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Research Article

Assessment of Acute Toxicity and Behavioral Disruptions in Indian Major Carp (*Labeo Rohita*) Under Pyrethrum Stress

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ABSTRACT

The extensive use of pesticides in agriculture has raised serious concerns regarding their inadvertent entry into aquatic ecosystems and subsequent toxicity to non-target organisms. Pyrethrum, a botanical insecticide derived from *Tanacetum cinerariifolium*, is often regarded as an eco-friendly alternative to synthetic pesticides; however, its toxic effects on aquatic fauna remain insufficiently explored. The present study evaluates the acute toxicity and associated behavioral alterations induced by pyrethrum in the Indian major carp *Labeo rohita*. Acute toxicity bioassays were conducted under laboratory conditions following APHA guidelines, and median lethal concentrations (LC₅₀) were determined for 24, 48, 72, and 96 hours of exposure. The LC₅₀ values exhibited a time-dependent decline, indicating increased toxicity with prolonged exposure, with the lowest LC₅₀ recorded at 96 hours (21.40 µg/L). Mortality increased with both concentration and exposure duration, reaching 100% at higher concentrations. In addition to lethality, pronounced behavioral and physiological disturbances were observed, including erratic swimming, surfacing and gulping, avoidance responses, excessive mucus secretion, reduced opercular movement, fin damage, and body discoloration. Dissolved oxygen levels declined significantly in exposure tanks, suggesting elevated metabolic stress and impaired respiration. These findings demonstrate that pyrethrum is highly toxic to *Labeo rohita* even at low concentrations and induces severe behavioral disruptions. The study underscores the ecological risks associated with botanical insecticides and highlights the need for cautious application and stringent regulation to protect aquatic ecosystems.

INTRODUCTION

Pesticides have become an evil necessity in agricultural practices due to their efficacy in pest control and enhancing crop production. The application of pesticides has increase sharply over the past few decades. From 1990 to 2022, the worldwide agricultural use of pesticides has increased gradually, reaching 3.69 million metric tons in 2022 (URL1).

However, the liberal and often unsystematic application of these chemicals has raised serious environmental

concerns, particularly with respect to their entry into aquatic ecosystems. Surface runoff from agricultural fields is the major pathway through which pesticides are transported to aquatic environments, where they come into direct contact with water bodies and bio-accumulate in aquatic organisms, including fish (Pandey et al., 2009). Consequently, pesticides have emerged as a major threat to aquatic biodiversity; disorganize food webs and ecological balance (Aydinalp and Porca 2004; Antwi and Reddy 2015; Baweja et al. 2020; Saha and Dutta 2024).

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Chronic pesticide exposure has been associated with physiological disturbances, behavioral abnormalities, histopathological damage, hematological alterations, biochemical disruptions, endocrine interference, immunosuppression, reproductive disorders, and even carcinogenic outcomes (Pandey *et al.*, 1995, 2014; Crisp *et al.*, 1998; Brouwer *et al.*, 1999; Mishra *et al.*, 2006, 2008; Ullah *et al.*, 2014; Ullah & Zorriehzahra, 2015).

Fishes are very sensitive to water quality alterations, so they are considered definitive bioindicators of aquatic contamination. Protecting fish health is fundamental to global food security and ecological stability, reflecting their critical role as both economic commodities and essential nutrient sources (Srivastava & Singh, 2013a).

In contemporary era, botanical insecticides have acquired increasing attention as environmental friendly substitute to synthetic pesticides. Among these, pyrethrum (*Tanacetum cinerariifolium*), a perennial plant of the family *Asteraceae*, has been extensively used for the extraction of pyrethrins (Moslemi *et al.*, 2018).

Pyrethrins are natural neurotoxic compounds obtained from the flower heads of pyrethrum (fig-1,2), that represent one of the most important groups of natural insecticides because of their rapid assault effects against various insect pests (Yang *et al.*, 2014). Historically, the insecticidal assets of pyrethrum were acknowledged in Central Asia nearly two centuries ago, and its use gained prestige during the Napoleonic wars when French soldiers employed it for the control of flea and lice infection. Its spread into Europe was facilitated through ancient trade routes (Mkwale, 2001).

Chemically, pyrethrins are grouped into two classes, i.e. pyrethrin I (comprising pyrethrin I, cinerin I, and jasmolin I) and pyrethrin II (including pyrethrin II, cinerin II, and jasmolin II)—which differ in their acid moieties (chrysanthemic acid and pyrethric acid respectively) (Chesang *et al.* 2017).

These esters exert their insecticidal effects by binding to sodium channels in the nerve membrane, causing repetitive nerve discharges, paralysis, and ultimately death. Although naturally derived, pyrethrins are not without environmental hazards. They are frequently formulated with synergists such as piperonyl butoxide to amplify potency, and their mode of action carries similarity to that of synthetic pyrethroids and organochlorines (Cox, 2002).

Critically, pyrethrins are classified as acutely toxic to fish, with described median lethal concentrations (LC50) in the range of 9–58 ppb, on the basis of species and water temperature. Their toxicity tends to increase in warm water, making tropical fish species particularly endangered (Johnson *et al.*, 1980).

Deltamethrin, first marketed in 1977, and cypermethrin are among the most widely used pyrethroids due to their high strength and photostability (Bradbury & Coats,

1989). However, their ecotoxicological impacts are intense. Terrible fish kills, such as the massive eel mortality in Lake Balaton, Hungary, have been linked to deltamethrin applications for mosquito control (Bálint *et al.*, 1995). Similarly, cypermethrin discloses high affinity for gill membranes, furnishing fish extremely susceptible even at trace concentrations (Shalwei *et al.*, 2012). Different from mammals, fish lack efficient metabolic pathways to detoxify pyrethroids, making them highly endangered to bioaccumulation and neurotoxic consequences (Borges *et al.*, 2007).

Behavioral changes are frequently the preliminary and most sensitive indicators of pesticide induced stress in fish. Pyrethroids and pyrethrins disrupt acetylcholinesterase activity, leading to hyperactivity, loss of equilibrium, erratic swimming, increased surfacing activity, and eventually lethargy prior to death. These behavioural inconsistencies are not only useful in ecotoxicological estimations but also provide understandings into sub-lethal impacts that could compromise feeding efficiency, predator avoidance, and reproductive success in natural populations. (Borges *et al.*, 2007; Ullah *et al.*, 2019; Tsai & Lein, 2021).

Within these circumstances, *Labeo rohita* (rohu), a freshwater major carp species of the family *Cyprinidae*, is of particular interest. As one of the most commercially predominant aquaculture species in South Asia, it contributes significantly to food supply and rural income. Its omnivorous feeding habits and sensitivity to waterborne pollutants mark it as an ideal representative organism for aquatic toxicology studies. Assessing the lethal concentration (LC50) of pyrethrum in *Labeo rohita* and analyzing associated behavioural alterations is therefore essential for griping the ecological indications of pyrethrin use and for establishing safe approaches that minimize threat to aquaculture and biodiversity.

Thus, the current study is designed to assess the acute toxicity of pyrethrum in *Labeo rohita* by finding out its LC50 value and reporting behavioural alterations under experimental conditions. consequently, the research aims to provide a scientific basis for environmental-toxicological risk assessment of plant based insecticides and contribute to sustainable pesticide management approaches that protect aquatic ecosystems.

MATERIALS AND METHODS

Fish (*Labeo rohita*) were collected from the local fish farms of Sultanpur, U. P. India and sensibly carried to the laboratory. Fishes were sanitized first in 0.05% KMnO₄ solutions for 2-3 minutes to avoid any cutaneous infection. The sanitized fishes were set aside in 300 litre plastic tank for 14 to 15 days for acclimatization in in-vitro condition. Fishes were fed with wheat flour and mustard cake in the ratio of 3:1 on alternate days. Water with food waste was replaced with freshwater in every 24hrs. Water tanks were



covered with transparent nylon net. When toxicity test was performed the feeding were halted before 24 hours. Fish were exposed to natural photoperiods and were set aside at room temperature. The physicochemical parameters such as dissolved oxygen (DO), temperature, and pH of the test media were recorded. The experiments were carried out in glass aquaria of 15L capacity in stationary laboratory conditions.

The DO of water was determined by modified Winkler's method in ppm before and after the transfer of fish for 24hrs, 48hrs, 72hrs and 96hrs in glass jars. The fish approximately of uniform length (16.75 ± 1.20 cm) and weight (71.9 ± 4.5 gm) were designated for the bioassay (fig-3). Fish were not given any food through the experiment. Besides mortality, fish behaviour and other external changes in the body of fish during the bioassay were observed and recorded. The test was performed during the month of May at room temperature ($37.78 \pm 1.3^\circ\text{C}$). The physicochemical parameters were maintained as per the guidelines of APHA (2005). The physicochemical properties of the water used in the experiments were as follows; temperature ($29.85 \pm 0.61^\circ\text{C}$), pH (6.96 ± 0.14), DO (7.7 ± 0.5 mg/L).

Pyrethrum 2% v/v technical grade procured online from the manufacturer Sabari Crop Care Sciences Pvt. Ltd, Thiruvallur, Tamilnadu, India for use in bioassay. The Stock solution of pyrethrum was prepared by mixing in absolute alcohol. The range finding tests or exploratory tests as prescribed by APHA (2005) were used to determine pyrethrum dose and mortality in fish. The range finding tests showed no mortality at concentration up to $5\mu\text{g/L}$, 70% mortality at $40\mu\text{g/L}$ and 100% mortality at $50\mu\text{g/L}$. After determining the test range ($5\mu\text{g/L}$, $10\mu\text{g/L}$, $15\mu\text{g/L}$, $20\mu\text{g/L}$, $25\mu\text{g/L}$, $30\mu\text{g/L}$, $35\mu\text{g/L}$, $40\mu\text{g/L}$, $45\mu\text{g/L}$, $50\mu\text{g/L}$), 30 acclimatized fishes in three replicates for each concentration, were released in 15L water tank for 24hrs, 48hrs, 72hrs and 96hrs acute toxicity test. A control set with equal number of fishes was also simultaneously run. Prior to the exposure, solution of desired concentrations of the pyrethrum were prepared and thoroughly mixed in tap water containing troughs. Pyrethrum induced fish mortality data were analyzed using IBM SPSS Student Version 21.

Behavioural responses, such as swimming pattern, opercular movement (OCM), surfacing and gulping, avoidance behaviour, mucus secretion, skin colour and fin posture were also observed.

RESULTS

LC₅₀ Value of Pyrethrum and behaviour of fish:

The table (Table 1) provides estimated LC (lethal concentration) values and their corresponding 95% lower and upper confidence limits (C.L.) for Pyrethrum exposure at different time points (24hrs, 48hrs, 72hrs, and 96hrs)

and Plot of adjusted Probits for LC₅₀ of pyrethrum are represented in graphs (GRAPH-1, 2, 3, and 4) for these hours respectively.

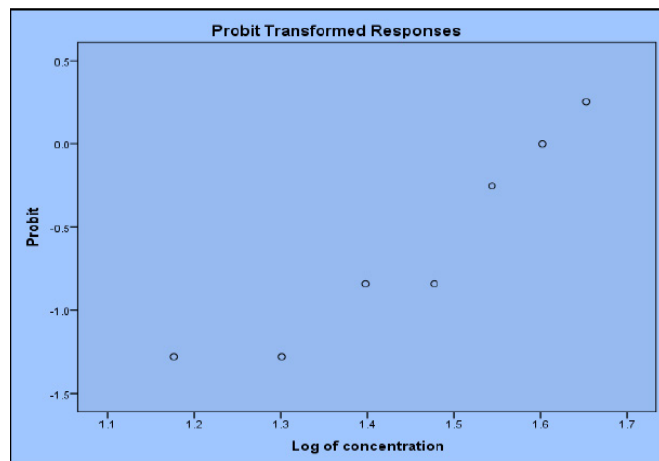
The LC values represent the concentration of Pyrethrum at which a certain percentage of the experimental system is expected to exhibit a lethal response. The 95% confidence limit (C.L.) reflects the range within which we can be 95% confident that the true LC value lies. At 24h, the estimated LC values ranged from 12.45 (LC 1.00) to 106.75 (LC 99.00) μg , with corresponding 95% confidence limit (C.L.), lower limits ranging from 5.89 to 73.59 μg , and upper limits ranging from 17.06 to 261.20 μg . This indicates variability in the estimated LC values, with higher concentrations having wider confidence intervals. At 48hrs, the estimated LC values continued to vary, ranging from 6.72 to 116.58 μg , with corresponding 95% confidence limit (C.L.), lower limits ranging from 2.57 to 75.88 μg , and upper limits ranging from 10.35 to 302.39 μg . The variability observed in the LC values and confidence intervals persisted, suggesting ongoing changes in the response to Pyrethrum exposure over time. Similarly, at 72hrs and 96hrs, the estimated LC values and confidence intervals continued to fluctuate, indicating a dynamic response to Pyrethrum exposure. This variability underscores the importance of considering both the concentration of the substance and the time of exposure when assessing its potential lethality in the experimental system. These data are essential in toxicology studies for understanding dose-response relationships and determining safe exposure levels.

Plot of adjusted Probits- (PYRETHRUM)

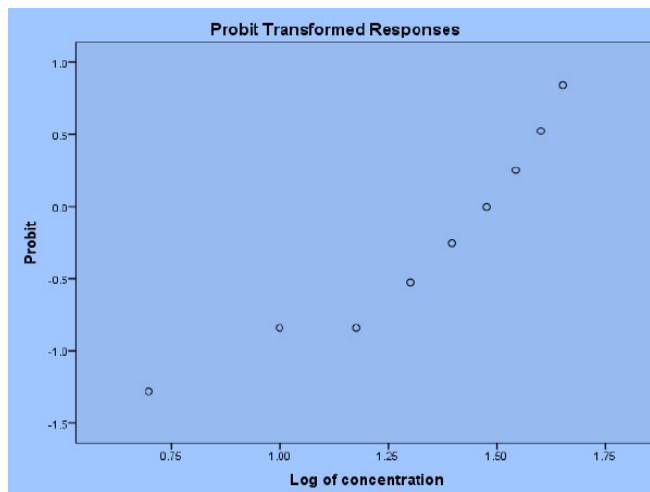
The Table (Table 2) presents data on the mortality of fish after short-term exposure to Pyrethrum at various concentrations ($\mu\text{g/L}$) over a 96h period. The "Control" group, without exposure to Pyrethrum, showed no mortality throughout the duration of the experiment. In contrast, the "Exposed" groups, which were exposed to increasing concentrations of Pyrethrum ranging from $5\mu\text{g/L}$ to $50\mu\text{g/L}$, exhibited varying levels of mortality. At 24hrs, there were no observed mortalities in the exposed groups, indicating no immediate lethal effects of Pyrethrum at these concentrations within the first day of exposure. However, as the exposure duration increased to 48hrs, 72hrs, and 96hrs, mortality rates in the exposed groups started to rise. The number of responding fish, which signifies mortality, increased with higher concentrations of Pyrethrum and longer exposure times. At 48hrs, mortalities were reported in the groups exposed to $9\mu\text{g/L}$ and above, with the highest mortality rate in the $30\mu\text{g/L}$ group. This trend continued at 72hrs and 96hrs, with increasing mortalities in the exposed groups compared to earlier time points. The highest mortality rates were consistently observed in the group exposed to $30\mu\text{g/L}$, where all fish succumbed to the effects of Pyrethrum by the end of the 96hrs period.

Table 1: Estimated LC value and 95% lower and upper confidence limits (C.L.) for Pyrethrum exposure

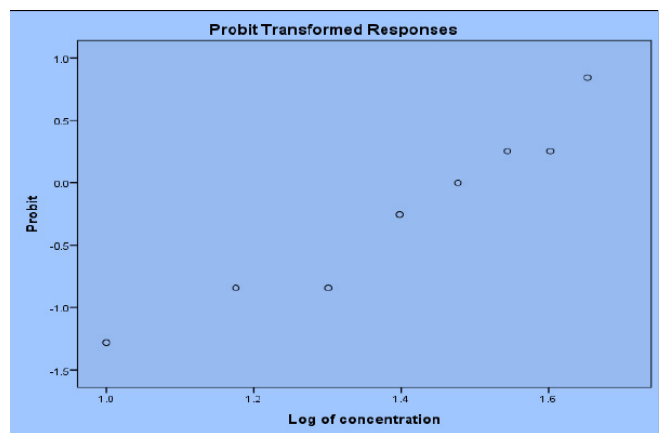
POINT	24 HOURS			48 HOURS			72 HOURS			96 HOURS		
	EXP.	95% C.L.		EXP.	95% C.L.		EXP.	95% C.L.		EXP.	95% C.L.	
	CON.	LOWER	UPPER	CON.	LOWER	UPPER	CON.	LOWER	UPPER	CON.	LOWER	UPPER
LC 1.00	12.45	5.89	17.06	6.72	2.57	10.35	3.31	0.78	6.17	3.75	1.20	6.40
LC 10.00	20.17	13.39	24.44	12.76	7.27	16.72	8.18	3.54	12.01	8.20	4.11	11.57
LC 50.00	36.46	31.54	44.10	28.00	23.08	33.90	24.85	19.16	32.10	21.40	16.64	26.49
LC 95.00	77.92	58.61	151.6	76.76	55.64	153.29	103.38	65.38	290.45	73.34	51.56	149.12
LC 99.00	106.75	73.59	261.20	116.58	75.88	302.39	186.60	100.67	781.18	122.18	75.93	330.98



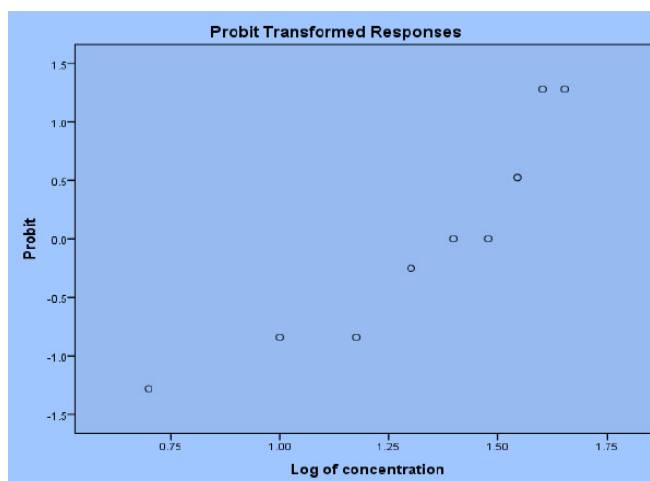
Graph 1: 24 hrs LC₅₀ value of Pyrethrum



Graph 3: 72 hrs LC₅₀ value of Pyrethrum



Graph 2: 48 hrs LC₅₀ value of Pyrethrum



Graph 4: 96 hrs LC₅₀ value of Pyrethrum

Fish exposed with different concentrations of pyrethrum induces adverse behavioural changes in fish, including restlessness, erratic swimming, and avoidance behaviour. Physiological manifestations include decreased opercular movement, increased mucus secretion, and discoloration with fin deterioration. The opercular movement (OCM) of the fish exhibits a decrease at all exposure concentrations (Table 3).

These symptoms signify heightened stress, respiratory distress, and physical damage, potentially culminating

in death if exposure persists. Monitoring and regulating pesticide levels in aquatic habitats are vital to safeguarding fish and maintaining ecosystem health. Outcome of DO has been given in (Tables 4 and 5).

DISCUSSION

Toxicity experiments were conducted in accordance with APHA (2005) guidelines. The water used in the bioassays



Table 2: Mortality of fish after short term exposure to Pyrethrum

Conc. ($\mu\text{g}/\text{l}$)	Exposed number	Responding numbers			
		24 hours	48 hours	72 hours	96 hours
Control	30	0	0	0	0
5 $\mu\text{g}/\text{L}$	30	0	0	3	3
10 $\mu\text{g}/\text{L}$	30	0	3	6	6
15 $\mu\text{g}/\text{L}$	30	3	6	6	6
20 $\mu\text{g}/\text{L}$	30	3	6	9	12
25 $\mu\text{g}/\text{L}$	30	6	12	12	15
30 $\mu\text{g}/\text{L}$	30	6	15	15	15
35 $\mu\text{g}/\text{L}$	30	12	18	18	21
40 $\mu\text{g}/\text{L}$	30	15	18	21	27
45 $\mu\text{g}/\text{L}$	30	18	24	24	27
50 $\mu\text{g}/\text{L}$	30	30	30	30	30

had the following physicochemical properties: temperature $29.85 \pm 0.61^\circ\text{C}$, pH 6.96 ± 0.14 , and dissolved oxygen (DO) 7.7 ± 0.44 ppm. These conditions fell within the normal range for conducting bioassays. Acute toxicity tests are designed to establish the concentration of a toxicant that results in 50% mortality of the test organisms (LC50) over a defined period. Such assays generate reproducible dose-response curves, which are essential for evaluating the toxic effects of chemicals. The LC50 is widely accepted as a reliable measure to assess the potential ecological risks of contaminants on aquatic organisms.

The present study examined the effects of pyrethrum on *Labeo rohita*, revealing significant toxic impacts. Monitoring of water quality showed variations in dissolved oxygen and temperature both in the presence and absence of fish. Without fish, DO declined from an initial 7.7 ppm to 5.02 ppm over 96 hours, while the temperature increased from 28.03°C to 33.48°C . In contrast, when fish were introduced, DO dropped more sharply from 7.83 ppm to 3.37 ppm, while temperature rose from 29.36°C to 33.98°C . These findings suggest that the stressed fish, under

pyrethrum exposure, accelerated DO depletion through heightened metabolic activity. Consequently, the chemical not only directly affected fish health but also altered the aquatic environment, amplifying stress conditions and toxic effects.

The LC50 values indicated that higher concentrations were required to produce lethal effects at shorter exposures, while lower concentrations became lethal with prolonged exposure. At 24 hours, LC values ranged from $12.45 \mu\text{g}$ (LC1.00) to $36.46 \mu\text{g}$ (LC50) and $106.75 \mu\text{g}$ (LC99.00). By 48 hours, these shifted to $6.72 \mu\text{g}$, $28 \mu\text{g}$, and $116.58 \mu\text{g}$, respectively. At 72 hours, LC values were $3.31 \mu\text{g}$, $24.85 \mu\text{g}$, and $186.60 \mu\text{g}$, while at 96 hours they were $3.75 \mu\text{g}$, $21.40 \mu\text{g}$, and $122.18 \mu\text{g}$. The progressive reduction in LC50 values indicates increased sensitivity of fish to pyrethrum over time. Mortality analysis supported these observations, with higher death rates occurring at elevated concentrations and longer exposures, culminating in 100% mortality at $50 \mu\text{g}/\text{L}$ after 96 hours.

Compared with other studies, these LC50 values were relatively higher. For example, Sapna et al., (2023) reported a 96-hour LC50 of $30 \mu\text{g}/\text{L}$ for cyphenothrin in *Cirrhinus mrigala*, and Saha and Kaviraj (2003) recorded a 96-hour LC50 of $1.27 \mu\text{g}/\text{L}$ for cypermethrin in *Heteropneustes fossilis*. Such variability in LC50 values may stem from environmental factors, particularly temperature (Pandey et al., 2008). Pandey et al. (2008) demonstrated that temperature significantly influences the physiological and chemical responses of *Heteropneustes fossilis* to dimethoate. Using a 96-hour static bioassay, they reported LC50 values of $14.39 \text{ mg}/\text{L}$ at lower temperatures ($17.16 \pm 0.78^\circ\text{C}$) and $2.98 \text{ mg}/\text{L}$ at higher temperatures ($27.50 \pm 1.50^\circ\text{C}$). In addition, behavioural changes including altered schooling, frequent surfacing, mucus secretion, and abnormal opercular activity were noted. Their results highlighted that increased temperatures intensified mortality, emphasizing the importance of considering seasonal temperature variations when assessing toxicant impacts.

Table 3: Movement of operculum in *Labeo rohita* after exposure with pyrethrum at different concentration levels and different time intervals

Concentration ($\mu\text{g}/\text{l}$)	0 hrs	24 hrs	48hrs	72hrs	96 hrs
Control	139.80 ± 2.85	145.00 ± 1.58	136.80 ± 1.31	146.40 ± 2.07	147.00 ± 1.41
5 μg	118.40 ± 2.72	133.40 ± 2.30	114.00 ± 1.58	92.80 ± 6.14	84.80 ± 2.78
10 μg	125.40 ± 5.23	131.40 ± 2.31	107.40 ± 4.34	92.20 ± 1.30	83.6 ± 1.63
15 μg	121.20 ± 2.56	125.00 ± 3.08	109.20 ± 2.38	90.00 ± 1.58	76.00 ± 2.28
20 μg	131.80 ± 2.78	130.40 ± 1.67	105.00 ± 1.58	84.80 ± 1.92	72.40 ± 1.85
25 μg	122.40 ± 3.93	135.00 ± 1.58	102.00 ± 1.58	82.20 ± 1.78	71.00 ± 1.41
30 μg	126.00 ± 5.29	130.80 ± 1.64	98.60 ± 1.67	80.60 ± 3.13	67.80 ± 1.72
35 μg	123.40 ± 4.22	134.40 ± 2.70	96.00 ± 1.58	75.80 ± 1.30	64.20 ± 1.72
40 μg	129.20 ± 3.70	128.00 ± 2.64	91.20 ± 4.55	69.40 ± 2.07	61.4 ± 1.85
45 μg	134.00 ± 1.67	140.00 ± 2.86	82.00 ± 1.58	64.80 ± 3.13	57.00 ± 2.00
50 μg	115.6 ± 3.77	Mortality	Mortality	Mortality	Mortality

Table 4: Estimation of DO at different time intervals of tap water used for fish experiment in PPM (Without fish)

Tests	Initial		24 hrs		48 hrs		72 hrs		96 hrs	
	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)
Mean	7.7	28.03	6.99	29.89	6.35	31.77	5.69	32.62	5.02	33.48
SD	0.44	0.48	0.43	0.45	0.32	0.58	0.31	0.46	0.27	0.44

Table 5: Estimation of DO at different time intervals of tap water used for fish experiment in PPM (With 5 fish in each tank).

Tests	Initial		24 hrs		48 hrs		72 hrs		96 hrs	
	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)	DO (ppm)	Temp. (°C)
Mean	7.83	29.36	6.22	30.38	5.53	31.31	4.46	33.30	3.37	33.98
SD	0.73	0.39	0.71	0.54	0.59	0.41	0.43	0.68	0.42	0.31

Pandey et al. (2022) further reported pyrethrum LC50 values of 0.75 µg/L (24h), 0.70 µg/L (48h), 0.62 µg/L (72h), and 0.55 µg/L (96h) for *Labeo rohita*. Soderlund et al. (2002) investigated oral LC50 values of pyrethroids in rats, finding 710 mg/kg (males) and 320 mg/kg (females) for pyrethrins, while permethrin had values of 1200 mg/kg for both sexes. Similarly, Cox (2002) reported high toxicity of pyrethrins to fish, with LC50 values ranging from 9 to 58 ppb. Bradbury and Coats (1989) also noted median lethal concentrations of pyrethroids typically below 10 µg/L. Toxicity of pyrethroids tends to increase at lower temperatures and is influenced by factors such as fish species, body size, and environmental conditions like sediment presence (Haya, 1989).

Several studies corroborate the acute toxicity of pyrethroids in freshwater fish. Sahoo et al. (2017) studied deltamethrin toxicity in *Labeo rohita* fingerlings, reporting 50% mortality at 0.38 µg/L after 72 hours, 0.39 µg/L after 60 hours, and 0.40 µg/L after 36 hours. Similarly, Sarkar et al. (2018) assessed cypermethrin toxicity in *Cyprinus carpio* fry, reporting LC50 values of 1.04 mg/L (24h), 0.92 mg/L (48h), 0.89 mg/L (72h), and 0.79 mg/L (96h), alongside noticeable behavioural changes.

The low LC50 values recorded in this study authenticate that pyrethrum is extremely toxic to *Labeo rohita*. Exposed fish exhibit several behavioural disorders, including avoidance reactions, erratic swimming, decreased opercular activity, excessive mucus secretion, fin damage, and periodic surfacing. These traits indicate drastic stress and reduced respiration, highlighting the strong toxicological impact of pyrethrum. Comparable detection were reported in *Anabas sp.* exposed to monocrotophos (Santhakumar and Balaji, 2000). Increased surfacing has also been linked to hypoxic conditions (Radhaia et al.), while the observed reduction in opercular movement may serve to limit toxin absorption through the gills, leading to reduced oxygen uptake. Similar reductions in oxygen utilization have been registered in *Cyprinus carpio* exposed to sub-lethal copper concentrations (Boeck et al., 1995). In contrast, Ganeshwade et al., 2006 reported elevated

opercular activity and coughing in common carp subjected to industrial effluents. Disorganized swimming patterns likely result from impaired neuromuscular coordination caused by acetylcholine accumulation at synaptic and neuromuscular junctions (Rao et al., 2005). Changes in body coloration have also been reported in *Cyprinus carpio* following exposure to HgCl₂ (Masud et al., 2005).

CONCLUSION

The present investigation clearly demonstrates that pyrethrum, despite its botanical origin, exerts strong acute toxic effects on the freshwater fish *Labeo rohita*. The progressive decrease in LC₅₀ values with increasing exposure duration confirms the cumulative and time-dependent nature of pyrethrum toxicity. High mortality rates at relatively low concentrations, coupled with marked behavioral and physiological disturbances, indicate that *L. rohita* is extremely sensitive to pyrethrum stress.

Behavioral responses such as erratic swimming, frequent surfacing, reduced opercular movement, mucus hypersecretion, fin deterioration, and discoloration served as early and sensitive indicators of pyrethrum-induced stress. These alterations reflect severe neurotoxic and respiratory impairment, which may compromise feeding efficiency, predator avoidance, growth, and reproductive fitness in natural populations. Additionally, the observed depletion of dissolved oxygen in exposure tanks further intensified physiological stress, exacerbating toxic outcomes. Overall, the findings challenge the assumption that botanical insecticides are inherently safe for aquatic organisms and emphasize that pyrethrum poses a significant ecological threat to freshwater fish. The study highlights the necessity for comprehensive ecotoxicological evaluation of plant-based pesticides before their widespread use. Further research involving chronic exposure, combined pesticide effects, and field-based assessments is strongly recommended to develop sustainable pest-management strategies that safeguard aquatic biodiversity and aquaculture productivity.



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