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Cypermethrin and Chlorpyrifos toxicity on enzyme activities in muscle and heart tissues of Indian major carp *Labeo rohita* (Hamilton, 1822)

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ABSTRACT
The study focuses on investigating the impact of cypermethrin and chlorpyrifos, commonly used pesticides in agriculture, on the Indian major carp Labeo rohita. The research specifically examines the toxicity of these pesticides when applied individually and in combination over a 7-day period. The concentrations used were 1/10th of the LC_{50} dosage for each pesticide individually (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 monitored in muscle and heart tissues of Labeo rohita. Results indicated an upward trend in Glucose-6-phosphate dehydrogenase (G6PDH) and Lactate Dehydrogenase (LDH) activities in all tissues. Conversely, Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH) activities showed a decrease in all tissues following exposure to cypermethrin and chlorpyrifos. Notably, these changes were more pronounced in the combined treatment, suggesting a potential synergistic effect between cypermethrin and chlorpyrifos. This emphasizes the importance of considering the combined impact of multiple pesticides on aquatic organisms in assessing water quality and potential ecological risks.
Keywords: *Cypermethrin, Chlorpyrifos, Labeo rohita, G6PDH, LDH, SDH, MDH, Muscle, Heart*

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INTRODUCTION

Pesticides are essential components of modern agricultural practices, serving vital roles in pest control, crop loss prevention, and the management of vector-borne diseases [1]. The global use of pesticides has surged to approximately 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, according to World Meter data [2]. This increasing reliance on pesticides poses a significant risk, with 64% of the world's agricultural land facing potential pesticide pollution from multiple active ingredients, and 31% at a high-risk level [3]. The widespread application of pesticides has detrimental consequences for lakes and rivers, primarily due to runoff from agricultural fields. This runoff poses risks to both animals and humans due to the pesticides' ability to bioaccumulate and disrupt the food chain [4]. Fish, crucial components of freshwater and marine ecosystems, play a vital role in maintaining ecological balance [5]. Synthetic pyrethroids, including the insecticide cypermethrin, have seen widespread use, raising concerns due to their impact not only on targeted pests but also on non-target species' biology [6]. Particularly, these insecticides can be highly toxic to fish and aquatic invertebrates, even at minimal concentrations. While demonstrating low toxicity to mammals, they pose a significant threat to invertebrates, fish, and amphibians [7]. This heightened toxicity has substantial implications for ecosystem health and biodiversity [8]. Organophosphates (OP) are favored pesticides due to their effectiveness and minimal persistence in the environment. Chlorpyrifos (CPF), an extensively used organophosphorous insecticide, falls into this category and is widely applied in agricultural areas [9]. In India, chlorpyrifos holds the position of the second-largest selling pesticide and has been used for over a decade to manage pests in various crops such as cotton, paddy fields, pasture, and vegetables [11]. The extensive application of chlorpyrifos raises concerns about increased toxicity in aquatic environments, leading to adverse effects on non-target organisms, particularly fish [12]. These pesticides directly inhibit the activity of acetylcholinesterase enzymes in both fish and invertebrates [10]. Changes in the enzymatic system can

significantly influence metabolic processes, attracting increased attention from toxicologists exploring how individual enzymes or enzyme groups respond to toxic insults. Numerous studies have addressed the impact of insecticides on various metabolic aspects, revealing that the pattern of enzymatic changes during toxic stress can vary among different tissues. Some enzymes show increased activity, while others undergo a progressive decrease [13]. Cypermethrin and chlorpyrifos, widely used pesticides with distinct chemical compositions and mechanisms of action, have the potential for synergistic effects on the physiology and metabolism of aquatic organisms when present together in aquatic environments. Synergistic effects occur when the combined toxicity of two substances exceeds the cumulative impact of their individual toxicities. In the case of cypermethrin and chlorpyrifos, their simultaneous presence may lead to heightened adverse effects on the metabolism of *Labeo rohita.* This could result in disruptions in metabolic pathways, enzyme activities, and overall physiological functions. The present study delves into the collective influence of cypermethrin and chlorpyrifos on selected enzymes in muscle and heart tissues of *Labeo rohita*, a freshwater Indian major carp species.

MATERIAL AND METHODS

Test Species: The fresh water fishes *Labeo rohita* (Hamilton) ranging from 8 to 12 cm in length and weighing 60-80g, were procured from a fish seed rearing center, Tirupati, Andhra Pradesh. The fish underwent a 10-day acclimatization period in large plastic water tanks within laboratory conditions. The room temperature was maintained at 28-30°C, pH of the water 8.1 (slightly alkaline), and dissolved oxygen levels of 8–10 ppm, with a 12-12-hour dark and light cycle. Throughout acclimatization, the water was changed daily, and the fish were fed a diet consisting of rice bran and groundnut oil cake All recommended precautions for the toxicity testing of aquatic organisms, as outlined by APHA in 1998, 2005, and 2012 were followed.

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

Experimental Design: An acute toxicity experiment lasting 48 hours was conducted using Finney's probit analysis [14]. The LC_{50} values were determined to be 0.15 ppm for Cypermethrin and 0.42 ppm for Chlorpyrifos. Sublethal concentrations for this study were set at $1/10^{th}$ of the LC_{50} values, resulting in concentrations of 0.015 ppm for Cypermethrin and 0.042 ppm for Chlorpyrifos. The fish were divided into distinct groups, each consisting of 10 individuals. Group I served as the control and was maintained in tap water. Group II was exposed to 1/10th 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 housed in separate 10-liter plastic containers. After a 7-day exposure period, the fishes were sacrificed, and tissues such as the muscle and heart were collected for the assessment of specific enzyme activities. The selected enzymes, including Glucose-6-Phosphate dehydrogenase (G6PD), Lactate dehydrogenase (LDH), Succinate Dehydrogenase (SDH), and Malate Dehydrogenase (MDH), were assessed using the methods of Lohr and Waller, 1965 [15], as modified by Mastanaiah *et al*., [16], Srikanthan and Krishnamoorthy [17], Nachlas *et a*l., [18], and Nachlas *et al.,* [18], respectively.

RESULTS

The study assessed alterations in the activity levels of Glucose-6-phosphate dehydrogenase (G6PDH), Lactate Dehydrogenase (LDH), Succinate Dehydrogenase (SDH), and Malate Dehydrogenase (MDH) enzymes in both control and experimental fish after exposure to cypermethrin and chlorpyrifos individually and in combination over a seven-day period. The enzyme activities were observed in both muscle and heart tissues (Graph 1 & 2). In all tissues, the activities of Glucose-6-phosphate dehydrogenase (G6PDH) and Lactate Dehydrogenase (LDH) exhibited an increasing trend, while Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH) activities showed a decrement. The observed changes were more pronounced in the group exposed to the combination of cypermethrin and chlorpyrifos (Group-IV), followed by fish treated individually with cypermethrin (Group-II) and chlorpyrifos (Group-III).

Table- 1: Alteration in enzyme activities in muscle tissues of *Labeo rohita* after acute exposure to cypermethrin and chlorpyrifos (µ moles of formazan/mg protein/hr)

Enzymes (µ moles of formazan/mg protein/hr)	Groups			
	Group I (Control)	Group II (Treated with Cypermethrin)	Group III (Treated with Chlorpyrifos)	Group IV (Treated with Cypermethrin + Chlorpyrifos)
G6PDH	0.645±0.008	0.856±0.006	0.732±0.008	1.024±0.004
LDH	1.156±0.001	1.483±0.003	1.359±0.001	1.541±0.004
SDH	0.443±0.002	0.375±0.004	0.410±0.003	0.302±0.006
MDH	0.586±0.003	0.320±0.004	0.437±0.002	0.284±0.005

Each value is the mean of five observations. (Values expressed in µ moles of formazan/mg protein/hr) ± SD, Values are significant at P < 0.05

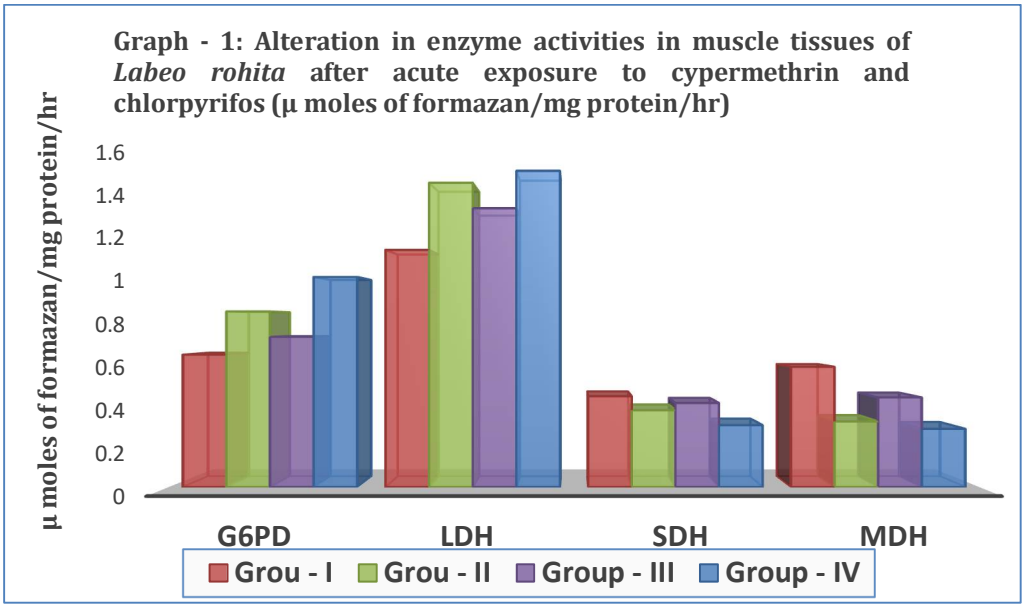


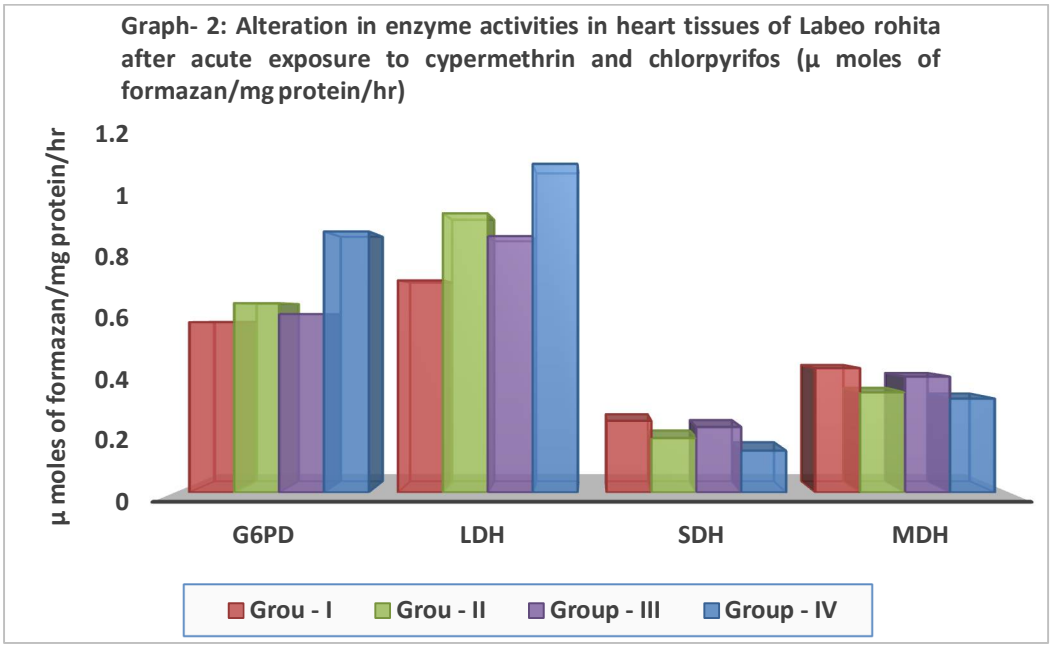
Table- 2: Alteration in enzyme activities in heart tissues of *Labeo rohita* after acute exposure to cypermethrin and chlorpyrifos (µ moles of formazan/mg protein/hr)

Enzymes (µ moles of formazan/mg protein/hr)	Groups			
	Group I (Control)	Group II (Treated with Cypermethrin)	Group III (Treated with Chlorpyrifos)	Group IV (Treated with Cypermethrin + Chlorpyrifos)
G6PDH	0.582±0.004	0.647±0.005	0.610±0.002	0.892±0.004
LDH	0.725±0.002	0.954±0.004	0.876±0.008	1.123±0.006
SDH	0.245±0.003	0.186±0.005	0.224±0.003	0.143±0.004
MDH	0.425±0.004	0.342±0.006	0.396±0.008	0.321±0.004

Each value is the mean of five observations. (Values expressed in µ moles of formazan/mg protein/hr) ± SD, Values are significant at P < 0.05

DISCUSSION

Glucose-6-phosphate dehydrogenase (G6PD) is a pivotal enzyme in the pentose phosphate pathway, a metabolic route generating NADPH and pentoses. NADPH is vital for cellular processes, maintaining redox balance, and shielding cells from oxidative stress. G6PD facilitates the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone while concurrently reducing $NADP^+$ to NADPH. The NADPH produced by G6PD is crucial for cells to combat oxidative stress, and deficiency in G6PD can result in hemolytic anemia, particularly under oxidative stress conditions. Individuals with G6PD deficiency may undergo hemolysis in response to specific drugs, infections, or exposure to certain substances [19].



The current study observed an increased activity of glucose-6-phosphate dehydrogenase (G-6-PDH) in the tissues of *Labeo rohita* exposed to individual and combined treatments of cypermethrin and chlorpyrifos. During conditions of elevated energy demand, this pathway generates glycolytic intermediates for energy production [20]. The heightened glucose oxidation, facilitated by the upregulated Hexose Monophosphate (HMP) shunt through G-6-PDH, is associated with prevalent anaerobic conditions [21]. The increase in G-6-PDH activity aligns with findings from previous studies [22]. This heightened activation of the HMP shunt is presumed to be a crucial mechanism in response to tissue repair, cell regeneration, and proliferation during inflammatory responses [23]. Lactate dehydrogenase (LDH) is a pivotal enzyme involved in cellular metabolism, specifically in the interconversion of lactate and pyruvate. This enzymatic reaction is integral to both anaerobic and aerobic energy production pathways within cells. LDH plays a crucial role in balancing the production and utilization of lactate and pyruvate, essential components of energy metabolism. Changes in lactate dehydrogenase (LDH) activity indicate alterations in the conversion of pyruvate to lactate during anaerobic conditions, aiding in the reoxidation of NADH. LDH is responsive to oxidative stress and can be induced accordingly, catalyzing the conversion of pyruvate to lactate under anaerobic conditions [24]. Consequently, the activity of various regulatory enzymes may be modified to meet heightened energy demands imposed by toxic stress. The increase in LDH activity could be attributed to a repressive influence on their synthesis or to the direct impact of pesticides on the enzymes. The present study observed an elevated LDH activity, a finding supported by several authors. Gambusia affinis exposed to chlorpyrifos showed increased LDH activity in liver and kidney tissues [25]. In response to stress, fish often meet their energy needs through anaerobic oxidation [26]. Cypermethrin significantly impacts overall oxygen consumption in the organism by reducing the activity of tricarboxylic acid (TCA) cycle enzymes [27]. Studies by Kamalaveni *et al*. [28] noted an increase in LDH activity in the liver of *Cyprinus carpio* under environmental stress pollution. Similarly, elevated LDH activity was observed in the freshwater fish Labeo rohita exposure to cypermethrin. Banaee *et al*. [29]. An increased level of LDH activities in *Cyprinus carpio* exposed to chlorpyrifos [30]. Succinate dehydrogenase (SDH) is an enzyme with 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 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. *Labeo rohita* fish treated with sub-lethal doses of cypermethrin and chlorpyrifos show 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. Various reports have emphasized a decrease in SDH activity in mouse muscles [31]. Jacob Doss *et al.* [32] noted a reduction in SDH activity in the liver and brain of Labeo rohita exposed to cypermethrin. Similarly, Satyaparameshwar *et a*l. [33] 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 [34]. A prior study showcased a significant decrease in SDH activity in the gastrocnemius muscle of mice treated with sodium fluoride compared to control groups [35]. 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, contributing to overall energy production within cells. The conversion of malate to oxaloacetate is crucial for the cycle to continue, as oxaloacetate serves as a substrate for the condensation with Acetyl-CoA, initiating a new cycle. In the present investigation, the activity levels of malate dehydrogenase (MDH) exhibited an inhibited pattern in muscle and heart tissues of *Labeo rohita* under the stress induced by cypermethrin and chlorpyrifos. As an NAD-dependent enzyme, MDH is responsible for converting malate to oxaloacetate and facilitating the reversible oxidation of fumarate to malate. Oxaloacetate also plays a crucial role in CO2 fixation and gluconeogenesis [36]. The decrease in malate dehydrogenase (MDH) activity indicates variations in oxidative metabolism, reflecting changes in carbohydrate turnover and energy output [37]. Changes in mitochondrial structure are recognized to impede MDH activity [38]. The decline in MDH activity may also result from oxaloacetate inhibition, as reduced TCA cycle dehydrogenase activity correlates with mitochondrial disintegration, hindering the conversion of acetate to CO2. 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.* [39] under the toxicity of fenitrothion in *Labeo rohita.* Cypermethrin hinders the activities of succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in fish [34].

CONCLUSION

Cypermethrin and Chlorpyrifos, widely employed pesticides, are recognized for their potential harm to aquatic organisms, with Cypermethrin demonstrating higher toxicity in fish compared to Chlorpyrifos. The combination of these pesticides poses a risk of synergistic effects, where their combined toxicity surpasses the sum of their individual impacts. These synergistic effects 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*. 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 agricultural fields and their release into water bodies poses a significant hazard to freshwater ecosystems. Given the aforementioned findings, it is advisable to discourage the indiscriminate use of these pesticides in water bodies.

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