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Effect of Agricultural Pesticide, Cypermethrin on Changes in Behavioural and Protein Profile of Larvivorous Fish *Aplocheilus lineatus*

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ABSTRACT
Cypermethrin is a synthetic pesticide that is widely used in agricultural fields and domestic areas to control pests and insect vectors. Cypermethrin considered to be a synthetic pyrethroid is extremely lethal on insects, and fishes and has a low impact on mammals and Aves. The present study aimed to investigate the acute toxicity of cypermethrin on the larvivorous fish *Aplocheilus lineatus,* which is the most common fish seen in pools, ponds, streams, paddy fields, and similar wetlands. The fish is highly significant in controlling vector-borne diseases prevailing in tropical countries. The LC_{50} value was determined to be 1.8 µg/L after 96 hrs of exposure to cypermethrin and the lethal value was 4 µg/L. The fish showed various behavioral changes during the experiment, including convulsions, vigorous gasping for air, erratically swimming, and loss of equilibrium. The results showed that even low concentrations of cypermethrin can exert acute effects on the biochemical constituents and behavioral aspects of *A. Lineatus*. Total protein, structural protein, and soluble protein decreased from 1.33 ± 0.003 to 0.746 ± 0.015 mg/g, 0.967 ± 0.024 to 0.476 ± 0.0272 mg/g, and 0.444 ± 0.09 to 0.360 ± 0.018 mg/g, respectively, at 96 hrs of exposure to 1 µg/L of cypermethrin. At the same time glycogen levels also decreased from 1.11 ± 0.054 to 0.51 ± 0.019 mg/g. These variations in the behavioral and biochemical content of the fish after exposure indicate the toxic effect of cypermethrin. Hence the present study suggests monitoring and regulatory use of this pesticide.

Key words: Sublethal, toxic, Pyrethroids, Larvivorous fish, Biochemical constituent

INTRODUCTION

Pesticides are extensively used in agriculture, forestry, public health, and veterinary practices to control pests. Synthetic pyrethroids are a popular choice among farmers due to their quick degradation and lack of persistence (Bradbury and Coats 1989). Larger amount of such lethal pesticides released from agricultural fields in to nearby aquatic ecosystems causes adverse effects on living organisms. In the last decades, these types of synthetic pyrethroid pesticides were developed for use in the household and agriculture sectors.

Pyrethrins, Pyrethroids, Carbamate, Organochlorine, and Organophosphate are the principal chemical groups of pesticides that are frequently utilized for pest control. The pesticidal residue that contaminates water comes from surface runoff, surface drainage, and intensive framing. The residual contamination is usually more effective few weeks after pesticide treatment in agricultural fields (Kumari 2020). For usage in agriculture and home applications, synthetic pyrethroids have been available for the past 20 years as an alternative to more toxic pesticides such as hydrocarbons, organophosphates, and carbamates. Numerous studies have demonstrated that pyrethroids are exceedingly hazardous to a range of non-target species, including freshwater fish, aquatic arthropods, and honeybees, even at very low doses (Siegfried 1993).

Most aquatic living organisms are vulnerable to toxic chemicals like heavy metals, pesticides, or both organic and inorganic residues. However, these chemical compounds are deadly poisonous to fish. Even though some chemicals require a few weeks for degradation, its adverse effect on fish is shown within a few hours of exposure. Consequently, these chemicals have great impact on the physiological functioning of the fish. Biochemical processes change in the tissues of fish due to the impact of various agricultural pesticides and chemicals has been reported (Halappa and David 2009). Cypermethrin is an artificial substance lethal to insects that falls under the category of pyrethroids which show strong adverse effects on fish health.

Aplocheilus lineatus lives in streams and reservoirs of high altitudes; rivers, ponds, paddy fields, swamps of plains, and brackish waters (Fig. 1). It is a larvivorous, indigenous fish living in small groups found close to the water surface, feeding on aquatic insects and their larvae. *A lineatus* is the common dominant species found in the Kerala region and represent more than 1% of the total fish population (George and Mathew 2022). The larvivorous feeding nature of this fish had significance in the agricultural sector in ecological and economic aspects.

Behavioral, hematological, and histological alterations are the prominent outcomes of pesticide exposure in higher concentrations in fish (Okogwu et al. 2022, Ullah et al. 2019). Biochemical and physiological alterations are linked to structural and functional modifications in cell proteins and glycogen. One of the numerous compensatory strategies for maintaining metabolic homeostasis under any stress situation is protein and glycogen metabolism. These changes adversely affect the behavioral patterns of the effected fish. The present study aims to find out the LC_{50} value for cypermethrin, and its impact on the behavioral changes and biochemical characteristics of common fish, *A. Lineatus*.

MATERIAL AND METHODS

Experimental animals

Healthy individuals of *A. Lineatus* were gathered from ponds and paddy fields of the Kariyavattom campus, University of Kerala, Thiruvananthapuram, Kerala. Collected individuals were first treated with potassium permanganate solution (0.5% w/v) to remove any possible infectious organisms. They were then allowed to habituat to lab settings in a glass tank with a volume of roughly 150 L of tap water for 14 days. Acclimation was done at room temperature (28-35°C) and the fish were exposed to the natural day and night cycle (12-14 hr photoperiod) and fed with standard food pellets. Water in the tanks was renewed daily and the physical and chemical parameters of water were examined according to the guidelines of Anonymous (2018)

Figure 1. *Aplocheilus lineatus*

Experimental pesticide

A stock solution of 1 mg/L of cypermethrin (Cyper25-Cypermethrin 25% EC manufactured by National Pesticides and Chemical, Mumbai purchased from the agricultural market of Thiruvananthapuram Kerala India) prepared in acetone. To test concentrations for the LC_{50} value and sub-lethal biochemical toxicity, test series of 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 µg/L were prepared by serial dilution of the stock solution (Neglur et al. 2020).

Exposure to cypermethrin

Fishes of size range with length (5 to 8 cm) and weight (10 to 15 g) were used for the experiment. The fish tanks were aerated to prevent low oxygen levels. Ten fish were exposed to concentrations of cypermethrin (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 µg/L) prepared in 20 L capacity aerated glass tanks. Mortality rates were recorded at 24, 48, 72, and 96 hrs after the start of the experiment, and any deceased fish were promptly removed and the LC_{50} for 96 hours was calculated (Finney 1971).

Behavioral studies and biochemical characteristic analysis

In behavioral studies, healthy *A. Lineatus* was introduced into an aquarium and 96 hrs exposed to cypermethrin's sublethal concentrations (0.5 and 1 µg/L). The fish were frequently observed for behavioral changes during each experimental period. Behavioral changes were recorded for further interpretation of the effect of the toxicant. Whole muscle tissue of experimental fish exposed to two sublethal concentrations was homogenated with 1% 0.25 M ice-cold sucrose solution for the analysis of soluble, structural, and total proteins in the tissue by using the Folin phenol reagent method (Lowry et al. 1951). Tissue glycogen was also estimated using the Anthrone method (Carroll et al. 1956)

Statistical analysis

All the experiments were performed in triplicates for the accuracy of the results. A probit model with a logarithmic base 10 concentration transformation was used to analyze the total number of animals used, exposure concentrations, and mortality rates in each experiment in statistical package SPSS 17.0. The P value is less than 0.05, which denotes that the values were statistically different.

RESULTS AND DISCUSSION

The average values of physio-chemical characteristics of water used in aquaria during the experiment were shown in Table 1. Fishes exhibited no mortality up to 0.3 µg/L cypermethrin during the study period. After 96 hrs, 5% mortality was observed at 0.5 µg/L, while 15, 30, 55, 65, 80, and 90% mortality was recorded at 1, 1.5, 2, 2.5, 3 and 3.5 µg/L cypermethrin, respectively. Mortality (%) of *Aplocheilus lineatus* increases with increase in pesticide (cypermethrin) concentration and 50% fish mortality was observed between the concentrations of 1.5 and 2 µg/L (Table 2). The derived LC_{50} value at 96 hr was 1.8 µg/L. Probit analysis of 95% confidence limits for the concentrations of cypermethrin indicates it is highly poisonous to fish (Table 3), and two sub-lethal concentrations (0.5 and 1 µg/L).

Table 1. Physico-chemical parameters of water

Parameters	Value
Temperature (°C)	32.1
pH	6.27
DO (mg/L)	7.8
Turbidity (NTU)	64.7
TDS (ppt)	26.28
Conductivity (µS/cm)	43.8
Sodium (mg/L)	45.88
Potassium (mg/L)	3.65
Chloride (mg/L)	61
Sulphate (mg/L)	56.7
Nitrate (mg/L)	0.12

Table 2. Mortality (%) of *Aplocheilus lineatus* exposed to different concentrations of cypermethrin for 96 hrs

Concentration (µg/L)	Mortality (%)
0	0
0.5	5
1.0	15
1.5	30
2.0	55
2.5	65
3.0	80
3.5	90
4.0	100

Table 3. Probit analysis of 95% confidence limits for the concentrations of cypermethrin in *Aplocheilus lineatus*

Probability	95% Confidence limits for concentration		
	Estimate	Lower bound	Upper bound
0.1	0.866	0.602	1.081
0.15	0.996	0.726	1.213
0.2	1.113	0.842	1.331
0.25	1.224	0.954	1.444
0.3	1.334	1.066	1.556
0.35	1.444	1.179	1.67
0.4	1.557	1.295	1.79
0.45	1.674	1.415	1.918
0.5	1.799	1.54	2.058
0.55	1.933	1.671	2.215
0.6	2.079	1.81	2.394
0.65	2.241	1.958	2.604
0.7	2.426	2.12	2.856
0.75	2.643	2.302	3.166
0.8	2.908	2.513	3.565
0.85	3.249	2.773	4.109
0.9	3.737	3.127	4.933
0.99	6.783	5.098	10.995

Table 4. Effects of cypermethrin on the behavioural pattern of *Aplocheilus lineatus* exposed to the pesticides up to 96 hrs

Parameters	**Control**	**Cypermethrin dose (µg/L)**		
		0.5	**1**	**4**
Hyperactivity	-	-	+	+++
Loss of balance	-	-	-	++
Air gasping	+	+	++	+++
Surfacing activity	+	+	+	+++
Rate of swimming	+	+	++	++
Schooling behavior	+++	++	+	-
Opercular activity	+	+	+	+++
Convulsions	-	-	+	++

- = None, + = mild, ++ = moderate, +++ = Slightly higher

When *A. Lineatus* was exposed to two sub-lethal concentrations (0.5 and 1 µg/L) and one lethal concentration (4 µg/L) of cypermethrin, it resulted in various behavioral changes. While the control group exhibited normal behavior throughout the experimental period, in the experimental group behavioural changes began 30 min after the exposure of fish to pesticide. Fish exposed to the highest concentration of 4 µg/L showed the changes first. The fish under exposure displayed symptoms such as loss of equilibrium, decreased mobility, and spending more time at the bottom of the tank. From time to time, the affected fish began to swim constantly sideways, and they displayed increased opercular movement and gasping for air. Additionally, some fish exhibited lordosis, hanging vertically in water, and motionlessness. Ultimately, the affected fish died with their mouths and operculum open. In contrast, fish exposed to a sublethal concentration of 0.5 µg/L displayed no such behaviour and seemed to behaviour similar to the control group (Table 4).

Agricultural aquatic ecosystems can be considered as the sink of several pesticides and contaminants. The half-life of cypermethrin in aquatic ecosystems is about 2 weeks (Anonymous 1989). Datta and Kaviraj (2003) observed that Cypermethrin produces oxidative stress in fish, initially showing frequent surfacing, increased opercular movement, and faster swimming activity though within 5-9 hrs of treatment, gradually showing lethargy in their movement. Deposition of thick mucus layer was found in the buccal cavity and gills of exposed fish probably to minimize the irritating effects of toxicant coming in close contact with the body. A sign of heavy internal haemorrhage around the pharynx was noticed in the dead fish. Cypermethrin is very toxic for fish (in laboratory tests 96 hrs LC_{50} were generally within the range of 0.4-2.8 µg/L), and aquatic invertebrates (LC_{50} in the range of 0.01-5 µg/L) (Sarkar et al. 2005). They also showed exposure to this chemical can cause abnormal swimming patterns, reduced opercular activity, and altered air-gasping behaviors. Kavitha and Rao (2007) also found that fish under pesticide stress showed symptoms of dullness, loss of equilibrium, loss of feeding, and erratic swimming. In an apparent effort to avoid breathing in the impure water, there has been an increase in surfacing and gulping of surface water. The fish eventually covers its entire body, gills, and mouth cavity in large amounts of mucus as a defense mechanism to counter the damaging effects of pollutants as also reported by Prakash and Verma (2019). Nevertheless, according to Ural and Koprucu (2006), breathing air at the surface of the water, swimming on the water surface, and disturbed swimming behavior were observed on the first day itself during sublethal exposure. When fish are exposed to toxic substances they go under stress, and thus need higher oxygen levels and thu come to surface water which still rich with oxygen (Wiemer-hastings and Xu 2005).

In the present study, 96 hr exposure to sub-lethal concentrations of cypermethrin led to a rapid decrease in protein content. Total protein decreased from 1.33 ± 0.003 to 0.746 ± 0.015. mg/g for the pesticide concentration of 0.5 µg/L and from 0.967 ± 0.024 to 0.476 ± 0.0272 mg/g for 1 µg/L concentration as compared to the control group (1.313 ± 0.036 and 1.183 ± 0.056 mg/g) (Table 5). Soluble protein levels decreased from 0.481 ± 0.025 to 0.397 ± 0.011 mg/g and 0.444 ± 0.009 mg/g to 0.36 ± 0.018 mg/g, respectively, for 0.5 and 1 µg/L concentration of cypermethrin while the control group maintained a range of 0.63 ± 0.009 to 0.623 ± 0.014 mg/g for the same period (Table 6). Similarly, structural protein levels in the sublethal concentration

Table 5. Effect of sub-lethal dose exposure of cypermethrin on total protein content (mg/g fresh weight) of *Aplocheilus lineatus*

Cypermethrin	Duration of exposure			
concentration (µg/L)	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
Control	1.31 ± 0.036	1.06 ± 0.064	1.28 ± 0.026	1.183 ± 0.056
0.5 µg/L	1.33 ± 0.003	0.999 ± 0.014	0.82 ± 0.0143	0.746 ± 0.015**
1 µg/L	0.967 ± 0.024	0.82 ± 0.17	0.637 ± 0.007	0.476 ± 0.0272**

**Significant at P≤0.05.

Table 6. Effect of sub-lethal dose exposure of cypermethrin on soluble protein content (mg/g fresh weight) of *Aplocheilus lineatus*

Cypermethrin	Duration of exposure			
concentration (µg/L)	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
Control	0.63 ± 0.009	0.667 ± 0.005	0.607 ± 0.01	0.623 ± 0.014
0.5 µg/L	0.481 ± 0.025	0.439 ± 0.007	0.403 ± 0.005	0.39 ± 0.011**
1 µg/L	0.444 ± 0.09	0.437 ± 0.005	0.426 ± 0.039	0.360 ± 0.018**

**Significant at P≤0.05.

Table 7. Effect of sub-lethal dose exposure of cypermethrin on structural protein content (mg/g fresh weight) of *Aplocheilus lineatus*

Cypermethrin	Duration of exposure			
concentration (µg/L)	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
Control	1.05 ± 0.021	1.09 ± 0.085	0.969 ± 0.008	1.12 ± 0.091
0.5 µg/L	0.955 ± 0.015	0.869 ± 0.005	0.67 ± 0.004	0.62 ± 0.029**
1 µg/L	0.911 ± 0.004	0.77 ± 0.005	0.707 ± 0.007	0.594 ± 0.007**

**Significant at P≤0.05.

of 0.5 µg/L declined from 0.955 ± 0.015 to 0.62 ± 0.029 mg/g and for 1 µg/L from 0.911 ± 0.004 to 0.594 ± 0.007 mg/g. In contrast, the control group had the structural protein between 1.05 ± 0.022 and 1.12 ± 0.091 mg/g for the same period (Table 7). No statistically significant differences were detected among fish exposed to different concentrations of cypermethrin (P≤0.05).

Exposure to sub-lethal concentrations of cypermethrin caused a decrease in total, soluble, and structural protein possibly due to increased metabolic activity to cope with environmental stress. This may have resulted from tissue breakdown, cellular fraction disturbance, and impairment of protein synthesis machinery. Kong et al. (2021) and Anigol et al. (2023) also reported fish tissues exhibit a decrease in enzyme, immunity and biochemical contents due to oxidative stress. Vijayan and Thomas (2018) reported that the most important biochemical component found in significant amounts in a fish's body is protein. Due to increased proteolytic activity or recurrent protein breakdown to produce energy under stress, there was a reduction in muscle protein in *A. Lineatus*. However, in earlier studies when *Channa punctatus* was exposed to cypermethrin and 2-cyhalothrin for 96 hours LC_{50} values were 0.4 mg/L for cypermethrin and 7.92 µg/L for 2-cyhalothrin. Hence it was concluded that low concentrations of these compounds can be toxic (Kumar et al. 2007). Revathi et al. (2020) also found a reduction in protein in fish on exposure to pesticides. Susan Jacob et al. (1982) concludes that *A. lineatus,* a larviorous fish seen in agricultural land, is extremely sensitive to pesticides. Moore and Waring (2001) have suggested that pyrethroids may have a greater harmful effect on biochemical and physiological structure. It is also suggested that low levels of cypermethrin in the aquatic environment may have a significant effect on the reproduction and development of invertebrates (Aydýn et al. 2005). Kumar et al. (2022) showed the importance of biomarkers when fish experience toxic environmental stress.

CONCLUSION

In summary, the effects of cypermethrin on *Aplocheilus lineatus* are significant. The pesticide has been observed to cause acute toxicity in fish and lead to changes in behavioral and protein constituents. There was a serious decrease in the level of protein content in the muscle tissues of *A. Lineatus*. Overall, the research highlights the potential risks posed by cypermethrin to fish populations and emphasizes the need for careful regulation and monitoring to mitigate its adverse effects on aquatic ecosystems.

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