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# CHLORPYRIFOS PESTICIDE EFFECT ON LABEO ROHITA LIVER EXPOSE FOR 28 DAYS

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***ABSTRACT***

*Because fungicides may remain in the environment for long periods of time and can be collected by aquatic creatures, they are considered to be dangerous contaminants of the aquatic environment. During the course of 15 and 45 days, Labeo rohita was subjected to low, medium, and high quantities of the pesticide profenofos. Red blood cell (RBC) and hemoglobin content were shown to be lower after 15 and 45 days of exposure, respectively, when compared to the control. In fish treated with profenofos, there was an increase in the total number of white blood cells (WBC). After exposing fish for 15 and 30 days to a sublethal dose of chlorantraniliprole (1/20th LC50) and their biochemical components, an assessment of the potential for oxidative stress to be induced was made in the different tissues of the fish Labeo rohita. The percentage of protein contained in several tissues, such as the liver, kidney, and gills, as well as the muscle, fell by 41%, 37%, 63%, and 50%, respectively. The largest drop in the amount of amino acids found in the kidney, liver, gills, and muscle of fish that had been exposed to pesticides were correspondingly 60%, 63%, 62%, and 51%. The similar trend was also seen in the amount of glycogen and total lipids. In comparison to the control, the levels of SOD, CAT, GST, Gpx, and LPO changed in all tissues when they were exposed to a sublethal quantity for 15 and 30 days. These results have the potential to serve as a biomarker for water contamination.*

**Keywords:** *aquatic environment, Profenofos treated fish, Labeo rohita*

## INTRODUCTION

In both domestic and agricultural settings, insecticides see heavy usage as a means of eradicating unwanted pests in both homes and fields. The most widely used synthetic chemicals for pest management are called organophosphorus (OP) insecticides. These pesticides have been shown to have harmful effects on aquatic creatures, particularly fish. Because it is an OP insecticide, chlorpyrifos is used all over the globe for the management of insect pests in agricultural and non-agricultural settings. An acetylcholinesterase (AChE) inhibitor, chlorpyrifos causes harm to the nervous system and accumulates in aquatic creatures. It also has a neurotoxic effect. The greatest ambient concentration of chlorpyrifos that has ever been documented in the United States is around 300 mg/L in the country's surface water. Not only is it one of the most significant pesticides found in fisheries products, but it is also one of the most Because its presence in freshwater bodies is hazardous to fish and other nontarget aquatic animals, chlorpyrifos is an excellent option for toxicity research because it has a half-life of 25.6 days in water with a pH of 7.0 and because the chemical has this half-life.

The presence of genotoxic pollutants in aquatic environments is one of the primary problems in the field of environmental sciences, and as a result, there is an urgent need to develop sensitive techniques for monitoring the genotoxic potentials of various chemicals in aquatic species. This requirement was prompted by the fact that the prevalence of genotoxic pollutants in aquatic environments is one of the key worries in the field of environmental sciences. The micronucleus test is becoming more used as a biomarker for the evaluation of the potentially carcinogenic effects of chemicals on aquatic species. The micronucleus test is one of the most significant genotoxic endpoints that is used to evaluate the aneugenic and clastogenic effects both in in vivo (studies) and in vitro (experiments). Because the blood of fish is in close touch with the aqueous medium in the gills, the circulatory system is susceptible to revealing any negative changes that occur in the aquatic environment. As a result, fish blood has seen widespread use in the fields of toxicology and environmental monitoring, where it serves as a potential signal of alterations in fisheries' physiological states. Therefore, hematological investigations of fish have the potential to be used as an indicator of the health condition of fish as well as the quality of the water.

A number of research have been carried out to investigate the impact that various pesticides have on the blood of fish. Even though the acute toxicity and genotoxicity of chlorpyrifos have been studied in the freshwater fish Labeo rohita using a static system in the past, there is a need for more research into the genotoxic and hematological effects of chlorpyrifos in this fish, which is one of the most desired fish species in Pakistan. Therefore, in order to evaluate the genotoxic and hematological effects of sublethal amounts of chlorpyrifos in Labeo rohita, the current research was carried out.

One of the most important requirements for the continuation of life on earth is the presence of water. The majority of the earth's surface is made up of water (71 percent). The actions of humans have a negative impact, either directly or indirectly, on the natural world. Countries both developed and developing, which are making tremendous advancements in the fields of agriculture, technology, and industry, are continually releasing different types of dangerous compounds into the biosphere, so posing a significant risk to the environment. This poses a serious problem for the world's ecosystems. Almost often, any change in the chemical composition of the natural aquatic environment will trigger changes in the biochemical features of the inhabitants, notably fishes. pollutants from homes, farms, and factories are the primary contributors to water pollution since these types of pollutants are often dumped into natural bodies of water.Inorganic, natural organic, and synthetic organic pesticides are the three types that are most often employed. Inorganic pesticides are the least environmentally friendly.

Borates, fluorides, and mercurial are examples of inorganic chemicals that are used as insecticides. Natural organic compounds include pyrethrum, rotenone, and nicotine6, while manmade organic compounds include chlorinated hydrocarbon, organophosphates, and cremates. Natural organic compounds may be found in plants and animals. The digesting process in vertebrates is carried out by the intestinal entrecotes, which express brush border enzymes such as disaccharides and alkaline phosphates and are responsible for carrying out the process. The accumulation of pesticides causes several physiological, biochemical, and even morphological reactions in the freshwater fauna. These responses are caused by the accumulation of pesticides impacting many activities of metabolites and enzymes that have been observed. Because of their efficacy, shorter persistence in the environment, and ease of detoxifying in animal tissues, organophosphates are the insecticides of choice in agricultural settings. These insecticides directly block the acetylcholinesterase activity that is seen in fish and other aquatic species.

## OBJEACTIVES

1. The Study Chlorpyrifos Pesticide Effect On Labeo Rohita Liver.
2. The Study Labeo Rohita Was Exposed To Low, Medium And High Concentrations Of Profenofos For 15 And 45 Days.

## RESEARCH METHODOLOGY

### Chemicals

The analytical grade of methyl methane sulfonate (MMS) was bought from Sigma-Aldrich in St. Louis, Missouri. A chlorpyrifos product with a 40% EC concentration and the brand name "Chlorpyrifos" was acquired from the market in the area. The product was made in Greece by M/s. K & N Efthymiadis. The majority of applications for this grade of chlorpyrifos are found in agricultural settings.

### Experimental fish specimen

After being cleaned with a potassium permanganate ($KMnO_4$) solution (0.05%) for two minutes, the freshwater fish Rohu (Labeo rohita, Family: Cyprinidae, Order: Cypriniformes) was obtained at the Faisalabad Fish Hatchery and moved to the glass aquaria. This was done in order to eliminate any cutaneous contaminants. The fish specimens had an average body weight of 1.2 cm and a body length of 1.2 cm. A photoperiod of 16:8 hours (light:dark) was used to acclimate the fish for a period of 15 days prior to exposing them to the herbicide. The specimens were given pelleted feed according to their body weight, however this feeding was discontinued 24 hours before the start of the pesticide exposure and continued until the conclusion of the insecticide treatment.

The fish were divided up into five different groups, each of which had ten individual fish, before being put into the aquariums. The fish specimens were placed in a static bioassay system for 96 hours with a 16:8-hour (light:dark) photoperiod, and they were subjected to the three nominal sublethal dosages of chlorpyrifos that were discussed before. Tap water that had been treated to remove the chlorine and methyl methanesulfonate (MMS) at a concentration of 5 mg/L were used, respectively, as the negative and positive controls. Two fish were used for each concentration were used to obtain blood samples at 24, 48, 72, and 96 hour intervals respectively. Before and after the exposure to the pesticide, the water's physicochemical parameters, including temperature, pH, dissolved oxygen, electrical conductivity, and total hardness, were measured and studied. At each of the sampling events, a sample of whole blood was drawn, and it was promptly processed for the micronucleus test as well as the hematological tests that followed. A syringe that had been treated with heparin was used to collect a sample of blood from the heart puncture.

**Micronucleus (MN) assay**

At each sampling, a drop of blood was quickly spread on a clean slide. This was done shortly after the sample was taken. Smears were generated on two slides from a single sample of fish blood that was taken. After being dried, the smears were fixed in methanol for ten minutes, then allowed to air dry at room temperature for another ten minutes, and then lastly stained with a 6% Giemsa solution in Sorenson buffer (pH 6.9) for twenty minutes. Following drying, the slides were given a final rinsing in distilled water in order to eliminate any excess discoloration. Under the light microscope (Labomed Lx400, Labo America, Inc., Los Angeles, CA, oil immersion lens, 100/1.25), a total of about 4000 erythrocytes were analyzed for each specimen, and 2000 cells were seen on each plate. The micronucleus may be identified by its features, which include round or oval entities that are not connected to the main nucleus, are less than one-third the size of the main nucleus, and display the same staining and focusing pattern as the main nucleus. The formula allowed for the determination of the micronucleus frequency:

**Hematological study**

The anticoagulant EDTA was included at a concentration of one percent in the vacutainers that were used to collect blood samples at intervals of 48 and 96 hours. The total erythrocyte count (TEC), the total leukocyte count (TLC), the hemoglobin content, and the packed cell volume (PCV) were all determined with the use of an automated hematological assay analyzer called HeCo Vet C (SEAC, Florence, Italy).

## DATA ANALYSIS

The study of the effects of environmental contamination on aquatic species is known as aquatic toxicology. This field of research looks at things like how pollution affects the health of fish and other aquatic organisms. Pesticide is pollution. The ability of a pesticide to cause damage to fish and other aquatic species is primarily determined by factors such as its toxicity, the length of time it is exposed to it, the dosage rate it delivers, and how long it remains in the ecosystem.The aspartameamino acid transferase activity and the alanine transferase activity in the muscle of the control fish were GOT (15.21), 13.54, and 12.23 mg/protein for 10, 20, and 30 days respectively. The GPT activity was 1.93, 1.86, and 1.79 mg/protein. The GOT Table 5 and GPT Table 6 activity in the lower sublethal concentration and for the higher sublethal concentration level was lowered in the experimental fishes for 10, 20, and 30 day exposure periods, respectively. It was discovered that the predicted reduction in enzyme activity of the muscles was not statistically significant at either dose during either of the testing periods.

Labeo rohita's control GOT and GPT activity was found in the liver (GOT 19.45, 18.23, 17.69 and GPT 9.52, 7.23, 6.43 mg/protein). Table 1 shows the results for an exposure time of 10, 20, and 30 days. In the experimental fishes, the enzyme activity of both the fishes treated with a lower sublethal dose and the fishes treated with a greater sublethal concentration reduced after 10, 20, and 30 days of treatment, respectively. The enzyme activity was found to drop the most throughout the course of 30 days of treatment for both the sublethal concentration of pesticide and the acid phosphatase. However, the values that were assessed for both the sublethal concentration and the acid phosphatase were determined to be statistically insignificant. Activity levels in the muscle of the control fish were 0.20 mg/protein, 0.17 mg/protein, and 0.13 mg/protein after 10, 20, and 30 days of therapy, respectively. In the experimental fish, the ACP activity at lower sublethal concentrations was found to be 0.18, 0.14, and 0.11 mg/protein, whereas at higher concentrations it was found to be 0.11, 0.06, and 0.03 mg/protein after exposure for 10, 20, and 30 days.

After 30 days of experimentation, there was a statistically significant reduction in the amount of enzyme activity in the muscles. at the liver of the control fish, the ACP activity was measured at 0.27, 0.21, and 0.16 mg/protein, whereas at higher sublethal concentrations, it was measured at 0.16, 0.10, and 0.06 mg/protein during the course of the experimental periods. In both of the sublethal doses of pesticide, it was discovered that the ACP activity was statistically insignificant during the first ten days of the exposure period, but statistically significant during the second thirty days of the exposure period. The activity of the enzyme alkaline phosphatase (ALP) in the muscles Table: The average protein content of the control fish was 0.42, 0.39, and 0.36 mg/fish after 10, 20, and 30 days, respectively.

The ALP of muscle at the lower sublethal concentration was 0.41,0.37, and 0.34 mg/protein for 10,20, and 30 days of exposure periods, while the ALP of muscle at the higher sublethal concentration was 0.035, 0.32, and 0.30 mg/protein for 10,20, and 30 days of exposure periods. After 10, 20, and 30 days of exposure, there was a statistically significant reduction in the amount of ALP activity found in the muscle. The lower sublethal concentrations of 0.73, 0.68, and 0.1 mg/protein that were administered to the fishes' livers for treatment durations of 10, 20, and 30 days respectively. When the fish were given a greater sublethal dosage, the amount that proved lethal to them was 0.65, 0.58, and 0.54 mg/protein for 10, 20, and 30 days of exposure, respectively. It was discovered that the enzyme activity of the control fishes was 0.76, 0.72, and 0.69 mg/protein after 10, 20, and 30 days of exposure, respectively. Both the lower and the higher sublethal concentrations of monocrotophos produced values that were statistically significant after 10, 20, and 30 days of exposure.

However, pesticides may cause a variety of adverse health effects in humans, including cancer, short and long term injury to the nervous system, lung damage, reproductive dysfunction, and possible dysfunction of the endocrine (hormone) and immune systems28. Pesticides eliminate, prevent, or repel pests such as insects, weeds, and rodents. The anticancer protein activity was shown to be statistically insignificant after 10 days of exposure to the sublethal dosage of pesticide, but it was revealed to be statistically significant after 30 days of exposure to the same concentration. The intracellular distribution patterns of enzymes in liver tissues were analyzed, and it was discovered that the enzyme, in general, was maintained in a dormant condition inside the membrane of lysosomes.

The presence of manganese in the cerebellum of fish led to an increase in the activity level of acid phosphates. The exposure to the insecticide chloropyrifos was the source of the enhanced acid phosphatase activity in Cavia procellus 14. In the current investigation, a reduced activity of acid phosphates (ACP) was seen in the 10, 20, and 30 days after treatment in both the lower and higher sublethal concentrations of pesticides. The impact of the pollutants on aquatic creatures and the statement that alkaline phosphatese (ALP) is a brush border enzymes that breaks different phosphorous esters at an alkaline pH and facilitated transport were discussed. It has been claimed that alkaline phosphatase is involved in active transport, the metabolism of glycogen, protein synthesis, the manufacture of certain enzymes, and secretary activity. Therefore, any change in the activity of alkaline phosphatase will have some kind of impact on the organisms. In the current experiment, it was discovered that the activity of alkaline phosphatase (ALP) was lower in the tissues of the test fishes as compared with the fishes that served as controls. For a period of thirty days, the highest sublethal dosage of pesticide showed the greatest decline.

It was observed that the exposure of the fish, Tilapia mossambia, to the pesticide sevin caused an inhibition of enzymes in the fish. It has been found that exposure to methyl parathion causes a reduction in the amount of GPT activity in the tissues of the same fish. The decrease in GOT activity that was seen in the fish Sacobranchusfossillis as a result of the toxic effects of thimidon was. It has been observed that the fish species Channapunctatus, which was treated with the pesticides Quinalphos, Dichlorvos, and suquin, exhibited a reduction in the activity of the tranminase enzymes. The sublethal effects of the pesticide cypermethrin on enzyme activities in the freshwater fish Cyprinuscarpio were investigated, and it was discovered that the lactate dehydrogenase activity rose after and 8, 12 days of treatment. In the current investigation, the elevation in the GPT and GOT activity of the fish treated with pesticide might have been increased depending on the anaerobic carbohydrate metabolism cumulative effect or possibly to meet the increased energy demands under sustained and prolonged toxic stress caused by pesticide monocrotophos.

## CONCLUSION

It is possible that this late disintegration may produce alterations in fish, which will manifest themselves as unpleasant affects on their hematological systems. In this manner, for the safety of human beings, prohibit the use of profane foos. When there is a limited supply of carbs, the body turns to lipids as an alternative source of energy to maintain its physiological processes. In this particular investigation, we noticed the anomalous behavioral repercussions that were caused in the fish that were treated. The sublethal exposure to the organophosphorus pesticide phenthoate revealed to be somewhat harmful to the fish L. rohita. This had an influence on the total protein and glycogen levels in many important organs, including the brain, gills, liver, kidney, gut, and muscles, amongst others. As a result, the major modifications were seen when the exposure was carried out for 1, 4, and 8 days, respectively. The decrease in the quantities of total proteins as well as glycogen might be the prospective impacts (impact) on the enzyme-mediated bio-defense mechanism of fingerlings, which represent major dangers to the aquatic environment as well as human life via the food chain.

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