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Enzymatic Responses in Liver and Kidney Tissues of Freshwater Carp *Labeo rohita* (Hamilton) Exposed to the Pesticides Cypermethrin and Chlorpyrifos

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Abstract: Pesticides used in agricultural practices can enter water bodies through runoff, leading to water contamination. This contamination can adversely affect the water quality, making it toxic for aquatic organisms. The present study was aimed to investigate the toxicity of cypermethrin and chlorpyrifos on a freshwater carp fish species *Labeo rohita*. The fish *Labeo rohita* was exposed to sublethal concentrations of cypermethrin and chlorpyrifos individually and in combination for a duration of 7 days. The concentrations used were 1/10th of the LC_{50} dosage for individual treatments (0.015 ppm for cypermethrin and 0.042 ppm for chlorpyrifos) and 1/20th of the LC_{50} for the combined treatment (0.0075 ppm for cypermethrin and 0.021 ppm for chlorpyrifos). The enzyme activities were observed in liver and kidney. The activities of Glucose-6-phosphate dehydrogenase (G6PDH), Lactate Dehydrogenase (LDH), showed an increased trend in all tissues, Succinate Dehydrogenase (SDH), and Malate Dehydrogenase (MDH) activities showed decrement in all tissues after exposure to cypermethrin and chlorpyrifos. Notably, these changes were more pronounced in the combined treatment compared to individual exposures, indicating a potential synergistic effect of cypermethrin and chlorpyrifos.

Keywords: Cypermethrin, Chlorpyrifos, *Labeo rohita*, Enzymatic responses, Liver, Kidney, Glucose-6-phosphate dehydrogenase, Lactate Dehydrogenase, Succinate Dehydrogenase, Malate Dehydrogenase

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Introduction

Pesticides play a crucial role in modern agriculture and constitute a major category of chemicals employed in diverse agricultural practices, encompassing pest control, prevention of crop losses, and the management of vector-borne diseases (Agarwal *et al.,* 2015; Naughton *et al*., 2018). Global usage of pesticides has reached around 4 million tons annually, with China, the United States, Brazil, Argentina, Canada, Ukraine, France, Malaysia, Australia, and Spain ranking among the top 10 countries in pesticide consumption, as per World meter data (Worldometer Pesticide Use by Country, 2022). This escalating trend in pesticide utilization is a key factor, with 64% of global agricultural land facing the risk of pesticide pollution from multiple active ingredients, and 31% at high risk (Tang *et al*., 2021). The widespread use of pesticides has detrimental effects on lakes and rivers due to runoff from fields, posing risks to both animals and humans owing to their ability to bioaccumulate and disrupt the food chain (Lushchak *et al.,* 2018; Bodnar *et al.,* 2022). Fish, integral to freshwater and marine ecosystems, play a crucial role in maintaining ecological balance (Okwuosa *et al*., 2019). Due to their mobility and relatively long lifespan, fish are considered effective bioindicators of long-term toxic effects and various habitat conditions (Falfushynska *et al.,* 2014; Falfushynska *et al.,* 2019). Reports suggest that certain teleost and zebrafish species exhibit metabolic traits resembling those of humans, making them potential alternative candidates for mechanistic research into cellular events triggered by physical and chemical stimuli (Hahn and Sadler, 2020).

Synthetic pyrethroids, including the newly synthesized insecticide called cypermethrin, is a type of pesticide that has been widely used. However, the use of these insecticides has raised concerns as they not only affect targeted pests but also impact the biology of non-target species (Elliot and Janes, 1978; Reddy and Yellamm, 1991). In particular, they can be highly toxic to fish and aquatic invertebrates even at very low concentrations. While these insecticides exhibit low toxicity to mammals, they pose a significant threat to invertebrates, fish, and amphibians (Robert Edwards, 1986). This heightened toxicity has notable repercussions on the health of ecosystems and their biodiversity (Madara Ranatunga *et al.,* 2023).

Organophosphates (OP) are highly favored pesticides owing to their efficacy and minimal persistence in the environment. These pesticides directly impede the activity of acetylcholinesterase enzymes in both fishes and invertebrates (Fulton and Key, 2001; Agrhari, *et al*., 2006). Chlorpyrifos (CPF), an extensively employed organophosphorous insecticide, falls within this category and is widely utilized in agricultural areas (Chandler *et al*., 1997; Hill *et al*., 1994). Chlorpyrifos, a widely employed organophosphate pesticide, holds the position of the second-largest selling pesticide in India. It has been utilized for over a decade to manage pests in various crops such as cotton, paddy fields, pasture, and vegetables (Rao *et al.,* 2003). The extensive application of chlorpyrifos raises concerns about heightened toxicity in aquatic environments, posing adverse effects on non-target organisms, particularly fish (Padmanabha *et al*., 2015; Banaee *et al*., 2013).

Alterations in the enzymatic system can have an impact on metabolic processes. Toxicologists are increasingly interested in examining how individual enzymes or groups of enzymes respond to toxic insults. Numerous reports discuss the effects of insecticides on various metabolic aspects. Researchers have demonstrated that the pattern of enzymatic changes during toxic stress can differ from one tissue to another. Some enzymes exhibit increased activity, while others demonstrate a progressive decrease (Durkin and Nishikavava, 1971).

Cypermethrin and chlorpyrifos are extensively utilized pesticides, each possessing unique chemical compositions and mechanisms of action. When these pesticides coexist in aquatic environments, there is a potential for synergistic effects on the physiology and metabolism of aquatic organisms. Synergistic effects manifest when the combined toxicity of two substances surpasses the cumulative impact of their individual toxicities. In the context of cypermethrin and chlorpyrifos, their concurrent presence may result in heightened adverse effects on the metabolism of *Labeo rohita*. This could entail disruptions in metabolic pathways, enzyme activities, and overall physiological functions. The current study explores the collective influence of cypermethrin and chlorpyrifos on selected oxidative enzymes in *Labeo rohita*, a freshwater Indian major carp fish species.

Materials and Methods

Test Species:

The freshwater fish *Labeo rohita* (8 to 12 cm; 60-80 g) were procured from a fish seed rearing center. The fish underwent a 10-day acclimatization period in a laboratory setting at 28-30°C, pH 8.1 (slightly alkaline), and dissolved oxygen levels of 8–10 ppm. Throughout acclimatization, the water was changed daily, and the fish were fed a diet consisting of rice bran and groundnut oil cake.

Test chemicals:

Cypermethrin technical grade (92% purity, cis:trans isomers ratio 40:60) obtained from Tagros Chemicals India Limited, Chennai, India and Chlorpyrifos technical grade insecticide with 97.5% purity was obtained from Nagarjuna Agri Chem Limited, Ravulapalem, East Godavari (Dt), AP, India.

Experimental Design:

Acute toxicity experiment was conducted for 48hrs by Finney (1971). Probit analysis and the LC_{50} value for Cypermethrin (0.15ppm) and Chlorpyrifos (0.42 ppm) were determined. $1/10^{th}$ of the LC_{50} value of cypermethrin (0.015 ppm) and chlorpyrifos (0.042 ppm) were taken as sublethal concentrations for this study. The fish were divided into distinct groups, each comprising 10 individuals. Group I served as the control, maintained in tap water. Group II was exposed to $1/10^{th}$ of the LC_{50} concentration of Cypermethrin, Group III to $1/10^{th}$ of the LC_{50} concentration of Chlorpyrifos, and Group IV to a combination of Cypermethrin ($1/20^{th}$ of LC_{50}) and Chlorpyrifos ($1/20^{th}$ of LC_{50}). All groups were maintained in separate 10-liter plastic containers. After 7-day exposure period, the fish were sacrificed, and liver and kidney were collected for the assessment of specific enzyme activities. The chosen enzymes included Glucose-6-Phosphate dehydrogenase (G6PD), Lactate dehydrogenase (LDH), Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH), which were estimated by the method of Lohr and Waller (1965) modified by Mastanaiah *et al*. (1978), Srikanthan and Krishnamoorthy (1955), Nachlas *et al*. (1960) and Nachlas *et al*. (1960), respectively.

Results and Discussion

The levels of Glucose-6-phosphate dehydrogenase (G6PDH), Lactate Dehydrogenase (LDH), Succinate Dehydrogenase (SDH), and Malate Dehydrogenase (MDH) enzymes were determined in the control as well as experimented fishes after exposure to cypermethrin and chlorpyrifos individually and in combination for seven days. The enzyme activities were observed in liver and kidney tissues (Figs. 1, 2). The activities of Glucose-6-phosphate dehydrogenase (G6PDH), Lactate Dehydrogenase (LDH), showed an increased trend in all tissues, Succinate Dehydrogenase (SDH), and Malate Dehydrogenase (MDH) activities showed decrement in all tissues. The changes were greater in combination treatment of cypermethrin and chlorpyrifos (Group-IV), followed by cypermethrin individually treated fishes (Group-II) and chlorpyrifos individually treated fishes (Group-III).

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that plays a crucial role in the pentose phosphate pathway, which is a metabolic pathway that generates NADPH and pentoses. NADPH is essential for various cellular processes, including the maintenance of cellular redox balance and the protection of cells from oxidative stress. G6PD catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone, while reducing $NADP^+$ to NADPH. NADPH produced by G6PD is critical for the cell to combat oxidative stress. G6PD deficiency can lead to hemolytic anemia, especially under conditions of oxidative stress. Increased activity of glucose-6-phosphate dehydrogenase (G-6-PDH) was observed in the present study in the tissues of *Labeo rohita* exposed to individual and combined

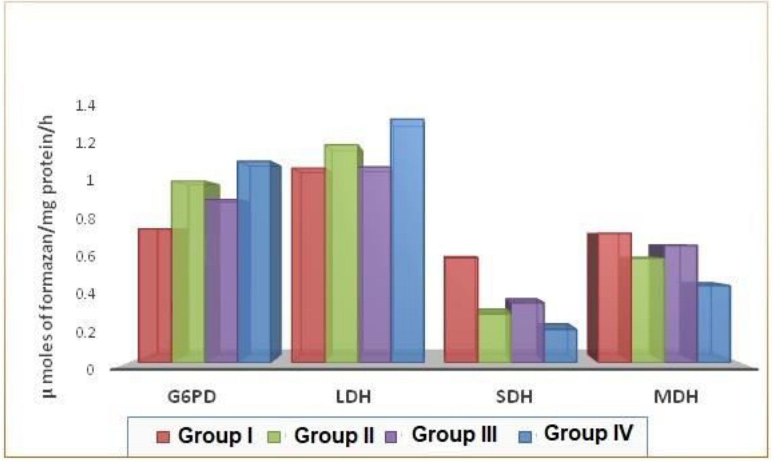

Fig. 1: Alteration in enzyme activity in liver of *Labeo rohita* after exposure to cypermethrin and chlorpyrifos. Each value represents mean ± SD (n=5), Values are significant at $P < 0.05$.

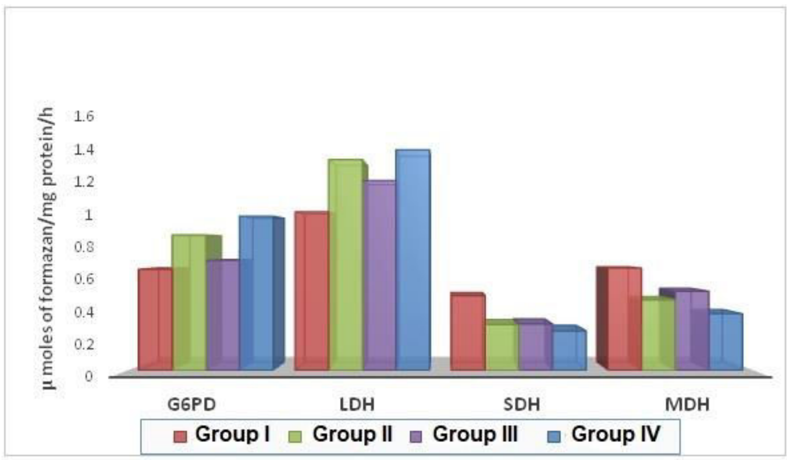

Fig. 2: Alteration in enzyme activity in kidney of *Labeo rohita* after exposure to cypermethrin and chlorpyrifos. Each value represents mean ± SD (n=5), Values are significant at $P < 0.05$.

treatments of cypermethrin and chlorpyrifos. Individuals with G6PD deficiency may experience hemolysis in response to certain drugs, infections, or exposure to certain foods or substances (Murray *et al*., 1995). In conditions of high energy demand, this pathway generates glycolytic intermediates for energy production (Voet and Veot, 1955). The heightened glucose oxidation, facilitated by the upregulated HMP shunt through G-6-PDH, is associated with prevailing anaerobic conditions (Bhatia *et al*., 1972). The rise in G-6-PDH activity aligns with findings from previous studies (Vani, 1991). This increased activation of the HMP shunt is speculated to be a crucial mechanism in response to tissue repair, cell regeneration, and proliferation during inflammatory responses (Beaconsfield and Carpi, 1964).

Lactate dehydrogenase (LDH) is a crucial enzyme involved in cellular metabolism, specifically in the interconversion of lactate and pyruvate. This enzymatic reaction is part of the anaerobic and aerobic energy production pathways in cells. LDH plays a key role in maintaining the balance between the production and utilization of lactate and pyruvate, which are important for energy metabolism. Changes in lactate dehydrogenase (LDH) activity signify variations in the conversion of pyruvate to lactate during anaerobic conditions, facilitating the reoxidation of NADH. Elevated LDH activity in tissues under diverse toxic conditions signifies the significance of LDH as a crucial glycolytic enzyme in biological systems. LDH is known to be responsive to oxidative stress and can be induced accordingly. LDH catalyzes the conversion of pyruvate to lactate under anaerobic conditions (Lehninger, 1993). Consequently, the activity of various regulatory enzymes may be modified to fulfill the heightened energy demands imposed by toxic stress. The increase in LDH activity could be ascribed to a repressive influence on their synthesis or to the direct impact of pesticides on the enzymes.

An increased LDH activity was observed in the present study. *Gambusia affinis* exposed to chlorpyrifos showed an increased LDH activity in liver and kidney tissues (Sharma *et al.,* 2016). When a fish experiences stress, it tends to fulfill its energy needs through anaerobic oxidation (Wallace Luiz, 2004). The activity of LDH is influenced by its five isoenzymes, and these activities can undergo changes in response to pathological conditions (Martin *et al.,* 2005). Cypermethrin significantly affects the overall oxygen consumption in the entire organism by reducing the activity of TCA cycle enzymes (Tripathi and Singh, 2002). Studies conducted by Kamalaveni *et al*. (2003) noted an increase in LDH activity in the liver of *Cyprinus carpio* under environmental stress pollution. Similarly, Jacob Doss *et al*. (2007) reported elevated LDH activity in the freshwater fish *Labeo rohita* following exposure to cypermethrin. Banaee *et al.* (2013) reported an increased level of LDH activities *Cyprinus carpio* exposed to chlorpyrifos. An increased LDH activity was observed at different concentrations of chlorpyrifos in fish (Topal *et al*., 2014).

Succinate dehydrogenase (SDH) is an enzyme that plays a crucial role in both the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) of cellular respiration. SDH catalyzes the oxidation of succinate to fumarate in the TCA cycle and transfers electrons to the ETC. The enzymatic activity of SDH connects the TCA cycle, which oxidizes Acetyl-CoA derived from nutrients, to the electron transport chain, where the electrons are transferred to oxygen to produce water.

The decreasing trend in succinate dehydrogenase (SDH) activity in the present study indicates alterations in oxidative metabolism, reflecting changes in carbohydrate turnover and energy output. The reduced functionality of the glycolytic pathway and the decreased entry of pyruvate into the tricarboxylic acid (TCA) cycle align with the observed decline in SDH activity levels. The fish *Labeo rohita* treated with sub-lethal doses of cypermethrin and chlorpyrifos exhibit a notable reduction in SDH activity, suggesting that the pesticides induced damage to cellular architecture and components may contribute to the observed increase in G6-PDH activity. A prior study showed a significant decrease in SDH activity in the gastrocnemius muscle of mice treated with sodium fluoride compared to control groups (Lakshmi and Pratap Reddy, 2000). Various reports have emphasized a decrease in SDH activity in mouse muscles (Chinoy *et al*., 1996). Jacob Doss *et al.* (2007) noted a reduction in SDH activity in the liver and brain of *Labeo rohita* exposed to cypermethrin. Similarly, Satyaparameshwar *et al*. (2006) documented diminished SDH activity in selected tissues of the freshwater mussel *Lamellidens marginalis* exposed to copper sulfate. Cypermethrin hinders the activities of oxidative enzymes like succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in fish (Leela Rani, 2006).

Malate dehydrogenase (MDH) catalyzes the reversible conversion of malate to oxaloacetate in the presence of NAD^+ or $NADP^+$. This enzymatic reaction is an integral part of the citric acid cycle and contributes to the overall energy production within cells. The conversion of malate to oxaloacetate is crucial for the cycle to continue, as oxaloacetate is a substrate for the condensation with Acetyl-CoA, initiating a new cycle. In the present investigation, the levels of malate dehydrogenase (MDH) displayed an inhibited pattern in specific tissues of *Labeo rohita* under the stress induced by cypermethrin and chlorpyrifos. MDH, as an NAD-dependent enzyme, is responsible for converting malate to oxaloacetate and facilitating the reversible oxidation of fumarate to malate. Oxaloacetate also plays a crucial role in CO_2 fixation and gluconeogenesis (Martin, 1983).

The decreased in malate dehydrogenase (MDH) activity indicates variations in oxidative metabolism, reflecting changes in carbohydrate turnover and energy output (Murray *et al*.,1995). Changes in mitochondrial structure are recognized to impede MDH activity (Lieber, 1984). The decline in MDH activity may also stem from oxaloacetate inhibition, as reduced TCA cycle dehydrogenase activity correlates with mitochondrial disintegration, hindering the conversion of acetate to CO_2. The diminished MDH levels imply a shift in respiratory metabolism towards anaerobiosis. Similar shifts towards anaerobic metabolism, leading to decreased oxidative metabolism and MDH activity, have been reported by Murthy *et al.* (1983) under the ttoxicity of fenitrothion in *Labeo rohita*. Samson Raju (2000) reported reduced MDH activity in the tissues of albino rats due to sodium selenate intoxication.

Conclusion

Cypermethrin and chlorpyrifos are commonly used pesticides known for their potential harm to aquatic organisms. Cypermethrin demonstrates notably higher toxicity in fish compared to chlorpyrifos. When these pesticides are combined, there is a risk of synergistic effects, where their combined toxicity exceeds the sum of their individual impacts. The synergistic effects of cypermethrin and chlorpyrifos disrupt various metabolic activities, leading to observable alterations in the activity levels of Glucose-6-phosphate dehydrogenase, lactate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase in the experimental fish *Labeo rohita*. The research also emphasizes that these chemicals exhibit synergistic effects across all parameters, with the combined effects being more severe than those resulting from independent exposure. Balancing the benefits of pesticide use in agriculture with potential risks to aquatic ecosystems is crucial. Implementing sustainable agricultural practices, adopting integrated pest management strategies, and developing less toxic alternatives are essential steps to minimize the environmental impact of pesticides on aquatic ecosystems. The frequent application of these pesticides in agriculture fields and their release into water bodies poses a significant hazard to freshwater ecosystems. Considering the aforementioned findings, it is advisable to discourage the indiscriminate use of these pesticides in water bodies.

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