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Research Article

Study Of Toxic Effect Of Cypermethrin EC (10%) On Slender Rasbora, Rasbora Daniconius (Hamilton, 1882)

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ABSTRACT

Cypermethrin, a type II synthetic pyrethroid insecticide is commonly utilised in agricultural and domestic applications. The excessive use of this can infiltrate freshwater ecosystems, impacting non-target species such as the slender rasbora Rasbora daniconius (Hamilton, 1882). This study evaluated experimentally the lethal and sublethal effects of cypermethrin. The LC50 value was determined as 3 ppm (96 hours) using probit analysis. The effects of a sublethal dose of cypermethrin 3 ppm after 10 and 15 days post-exposure showed significant protein depletion in the fish. Behavioural changes were also noted after treating the fish at sub-lethal concentrations.

INTRODUCTION

Cypermethrin is a pyrethroid insecticide widely used in homes, agriculture, and industrial settings for controlling various insect pests [1](Kakko et al., 2003). Cypermethrin exhibited stability against photochemical and microbial degradation and had very low toxicity to humans and animals. This insecticide has become more widely used than organochlorine, organophosphorus, and carbamate insecticides [2] (Coats et al., 1989).. It is significantly utilized in both agricultural and domestic contexts. Currently, approximately 30% of pesticides used globally are synthetic pyrethroids [3] (Oda and Maddawy, 2012). The excessive and immoderate use of cypermethrin can lead to runoff into natural water bodies, posing significant risks to non-target aquatic organisms. Behavioural, haematological, and histological changes are critical effects of pesticide exposure at higher concentrations in fish. The toxic effects of cypermethrin pose a significant threat to aquatic ecosystems.

The slender rasbora, *Rasbora daniconius* (Hamilton, 1882), is one of the common fish

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species inhabiting the freshwater environments of paddy fields. The study investigates the acute toxicity of the pyrethroid cypermethrin on slender rasbora collected from paddy wetlands in the Pannissery area near Sreekrishna College, Guruvayur. Toxicity tests revealed that LC_{50} of cypermethrin for the fish to be 4 ppm using probit analysis. Toxicity study at sublethal concentration of 2ppm for 10 days and 15 days post-exposure to slender rasbora demonstrates the significant behavioural changes and a substantial decrease in protein content in the treated fish. The comprehensive data of protein concentration depletion due to pesticide toxicity in this species underscore the need for responsible pest management practices in both domestic and agricultural settings.

SUBJECTS AND METHODS:

Experimental set up (Test specimen, Collection method and Insecticide formulation)

The fish used for the experiments were collected from the paddy wetlands in Pannissery near Sreekrishna College . They were collected using cast and drag nets. Twenty healthy freshwater fish, *Rasbora daniconius*, with an average length of 5-8 cm and a weight of 20 ± 2 g, were used for the study. The fish were cleaned using a 0.5% potassium permanganate solution to remove any contaminants. The fish were acclimatised for 7 days in an aquarium of 50 L capacity in dechlorinated aerated water at room temperature (26 ± 2 °C).

Study of Lethal dose - LD_{50}

Cypermethrin (10% EC), manufactured by National Pesticides and Chemical, Mumbai, was purchased from the agricultural market in Thrissur, Kerala, India. A stock solution of cypermethrin was prepared in distilled water for experiment. Test series concentrations of 1, 2, 3, and 4 µg/l were prepared by serial dilution of the stock solution [4] (Neglur et al., 2020). The fish were in pesticide-free water served as control. The treated water in the aquaria was changed after 24 hours, and freshly prepared concentrations of cypermethrin were used for the experiment. The test was conducted for 96 hours, during which dead fish were removed. Probit analysis was employed to determine the median lethal concentration [5] (Finney, 1971).

Sublethal toxicity study

For the acute toxicity study, the fish were maintained in aquaria up to 10 days and 15 days. For Control experiment the aquarium was filled with dechlorinated, aerated water at room temperature (26 ± 2 °C). For experiment the aquarium was filled with water containing 3 µg/l cypermethrin concentration. The treated water was changed every 24 hours and a fresh concentration of 3 µg/l was maintained throughout the period. The behaviour of the fish, i.e., swimming, opercular movement, etc., was recorded after 96 hours of observation. Protein concentration was assessed after 10 and 15 days post-exposure using a standard procedure [6]Lowri (1957).

Protein Concentration study

The fish were sacrificed periodically, and whole muscle tissues from both treated and untreated fish were homogenised using a mortar and pestle. Protein concentration in fish whole-body homogenates is typically determined by extracting protein using buffer (e.g., 0.1M KCl buffer, centrifuging to obtain a clear supernatant and analysing it with colorimetric method of against a BSA standard curve, The process involves precise weighing, homogenization at 4∘C. Data obtained from both the control and experimental groups were analysed and presented graphically.

RESULTS:

Lethal study

The LC_{50} of cypermethrin was determined using Probit analysis. Mortality was observed for 24 hours , 48 hours and 96 hours of study. The observation of experiment is presented in Table 1. All fishes died when treated with 4 µg cypermethrin after 96 hours of observations. The LD_{50} of cypermethrin is 3 µg concentration after 96 hours of observations.

Table 1. Observation (96 hours) of Mortality in *Rasbora daniconius* (n=10) when treated with different concentrations of (EC 10%) Cypermethrin

Experiment	Cypermethrin Concentration	Mortality Observed after 24 hours	Mortality Observed after 48 hours	Mortality Observed after 96 hours
1	1 µg/l	0	0	0
2	2 µg/l	0	0	2 died
3	3 µg/l	0	0	3 died
4	4 µg/l	0	0	10 died

Sublethal toxicity study

Exposure of slender rasbora to a sublethal dose of cypermethrin 3 µg/l for 10 days and 15 days resulted in significant behavioural changes and biochemical alterations, particularly a depletion of protein concentration. This depletion indicates dose-response pressure or metabolic disturbances following pesticide exposure.

Behavioural modifications were also observed along with the biochemical changes. The observations of behvariour were presented in Table 4. The behaviours changes were recorded for hyperactivity, loss of balance, rate of swimming, opercular activity and surfacing activity and air gasping activity. Such changes contribute to the overall compromise of the fish's health and survival.

Behavioural Studty

Table 2. Impact of Cypermethrin (10% EC) on the behaviour pattern of *Rasbora daniconius*

Parameter's	**Control**	**Sublethal dose 3 µg/l Sublethal concentration**	
		96 hours (3 days)	**5 days**
Hyperactivity	−	++	+++
Loss of balance	−	+	++
Rate of swimming	−	++	+++
Opercular activity	−	++	+++
Surfacing Activity	−	++	+++
Air gasping activity	−	+	++

− indicate normal behaviour, + indicate increasing behaviour

Protein depleti

Statistical analysis of the protein depletion after 10 days and 15 days exposure to the slender rasbora was done using ‘t’ value. After 10 days protein concentration was depleted to 83% in test specimen while in 15 days it is 88.5 %. This strongly suggests that the toxicant has a severe adverse biochemical effect, likely due to: Protein degradation, Metabolic stress and Impaired protein synthesis.

Statistiical analysis (Table 3)

Table 3. Changes in the Protein Concentration when exposure to different periods of sublethal toxicity 10 days and 15 days

Days of exposure	Protein Concentration	
	Control	Treated
10 days	3.08+.05	0.50+.03
% Changes	83.8	
‘t’ value	75.9	
15 days	3.74±.07	0.113±.005
% Changes	97%	
‘t’ value	88.5	

DISCUSSION :

The present study determines the LC_{50} value of cypermethrin to the Slender Rasbora, *Rasbora daniconius* is 3 µg/l. [7]Adhikari et al. (2005) reported the toxicity of cypermethrin in laboratory tests. In aquatic invertebrates LD_{50} toxicity ranging from 0.01 to 5 µg/l. In Table 4 the study of the effects of cypermethrin on various freshwater fishes were presented.

In Table 4, the study of the effects of cypermethrin on various freshwater fish species was presented. From the data in Table 4, it is evident that cypermethrin impacts freshwater fish at both genetic and physiological levels. The table also discusses behavioural, biochemical, and histopathological changes in the affected fish. This indicates that cypermethrin exhibits high toxicity even at low concentrations. To better understand the toxic effects of cypermethrin on fish, it is recommended to examine behavioural changes in conjunction with biochemical parameters, such as protein concentration, and other metabolic functions.

Table 4.The toxic effects of Cypermethrin invarious fish species with reference

SI.No.	Model Organism	Changes Observed	Reference
1	Channa punctata	Genotoxicity and Oxidative stress in fish erythrocytes	Ansari and Kumar (1988) [8]
3	Oncorhynchus mykiss trout	Biochemical parameters	Atamanalp et al (2002) [9]
4	Rohu (Labeo rohita)	Haemato-biochemical alterations	Das and Mukherjee (2003) [10]

5	Rainbow Trout	Paramount effects on different haematological parameters	Cakmak(2003) [11]
6	Labeo rohita	Haematological changes	Adhikari et al (2004) [12]
7	Cirrhinus mrigala	Protease activity and free amino acid levels have significantly increased	Prashanth and David (2006) [13]
8	Cirrhinus mrigala	significant changes in the ammonia, urea, and glutamine levels	Prashanth (2007) [14]
11	Clarias gariepinus	Histopathological effects	Ayoola and Ajani (2008) [15]
12	Heteropneustes fossilis	Disturbance to spermatogenic cells and follicular wall	Carriquiriborde et al. (2009) [16]
13	Clarias batrachus	Biochemical alterations	Begum G (2009) [17]
15	Labeo rohita	Hyper excitability, erratic swimming, loss of equilibrium and sinking to the bottom	Marigoudar et. al. (2009) [18]
16	Channa punctata	Cytogenetic and oxidative stress	Ansari et al (2011) [19]
17	Zebrafish (Danio rerio) embryos	Developmental toxicity	Shi et al., (2011) [20]
18	Catla catla	Alterations in anti-oxidative enzymes	Akinrotimi et al (2012) [21]
19	Clarias gariepinus	Haematological changes	Akinrotimi et al (2012) [21]
20	Catla catla	Biochemical and Haematological parameter changes	Kannan et al., (2014) [22]
21	Clarius batrachus	Marked alterations in the level of LPO	Kumar et al.,(2014) [23]
22	Jenynsia multidentate	Changes in acetylcholinesterase activity and erratic swimming	Bonansea et al., (2016) [24]
23	Anabas testudineus	Haematological changes	Velmurugan et al (2016) [25]
24	Danio rerio	Oedema of and yolk sac and pericardium	Xu C et al (2018) [26]
25	Heteropneustes fossils	Glutamate dehydrogenase (GDH) enzyme activity was elevated in the liver and gill tis- sues.	Sulthana RN (2021) [26 [27]
26	Channa punctata	Gill toxicity	Dubey and Sharma (2022) [28]
27	Channa punctata	Alterations in the haematological parameter	Ghosh et al. (2022) [29]
28	Catla catla	DNA damage	Sharma and Jindal (2022) [30]
29	Mystus cavasius	Negative impact on the haemato-biochemical parameters and female gonad	Uddin et al (2022) [31]

30	Aplocheilus lineatus	Behavioural and Protein constituents	Sai Krishna et al (2024) [32]

Cypermethrin exposure causes abnormal swimming patterns, reduced opercular activity, and altered air-gasping behaviour in fish. Fish subjected to pesticide stress sometimes exhibit symptoms of dullness, loss of equilibrium, loss of appetite, and erratic swimming [33] (Kavitha and Rao, 2007). [34]Ural and Koprucu (2006) also reported that fish began breathing air at the surface, swimming on the water's surface, and displaying altered swimming behavior from the first day when exposed to sublethal concentrations. [35]Kaviraj and Gupta (2014) observed that cypermethrin induces oxidative stress in fish, initially leading to frequent surfacing, increased opercular movement, and faster swimming activity. However, within 24 hours of treatment, the fish gradually displayed lethargy in their movements.

The acute toxicity of this pesticide at sublethal concentrations was examined through observation of behavioural and biochemical changes. After 10 and 15 days of cypermethrin exposure, protein concentrations in the muscle tissue of the fish decreased by more than 80%. The sublethal effects of cypermethrin on protein metabolism suggest that the reduction in protein content in the experimental fish may be attributed to several factors. The effect of cypermethrin on protein concentration has been studied by[36] Sambasiva Rao (1999), [37]Li et al. (2005) and [38]Thenmozhi et al. (2011). These reductions in protein levels may be due to increased energy demands and the breakdown of biomolecules as the fish cope with pesticide-induced metabolic stress [39] (Kumari et al., 2024).

CONCLUSION:

The effects of cypermethrin on the slender rasbora, *Rasbora daniconius* cause acute toxicity in the fish and lead to changes in behavioral and protein aspects. There was a higher degree of decrease in the level of protein content in the whole-body extract. The present research highlights the harmful effect caused by cypermethrin to the fish populations and emphasizes the need for careful regulation and monitoring to adjudicate its effective use on aquatic ecosystem.

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Author's statements

Authors clearly states that there are no potential conflicts of interest in the article. No funding agency resources are available for this study. While handling the specimens, ethical procedures and standards are maintained.

Authors' Contributions-

Brinesh R prepared the manuscript, conceived the study, Dilsha Parvin and Devika K researched the literature, and contributed in the lab work.

Ethical Issues

There were no ethical issues and concerns in the experiment and result in my original research paper.

Conflict of Interest

Author declares that there is no conflict of interest while doing the experiment and preparing the manuscript.

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