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ORIGINAL ARTICLE

Pesticide toxicity induced oxidative stress in freshwater fish *Labeo rohita*

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ABSTRACT
Pesticides can have significant impacts on aquatic ecosystems, and their toxicity can pose serious threats to aquatic organisms and the overall health of water bodies. This study examined the acute toxicity of the synthetic pyrethroid pesticide cypermethrin and the organophosphate pesticide chlorpyrifos, both separately and in combination, on the antioxidant enzyme activities Viz., Xanthine oxidase (XOD), Superoxide dismutase (SOD) and Catalase (CAT) in the kidney and muscle tissues of the carp fish species Labeo rohita. The fish were subjected to sublethal concentrations of cypermethrin and chlorpyrifos individually and in combination over a 7-day period. Individual treatments were administered using 1/10th of the LC_{50} dosage, equivalent to 0.308 µg/L for cypermethrin and 44.28 µg/L for chlorpyrifos. In the case of the combined treatment,1/20th of the LC_{50} in concentrations of 0.154 µg/L for cypermethrin and 22.14 µg/L for chlorpyrifos. A significant (P<0.05) increase was observed in the activities of XOD, SOD, and CAT in both kidney and muscle tissues compared to the control group. Notably, these alterations were more pronounced in the combined treatment, indicating a potential synergistic effect of cypermethrin and chlorpyrifos. This underscores the importance of considering the combined impact of these pesticides when evaluating their effects on the antioxidant defence system in aquatic organisms.
Keywords: *Cypermethrin, Chlorpyrifos, Labeo rohita, XOD, SOD, CAT, Kidney, Muscle.*

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INTRODUCTION

Pesticides play a vital role in Indian agriculture, safeguarding crops from pests, diseases, and weeds [1]. Pesticides are indispensable tools for farmers, ensuring a stable and ample food supply. Pesticide application is crucial for sustaining high crop yields by preventing losses caused by pests and diseases [2]. However, the impact of pesticides on aquatic organisms is a significant concern, as these chemicals can enter water bodies through various pathways, such as runoff from agricultural fields, urban areas, and industrial discharges. This introduction of pesticides can have adverse effects on aquatic ecosystems, affecting not only targeted pests but also non-target organisms like fish [3]. Fish, among the diverse inhabitants of aquatic ecosystems, exhibit relatively higher sensitivity to changes in their environment. The concentration of pesticides in aquatic organisms often surpasses that in the ecosystem itself, a result of bioaccumulation where toxic substances are absorbed from the environment and accumulate in various organs and tissues. Additionally, the concentration of these substances tends to increase at higher trophic levels, possibly due to biomagnification [4]. The release of pesticides into aquatic ecosystems significantly impacts fish, with potential repercussions for human health [5]. The discharge of potentially harmful agrochemicals, including pesticides, into freshwater environments has notably adverse effects on non-target species, especially aquatic animals [6,7]. Pesticide residues in aquatic environments pose toxicological hazards to a diverse range of non-target organisms, ultimately entering the food chain [8]. Cypermethrin belongs to the pyrethroids [9]. Its widespread use in tropical countries includes the management of insect pests affecting crops. Type II pyrethroids, like cypermethrin, are preferred in pest

management due to their relatively low toxicity to birds and mammals, although they have been identified as highly toxic to fish [10,11]. Consequently, the vulnerability of fish to agricultural run-offs has led to cypermethrin being a focal point in numerous research studies. Chlorpyrifos, classified as an organophosphate with broad-spectrum properties, is extensively used in agriculture and demonstrates varying levels of toxicity among different species. Notably, it stands as the second-largest selling organophosphate agrochemical in India [12]. The widespread application of chlorpyrifos raises concerns about increased toxicity in aquatic environments, potentially causing adverse effects on non-target organisms, with a particular emphasis on fish [13]. Pesticides have a significant capability to induce oxidative stress in aquatic organisms by generating free radicals and reactive oxygen species (ROS). This process disrupts the balance between intracellular ROS levels and antioxidant protection, leading to oxidative stress in organisms [14]. The presence of toxicants in aquatic organisms exposed to pesticides is linked to the generation of ROS, resulting in oxidative stress, proposed as a plausible mechanism of toxicity [15]. Pesticides can induce the production of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals [16]. Antioxidant enzymes play a crucial role in mitigating the detrimental effects of free radicals and reactive oxygen species, offering protection to cells against damage. Superoxide dismutases (SODs), among these enzymes, play a pivotal role in breaking down superoxide anions into oxygen and hydrogen peroxide. Alterations in the enzymatic system can exert influence over metabolic processes, as indicated by research demonstrating that changes during toxic stress may manifest differently among tissues. Some enzymes may exhibit heightened activity, while others may undergo a gradual decrease [17]. The pesticides cypermethrin and chlorpyrifos, both widely used, possess distinctive chemical structures and mechanisms of action. In aquatic environments where these pesticides coexist, the potential for interaction exists, giving rise to synergistic effects on the physiology and metabolism of aquatic organisms. This interaction has the potential to disrupt metabolic pathways, enzyme activities, and overall physiological functions. Despite individual toxicological studies on cypermethrin and chlorpyrifos, there is a noticeable gap in research regarding their combined toxic effects. Consequently, this study aims to explore the cumulative impact of cypermethrin and chlorpyrifos on oxidative stress in *Labeo rohita.*

MATERIAL AND METHODS

Test Species: *Labeo rohita* (Hamilton), freshwater fishes measuring 8 to 12 cm in length and weighing 60-80g, were obtained from a fish seed rearing center in Tirupati, Andhra Pradesh. The fishes underwent a 10-day acclimatization period in large plastic water tanks under laboratory conditions. The room temperature was maintained at 28-30°C, water pH at 8.1 (slightly alkaline), and dissolved oxygen levels ranged from 8 to 10 ppm, adhering to a 12-12-hour dark and light cycle. During acclimatization, the water was changed daily, and the fish were fed a diet comprising rice bran and groundnut oil cake. All recommended precautions for the toxicity testing of aquatic organisms, as outlined by APHA (American Public Health Association) in 2005 and 2012 [18], were diligently followed. **Test chemicals:** Cypermethrin technical grade (92% purity, cis:trans isomers ratio 40:60) obtained from Tagros Chemicals India Limited, Chennai, and Chlorpyrifos technical grade insecticide with 97.5% purity from Nagarjuna Agri Chem Limited, Ravulapalem, East Godavari (Dt), AP, India.

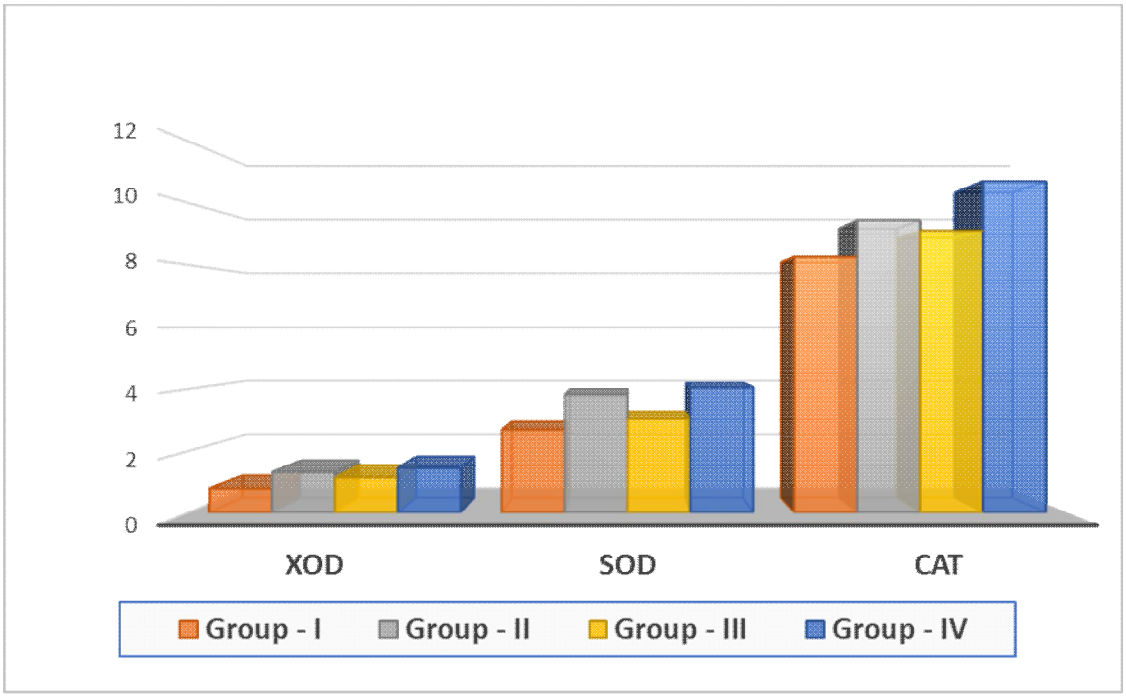
Experimental Design

The LC50 values for Cypermethrin and Chlorpyrifos were determined as 3.08 µg/L by Rajib Majumder *et al.,* [19] and 442.8 µg/L by Muhammad Ismail *et al.,* [20], respectively, and were utilized in the present study. The concentrations applied were $1/10^{th}$ of the LC_{50} dosage for individual treatments (0.308 µg/L for cypermethrin and 44.28 µg/L for chlorpyrifos) and $1/20^{th}$ of the LC_{50} for the combined treatment (0.154 µg/L for cypermethrin and 22.14 µg/L for chlorpyrifos) as sublethal concentrations. The fish were divided into four groups, each consisting of 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 and chlorpyrifos $1/20^{th}$ of LC_{50}. After stipulated 7 days of exposure period, the fishes were sacrificed, and tissues such as the kidney and muscle were collected for the assessment of oxidative enzyme activities. XOD activity was determined according to the method of Srikanthan and Krishnamoorthy [21]. SOD activity was measured using the method of Beauchamp and Fridovich [22], while Catalase (CAT) activity was determined following the method of Aebi [23].

RESULTS AND DISCUSSION

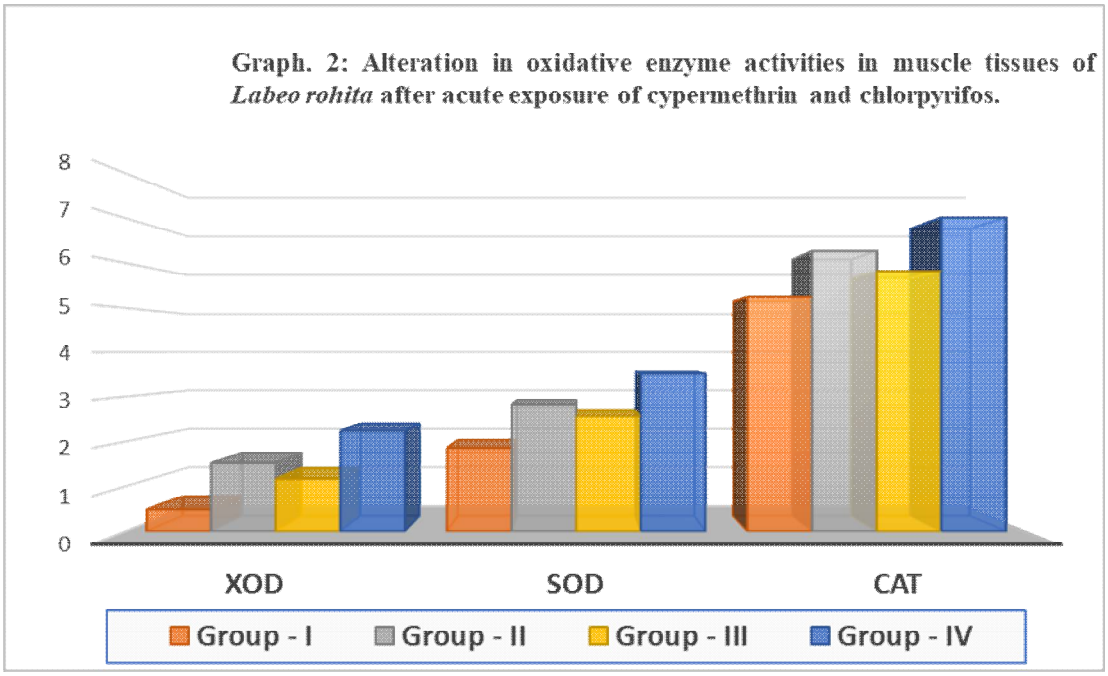
Modifications in oxidative enzyme activities were evaluated in both control and experimental fishes exposed to cypermethrin and chlorpyrifos, administered individually and in combination over a seven-

day period in an acute toxicity scenario. Remarkable alterations were evident in the experimental fish, showcasing a substantial and noteworthy increase in Xanthine oxidase (XOD), Superoxide dismutase (SOD), and Catalase (CAT) levels within the kidney and muscle tissues of *Labeo rohita.* The observed changes were more pronounced in the combined treatment compared to individual exposures, suggesting a potential synergistic effect of cypermethrin and chlorpyrifos (Graph. 1 & 2). XOD activities were quantified in terms of µ moles of formazan/mg protein/hr. SOD activities were measured in Units of Superoxide anion reduced/mg protein/min. CAT activities were determined by the µ moles of H_2O_2 decomposed/mg protein/min. Oxidative stress results from an imbalance between the generation of free radicals and an organism's ability to counteract their harmful effects through the actions of enzymes and small molecules acting as antioxidants. Numerous pollutants found in freshwater and marine ecosystems have been identified as contributors to a cellular environment that promotes pro-oxidant conditions, leading to the production of reactive oxygen species (ROS). Xanthine oxidase exhibits antioxidant properties by aiding in the neutralization of free radicals, reactive molecules capable of causing cellular damage. The observed elevation in xanthine oxidase levels in the current study suggests an increased production of superoxide anions (O^{-2}) in the kidney and muscle tissues of *Labeo rohita* exposed to cypermethrin and chlorpyrifos. The noteworthy rise in xanthine oxidase activity under pesticide-induced stress may be attributed to the conversion of xanthine dehydrogenase into xanthine oxidase. This change in enzyme activity is likely a response to maintain nitrogen balance within the tissues, as xanthine oxidase is produced when the native form of xanthine dehydrogenase undergoes alterations, such as sulphydryl oxidation or limited proteolysis [24]. Similar increased XOD activities were observed in the liver tissues of albino rats treated with aluminum chloride [25] and in liver tissues of albino rats treated with chlorpyrifos [26]. Increased Xanthine oxidase activities were observed in pesticide toxicity affected individuals [27]. Superoxide dismutase (SOD) holds a critical role as a primary antioxidant enzyme, crucial in managing oxidore radicals and providing a fundamental defense against the deleterious impacts of reactive oxygen species (ROS) [28]. Both Superoxide dismutase (SOD) and catalase (CAT) play essential roles in neutralizing superoxide anion radicals generated by xanthine oxidase. These enzymes are pivotal in protecting cells against the harmful effects of hazardous pollutants, counteracting detrimental oxidative processes within cells [29]. The increase in Superoxide dismutase (SOD) activity observed during oxidative stress induced by cypermethrin and chlorpyrifos can be attributed to the conversion of superoxide radicals into hydrogen peroxide. Subsequently, this hydrogen peroxide may undergo transformation by Catalase (CAT) into oxygen and water [30]. In this investigation, the observed elevation in superoxide dismutase activity (Graph 1 & 2) aligns with findings from similar studies, the changes in superoxide dismutase (SOD) activities could indicate cellular oxidative stress resulting from exposure to the toxicant. Comparable observations have been reported in studies investigating the impact of pesticides on other fish species exposed to chlorpyrifos [31]. Additionally, Kumar *et al.,* [16] reported an increase in SOD activity in bronchi and hepatic tissue of *Oreochromis mossambicus* following acute exposure to endosulfan. A substantial increase in SOD activity was noted in liver, gill, kidney, muscle, and brain tissues of *Labeo rohita* exposed to a pesticide mixture of endosulfan and chlorpyrifos [32]. Increased SOD activity was also observed in the liver and gill tissues of *Ctenopharyngodon idellus* during acute toxicity induced by chlorpyrifos [33]. Catalase plays a pivotal role in scavenging reactive oxygen species (ROS), converting them into less reactive forms, and preventing lipid peroxidation. Additionally, Catalase is involved in the transformation of hydrogen peroxide into oxygen and water. The heightened level of Catalase (CAT) observed may be a result of the elimination of reactive oxygen species (ROS) generated within the cell due to exposure to insecticides [34]. Increased Catalase (CAT) activity leads to an elevated production of hydrogen peroxide (H_2O_2). A significant rise in CAT activity in the liver of common carp in the presence of endosulfan has been reported [35], aligning with findings by Oruc and Usta [36]. Furthermore, a notable increase in catalase (CAT) activity was observed in the gills and liver of both *R. rita* and *C. carpio* in pesticide-polluted areas of the river Ganga [37]. Similar outcomes were documented by Kavitha and Rao [38], who observed heightened CAT activity in *Gambusia afnis* afer following exposure to the lethal effects of the organophosphate pesticide monocrotophos. Khare *et al.,* [39] noted a significant increase in *Catla catla* after pesticide exposure, while Clasen *et al.,* [40] reported an increase in liver catalase activity in *C. carpio* from a pesticide-contaminated environment. The rise in CAT activity reflects oxidative damage, responses to contaminants, and the organism's mechanism of repair to protect itself from the toxicity of chemicals.



Graph. 1: Alteration in oxidative enzyme activities in kidney tissues of *Labeo rohita* after acute exposure of cypermethrin and chlorpyrifos.

Each value is the mean of five observations. ± SD, Values are significant at P < 0.05



Graph. 2: Alteration in oxidative enzyme activities in muscle tissues of *Labeo rohita* after acute exposure of cypermethrin and chlorpyrifos.

Each value is the mean of five observations. ± SD, Values are significant at P < 0.05

CONCLUSION

The outcomes of this study underscore that simultaneous exposure to insecticides can induce significant alterations in the antioxidant enzymes of fish. These enzymes emerge as valuable biomarkers, offering a means to detect pesticide pollution in aquatic environments. The findings further imply that the presence of pesticides in water bodies may present health risks to aquatic organisms, particularly fish. Addressing the environmental repercussions of pesticides on aquatic ecosystems necessitates the adoption of sustainable agricultural practices, the implementation of integrated pest management strategies, and the

exploration of less toxic alternatives. The extensive application of these pesticides in agricultural fields and their release into water bodies poses a considerable threat to freshwater ecosystems. In light of these findings, it is recommended to discourage the indiscriminate use of these pesticides in water bodies.

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