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# Pesticide Induced Oxidative Stress on Non-target Organism *Labeo rohita*

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**ABSTRACT**
The application of pesticides in agricultural activities can result in their runoff into water bodies, causing water contamination. This contamination poses a threat to water quality, rendering it harmful to aquatic organisms. This study investigated the acute toxic effects of the synthetic pyrethroid pesticide cypermethrin and the organophosphate pesticide chlorpyrifos, both individually and in combination, on the antioxidant enzyme activities viz., Xanthine oxidase (XOD), Superoxide dismutase (SOD) and Catalase (CAT) in the liver and gill tissues of the fish, *Labeo rohita*. *L. rohita* were exposed to sub-lethal concentrations of cypermethrin and chlorpyrifos individually and in combination for 7 days. The concentrations used 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 (0.154µg/L for cypermethrin + 22.14µg/L for chlorpyrifos). The findings of this study revealed a significant ($P<0.05$) increase in the activities of Xanthine oxidase (XOD), Superoxide dismutase (SOD) and Catalase (CAT) in both liver and gill tissues compared to the control group. The observed alterations were more pronounced in the combined treatment as compared to individual exposures, suggesting a potential synergistic effect of cypermethrin and chlorpyrifos.

**Key words:** Cypermethrin, Chlorpyrifos, *Labeo rohita*, XOD, SOD, CAT, Liver, Gill.

## INTRODUCTION

Pesticides play a crucial role in Indian agriculture by helping to protect crops from various pests and diseases (Yadav and Gopinathan 2013). They are essential tools for farmers to ensure a reliable and sufficient food supply. The application of pesticides helps in maintaining high crop yields by preventing losses due to pests and diseases (Pingali and Pandey 2001). Pesticide toxicity on aquatic organisms is a significant concern as these chemicals can enter water bodies through various pathways, including runoff from agricultural fields, urban areas, and industrial discharges. Pesticides can have detrimental effects on aquatic ecosystems, affecting not only the target pests but also non-target organisms, including fish, invertebrates, and aquatic plants (Hazarika and Das, 1998). Among the diverse organisms inhabiting aquatic ecosystems, fish exhibit a relatively higher sensitivity to alterations in their surrounding environment. The concentration of pesticides in aquatic organisms is observed to be several times greater than the concentration found in the ecosystem itself. This phenomenon is attributed to bioaccumulation, wherein toxic substances are absorbed from the environment by the organism and accumulate in various organs and tissues. Additionally, the concentration of toxic substances tends to increase at higher trophic levels, likely due to biomagnification (Martin and Knaeur 1973). The release of pesticides into aquatic ecosystems significantly impacts fish, with potential implications for human health (Metelev et al. 1983). The release of potentially harmful agrochemicals, including pesticides, into freshwater environments has resulted in notably adverse impacts on non-target species, particularly aquatic animals (John 2007, Naz and Javed 2012). Residues of pesticides in aquatic environments present toxicological hazards to a diverse range of non-target organisms (Dar et al. 2015), ultimately entering the food chain.

Cypermethrin is a fourth-generation halogenated type II pyrethroid (Kaviraj and Gupta 2014). It is widely used in tropical countries to manage insect pests affecting various crops such as cotton, cabbage, okra, brinjal, sugarcane, wheat, sunflower, and others. Type II pyrethroids find widespread use in pest management due to their comparatively low toxicity to birds and mammals, but it has been identified as highly toxic to fish (Kumar et al. 2007, Saha and Kaviraj 2008). Consequently, the susceptibility of fish to agricultural run-offs has made cypermethrin a focal point in numerous research studies.

Chlorpyrifos, an organophosphate with broad-spectrum properties, finds extensive application in agriculture, exhibiting varying levels of toxicity among different species. Notably, it ranks as the second-largest selling organophosphate agrochemical in India United States Environmental Protection Agency (Anonymous 1992). The widespread use of chlorpyrifos gives rise to concerns regarding increased toxicity in aquatic environments, potentially causing adverse effects on non-target organisms, with a particular emphasis on fish (Padmanabha et al. 2015).

These pesticides possess a high capacity to instigate oxidative stress in aquatic organisms by generating free radicals and reactive oxygen species (ROS). This process leads to an imbalance between intracellular ROS levels and antioxidant protection, resulting in oxidative stress in organisms (Toni et al. 2011). The presence of toxicants in aquatic organisms exposed to pesticides is associated with the production of ROS, leading to oxidative stress, which is proposed as a plausible mechanism of toxicity (Oropesa et al. 2008). Pesticides have the potential to trigger the generation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hydroxyl radicals (Kumar et al. 2011). Antioxidant enzymes play a vital role in counteracting the harmful effects of free radicals and reactive oxygen species, providing protection to cells against damage. Superoxide dismutases (SODs), among these enzymes, play a key role in breaking down superoxide anions into oxygen and hydrogen peroxide. These enzymes are widely distributed in aerobic cells and extracellular fluids. In contrast, catalases are enzymes that accelerate the transformation of hydrogen peroxide into water and oxygen, utilizing either iron or manganese cofactors (Ananya 2019).

Changes in the enzymatic system can influence metabolic processes, researchers have illustrated that the enzymatic alterations during toxic stress may vary between tissues, with some enzymes displaying heightened activity and others showing a gradual decrease (Durkin and Nishikavava 1971). Both cypermethrin and chlorpyrifos are extensively used pesticides with distinctive chemical structures and mechanisms of action. In aquatic environments where these pesticides coexist, there is a potential for interaction, leading to synergistic effects on the physiology and metabolism of aquatic organisms. This interaction could result in disruptions in metabolic pathways, enzyme activities, and overall physiological functions. While there have been individual toxicological studies on cypermethrin and chlorpyrifos, there is a lack of understanding on their combined toxic effects. Therefore, the present study aimed to investigate the collective impact of cypermethrin and chlorpyrifos on oxidative stress in *L. rohita*.

## MATERIALS AND METHODS

The fresh water fishes, *Labeo rohita* (Hamilton) ranging from 8 to 12 cm in length and weighing 60-80 g, 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 American public health association (Anonymous 2012) were followed. 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, India. The $LC_{50}$ value for Cypermethrin was 3.08 µg/L as determined by (Majumder et al. 2018) and for Chlorpyrifos was 442.8 µg/L as determined by (Ismail et al. 2018) were used for present study. The concentrations used were $1/10^{th}$ of the $LC_{50}$ dosage for individual treatments (0.308 µg/L cypermethrin and 44.28 µg/L chlorpyrifos) and $1/20^{th}$ of the $LC_{50}$ for the combined treatment (0.154 µg/L cypermethrin + 22.14 µg/L chlorpyrifos) as sub-lethal concentrations for this study. Ten individuals of fish were exposed to each of the treatment for 7 days i.e., $T_o$ control, maintained in tap water, $T_1$ exposed to $1/10^{th}$ of the $LC_{50}$ concentration of Cypermethrin, $T_2$ exposed to $1/10^{th}$ of the $LC_{50}$ concentration of Chlorpyrifos, and $T_3$

Table 1. Change in oxidative enzyme activities in liver tissues of *Labeo rohita* after sub-lethal exposure of cypermethrin and chlorpyrifos

| Enzyme | Control | Treated with Cypermethrin | Treated with Chlorpyrifos | Treated with Cypermethrin + Chlorpyrifos |
|---|---|---|---|---|
| XOD (µ moles of formazan | 0.87±0.02 | 1.32±0.01 | 1.20±0.01 | 1.76±0.03 |
| /mg protein/hr) | PC | 51.72 | 37.93 | 102.29 |
| SOD (Units of Superoxide | 6.12±0.03 | 6.83±0.02 | 6.48±0.02 | 7.36±0.04 |
| anion reduced/mg protein/min) | PC | 11.60 | 5.88 | 20.26 |
| CAT (µ moles of $H_2O_2$ | 8.20±0.08 | 9.55±0.04 | 9.42±0.02 | 9.87±0.07 |
| decomposed/mg protein/min) | PC | 16.46 | 14.87 | 20.36 |

Each value is the mean of five observations ± SD, Values are significant at $P < 0.05$; PC - % change

exposed to a combination of Cypermethrin (1/20th of $LC_{50}$) + Chlorpyrifos (1/20th of $LC_{50}$). After a 7-day exposure period, the fishes were sacrificed and tissues of liver and gills collected for the assessment of specific enzyme activities. The XOD activity was assessed following the method of (Srikanthan and Krishnamurthy 1955). SOD activity was measured by the method of (Beachamp and Fridovich 1971). The activity of Catalase (CAT) was determined by Aebi's method (Aebi 1974).

## RESULTS AND DISCUSSION

Alterations in Oxidative enzyme activities were assessed in control and experimental fishes exposed to cypermethrin and chlorpyrifos, both individually and in combination for seven days in acute toxicity. Substantial changes were noted in the experimental fish, with a significant increased trend observed in Xanthine oxidase (XOD), Superoxide dismutase (SOD) and Catalase (CAT) in liver and gill tissues of *Labeo rohita*. The combined treatment resulted in more noticeable changes compared to individual treatments, with cypermethrin toxicity exhibiting greater effects than chlorpyrifos toxicity. This suggests that cypermethrin may be more toxic to aquatic organisms than chlorpyrifos. Furthermore, the combination of both substances showed a synergistic effect (Tables 1, 2).

Oxidative stress arises from an imbalance between the generation of free radicals and an organism's capacity to mitigate their detrimental effects through the actions of enzymes and small molecules functioning as antioxidants. Numerous pollutants present in freshwater and marine ecosystems have been identified as contributors to a cellular environment favoring pro-oxidant conditions, leading to the generation of reactive

Table 2. Alteration in oxidative enzyme activities in gill tissues of *Labeo rohita* after sub-lethal exposure of cypermethrin and chlorpyrifos

| Enzyme | Control | Treated with Cypermethrin | Treated with Chlorpyrifos | Treated with Cypermethrin + Chlorpyrifos |
|---|---|---|---|---|
| XOD (µ moles of formazan | 0.645±0.04 | 1.013±0.02 | 0.826±0.08 | 1.124±0.01 |
| /mg protein/hr) | PC | 57.05 | 28.06 | 74.26 |
| SOD (Units of Superoxide | 1.67±0.06 | 1.98±0.004 | 1.82±0.008 | 2.02±0.07 |
| anion reduced/mg protein/min) | PC | 18.56 | 8.98 | 20.95 |
| CAT (µ moles of $H_2O_2$ | 5.48±2.01 | 5.87±0.004 | 5.72±0.008 | 6.23±11.69 |
| decomposed/mg protein/min) | PC | 7.11 | 4.37 | 13.68 |

Each value is the mean of five observations ± SD, Values are significant at $P < 0.05$; PC - % change oxygen species (ROS).

XOD has antioxidant properties. It can help neutralize free radicals, which are reactive molecules that can cause cellular damage. The observed increase in XOD levels in the present study indicates an elevated production of superoxide anions ($O^{-2}$) in the liver and gill tissues of *L. rohita* exposed to cypermethrin and chlorpyrifos. The significant increase in XOD activity under pesticides induced stress (Tables 1, 2) may be attributed to the conversion of xanthine dehydrogenase into XOD. This alteration in enzyme activity is likely a response to maintain nitrogen balance within the tissues, as XOD is produced when the native form of xanthine dehydrogenase undergoes changes, such as sulphydryl oxidation or limited proteolysis (Dellacorte and Stripe 1972). Elevates of XOD activity was observed in liver tissues of albino rat treated with chlorpyrifos (Savithri et al. 2016, 2023).

SOD stands as a primary and crucial antioxidant enzyme, playing a vital role in managing oxiradicals and forming a fundamental defense against the lethal impacts of reactive oxygen species (ROS) (Kohen and Nyska 2002). SOD and CAT are playing essential roles in neutralizing superoxide anion radicals produced by XOD. These enzymes are pivotal in safeguarding cells against the harmful effects of hazardous pollutants, mitigating the detrimental oxidative processes within cells (Kuthan et al. 1986). The elevation in SOD activity during oxidative stress induced by cypermethrin and chlorpyrifos could be attributed to the conversion of superoxide radicals into hydrogen peroxide. Subsequently, this hydrogen peroxide may undergo transformation by CAT into oxygen and water (Sanchez et al. 2005). In the present study, the noted elevation in SOD activity (Tables 1, 2) aligns with the findings of similar findings by in other studies. Elevation in SOD activity in fish subjected to chlorpyrifos exposure was reported by Oruc (2010). Similarly, Kumar et al. (2011) reported an increase in SOD activity in bronchi and hepatic tissue of *Oreochromis mossambicus* following acute exposure to endosulfan. A significant increase in SOD activity was observed in liver, gill, kidney, muscle and brain tissues of *L. rohita* exposed to pesticide mixture of endosulfan and chlorpyrifos (Naz et al. 2019). Increased SOD activity observed in liver and gill tissues of Ctenopharyngodonidellus in acute toxicity of chlorpyrifos (Kaur and Jinda 2017).

CAT plays a crucial role in scavenging reactive oxygen species (ROS), transforming them into less reactive forms, and preventing lipid peroxidation. It plays a role in transforming hydrogen peroxide into oxygen and water. The enhanced level of CAT may result from the elimination of reactive oxygen species (ROS) generated within the cell due to exposure to insecticides (Stara et al. 2012). Elevated CAT activity leads to increased production of hydrogen peroxide ($H_2O_2$). A significant increase in CAT activity in the liver of common carp in the presence of endosulfan was reported by Salvo et al. (2012) which aligns with the findings reported by Oruc and Usta (2007). A significant rise in CAT activity was noted in the gills and liver of both *R. rita* and *C. carpio* in pesticide polluted areas of river Ganga (Shah and Parveen 2022). Similar outcomes have been documented by Kavitha and Rao (2007) who observed heightened CAT activity in *Gambusia afnisafer* following exposure to the lethal effects of an organophosphate pesticide, Monocrotophos. Khare et al. (2019) noted a significant increase of CAT in *Catla catla* after pesticide exposure, while Clasen (2018) reported an increase in liver catalase activity in *C. carpio* from a pesticide-contaminated system. 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.

## CONCLUSION

The findings of this study revealed that the combined exposure to insecticides can result in significant changes in the antioxidant enzymes of fish. These enzymes serve as valuable biomarkers for detecting pesticide pollution in aquatic environments. The results further suggest that the presence of pesticides in water bodies could pose health risks to aquatic animals, particularly fish. Mitigating the environmental impact of pesticides on aquatic ecosystems requires the adoption of sustainable agricultural practices, integrated pest management strategies, and the creation of less toxic alternatives. The widespread use of these pesticides in agricultural fields and their release into water bodies poses a substantial threat to freshwater ecosystems. In light of the above findings, it is recommended to discourage the indiscriminate use of these pesticides.Top of Form

**Authors' contributions**: Both the authors contributed equally.

**Conflict of interest:** Authors declare no conflict of interest.

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