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ACUTE TOXICITY OF CYPERMETHRIN AND ALPHA-CYPERMETHRIN-BASED INSECTICIDES ON CYPRINID FISH

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Abstract
This study investigates the acute toxicity of cypermethrin and alpha-cypermethrin-based insecticides on cyprinid fish species. A 96-hour static non-renewal bioassay was conducted in the Ecotoxicology Laboratory of R.D.I.P.P. Bucharest. Juveniles of common carp (*Cyprinus carpio*) and Prussian carp (*Carassius gibelio*) were exposed to a range of insecticide concentrations. Acute toxicity data were analyzed using probit analysis, and 96-hour LC_{50} values (Mean Lethal Concentration) as well as the NOEC (No Observed Effect Concentration) were determined. Exposure to cypermethrin and alpha-cypermethrin induced behavioral and anatomical changes, including respiratory distress, loss of balance, reduced response to tactile stimuli, and abnormal swimming patterns, which intensified with increasing concentration and exposure duration. Among the tested species, common carp juveniles were the most sensitive, highlighting species-specific differences in susceptibility to these insecticides.

Key words: cyprinid fish, acute toxicity, cypermethrin, alpha-cypermethrin, behavioral responses

INTRODUCTION

Chemical control remains the principal strategy employed in modern agriculture to reduce yield losses caused by pathogens, pests, and weeds. However, the intensive and often indiscriminate use of pesticides generates substantial environmental and ecological concerns. Addressing these issues requires comprehensive scientific investigations into the effects of pesticides on human health and ecosystem integrity (Rice et al., 2007; Ekström & Ekbom, 2011; Gill & Garg, 2014; Neelima et al., 2016).

Among these chemicals, synthetic pyrethroid insecticides are widely used for pest management across agricultural systems. Numerous studies have shown that non-target organisms-particularly aquatic invertebrates and fish are highly sensitive to the neurotoxic effects of pyrethroids (Reddy et al., 1991; Philip et al., 1995; Aydin et al., 2025). At comparable concentrations, these compounds may be up to 1000 times more toxic to fish than to mammals or birds (Edwards et al., 1986; Bradbury & Coats, 1989). This pronounced susceptibility is attributed not only to species-specific differences in metabolic detoxification pathways but also to the heightened sensitivity of the fish nervous system to pyrethroid exposure (Mondal et al., 2015; Moore & Waring, 2001). Among the most commonly used representatives of this class are cypermethrin and alpha-cypermethrin.

Within the European Union, environmental risk assessment constitutes a critical component of the authorization, marketing, and application of plant protection products. This process aims to determine toxicity thresholds for a wide range of non-target organisms, including birds, aquatic species, bees, microorganisms, and beneficial insects. The guidelines established by the Organisation for Economic Co-operation and Development (OECD), particularly the principles of Good Laboratory Practice (GLP), provide a harmonized and internationally recognized framework for conducting ecotoxicological assessments, including those evaluating impacts on aquatic organisms.

The Ecotoxicology Laboratory of the Research and Development Institute for Plant Protection (RDIPP) has extensive experience in assessing the environmental risks associated with pollutants, especially plant protection products and their effects on the biotic components of ecosystems.

In this context, the present study investigates the acute toxicity of two commonly used pyrethroids, cypermethrin and alpha-cypermethrin, to juvenile stages of common carp (*Cyprinus carpio*) and Prussian carp (*Carassius gibelio*). The results offer new insights into the ecotoxicological risks posed by these insecticides and provide scientific support for evidence-based environmental risk assessment.

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MATERIALS AND METHODS

Test organisms: Juvenile specimens of common carp (*C. carpio*) (Linneaus, 1758) and Prussian carp (*C. gibelio)* (Bloch*,* 1782), (Teleostei, Cyprinidae) were obtained from accredited fish farms.

Transport and acclimation: Fish were transported in 60 L plastic containers, at a density of ~2.5 kg per 50 L of source water, with oxygen supplied via manual aerators and supplemental oxygen cylinders. The reception of the fish batch was carried out according to specific procedures, with health status, mortalities, and acclimatization parameters being recorded in the reconstituted water. Fish were acclimated for two weeks under controlled conditions: 21-24°C water temperature, continuous aeration, daily feeding at 2% of total biomass, daily removal of organic waste, and immediate removal of mortalities.

Mortality was monitored after 48 h: ≥10% mortality - batch rejected; 5 - 10% – acclimation extended 7 days; <5% batch accepted for further experimentation. For this study, young fish around the same age and size were used. Prior to the start of testing the weight and length of a total of 10 fish was determined, the average body length was 6.5 cm and weight of 2.8 g, according to GLP technical procedures.

Test chemicals:

For the risk assessment, we used two commercial insecticide formulations: Cipermethrin 10 EC (containing 100 g/L of the active ingredient cypermethrin) and Ciperthrin 10 CE (containing 100 g/L of the active ingredient alpha-cypermethrin).

Experimental design and test conditions:

Acute toxicity tests were performed following OECD Guideline 203 (Fish, Acute Toxicity Test). Groups of seven fish were exposed to a series of cypermethrin and alpha-cypermethrin concentrations, along with a control group (without pesticide). Mortality and behavioral changes were recorded at 24, 48, 72 and 96 hours. Dead fish were immediately removed to prevent deterioration of water quality. Water temperature, pH, and dissolved oxygen were monitored daily throughout the experiment.

To establish the toxic concentrations of the test substances, a preliminary range-finding test was first conducted over 24-48 hours. Concentrations were selected in a logarithmic series (1000, 100, 10, 1, and 0.1 mg/L), with three fish randomly assigned to each solution. This preliminary test aimed to identify concentrations causing 100% mortality as well as those resulting in less than 50% mortality. Based on these results, the definitive acute toxicity test was performed using a geometric series of concentrations, typically with a ratio of 2 (e.g., 8, 4, 2, 1 mg/L), or, when necessary, with a ratio smaller than 2 to refine the concentration range and accurately determine the LC_{50}. A control test was conducted in parallel, in which fish were maintained in toxin-free water under the same experimental conditions as the treated groups. This ensured that any observed effects could be attributed specifically to the test substances.

During testing, fish were exposed to cypermethrin and alpha-cypermethrin in the following conditions:

- Test type: static
- Duration: 96 hours
- Number of fish: 7 individuals for each test concentration, including the control
- Water volume: 20 L per aquarium
- Dilution and test water: reconstituted water
- Light regime: 16 hours light and 8 hours darkness
- Dissolved oxygen: maintained above 60% of the air-saturation value
- Temperature: 21–24 °C, constant throughout the test, with a maximum deviation of ±1 °C
- pH: between 6 and 9, with fluctuations not exceeding one unit during the test
- Aeration: test solutions must not be aerated
- Feeding: no feeding during the exposure period
- Test validity: the test is considered invalid if more than one fish dies in the control group during the 96-hour exposure period
- Test concentrations: must remain within 80% of their initial measured values. The test concentrations were prepared by directly dissolving the substance in reconstituted water
- All test solutions were prepared immediately prior to the start of the exposure.

Data Analysis: The median lethal concentration (96-hour LC_{50} values) was statistically calculated using the probit method, which employs graphical relationships between the logarithm of the toxicant concentration and the mortality frequency, expressed as percentages and converted into probit units (Finney, 1962).

The median lethal concentration represents the concentration of a test substance in water that is expected to cause mortality in 50% of the exposed organisms over a defined exposure period.

Additionally, the maximum concentration that produced neither mortality nor signs of toxicity in fish during the test period (NOEC- No Observed Effect Concentration) was determined.

RESULTS AND DISCUSSIONS

For the environmental risk assessment of cypermethrin and alpha-cypermethrin-based insecticides according to GLP, the following steps were undertaken: a GLP-compliant test facility was established; appropriate space for the growth, acclimatization, and testing of fish was ensured; a complex water production system for performing the tests was installed; a control system for environmental conditions was implemented; and specific standard operating procedures were developed and accredited under GLP**.** For fish maintenance in the laboratory, as well as for conducting the tests, reconstituted water was used . The GLP testing unit is equipped with an automated system that produces the reconstituted water required for performing ecotoxicological tests, in accordance with the requirements of Appendix 2 to Method C2 of Regulation (EC) No. 440/2008. Reconstituted water is a saline solution used for conducting ecotoxicological tests. Key parameters including pH, hardness, and the ratios of Na^+/K^+ and Ca^{2+}/Mg^{2+} ions are continuously monitored and maintained within specified limits: pH 7.8 ± 0.2; hardness (Ca CO_3) 10-250 mg/L; Na^+/K^+ ratio 10:1 mmol; Ca^{2+}/Mg^{2+} ratio 4:1 mmol. The study was validated by comparison, demonstrating full compliance with the quality criteria specified in Method C1 of Regulation (EC) No. 440/2008. Validation was based on meeting the following quality criteria:

- no mortality was observed in the control group throughout the 96-hour exposure period;
- dissolved oxygen concentrations were maintained above 60% of the air-saturation value at all times;
- pH remained stable, with variations not exceeding one unit over the entire test duration.

Acute toxicity ((96-hour LC50 values):

Exposure of *C. carpio* and *C. gibelio* juveniles to cypermethrin and alpha-cypermethrin resulted in concentration and time dependent mortality. The test concentrations of cypermethrin and alpha-cypermethrin, applied under identical conditions, produced different toxic effects on the fish. No mortality or sublethal effects were observed in the control group. Both insecticides exhibit a clear concentration-mortality relationship, with correlation coefficients approaching approaching 1.0 ($r > 0.99$). However, due to optical isomerism, alpha-cypermethrin displays higher biological activity and increased affinity for the nervous system of aquatic organisms, which explains the high toxicity observed in juvenile common carp. The 96-hour LC_{50} values indicated that alpha-cypermethrin was slightly more toxic than cypermethrin for both species, suggesting higher biological activity of the alpha isomer. The observed LC_{50} values for *C. carpio* were generally lower than those for *C. gibelio*, indicating that common carp are more sensitive to pyrethroid exposure. Cyperthrin 10 CE causes complete mortality at a concentration of 0.025 mg/L, whereas Cypermethrin 10 EC reaches the same level of mortality only at 0.2 mg/L. By analyzing the interval between the onset of asphyxiation and fish death, we found that it ranged from a few minutes to tens of minutes in toxic solutions of alfa-cypermethrin, and from tens of minutes to hours in fish maintained in toxic solutions of cypermethrin, demonstrating the significantly higher acute toxicity of the α-isomer.The 96-h median lethal concentration (LC_{50}) for *C. carpio* was 0.023 a.i./L for cypermethrin and 4.92 µg a.i./L for alpha-cypermethrin (Tables 1 and 2). The temporal variation of mortality induced in common carp by alpha-cypermethrin is shown in Figure 1. Similar interspecific differences in susceptibility to pyrethroids have been previously reported by Singh et al. (2012) and Velisek et al. (2006). Alpha-cypermethrin is highly toxic to fish. It can affect aquatic ecosystems even at low concentrations, due to its moderate environmental persistence. Alpha-cypermethrin is practically non-toxic to birds but is highly toxic to fish and aquatic invertebrates (Sarıkaya, 2009; Yılmaz & Erbaşlı 2004).

Table 1.

The acute toxicity of Cypermetrin on juvenile *Cyprinus carpio*

Concentration mg a.i./L	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	-
0.0032	7	0	0	0	0	-
0.0064	7	0	0	0	1	Loss of balance (the fish were lying on the bottom of the aquarium lying on their sides) and slow respiratory movements, although the oxygen concentration in the aquarium water was within normal limits
0.0125	7	0	0	0	2	
0.025	7	0	1	1	2	
0.05	7	0	1	2	2	
0.1	7	1	1	2	2	
0.2	7	2	5	-	-	
Martor	7	0	0	0	0	-
NOEC (96 h) = 0.0032 mg a.i./L; LC_{50} (96 h) = 0.023 mg a.i./L; Correlation Coefficient R= 0.9930						

Table 2.

The acute toxicity of Alpha-Cypermethrin on juvenile *Cyprinus carpio*

Concentration mg a.i./L	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	-
0.00078	7	0	0	0	0	-
0.00156	7	0	0	0	1	Inactivity, sudden sinking, discoloring, weakness, spiral swimming, balance loss, spasms, slow and heavy breathing.
0.00312	7	0	0	1	1	
0.00625	7	0	1	1	2	
0.0125	7	1	1	2	2	
0.025	7	3	4	-	-	
0.05	7	7	-	-	-	
Martor	7	0	0	0	0	-
NOEC (96 h) = 0.00078 mg a.i./L; LC_{50} (96 h) = 0.00492 mg a.i./L; Correlation Coefficient R= 0.9918						

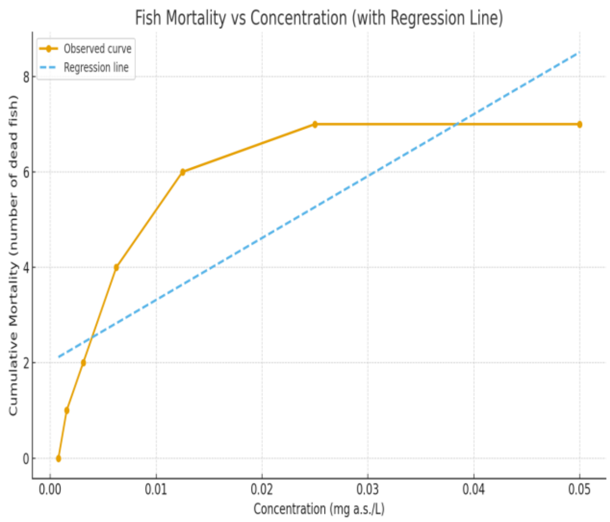

Figure 1. **Mortality time variation induced by lethal concentration of solution of alpha-cypermethrin in common carp**

Behavioral responses:

Sublethal concentrations of both pesticides induced noticeable behavioral alterations in fish prior to mortality. Exposed individuals exhibited hyperactivity, erratic swimming, loss of equilibrium, rapid opercular movement, excessive mucus secretion, and, at higher concentrations, lethargy accompanied and surface gasping. These behavioral symptoms are consistent with neurotoxic effects characteristic of pyrethroid exposure, resulting from disruption of sodium channel function in neuronal membranes (Soderlund & Bloomquist, 1989). Fish initially maintained normal schooling and alert behavior. Sublethal cypermethrin and alpha-cypermethrin caused gradual disruption of schooling and increased ventilation, while lethal exposure led to immediate sinking, erratic swimming, and surfacing behavior. By 72 h, fins were extended and mouth and opercula widely opened; by 96 h, fish lost equilibrium, died, and showed pale bodies, discolored gills, and coagulated gill mucus. Some fish sank intermittently to the bottom and temporarily regained normal posture and balance.

Comparative sensitivity:

The juvenile *C. gibelio*, which proved to be more resistant to the toxic effects of this substance, a resistance that could be explained by the species high ecological plasticity and adaptability, being present in all lakes and ponds in our country. The difference in sensitivity between *C. carpio* and *C. gibelio* may be attributed to species-specific variations in metabolic detoxification efficiency and gill permeability. Fish species with higher metabolic enzyme activity or thicker gill epithelia generally exhibit greater tolerance to lipophilic compounds such as pyrethroids (Bradbury & Coats, 1989; Jiang et al., 2025). Furthermore, lipid-rich tissues in fish serve as reservoirs for these compounds, potentially prolonging their toxic effects even after exposure cessation.

Table 3

The acute toxicity of Cypermetrin on juvenile *Carassius gibelio*

Concentration mg a.i./L	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	-
0.02	7	0	0	0	0	-
0.04	7	0	0	0	1	Loss of balance and slow respiratory movements
0.08	7	0	0	1	1	
0.16	7	0	0	1	2	
0.32	7	0	1	1	2	
0.64	7	1	2	4	-	
Control	7	0	0	0	0	-
NOEC (96 h) = 0.02 mg a.i./L ; LC_{50} (96 h) = 0.18 mg a.i./L; Correlation Coefficient R = 0.9922						

Table 4.
The acute toxicity of Alpha-Cypermethrin on juvenile *Carassius gibelio*

Concentration mg a.i./L	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	-
0.005	7	0	0	0	0	-
0.010	7	0	0	1	1	Exciting movement, balance loss disorder swimming movements. Some fish sank intermittently to the bottom and temporarily regained normal posture and balance.
0.015	7	0	0	1	2	
0.020	7	0	1	1	2	
0.025	7	1	1	2	2	
0.030	7	2	2	3	-	
Control	7	0	0	0	0	-
NOEC (96 h) = 0.005 mg a.i./L; CL_{50} (96 h) = 0.017 mg a.i./L; Correlation Coefficient R= 0.9918						

Compared to common carp juveniles, which showed clear differences in susceptibility between the two active ingredients, Prussian carp juveniles exhibited very similar tolerance to both cypermethrin and alpha-cypermethrin, with largely comparable results. The 96-h median lethal concentration (LC_{50}) for *C. gibelio* was 0.18 mg a.i./L for cypermethrin and 0.017 mg a.i./L for alpha-cypermethrin (tables 3 and 4). The 96-h No Observed Effect Concentration was 0.02 mg a.i./L for cypermethrin and 0.005 mg a.i./L for alpha-cypermethrin (Tables 3 and 4)

Environmental and ecological implications:

Although pyrethroids undergo rapid degradation in the environment through photolysis and hydrolysis, their strong adsorption to sediments can result in prolonged persistence within benthic habitats. Consequently, even short-term contamination events may generate long-lasting effects on aquatic organisms (Gürkan & Hayretdağ, 2016). The findings of this study support previous reports indicating that, despite being regarded as relatively safe for terrestrial applications, pyrethroid insecticides pose significant risks to aquatic life even at very low concentrations (Hill, 1989; Sandahl et al., 2005; Lidova et al., 2019; Farag et al., 2021; Tahir et al., 2024). Furthermore, Kumaraguru and Beamish (1981) reported a negative correlation between water temperature and the acute toxicity of synthetic pyrethroids to fish. Therefore, the presence of pyrethroids in aquatic environments during periods of declining temperatures - such as during the spawning season of cold - water fish - may exacerbate their toxic impact on reproductive processes (Moore & Waring, 2001). The clinical signs observed in fish exposed to lethal pesticide concentrations were atypical yet consistent with those described in the scientific literature (Grădilă, 2013; Majumder & Kaviraj 2017; Sinha & Shrivastava 2024) The progression of pesticide-induced toxicity typically began with an excitation phase, followed by partial loss of equilibrium, and culminated in a final stage characterized by complete loss of balance. Mortality occurred at time intervals that varied depending on the concentration of the pesticide solution.

CONCLUSIONS

1. The findings demonstrate that both cypermethrin and alpha-cypermethrin exhibit high acute toxicity to juvenile *C. carpio* and *C. gibelio*, with alpha-cypermethrin being slightly more potent.
2. Behavioral and physiological disturbances observed in exposed fish confirm the neurotoxic nature of pyrethroids.
3. These results highlight the necessity for cautious application of pyrethroid - based pesticides near aquatic environments and emphasize the importance of continuous monitoring to mitigate ecological risks.
4. The clinical signs observed in fish exposed to lethal pesticide concentrations were atypical yet consistent with those described in the scientific literature.
5. Due to their widespread use and potential ecological risks, understanding the toxicokinetics and neurophysiological mechanisms underlying pyrethroid toxicity in aquatic species is essential for accurate environmental risk assessment and the development of sustainable pest management strategies.
6. Biological model systems with fish, conducted under a GLP test facility in Research - Development Institute for Plant Protection have proven effective and validity of the environmental risk assessment of plant protection products according to Good Laboratory Practice Principles.

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