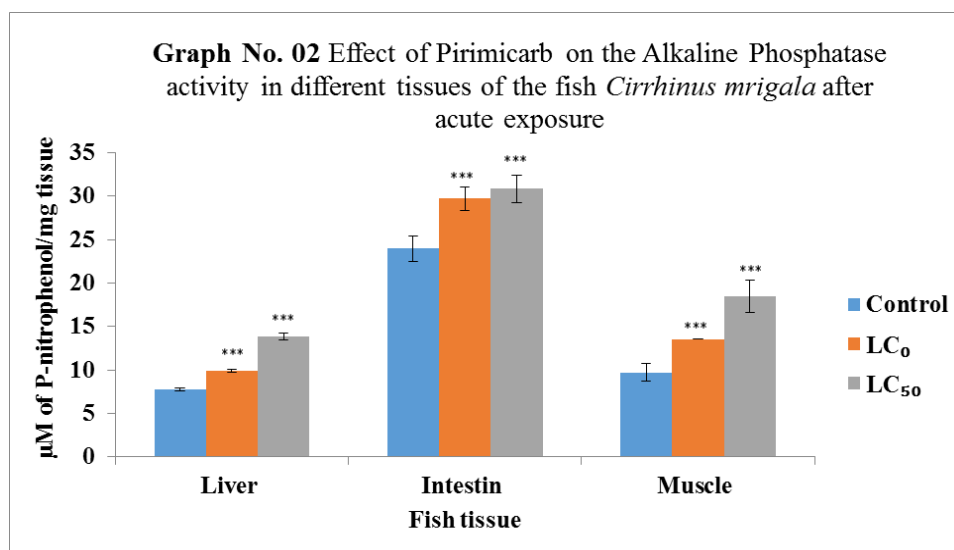
protein, while the LC₅₀ concentration group exhibited a further marked elevation of 25.73 ± 1.82 μmol /mg protein. Statistical analysis revealed that alkaline phosphatase activity in both LC₀ and LC₅₀ groups was highly significant compared to the control ($p < 0.001$), indicating a dose-dependent induction of enzymatic activity in response to pirimicarb exposure. Similarly, in the intestinal tissue, the alkaline phosphatase activity of control fish was 24.01 ± 1.47 μmol /mg protein. Following exposure to LC₀ concentration, the enzyme activity increased to 29.72 ± 1.36 μmol /mg protein and in the LC₅₀ group, it further rose to 30.89 ± 1.58 μmol /mg protein. Again, these increases were statistically significant ($p < 0.001$) when compared to the control, demonstrating that pirimicarb exposure triggers an upregulation of alkaline phosphatase activity in the intestine as well. The observed enhancement of alkaline phosphatase activity in both liver and intestine tissues suggests a biochemical response to pirimicarb-induced stress, possibly reflecting increased metabolic turnover, detoxification processes or membrane-bound enzyme mobilization in the affected tissues. The dose-dependent nature of these responses underscores the sensitivity of alkaline phosphatase as a biomarker for acute pesticide exposure in *Cirrhinus mrigala*. In the muscle tissue of *Cirrhinus mrigala*, the alkaline phosphatase activity in the control group was measured at 9.72 ± 1.03 μmol of p-nitro phenol/mg protein. Exposure to LC₀ concentration of pirimicarb led to a marked increase in alkaline phosphatase activity, reaching 13.58 ± 0.02 μmol /mg protein, while the LC₅₀ concentration group exhibited a further significant elevation to 18.46 ± 1.85 μmol /mg protein. Statistical analysis confirmed that these increases in both LC₀ and LC₅₀ groups were highly significant compared to the control ($p < 0.001$).

The dose-dependent enhancement of alkaline phosphatase activity in muscle tissue indicates a stress-induced enzymatic response to pirimicarb exposure. This increase may reflect elevated metabolic activity, enhanced membrane-bound enzyme mobilization or adaptive physiological mechanisms to cope with the toxicant. Together with the responses observed in liver and intestine, the results highlight the sensitivity of alkaline phosphatase as a reliable biochemical biomarker for assessing acute pesticide-induced stress in economically important freshwater fish.

Table No. 01: Effect of Pirimicarb on the Alkaline Phosphatase activity in different tissues of the fish *Cirrhinus mrigala* after acute exposure.

Groups	μmol of p-nitro phenol phosphate/mg protein in tissue		
	Liver	Intestine	Muscle
Control Group	7.82 ± 0.17	24.01 ± 1.47	9.72 ± 1.03
LC ₀	$9.89 \pm 0.19^{***}$	$29.72 \pm 1.36^{***}$	$13.58 \pm 0.02^{***}$
LC ₅₀	$13.87 \pm 0.43^{***}$	$30.89 \pm 1.58^{***}$	$18.46 \pm 1.85^{***}$

(Values expressed is mean of (n=5); $\pm$ SD) * P <0.05; ** P <0.01; *** P <0.001; NS = > 0.05



(Values expressed as Arithmetic Mean of (n=5); $\pm$ SD, *** indicate P <0.001).

DISCUSSION

Most aquatic organisms, including fishes, are ectothermic, meaning they are unable to regulate their internal body temperature independently of the surrounding environment (Kearney et al., 2009). Consequently, water temperature exerts a profound influence on the physiological and biochemical processes of fish and other aquatic species (Rand et al., 2020). For the normal growth, development and metabolism of fishes, as well as primary aquatic producers and consumers such as phytoplankton, zooplankton and aquatic insects, there exists a specific optimal temperature range (Cserh ti et al., 2002). Deviations above or below this range can lead to a decline in population density, sometimes resulting in the complete absence of certain species from particular habitats (Connell & Orias, 1964). For example, in shallow lakes and ponds, elevated water temperatures often limit the presence of some fish species due to physiological stress. Temperature also significantly affects the chemical properties of water. Higher temperatures increase the rate of chemical reactions, which can alter water chemistry and negatively impact biological processes (Akiya & Savage, 2002). Moreover, temperature influences the solubility of gases, including oxygen: while warmer water can hold more saturated oxygen, the actual dissolved oxygen may remain insufficient to meet the respiratory demands of aquatic organisms (Swann, 1997; P rtner, 2008).

In addition, the toxicity of certain aquatic pollutants is temperature-dependent, with some toxicants becoming more reactive and harmful at elevated temperatures (Camargo, 2003). Sudden fluctuations in water temperature can disrupt metabolic activity, placing fishes under stress conditions that may reduce their growth, reproduction, and survival, ultimately affecting population size and structure (Portz et al., 2006; Jobling

et al., 2006). Despite these challenges, fishes exhibit adaptive responses to cope with temperature fluctuations, including behavioural, physiological, and biochemical mechanisms that support survival, growth and reproduction under varying environmental conditions (Ficke et al., 2007).

Several studies have evaluated the acute toxicity (LC₅₀) of various insecticides in both freshwater and marine fish species, providing essential data for risk assessment and environmental management. Mhadhbi and Beiras (2015) determined the 48- and 96-hour LC₅₀ values of seven selected insecticides in the marine fish turbot (*Psetta maxima*). The reported LC₅₀ values (mg/L) for 48 and 96 hours of exposure were: chlorpyrifos (116.6, 94.65), dieldrin (146, 97), pirimiphos-methyl (560, 425), diazinon (1837, 1230), alachlor (2177, 2233), diuron (10076, 7826), and atrazine (11873, 9957). These results highlighted the differential toxic potency of these compounds, with organophosphates and organochlorines exhibiting higher toxicity compared to herbicides and herbicide-related chemicals. In freshwater systems, Singh et al. (2018) conducted comparative acute toxicity studies on the freshwater fish *Channa punctatus*, evaluating the insecticides triazophos and deltamethrin. The 96-hour LC₅₀ values were found to be 0.069 mg/L for triazophos and 7.33 μ g/L for deltamethrin, demonstrating the extremely high toxicity of deltamethrin even at microgram levels.

Focusing specifically on *Cirrhinus mrigala*, Nair et al. (2018) investigated the acute toxicity of the organophosphate insecticide quinolphos over multiple exposure durations (12, 24, 48, 72, 96, and 150 hours). The reported LC₅₀ values were 0.014, 0.135, 0.130, 0.125, and 0.124 mg/L, indicating a rapid onset of toxic effects with relatively low lethal concentrations. Similarly, Islam et al. (2019) studied the toxicity of the

organophosphate insecticide sumithion on striped catfish, exposing them to concentrations of 0, 3, 4, 5, and 6 mg/L for 96 hours. The 96-hour LC_{50} value was reported as 5.886 mg/L, reflecting moderate acute toxicity under the experimental conditions. These studies collectively demonstrate that the acute toxic effects of insecticides vary widely depending on chemical class, concentration, exposure duration, and the sensitivity of the fish species. Such data provide crucial insights for establishing safe exposure limits and for understanding species-specific susceptibility to commonly used pesticides in both freshwater and marine ecosystems.

Acute toxicity studies have been extensively conducted to evaluate the lethal effects of insecticides on different freshwater fish species, providing critical data for environmental monitoring and risk assessment. De Souza et al. (2020) reported the 96-hour LC_{50} values of the insecticide deltamethrin in five Amazonian fish species: *Paracheirodon axelrodi*, *Colossoma macropomum*, *Hemigrammus rhodostomus*, *Corydoras schwartzii*, and *Carnegiella strigata*. The LC_{50} values ranged between 6.69 and 23.63 $\mu\text{g/L}$, indicating species-specific sensitivity to deltamethrin. Similarly, Amaeze et al. (2020) investigated the acute toxicity of multiple insecticides in African catfish (*Clarias gariepinus*). The reported 96-hour LC_{50} values were as follows: deltamethrin, 21.24 $\mu\text{g/L}$; abamectin, 8.939 $\mu\text{g/L}$; carbofuron, 37.675 $\mu\text{g/L}$; chlorpyrifos, 34.55 $\mu\text{g/L}$; cypermethrin, 9.461 $\mu\text{g/L}$; dimethoate, 21,112.39 $\mu\text{g/L}$; dichlorvos, 884.33 $\mu\text{g/L}$; fipronil, 6.148 $\mu\text{g/L}$; lambda-cyhalothrin, 2.043 $\mu\text{g/L}$; and paraquat, 10,284.288 $\mu\text{g/L}$. These results highlight the wide variability in toxic potency among different chemical classes, with pyrethroids such as deltamethrin and lambda-cyhalothrin exhibiting high acute toxicity at microgram levels, whereas organophosphates and herbicides demonstrated comparatively lower acute toxicity. In another study, Saha and Saha (2021) evaluated the acute toxicity of the pyrethroid insecticide bifenthrin in adult *Clarias batrachus* (Linn.), reporting a 96-hour LC_{50} value of 3.464 mg/L. This study further underscores the high sensitivity of catfish species to pyrethroid insecticides, even at low concentrations.

Collectively, these studies demonstrate that the acute toxicity of insecticides is highly dependent on chemical type, concentration, exposure duration and fish species. Such data are crucial for determining safe exposure limits, understanding species-specific vulnerabilities, and implementing appropriate measures to protect aquatic ecosystems and non-target organisms from pesticide contamination. Several recent investigations have assessed the acute toxicity (LC_{50}) of various insecticides in different fish species, highlighting species-specific responses and the influence of environmental conditions on toxicity. Parveen et al. (2021) evaluated the acute toxicity of the insecticide flubendiamide in the freshwater fish *Catla catla*. Fish were exposed to different concentrations for 24, 48, 72, and 96 hours, and

the corresponding LC_{50} values were observed as 3.566, 3.456, 3.0221, and 2.892 mg/L, respectively. The results indicated a time-dependent increase in toxicity, with longer exposure resulting in lower LC_{50} values. Dake et al. (2021) studied the fry stage of flathead grey mullet (*Mugil cephalus*), reporting 96-hour LC_{50} values of 3.45 $\mu\text{g/L}$ for chlorpyrifos and 4.06 $\mu\text{g/L}$ for dimethoate, demonstrating the high acute sensitivity of early life stages to organophosphorus insecticides. In marine species, El Ayari et al. (2022) evaluated the toxicity of multiple insecticides, including carbaryl, permethrin, tebufenpyrad, and cypermethrin, in the larval and embryonic stages of European sea bass (*Dicentrarchus*). The reported LC_{50} values were 13.88 ppm (carbaryl), 72.7 ppm (permethrin), 92.00 ppm (tebufenpyrad), and 25.67 ppm (cypermethrin), indicating differential susceptibility depending on the chemical class and developmental stage. Yang et al. (2022) investigated the 96-hour acute toxicity of prometryn in juvenile *Tilapia* (*Oreochromis niloticus*), reporting an LC_{50} value of 5.49 mg/L, highlighting moderate toxicity at environmentally relevant concentrations. Furthermore, Sayadi et al. (2022) demonstrated that the acute toxicity of the herbicide paraquat is highly dependent on physicochemical water parameters, including temperature, pH, and hardness. These findings collectively underscore that insecticide toxicity in fish is influenced not only by chemical type and concentration but also by exposure duration, developmental stage and environmental conditions. Such studies are crucial for understanding species-specific sensitivity, optimizing safe pesticide use and predicting ecological risks in aquatic environments.

In the present study, fingerlings of the freshwater fish *Cirrhinus mrigala* were exposed to varying concentrations of the carbamate insecticide pirimicarb over exposure periods of 24, 48, 72, and 96 hours to determine its acute toxic effects. The 96-hour LC_{50} , representing the concentration causing 50% mortality, was determined to be 0.025 ppm, indicating that *Cirrhinus mrigala* is highly sensitive to pirimicarb exposure. No mortality was observed at a lower concentration of 0.0175 ppm, which is considered a non-lethal exposure level under the experimental conditions. At 0.019 ppm, the minimum mortality of 10% was recorded, demonstrating a dose-dependent increase in lethality. These findings suggest that even sub-micro molar concentrations of pirimicarb can have significant toxic effects on *Cirrhinus mrigala*, highlighting the high acute toxicity of carbamate insecticide to an economically important freshwater fish species. The data provide essential baseline information for establishing safe exposure limits and for assessing the ecological risks associated with pirimicarb contamination in freshwater ecosystems.

Alkaline phosphatase is a membrane-bound metalloenzyme comprising a cluster of isozymes that are structurally related but exhibit distinct tissue-specific

characteristics (Kaplan, 1972). Alkaline phosphatase catalyses the hydrolysis of organic phosphate esters in the extracellular environment, releasing inorganic phosphate, which is critical for numerous physiological processes (Sharma et al., 2012). The enzyme requires zinc and magnesium as cofactors for its catalytic activity. Alkaline phosphatase is widely distributed across various tissues, including the liver, kidney, bones and intestine and all isozymes share a common catalytic function despite having distinct physiological roles in different tissues (Moss, 1982; McComb et al., 2013). The enzyme exhibits broad substrate specificity, hydrolysing diverse phosphomonoesters, which underscores its versatility in metabolic processes (Lowe et al., 2017). Functionally, alkaline phosphatase plays a crucial role in multiple physiological processes, including embryonic development, lipid transport, renal phosphate handling and bone mineralization (Hahnel et al., 1990; Demeule et al., 1991; van Straalen et al., 1991; Hernández-Mosqueira et al., 2015). The levels of alkaline phosphatase in tissues and serum can serve as a sensitive marker of physiological, clinical and environmental influences, reflecting alterations in metabolic activity due to stress, disease, or xenobiotic exposure (Lallès, 2019). Given its widespread distribution and functional importance, alkaline phosphatase is commonly employed as a biochemical biomarker in environmental toxicology studies to assess the impact of pollutants, pesticides and other chemical stressors on aquatic organisms.

In the present investigation, acute exposure of freshwater fish *Cirrhinus mrigala* to the carbamate insecticide pirimicarb for 96 hours (at $LC_0 = 0.0175$ ppm and $LC_{50} = 0.025$ ppm) resulted in a significant elevation of alkaline phosphatase activity in liver, intestine and muscle tissues compared to the control group, which was not exposed to any insecticide. These findings are consistent with numerous previous studies that reported similar enzymatic responses under chemical stress. Pilo et al. (1972) suggested that an increase in alkaline phosphatase activity may be associated with enhanced abnormal protein synthesis. Al-Attar (2005) observed elevated alkaline phosphatase activity in cadmium-treated *Oreochromis niloticus*, attributing the increase to hepatic dysfunction. Likewise, Rao (2006) reported significant elevation of alkaline phosphatase activities in the plasma, gills, and kidney tissues of euryhaline fish (*Oreochromis mossambicus*) following 30-day exposure to the organophosphorus insecticide RPR-V (0.017 mg/L). Rao et al. further noted that the increased phosphatase activities could be due to enhanced lysosomal mobilization and tissue necrosis induced by insecticidal toxicity.

An elevation of alkaline phosphatase in plasma and tissues is widely recognized as a conventional biomarker of liver damage (Fernandez & Kidney, 2007). Damage to hepatocytes and other tissue cells caused by insecticidal toxicity can disrupt cell membrane integrity, leading to altered phosphatase enzyme activity, which serves as a

sensitive indicator of cellular and tissue-level injury (Gaskill et al., 2005).

Collectively, the observed increase in alkaline phosphatase activity in liver, intestine, and muscle tissues of *Cirrhinus mrigala* upon pirimicarb exposure reflects cellular stress, hepatic dysfunction, and potential tissue necrosis, confirming the enzyme's utility as a reliable biomarker of acute insecticide-induced toxicity in freshwater fish.

SUMMARY

Acute exposure of freshwater fish *Cirrhinus mrigala* to the carbamate insecticide pirimicarb for 96 hours resulted in a significant increase in alkaline phosphatase activity in liver, intestine, and muscle tissues compared to controls. This elevation reflects cellular stress, hepatic dysfunction and tissue damage induced by insecticidal toxicity. The observed enzymatic changes align with previous reports in fish exposed to various chemical stressors, where increased phosphatase activity was linked to abnormal protein synthesis, lysosomal mobilization and tissue necrosis. Therefore, alkaline phosphatase serves as a reliable biomarker for assessing acute pesticide-induced toxicity in freshwater fish.

CONCLUSION

The present study demonstrates that acute exposure to the carbamate insecticide pirimicarb significantly alters the alkaline phosphatase activity in the liver, intestine, and muscle tissues of freshwater fish *Cirrhinus mrigala*. The observed increase in enzyme activity indicates cellular stress, hepatic dysfunction and tissue-level damage, confirming the sensitivity of alkaline phosphatase as a biochemical biomarker for insecticide-induced toxicity. These findings underscore the potential ecological risks posed by pirimicarb contamination in freshwater ecosystems and highlight the importance of monitoring biochemical responses in non-target aquatic organisms.

Future Perspective

Future research should focus on sub-lethal and chronic exposure studies to better understand the long-term physiological and biochemical effects of pirimicarb on freshwater fish. Additionally, investigations integrating other enzymatic biomarkers, haematological parameters and histopathological assessments could provide a more comprehensive understanding of pesticide-induced toxicity. Such studies will be crucial for establishing environmentally safe exposure limits, improving risk assessment strategies and guiding sustainable pesticide management in aquatic habitats.

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