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Acute Toxicity and Behavioral Anomalies in *Labeo Rohita* Due to Exposure of Expired Permethrin, Malathion and Dichlorovos Mixture

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ABSTRACT

Evaluation of waterborne toxicity is essential to estimate aquatic pollution. Generally, hazardous contaminants are a greater threat to freshwater organisms. **Objectives:** To measure the tolerance limit of *Labeo rohita* to expired permethrin, malathion, and dichlorovos mixture at different concentrations. **Methods:** Freshwater fingerlings of *L. rohita* were exposed to sub-lethal (0.3mg/l) and lethal (2.3mg/l) concentrations of permethrin, malathion, and dichlorovos mixture, with three replications under constant water temperature (16-30°C), total water hardness (225mgL^{-1}) and pH (6.8-7.7) for 96-hr. Probit analysis was used to calculate the Lethal concentration (LC50) and (LC100) of 96-hour exposure of the pesticide mixture for fish. Correlation analyses were conducted to identify relationships between the variables under study. Physicochemical parameters of water (Electrical conductivity (EC), Dissolved oxygen (DO), Oxidation-reduction potential (ORP), and Salinity) were recorded on a 12-hr basis. **Results:** For 96 hours, the mean values of LC50 and LC100 of *L. rohita* were calculated as 1.53 ± 0.028 and 3.05 ± 0.083 mgL^{-1}, respectively. *L. rohita* exhibited behavioural changes such as erratic swimming, loss of equilibrium, heavy breathing, and abnormal posture due to the toxic effects of the pesticide mixture. Correlation analysis indicates that increasing concentrations of permethrin, malathion, and dichlorvos pesticides significantly degrade water quality, reducing DO while increasing ORP, salinity, and EC. **Conclusions:** It was concluded that an expired pesticide mixture is more lethal to aquatic life than an unexpired pesticide mixture. So, it is important to take precautionary measures before discarding expired pesticides in aquatic ecosystems.

INTRODUCTION

Evaluation of waterborne toxicity is essential to estimate aquatic pollution. Generally, hazardous contaminants are a greater threat to freshwater organisms [1]. The investigation of freshwater poisoning addresses the detrimental impacts of exposure to harmful substances, especially pesticides and insecticides, in aquatic ecosystems. These highly toxic substances are mainly chemical pesticides, that destroy aquatic ecosystems. Insecticides are highly lethal and despite their acute toxicity, they are commonly used throughout the agricultural and other private sectors [2]. The ability of a poisonous material to cause harm to an organism with only one, brief, encounter is known as acute toxicity. The primary purpose of the acute toxicity test for pesticides is to rapidly determine the doses that cause fish to suffer immediate and permanent harm [3]. The most popular acute toxicity assays, such as LC50 and LC100, show the danger to the test organisms concerning exposure time intervals and death rates [4]. Compared to many other pesticides, permethrin is seen to be a safer choice because it quickly metabolizes and is eliminated by living things, leaving it out of the environment. A recent study revealed that photo-degradation products of permethrin could prove a greater threat to aquatic creatures than the element itself [5]. Malathion pollution has propagated to many significant water sources. Therefore, people who

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inadvertently ingest fish infected with malathion possess a greater likelihood of suffering from health problems caused by malathion toxicity, notably Parkinson's disease, neurocognitive challenges, weight gain, and related metabolic diseases [6]. Organophosphates, like dichlorvos, behave as strong nerve poisons that can be highly dangerous to fish and other organism aquatic ecosystem. It works by limiting synaptic transmission in cholinergic neurons by inhibiting the acetylcholinesterase (Ache) activity. The organism perishes untimely as a result of parasympathetic challenges spurred due to this disruption of nerve activity [7]. Fish provide vital means of protein for human beings, but they may become affected by chemical pesticides, that may interfere with their capacity to evolve, endure, and propagate. The primary culprit of aquatic pollution is agricultural cultivation. Exposure to pollutants for a prolonged amount of time might harm fish tissues, reduce metabolism, and diminish disease resistance [2]. *L. rohita* grows best in temperatures over 14°C. It blooms quickly; in a year, under normal growing scenarios, and normally reaches a total length of about 35–45 cm and a weight of 700–800 g. Its native habitat is shallow external sections of flooded rivers, where spawning occurs under an ideal temperature range of 22 to 31°C [8]. Pesticides are used to get rid of a variety of pests, such as insects, and rodents in order to improve the quantity and caliber of agricultural output [9]. These pesticides are extremely toxic and harmful to aquatic life as well as the quality of the water [10]. When crops are sprayed to eradicate pests, pesticides pose a serious hazard to non-target creatures, especially fish. These substances have the potential to kill fish by affecting their metabolic functions and causing health problems [11]. Fish exposed to pesticides on a long-term basis suffer from oxidation, mutagenesis, suppression of acetylcholinesterase function, carcinogenic effects, and changes in histopathology and development [12]. Moreover, pesticides make fish habitats less suitable and cause behavioral changes in fish, which increases the likelihood that the fish will be preyed upon [13]. The presence of expired pesticides in ecosystems introduces unique toxicological challenges that are not observed with unexpired formulations [14]. The quantity of expired pesticides in Pakistan's North West Frontier Province is estimated to exceed 5,000 tons, based on inventories and surveys [15]. The degradation of pesticides can result in their movement through soil and water or cause them to volatilize, reaching neighboring or distant locations [16].

This study aims to assess the toxicity of expired pesticide mixtures to *Labeo rohita* under controlled laboratory conditions.

METHODS

An experimental study design was conducted at the Department of Zoology, University of Sialkot, from September 2024 to February 2025. The juveniles of freshwater fish Labeo rohita, measuring about 6-7 cm in length, were acclimatized for 7 days in the laboratory under constant conditions. During acclimatization, water parameters were checked twice daily, and fish were fed to satiation on the feed (3.00Kcalg-1 digestible energy and 37% digestible protein). Acute toxicity tests on *L. rohita* were conducted for 96 hours under controlled conditions to determine tolerance limits, recording LC50 and LC100 values at 4-hour intervals. All glassware and aquaria were washed before the experiment. Aquaria were filled with tap water, maintaining water hardness (225 mg/L), pH (6.8-7.7), and temperature (16-30°C). Ten *L. rohita* fish of similar weight were stocked per aquarium, with three replications for each pesticide concentration, while control fish were kept in pesticide-free water. Solutions of expired permethrin, malathion, and dichlorvos were mixed in a 1:1:1 ratio to prepare the pesticide mixture. To prepare a 75ml mixture, 25ml of permethrin, 25ml of malathion, and 25ml of dichlorvos solution were mixed in a measuring flask. The test concentration was started from a zero with an increment of $0.02mgL^{-1}$. During the acute toxicity tests, concentrations of permethrin, malathion, and dichlorvos pesticide mixtures in an aquarium were gradually increased. 96 hr LC50 and the lethal concentration value of the permethrin, malathion, and dichlorvos for *L. rohita* were checked by Probit analysis. The statistical analysis method was used to check the physical chemistry of water parameters and fish mortality rate. Tuckey's Student Newman Keul test, correlation was also applied to check the relationship of pesticide mixture concentration and physicochemical parameters of water [17].

RESULTS

Increased fish mortality occurred from a gradual rise in the concentration of pesticides in the mixture. In all replicated aquaria, however, 50% of fish death was found at a dosage of 1.5 mgL^{-1}, whereas 100% of fish mortality occurred at a concentration of 2.3 mgL^{-1}. The percent mortality of *Labeo rohita* at various concentrations of a mixture of pesticides was recorded during a 96-hour exposure period for all three replications. 96-hour LC50 and the lethal concentration (LC100) values of the permethrin, malathion, and dichlorvos mixtures for the *Labeo rohita* during the first replication were calculated at 1.40 ± 0.029 and 2.985 ± 0.084mgL^{-1}, respectively, with 95% confidence ranges of 1.344-1.458 and 2.833-3.167. Its Deviance Chi-square score, on the other hand, was calculated as 34.71, with the goodness of a fit test (p) 0.02. The 96 hr. LC50 and the lethal concentration values for the second replication were 1.61 ±

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0.028mgL^{-1} and 3.13 ± 0.085mgL^{-1}, respectively, with a computed Chi-square value of 48.50 and p-value of 0.02. 96hr LC50 and the lethal concentration (LC100) values for the 3rd replication was 1.588 ± 0.027mgL^{-1}, and 3.042 ± 0.080mgL-1, respectively. It had a deviance Chi-square score of 47.67 and a goodness of fit test p-value of 0.04 (Table 1).

Table 1: Calculated 96hr LC50 and The Lethal Concentration (Mean ± SD) of Pesticide Mixture for the *Labeo Rohita*

Fish Species		Mean 96-hr Lc50 (mg^{-1})	95% Confidence Interval (mg^{-1})	Mean Lethal Concentration (mg^{-1})	95% Confidence Interval (mg^{-1})
Labeo Rohita	Replication I	1.40 ± 0.029	1.344-1.458	2.985 ± 0.084	2.833-3.167
	Replication II	1.61 ± 0.028	1.560-1.670	3.13 ± 0.085	2.979-3.318
	Replication III	1.588 ± 0.027	1.534-1.644	3.042 ± 0.080	2.897-3.217
***Mean ± SD**		**1.53 ± 0.028**		**3.05 ± 0.083**	

In the present study, Labeo rohita indicated behavioural alterations such as erratic swimming and loss of equilibrium after being exposed to expired permethrin, malathion, and dichlorvos mixture. They were assembled in a single corner of the tank, then remained at the bottom, regularly rising to the surface, displaying heavy breathing, and heightened opercula movement, indicating distress and neurological impairment with their scales removed. These changes are likely due to the neurotoxic effects of the expired pesticide mixture, which disrupts normal nervous system function, leading to symptoms such as hyperactivity, lethargy, and abnormal posture. At the pesticide mixture concentrations of 2.3 and 0.1mgL^{-1}, the dissolved oxygen levels in test media (for *Labeo rohita*) were found lowest (5.14 ± 0.65mgL^{-1}) and maximum (7.69 ± 0.94mgL^{-1}) respectively. The dissolved oxygen (DO) content of the test media altered significantly as the pesticide mixture concentration increased. The DO content was decreased gradually as the concentration of the pesticide mixtures increased. At pesticide mixture concentrations of 0.1 and 2.3mgL^{-1}, maximum and minimum mean values of oxidation-reduction potential (ORP) in the test media were calculated as 67.11 ± 8.68 and 139.8 ± 16.9 mV, respectively. The lowest electrical conductivity value (917 ± 8.71µScm^{-1}) was found at a concentration of 0.1mgL^{-1}, while the highest value (968 ± 17.0µScm^{-1}) was recorded at a concentration of 2.3mgL-1 pesticide mixture. The salinity of the test medium fluctuated after acute pesticide mixture exposure due to varying exposure doses. At pesticide mixture exposure levels of 2.3 and 0.1mgL^{-1}, the maximum and minimum salinity values (for *Labeo rohita*) test medium were calculated as 0.56 ± 0.045 and 0.44 ± 0.005 mgL^{-1}, respectively. The maximum salinity in a medium was found at 2.3mgL^{-1} pesticide mixture concentration, while the lowest was found at 0.1mgL^{-1}(Table 2).

Table 2: Physicochemical Parameters (Means ± SD) of Test Media Used for the *Labeo Rohita* During the Acute Toxicity Test with Various Concentrations.

Conc. (mg^{-1})	Temperature (C$^{\circ}$)	pH	Hardness (mg^{-1})	EC (µScm^{-1})	DO (ppm)	ORP (mV)	Salinity (ppt)
0.1	16.8 ± 0.51	6.90 ± 0.25	222 ± 0.57	917 ± 8.71	7.69 ± 0.94	67.11 ± 8.68	0.44 ± 0.005
0.3	16.8 ± 0.51	6.90 ± 0.25	222 ± 0.57	917 ± 8.71	7.69 ± 0.94	67.11 ± 8.68	0.44 ± 0.005
0.5	16.8 ± 0.51	6.90 ± 0.25	222 ± 0.57	917 ± 8.71	7.69 ± 0.94	67.11 ± 8.68	0.44 ± 0.005
0.7	17.2 ± 1.15	7.41 ± 0.19	225 ± 0.01	958 ± 29.7	5.69±0.27	99.30 ± 29.3	0.47 ± 0.001
0.9	17.2 ± 1.15	7.41 ± 0.19	225 ± 0.01	958 ± 29.7	5.69 ± 0.27	99.30 ± 29.3	0.47 ± 0.001
1.1	17.2±1.15	7.41 ± 0.19	225 ± 0.01	958 ± 29.7	5.69 ± 0.27	99.30 ± 29.3	0.47 ± 0.001
1.3	18.3 ± 0.30	7.06 ± 0.25	223 ± 0.02	940 ± 22.9	7.01 ± 1.10	126.3 ± 8.59	0.55 ± 0.047
1.5	18.3 ± 0.30	7.06 ± 0.25	223 ± 0.02	940 ± 22.9	7.01 ± 1.10	126.3 ± 8.59	0.55 ± 0.047
1.7	18.3 ± 0.30	7.06 ± 0.25	223 ± 0.02	940 ± 22.9	7.01 ± 1.10	126.3 ± 8.59	0.55 ± 0.047
1.9	19.1 ± 0.11	6.96 ± 0.05	226 ± 0.03	968 ± 17.0	5.14 ± 0.65	139.8 ± 16.9	0.56 ± 0.045
2.1	19.1 ± 0.11	6.96 ± 0.05	226 ± 0.03	968 ± 17.0	5.14 ± 0.65	139.8 ± 16.9	0.56 ± 0.045
2.3	19.1 ± 0.11	6.96 ± 0.05	226 ± 0.03	968 ± 17.0	5.14 ± 0.65	139.8 ± 16.9	0.56 ± 0.045

DISCUSSION

Agricultural activities release a combination of pollutants into water sources including pesticides, fertilizers, manure from farm animals, and sediments. The widespread use of various pesticides and insecticides in farming activities leads to water contamination, causing environmental and health issues. Pesticide application has been reported to cause fatalities among aquatic animals [18]. In the current investigation, the mortality rate in fish was increased with increasing concentration of pesticide mixture and time exposure. The median lethal concentration (LC50/96h) and the lethal concentration (LC100/96h) for various toxicants were employed to assess the sensitivity and the survival capability of a test organism. In the current study, acute toxicity experiments have been performed on *Labeo rohita*

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at different concentrations, to determine LC50 and the lethal concentrations of permethrin, malathion, and dichlorvos expired mixture to fish. The median lethal concentration (LC50) suggests that expired formulations, such as dichlorvos, fenvalerate, lambda-cyhalothrin, pretilachlor, tebuconazole, hexaconazole, capta may exhibit higher toxicity than other formulations. pH changes in these expired pesticides can result in byproducts that cause acute toxicity to fish species like *L. rohita* [19]. Calculated 96-hr LC50 values of chlorpyrifos and dichlorvos of 0.753 mgL^{-1} and 12.964 mgL^{-1} for Tor putitora. The median lethal concentration (LC50) of dichlorvos and paraquat against juveniles of Clarias gariepinus were calculated as 730 μgl^{-1} and 50 μgl^{-1} respectively. In the present study, the LC50 and LC100 for *L. rohita* were 1.53 ± 0.028 and 3.05 ± 0.083 mgL^{-1}. As a comparative analysis, the current study verified that an expired pesticide mixture is highly lethal to aquatic life than an unexpired pesticide mixture[20].

CONCLUSIONS

It was concluded that increasing concentrations of the permethrin, malathion, and dichlorvos pesticides mixture significantly affect water quality parameters, characterized by a decrease in dissolved oxygen and increases in oxidation-reduction potential, salinity, and electrical conductivity, which collectively stress the aquatic environment and adversely impact the survival of *L. rohita*.

Authors Contribution

Conceptualization: ZA
Methodology: ZA
Formal analysis: NM
Writing review and editing: NM

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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