Single and Joint Acute Toxicity Effects of Lambda-cyhalothrin and Cypermethrin on Nile tilapia (*Oreochromis niloticus*) Fingerlings

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Abstract The extensive co-occurrence of pyrethroid insecticides such as lambda-cyhalothrin (LCT) and cypermethrin (CPM) in aquatic systems poses a potential risk, yet a significant research gap exists regarding their combined toxicological effects. In this study, the single and joint acute toxicity effects of lambda-cyhalothrin (LCT) and cypermethrin (CPM) on Nile tilapia fingerlings were investigated using 96-h bioassays. Results showed both were highly toxic, with LCT (96-h LC_{50} = 66.53 μg/L) being four-fold more potent than CPM (259.41 μg/L). Regression analysis confirmed positive correlation ($P < 0.01$) between pesticide concentration and observed mortality. The binary mixture exhibited synergistic effect with Additive Index (AI) > 0, indicating combined effects exceeded the sum of their individual actions. This synergism likely stems from mutual inhibition of metabolic detoxification pathways, leading to increased internal concentrations and amplified neurotoxicity. Generally, this study confirmed that single-compound risk assessments dangerously underestimate pyrethroid mixture hazards, necessitating their inclusion in regulatory frameworks for accurate aquatic biodiversity protection.

Key words Acute toxicity; Joint toxicity; Lambda-cyhalothrin; Cypermethrin; *Oreochromis niloticus*

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Pyrethroid pesticides are a class of insecticides widely applied in agriculture for controlling major pests to improve crop protection and production[1-2]. This class of insecticides is reported to cause detrimental effects on non-targeted terrestrial and aquatic organisms[3-4]. They can spread chiefly through spray drift, surface runoff and leaching[5-6]. Pyrethroids including lambda-cyhalothrin and cypermethrin demonstrate exceptional pesticidal potency, with approximately 1 000-fold greater toxicity to fish than to mammals and birds due to their high gill absorption and limited metabolic detoxification[7,8]. They usually affect brain, liver, muscles, kidneys, and gonads, which results in impaired reproduction, growth, behavior changes, and death[8-9]. Regulatory agencies such as the U.S. EPA and ECHA classify pyrethroids as major hazards to aquatic life[10-11]. Despite their frequent co-detection in water bodies[12-13], the combined toxicity data of these pyrethroids remained undefined. This study aimed to determine the individual 96-h LC_{50} values of these pyrethroids through probit analysis and assess their joint toxicity using the Additive Index method in Nile tilapia fingerlings. This species was selected as a model organism due to its global significance in aquaculture and prevalence in regions where these pesticides are extensively applied[14]. Furthermore, its environmental tolerance and handling robustness meet the OECD and ISO guidelines for test organisms[15]. The resulting data are critical for precise environmental risk assessment and developing strategies to safeguard aquatic ecosystems.

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Materials and Methods

Test organisms and adaptation

Healthy Nile tilapia (*Oreochromis niloticus*) fingerlings (12.4 ± 0.4 g, 8.2 ± 0.4 cm, n = 1 000) were acclimated for 14 d in a 5 000 L aerated tank. Fish were fed commercial feed twice per day and throughout acclimation period no mortality observed. The selected fish were fastened for 24 h before the trial day. A semi-static system was maintained with daily monitoring of DO, pH and water temperature.

Test water

Test water was dechlorinated via aeration unceasingly for 7 d. Water quality parameters (temp: 30 ± 0.6 ℃, DO: 5.30 ± 0.50 mg/L, pH: 7.5 ± 0.4) were monitored tri-daily. Ammonia levels remained below 0.05 mg/L, confirming OECD compliance. Continuous aeration was provided throughout the 96-h bioassay.

Preliminary test

Following acclimation, a preliminary range-finding test was conducted in 20 L dechlorinated water using 5 fish per 30 L tank. Nile tilapia fingerlings were exposed to test concentrations of 40, 60, 80, and 100 μg/L for lambda-cyhalothrin and 190, 245, 350, and 407 μg/L for cypermethrin. Water was renewed daily. Mortality was recorded and dead individuals were promptly removed during the 24 - 48 h exposure period.

Acute toxicity test

Following the preliminary test, definitive acute toxicity

concentrations were established. Ten Nile tilapia fingerlings of known size were exposed to seven concentrations of lambda-cyhalothrin (60.00, 65.19, 70.82, 76.94, 83.59, 90.81, 98.66 g/L) and cypermethrin (244.00, 265.70, 289.33, 315.06, 343.08, 373.59, 406.81g/L), plus a control. All treatments used 20 L of dechlorinated water and were replicated three times. The 96-h semi-static bioassay followed OECD guidelines with daily water renewal delivering fresh oxygen-rich water. Dead fish were promptly removed, with mortality recorded at 24 h intervals. The 96-h LC_{50} values and 95% confidence limits were determined through probit analysis using Linear Regression in Excel 2016.

Joint toxicity test

Based on individual acute toxicity results, a joint toxicity test was conducted. Nile tilapia (n = 10 per replicate) was exposed for 96 h in 20 L of dechlorinated water to seven joint concentrations of lambda-cyhalothrin (31.29, 34.08, 37.10, 40.40, 43.99, 47.91, 52.17 μg/L) and cypermethrin (122.00, 132.85, 144.66, 157.53, 171.54, 186.80, 203.40 μg/L), plus a control, both replicated three times. Half the test solution was replaced daily to maintain water quality. Mortality and behavior were recorded at 24 h intervals. The 1 ∶ 1 toxicity unit mixture was evaluated using Marking's Additive Index method, with mortality data tabulated per OECD guidelines for final analysis.

Results and Analysis

Acute toxicity of lambda cyhalothrin and cypermethrin on tilapia fingerlings

The 96-h acute toxicity of lambda-cyhalothrin (LCT) and cypermethrin (CPM) was evaluated in Nile tilapia fingerlings. The experiment revealed that the LC_{50} values for lambda cyhalothrin and cypermethrin at 24, 48, 72 and 96 h were 77.50, 72.59, 69.17, 66.53 μg/L and 335.30, 302.74, 265.06, 259.41 μg/L respectively. It was noted that LC_{50} values decreased with exposure time and concentration. Moreover, the 96-h LC_{50} of LCT (66.53 μg/L) was approximately four folds more toxic than CPM (259.41 μg/L). Mortality showed significant concentration and time dependence ($P < 0.01$), with a strong positive correlation between log concentration and probit mortality. Exposed fish exhibited distinct neurotoxic behaviors including hyperactivity, loss of equilibrium, and convulsions, while control groups remained unaffected. These results established clear dose-response relationships for both pyrethroids. Detailed data are presented in Table 1 and Table 2.

Table 1 Acute toxicity of lambda-cyhalothrin to tilapia fingerlings

Concentration // μg/L	Death rate // %			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
60.00	17	17	27	33
65.19	27	33	37	40
70.82	33	40	53	63
76.94	43	53	60	70
83.59	50	83	83	97
90.81	80	87	97	100
98.66	87	100	100	100
Regression equation	$Y = 9.504X - 12.956$	$Y = 11.825X - 17.005$	$Y = 13.063X - 19.035$	$Y = 15.028X - 22.396$
Correlation coefficient	0.970 9**	0.980 5**	0.964 0**	0.936 5**
LC_{50} // μg/L	77.5	72.59	69.17	66.53
95% confidence limit	71.52 – 83.98	67.70 – 77.83	64.94 – 73.67	62.65 – 70.65

Table 2 Acute toxicity of cypermethrin to tilapia fingerlings

Concentration // μg/L	Death rate // %			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
244.00	13	20	40	40
265.70	23	40	53	53
289.33	27	30	57	73
315.06	27	47	67	77
343.08	50	73	80	97
373.59	60	83	83	97
406.81	87	90	100	100
Regression equation	$Y = 9.044\,4x - 17.841$	$Y = 9.546\,3X - 18.685$	$Y = 6.594\,6X - 10.981$	$Y = 12.515x - 25.211$
Correlation coefficient®	0.947 8**	0.963 3**	0.988 3**	0.968 2**
LC_{50} // μg/L	335.3	302.74	265.06	259.41
95% confidence limit	308.18 – 364.81	279.49 – 327.92	233.93 – 300.34	242.88 – 277.06

Joint toxicity of lambda-cyhalothrin and cypermethrin on tilapia fingerlings

The binary mixture of lambda-cyhalothrin and cypermethrin demonstrated synergistic effect to tilapia fingerlings. Biological activity (S) values (0.97, 0.96, 0.95) and the Additive Index (AI =0.03, 0.04,0.05) for 24, 48 and 96 h respectively consistently exceeded zero, confirming synergism. The mixture was more lethal than individual exposures. A significant positive correlation ($P < 0.01$) showed mortality increased while time-to-death decreased with concentration. Neurotoxic behaviors included hyperactivity, loss of equilibrium, and reduced locomotion. Comprehensive joint toxicity data are presented in Table 3 and Table 4.

Table 3 Death rate of tilapia fingerlings under joint toxicity of lambda-cyhalothrin and cypermethrin

S/N	Concentrations // μg/L		Death rate // %			
	Cypermethrin	Lambda-cyhalothrin	24 h	48 h	72 h	96 h
1	Control	Control	0	0	0	0
2	122.00	31.29	13	27	31	49
3	132.85	34.08	23	40	50	57
4	144.66	37.10	37	47	67	77
5	157.53	40.40	63	73	87	87
6	171.54	43.99	70	87	88	89
7	186.79	47.91	87	97	97	100
8	203.40	52.17	100	100	100	100

Table 4 Joint toxicity effects of lambda-cyhalothrin and cypermethrin to tilapia fingerlings

Pesticides	Parameter	Time // h		
		24	48	96
Cypermethrin	Regression equation	$Y = 12.154X - 21.52$	$Y = 13.336X - 23.609$	$Y = 9.323\,9X - 14.491$
	Correlation coefficient®	0.994 1**	0.982 2**	0.977 9**
	LC_{50} // μg/L	152.05	139.72	123.15
	95% confidence limit	142.08 – 168.72	131.35 – 148.62	111.79 – 135.67
Lambda-cyhalothrin	Regression equation	$Y = 12.158X - 14.342$	$Y = 13.34X - 15.735$	Y = 9.329X – 8.989
	Correlation coefficient®	0.994 1**	0.982 2**	0.977 8**
	LC_{50} // μg/L	38.98	35.84	31.59
	95% confidence limit	36.43 – 41.71	33.69 – 38.12	28.68 – 34.80
Joint effects	Biological activity (S)	0.97	0.95	0.96
	Additive index (AI)	0.03	0.04	0.05
	Conclusion	Synergistic	Synergistic	Synergistic

Discussion

The 96-h LC_{50} values confirmed lambda-cyhalothrin (66.53 μg/L) as approximately four times more toxic than cypermethrin (259.41 μg/L), classifying both as "super toxic" to fish[10-11]. This potency difference was attributed to lambda-cyhalothrin's fluorine chemistry and enantiomeric purity, which enhanced sodium channel binding and metabolic stability[16-17]. Both compounds showed significant time- and-concentration dependent effects, confirmed by robust probit models ($R = 0.97$, $P < 0.01$). Critically, binary exposure demonstrated unequivocal synergistic effect with Additive Index (AI) > 0 across all exposures. These findings align with Kamutambuko *et al.*[18], who similarly reported pyrethroid synergism ($S < 1$, $AI > 0$) for 96-h exposures, validating the principal results of this study. This likely results from mutual inhibition of cytochrome P450 and carboxylesterase detoxification pathways, amplifying internal concentrations and neurotoxicity[17,19]. The observed neurobehavioral symptoms align with mode of action of type II pyrethroids which includes lambda-cyhalothrin and cypermethrin[17,20].

Conclusion

Generally, lambda-cyhalothrin and cypermethrin are individually highly toxic to tilapia, and their combination results in a synergistic that markedly exacerbates the lethal outcomes. This synergistic effect interaction increases the risk to non-target aquatic organisms like tilapia which are both economically important to aquaculture and serves as the ecological models for other fish species. This underscores the critical importance of incorporating mixture toxicity assessments into the environmental risk evaluation frameworks for pesticides to more accurately protect aquatic ecosystems.

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