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Research Article

Determination of Lethal Concentrations of Profenofos and Azadirachtin on *Labeo rohita* and Analysis of Electrolyte Imbalance Following Sub-Lethal Exposure

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ABSTRACT

This study explores the acute and sub-lethal toxic effects of two commonly used pesticides—Profenofos (an organophosphate) and Azadirachtin (a biopesticide)—on the freshwater fish *Labeo rohita*. The 96-hour LC_{50} values were determined through controlled laboratory exposures, revealing Profenofos to be suggestively more toxic (0.08 mg/L) than Azadirachtin (50.0 mg/L). Sub-lethal exposures at 50% of LC_{50} concentrations over 96 hours and 28 days were shown to assess their effect on fish electrolyte balance. Measurements of sodium, potassium, and calcium levels indicated a progressive decline, with Profenofos causing more severe disturbances compared to Azadirachtin. These findings suggest that even low concentrations of these pesticides can harm osmoregulation and physiological stability in aquatic organisms. The study highlights the ecological risks associated with pesticide contamination in aquatic ecosystems and the need for regulatory analysis on pesticide usage near freshwater bodies.

INTRODUCTION

Pesticides are prevalent pollutants that often enter aquatic ecosystems, posing significant risks to their health and stability (Bojarski & Witeska, 2020; Kanan et al.,2020). Globally, approximately three billion kilograms of pesticides are utilized annually for agricultural and domestic purposes, with an estimated expenditure of 40 billion (Sharma et al., 2020). Among the various pesticide classes, organophosphates and synthetic pyrethroids are among the most widely used (McKelvey et al., 2013). Organophosphates function by inhibiting cholinesterase activity (Costa, 2015).

Profenofos, a broad-spectrum organophosphate, is commonly applied in India for pest control in agriculture and households (Kavitha & Venkateswara Rao, 2009; Kushwaha et al., 2016). Similarly, λ-cyhalothrin, a synthetic pyrethroid classified as Class II, is extensively used to manage pests in crops such as vegetables, cotton, and cereals (He et al., 2008). Both profenofos and λ-cyhalothrin are known to exhibit high toxicity toward aquatic organisms, including fish and invertebrates (Nataraj et al., 2017; Sharafeldin et al., 2015).

In recent years, biopesticides like azadirachtin have gained attention due to their lower persistence and environmentally friendly biodegradability (Morgan,

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2009). However, azadirachtin has also been documented as toxic to certain aquatic species, including fish (Kumar et al., 2011). Although the toxic effects of these pesticides on various aquatic organisms are well-documented, there is limited research on their impacts on growth rates, autotomy, and oxidative stress biomarkers in aquatic species.

Qualitative toxicity assessments often focus on lethal outcomes, such as the median lethal concentration (LC50). However, sublethal toxicity evaluations are more relevant, as organisms are typically exposed to low, environmentally realistic levels of hazardous chemicals (Brahma and Gupta, 2020). Sublethal tests that combine behavioral changes with biochemical biomarkers have been proposed as a preferred ecotoxicological approach (Dhara et al., 2015; Hellou, 2011). As the severity of toxicity continues to increase, implementing an effective biomarker system for species has become essential for evaluating the effects of contaminants on aquatic organisms (Bhattacharya et al., 2021; Hook et al., 2014).

Fish, especially rohu (*Labeo rohita*), serve as important bio-indicators for evaluating the effects of toxicants, offering essential information for safety assessments and environmental quality monitoring (Hong et al., 2020). Among the six species of carp, *L. rohita* is highly favored and accounts for approximately 60% of the total carp production in Asian countries (FAO, 2018). In freshwater fish, water influx and ion loss to the environment are balanced by passive ion uptake from the surrounding water through the branchial epithelium (McCormick SD et al., 1995, Eyckmans M et al., 2010).

Inorganic ions are generally essential for cellular metabolism (Sangeetha J, et al., 2014). Sodium, the primary cation, plays a critical role in osmoregulation and acid-base balance [Muralidharan L 2014). Its active movement across the gills and external medium concentration makes sodium a reliable indicator of stress conditions (Bentley PJ et al., 1971). Similarly, potassium, the main extracellular cation, is vital for acid-base balance, osmotic pressure regulation, and the proper functioning of nerves and muscles (Larsson A, et al., 1981). Chloride ions, as significant anions, are crucial in regulating osmotic pressure (Singh D and Singh A 2002). The exchange of chloride ions with bicarbonate ions across the gill epithelium is essential for maintaining acid-base balance (Williams EM, Eddy FB 1988). Any disruption in these ion levels can impair various physiological processes in aquatic organisms (Baskin SI et al., 1981).

MATERIAL AND METHODS

Type of Study

The study described in the provided materials and methods is a laboratory-based experimental study with a focus on the toxicity and sub-lethal effects of two pesticides, Azadirachtin and Profenofos, on *Labeo rohita.*

Study Design

Labeo rohita, a member of the Cyprinidae family and a key carp species in South Asia, were sourced from a local fish farm in Sultanpur, Uttar Pradesh. To ensure the fish used in the study were healthy, they were carefully transported to the laboratory in plastic bags to minimize injury. Upon arrival, the fish were disinfected by immersing them in a 0.05% potassium permanganate ($KMnO_4$) solution for two minutes. Following disinfection, the fish were transferred to a 100-liter plastic tank and acclimatized to laboratory conditions for 15 days under natural light. During this acclimatization period, the fish were fed daily with a diet consisting of rice bran mixed with mustard oil cake. Uneaten food was removed daily, and the tank water was regularly replaced to maintain hygiene. Dead fish, if any, were promptly removed to prevent water fouling.

The acclimatization process allowed the fish to adapt to laboratory conditions, reducing stress and ensuring they were well-adjusted for subsequent experimental procedures. After acclimatization, the fish were fasted for 24 hours before exposure to profenofos and azadirachtin. Stock solutions of both pesticides were prepared, and varying amounts of these solutions were added to water in the experimental tanks. The pesticides were thoroughly mixed using a glass rod before introducing the fish into the tanks. Fish of uniform size and weight were divided into eight groups, each consisting of four fish. These groups were exposed to different concentrations of profenofos and azadirachtin to determine the LC_{50} values for 24, 48, 72, and 96 hours. Two replicates were included for each concentration, and a control group was maintained under identical conditions without pesticide exposure. Fish were not fed during the experiment, and mortality in each group was recorded. Dead fish were promptly removed to maintain water quality.

Water Quality and Parameters

- The water used for the experiments is subjected to a thorough assessment of its physiochemical characteristics. This assessment is conducted in accordance with the standard procedures outlined in the APHA (American Public Health Association) guidelines of 2005.
- Physiochemical characteristics of water might include parameters like pH, temperature, dissolved oxygen, ammonia, nitrate, and other relevant factors. This step ensures that the water conditions in the laboratory closely mimic the natural habitat of *Labeo rohita*, creating a suitable environment for the study.

Toxicity Testing (LC50)

- LC50 values refer to the concentration of a substance (in this case, Azadirachtin and Profenofos) that is lethal to 50% of the test organisms *(Labeo rohita).*
- The LC50 values for Azadirachtin and Profenofos are determined at different time intervals: 24 hours, 48

hours, 72 hours, and 96 hours. This involves exposing a group of fish to varying concentrations of the pesticides.

- Mortality rates of the exposed fish are recorded and analyzed. The Probit analysis method, as per Finney (1971), is employed to estimate the LC50 values. This method is commonly used for dose-response analysis in toxicology studies.

Sub-Lethal Exposure

- After establishing the LC50 values, the study continues by exposing another group of fish to sub-lethal concentrations 50% of LC50 of Azadirachtin and Profenofos.
- These exposures are carried out for both short-term (96 hours) and long-term (28 days) durations. Sub-lethal concentrations are typically lower than the LC50 values and may not result in immediate mortality but can still cause adverse effects on the fish.

Electrolyte Levels

- Electrolyte levels in the fish exposed to sub-lethal concentrations of the pesticides are measured.
- The results of these measurements are subjected to statistical analysis. This analysis can provide insights into how the pesticides impact the fish's physiological functions, including electrolyte balance.

RESULTS

Water Quality and Parameters

The water quality parameters for the study indicate optimal conditions for Labeorohita. The pH is slightly alkaline at 7.5, and the temperature is consistently maintained at 25°C. Dissolved oxygen levels are sufficient at 8.0 mg/L, and both ammonia (0.05 mg/L) and nitrate (0.10 mg/L) concentrations are low, indicating minimal nitrogenous waste. The Total Dissolved Solids (150 mg/L) and Total Suspended Solids (50 mg/L) levels reflect a clean water environment. Biochemical Oxygen Demand (2.0 mg/L) and Chemical Oxygen Demand (20 mg/L) suggest low organic pollution. The water has moderate hardness (120 mg/L), low turbidity (5.0 NTU), and moderate electrical conductivity (250 µS/cm). Chlorine (0.1 mg/L), bromine (0.05 mg/L), sodium (10 mg/L), and iodine (0.01 mg/L) levels are within safe limits, ensuring a healthy aquatic environment for the fish.

LC_{50} Values for Azadirachtin and Profenofos

Our study investigated the LC50 values for Azadirachtin and Profenofos over various exposure times to evaluate their acute toxicity. The results indicated a distinct difference in the toxicity levels of these pesticides over time. For Azadirachtin, the LC50 values decreased from 85.0 LC50 mg/L at 24 hours to 50.0 mg/L at 96 hours, demonstrating a significant increase in toxicity with prolonged exposure (Table 1). Conversely, Profenofos exhibited much lower LC50 values, starting at 0.12 mg/L at 24 hours and reducing to 0.08 mg/L at 96 hours (Table 2). This substantial decrease indicates that Profenofos is highly toxic even at minimal concentrations and its toxicity escalates with extended exposure.

The Figure 1 depicting LC50 values for Azadirachtin over changing exposure times shows a clear inverse relationship between exposure time and the concentration required to cause 50% mortality in the test population. As exposure time increases from 24 to 96 hours, the LC50 values gradually decrease from 85.0 mg/L to 50.0 mg/L. This trend indicates that Azadirachtin shows time-dependent toxicity, where prolonged exposure enhances its toxic effects, allowing lower concentrations to be lethal. Such a pattern suggests cumulative or late toxic action, where longer contact periods increase physiological damage in organisms.

The Figure 2 showing LC50 values for Profenofos determine a decreasing tendency in toxicity thresholds over time, signifying that the pesticide's lethal concentration decreases as the time of exposure increases. Definitely, the LC50 value drops from 0.120 mg/L at 24 hours to 0.100 mg/L at 48 hours, 0.090 mg/L at 72 hours, and lastly to 0.080 mg/L at 96 hours. This pattern indicates a time-dependent increase in toxicity, where prolonged exposure increases the toxic effects of Profenofos on the test organisms. Such behavior is typical of substances that exert cumulative toxic action and prolonged contact leads to greater physiological stress or damage even at lower concentrations.

Electrolyte Levels in Fish after Sub-Lethal Exposure

Our study examined the effects of sub-lethal exposure to Azadirachtin and Profenofos on the electrolyte levels in fish over different durations. The control group, with no pesticide exposure, had sodium, potassium, and calcium levels at 140 mmol/L, 4.0 mmol/L, and 2.2 mmol/L, respectively. After 96 hours of exposure to Azadirachtin,

Table 1: LC50 (mg/L) Values for Azadirachtin

Pesticide	*Exposure Time (hours)*	*LC5 mg/L)*
Azadirachtin	24	85.0
Azadirachtin	48	70.0
Azadirachtin	72	60.0

Table 2: LC50 (mg/L) Values for Profenofos

Pesticide	*Exposure Time (hours)*	*LC50 (mg/L)*
Profenofos	24	0.12
Profenofos	48	0.10
Profenofos	72	0.09
Profenofos	96	0.08

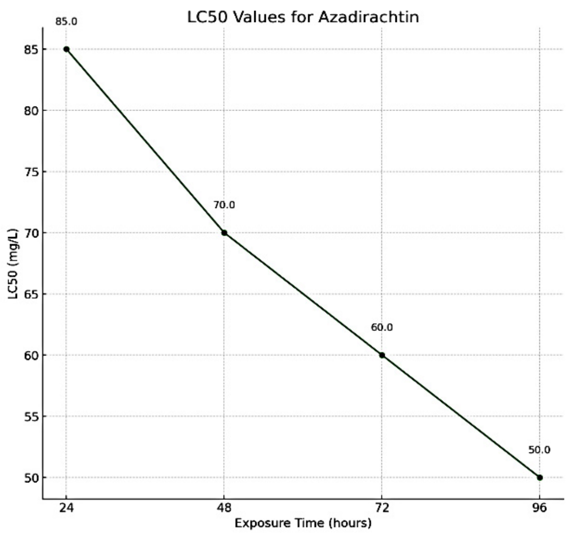

Figure 1: LC50 (mg/L) Values for Azadirachtin

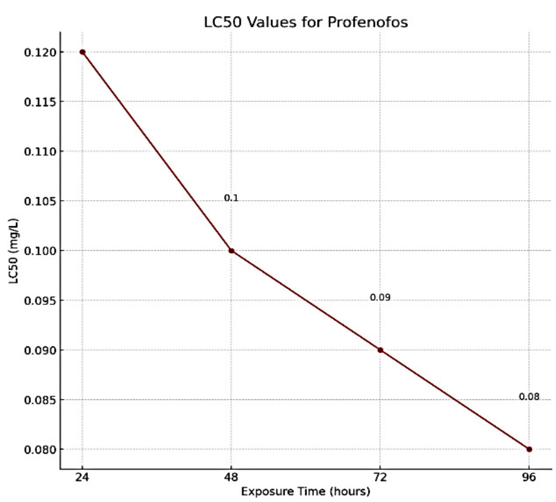

Figure 2: LC50 (mg/L) Values for Profenofos

sodium levels decreased to 135 mmol/L, potassium to 3.8 mmol/L, and calcium to 2.1 mmol/L. Prolonged exposure to 28 days resulted in further reductions, with sodium at 130 mmol/L, potassium at 3.5 mmol/L, and calcium at 2.0 mmol/L. Profenofos exposure showed even more pronounced effects, with sodium levels dropping to 125 mmol/L, potassium to 3.2 mmol/L, and calcium to 1.8 mmol/L after 96 hours. After 28 days, these levels further decreased to 120 mmol/L, 3.0 mmol/L, and 1.5 mmol/L, respectively (Table 3). These results indicate that both pesticides significantly disrupt electrolyte balance in fish, with Profenofos causing more severe alterations compared to Azadirachtin. The decrease in essential electrolytes such as sodium, potassium, and calcium suggest that prolonged pesticide exposure can adversely affect fish health, potentially leading to impaired physiological functions and increased susceptibility to environmental stressors.

Table 3: Electrolyte Levels (mmol/L) in Fish after Sub-Lethal Exposure

Pesticide	*Exposure duration*	*Sodium (mmol/L)*	*Potassium (mmol/L)*	*Calcium (mmol/L)*
Control	-	140	4.0	2.2
Azadirachtin	96 hours	135	3.8	2.1
Azadirachtin	28 days	130	3.5	2.0
Profenofos	96 hours	125	3.2	1.8
Profenofos	28 days	120	3.0	1.5

The sodium (Na^+) concentration in mmol/L under control conditions and after exposure to Azadirachtin and Profenofos for varying durations. The sodium level in the control group is 140 mmol/L, indicating the baseline concentration under normal, unexposed conditions. Upon exposure to Azadirachtin for 96 hours, sodium levels decreased to 135 mmol/L, suggesting a slight reduction in sodium regulation. This effect became more pronounced after 28 days of exposure, with sodium levels dropping further to 130 mmol/L. These results imply a cumulative impact of Azadirachtin on sodium levels with prolonged exposure. In the case of Profenofos exposure, sodium levels showed a more significant decline. After 96 hours, the concentration dropped to 125 mmol/L, indicating a greater immediate impact compared to Azadirachtin. After 28 days of exposure, sodium levels reduced further to 120 mmol/L, the lowest value recorded among all groups. This demonstrates a substantial and potentially toxic effect of prolonged Profenofos exposure on sodium homeostasis (Figure 3).

The potassium (K^+) levels in mmol/L under control conditions and after exposure to Azadirachtin and Profenofos for varying durations. In the control group, potassium levels are 4 mmol/L, representing the baseline concentration in normal, unexposed conditions. Exposure to Azadirachtin for 96 hours resulted in a slight decrease in potassium levels to 3.8 mmol/L, indicating a minor reduction in potassium regulation. After 28 days of exposure, potassium levels further declined to 3.5 mmol/L, showing a progressive impact with prolonged exposure. In the case of Profenofos exposure, potassium levels exhibited a more significant decline. After 96 hours, the

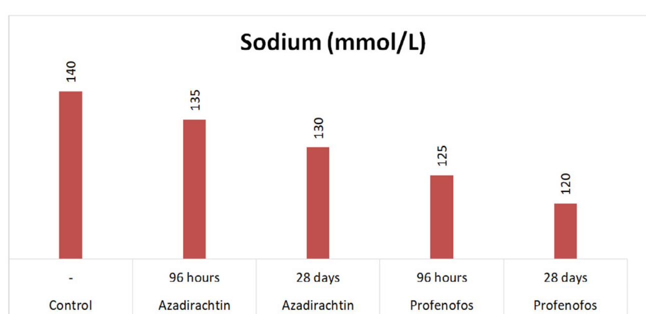

Figure 3: Showing Sodium Levels (mmol/L) in Fish after Sub-Lethal Exposure

concentration dropped to 3.2 mmol/L, suggesting a more substantial immediate effect compared to Azadirachtin. With 28 days of exposure, potassium levels decreased further to 3 mmol/L, the lowest value among all groups. This indicates a pronounced effect of Profenofos on potassium regulation over time (Figure 4).

Calcium (Ca^{2+}) levels, measured in mmol/L, varied under control conditions and after exposure to Azadirachtin and Profenofos over different durations. In the control group, calcium levels were 2.2 mmol/L, representing the baseline in unexposed conditions. Exposure to Azadirachtin resulted in a slight reduction, with levels decreasing to 2.1 mmol/L after 96 hours and further declining to 2.0 mmol/L after 28 days, indicating a gradual impact with prolonged exposure. In contrast, Profenofos caused a more significant decline, with calcium levels dropping to 1.8 mmol/L after 96 hours, reflecting a stronger immediate effect compared to Azadirachtin. Prolonged exposure to Profenofos for 28 days led to a further decrease to 1.5 mmol/L, the lowest value observed across all groups, demonstrating its pronounced effect on calcium regulation over time (Figure 5).

DISCUSSION

The study demonstrates the significant impact of Azadirachtin and Profenofos on *Labeo rohita* under controlled water quality conditions. Optimal pH, temperature, dissolved oxygen, and low pollutant levels ensured minimal external stress, isolating the effects of the pesticides. LC50 data reveal that Profenofos is substantially more toxic than Azadirachtin, with its LC50 values dropping sharply from 0.12 mg/L to 0.08 mg/L over 96 hours, compared to Azadirachtin's decrease from 85.0 mg/L to 50.0 mg/L over the same period. Sub-lethal exposure disrupted fish electrolyte homeostasis, with Profenofos causing greater reductions in sodium, potassium, and calcium levels than Azadirachtin. Sodium levels decreased significantly under Profenofos, from 140 mmol/L in the control group to 120 mmol/L after 28 days, while potassium and calcium showed similar declines, reaching their lowest at 3.0 mmol/L and 1.5 mmol/L, respectively. These findings indicate that Profenofos poses a greater risk to fish health, emphasizing the need for its cautious use to mitigate ecological harm.

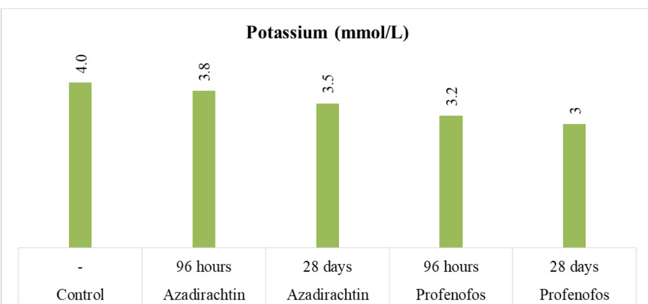

Figure 4: Showing Potassium Levels (mmol/L) in Fish after Sub-Lethal Exposure

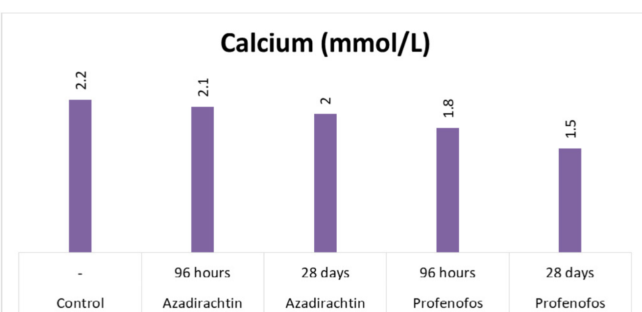

Figure 5: Showing calcium Levels (mmol/L) in Fish after Sub-Lethal Exposure

Profenofos is a non-systemic organophosphorus insecticide and acaricide that works through contact and stomach action. It is commonly used to control insects, especially Lepidoptera (Rajesh and Pilo, 2009). Fish are often utilized as bio-indicators to assess the quality of the aquatic environment because they are highly sensitive to changes in their surroundings, making them valuable for detecting potential pollution hazards caused by new chemicals (Lakra and Nagpure, 2009). In another study, the 96-hour LC50 value for Profenofos (25% EC) in *Labeo rohita* was found to be 0.1 mg/L, indicating that the pesticide is highly toxic to the test fish. Chatterjee, A., et al., (2021), found that the 96-hour LC50 values for Profenofos, λ-Cyhalothrin, and Azadirachtin in *Tubifex tubifex* were 0.59, 0.13, and 82.15 mg/L, respectively. Worms exposed to these pesticides exhibited several behavioral changes, including increased mucus secretion, erratic movements, wrinkling, and a reduced tendency to clump during acute exposure. When exposed to sublethal concentrations of Profenofos (0.059 and 0.118 mg/L), λ-Cyhalothrin (0.013 and 0.026 mg/L), and Azadirachtin (8.2 and 16.4 mg/L) for 14 days, there were significant effects on the growth rate and oxidative stress enzyme levels in *T. tubifex* (Chatterjee, A., et al., 2021). The variation in the LC50 value could be attributed to differences in ambient temperature (Pandey et al., 2008). Under stressful toxic conditions, fish reduce opercular movement to avoid water uptake, while simultaneously increasing the frequency of gulping (Pandey et al., 2009).

Changes in swimming behavior are characterized by sudden, quick movements followed by brief periods of calm where the fish settle at the bottom of the trough within the first two hours of toxicant exposure. While swimming, the fish vigorously shake their heads, likely in an attempt to rid themselves of excess mucus on their gills. The loss of balance and body tremors in fish, which increases with higher concentrations of cypermethrin and prolonged exposure, may be due to the toxicant's impact on Na+ channels in nerve membranes, disrupting nerve impulse transmission (Roberts and Hudson, 1998; Soderlund, 2002).

Hypocalcemia and hypophosphatemia have been observed in teleosts exposed to chemicals like cypermethrin, deltamethrin, Metacid-50, and chlorpyrifos (Mishra et al., 2001, Atamanalp et al., 2002; Logaswamy et al., 2007). Thangavel et al. (2005) found similar conditions in *Oreochromis mossambicus* exposed to dimecron and ziram (Thangavel et al., 2005). However, Velisek et al., (2006), reported no changes in calcium and phosphate levels in common carp exposed to deltamethrin. Previous studies suggest that hypocalcemia is likely caused by the inhibition of calcium uptake by the gills (Velisek et al., 2006). Fish exposed to pesticides typically show damage to the gill epithelium (Yildirim et al., 2006; Velmurugan et al., 2007; Peebua et al., 2008). An increase in inorganic phosphate levels in the blood and skeletal muscles has been noted in fish following exposure to endosulfan (Gill et al., 1990). Hypermagnesemia has been observed in several fish species exposed to various insecticides (Singh et al., 2002). However, Rangaswami and Padmanabha Naidu (1989) reported hypomagnesemia in tilapia exposed to endosulfan (Rangaswami and Padmanabha Naidu 1989). Dabrowska et al. (1991) suggested an inverse relationship between Mg+2 and Ca+2 levels in common carp, while Thangavel et al. (2005) found no change in serum magnesium levels. However, further evidence suggests that fish with hypocalcemia due to insecticide exposure show elevated magnesium levels, which may result from kidney damage (Thangavel et al. 2005).

Sunanda M et al.(2016) observed no significant changes in protein levels after 48 hours in *Labeo rohita* exposed to a pesticide concentration of 0.1891 ppm for 2 days. However, they found the greatest reduction in protein levels in the head, with the least depletion in the muscle tissue (Sunanda M et al. 2016). Similarly, Tilak et al. (2005), reported total protein depletion in the muscle, liver, kidney, brain, and gill tissues of *Labeo rohita* exposed to both technical and 20% EC formulations of chlorpyrifos (Tilak et al. 2005) Aquatic organisms require optimal conditions for healthy growth and well-being (Abdel-Wahab et al., 2021). A similar effect was observed in *Clarias batrachus* following exposure to Quinalphos (Shrivastava et al., 2012). A reduction in the Specific Growth Rate (SGR) of fish is linked to the disruption of metabolic processes within their bodies (Kim et al., 2018).

The increase in the Feed Conversion Ratio (FCR) is likely a result of the toxic effects of pollutants, which interfere with the fish's respiration and metabolic functions, disrupting the digestion process (Padmanabha et al., 2015; Sunanda et al., 2016). Pesticides can significantly impact the physiological health of fish. As a result, biochemical tests are commonly used in laboratories to detect both acute and chronic toxicity from insecticides (Banaee M. et al., 2008). These tests are valuable tools for diagnosing the toxic effects on specific organs and assessing the overall physiological condition of fish. Studies on chlorpyrifos have shown that it induces biochemical changes in fish, and these alterations serve as useful indicators to evaluate the effects of toxicants on fish metabolism (kajare A, et al., 2020), Padmini K, Rajaram P,1975).

COCLUSION

The study highlights the detrimental effects of Azadirachtin and Profenofos on *Labeo rohita*. Profenofos exhibited significantly higher toxicity, with lower LC50 values and greater disruption of sodium, potassium, and calcium levels compared to Azadirachtin. Prolonged exposure to both pesticides caused cumulative declines in electrolyte levels, with Profenofos showing the most severe impacts. These findings underscore the potential risks of pesticide contamination in aquatic environments, emphasizing the need for careful management to protect aquatic organisms.

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