Acute Single and Joint Toxicity Effects of Deltamethrin and Lambda-cyhalothrin on Zebrafish (*Danio rerio*)

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Abstract In this research, the single and combined toxicity effects of two commonly used pesticides, lambda-cyhalothrin and deltamethrin, were investigated on zebrafish at 20 ℃, with a weight of (1 ±0.1) g and a length of (3.5 ±0.35) cm. The study revealed that lambda-cyhalothrin exhibited higher toxicity compared to deltamethrin. Additionally, when used together, these pesticides showed significantly increased toxic effects on zebrafish. The 96-h LC_{50} values were determined to be 3.059 μg/L (confidence limits 0.077-0.351 μg/L) for lambda-cyhalothrin and 1.304 μg/L (confidence limits: 0.046-0.228 μg/L) for deltamethrin, both demonstrating a significant positive correlation ($P<0.05$). These results underscore the importance of regulating and managing pesticide use to safeguard aquatic organisms and uphold environmental sustainability.

Key words Acute toxicity; Joint toxicity; Lambda-cyhalothrin; Deltamethrin; Zebrafish (*Danio rerio*)
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Pyrethroids, a type of pesticide widely employed in agriculture for insect pest control[1], are organic toxic substances that specifically target the nervous system of insects. They achieve this by disrupting the normal functioning of voltage-gated sodium channels. In addition, certain types of pyrethroids interact with the chloride channels found in the gamma amino-butyric acid (GABA) receptor, resulting in disturbances in the initiation and propagation of action potentials in neurons and excitable cells. Ultimately, this leads to paralysis and eventual death of the insects[2]. The increased use of pyrethroid pesticides has been driven by recent bans on persistent organochlorine, carbamate, and organophosphate insecticides, which were found to have detrimental effects on the environment[3]. Consequently, the frequency and quantity of pyrethroid residues detected in the environment have risen[4]. Two widely utilized pyrethroids in the global market are deltamethrin and lambda-cyhalothrin, both recognized as prominent players in the pyrethroid industry[5-6].

Although studies have been conducted to investigate the individual toxicity of these chemicals, there are still gaps in our understanding of the combined effects of both chemicals on fish. This study was conducted to assess the impact of combined exposure to deltamethrin and lambda-cyhalothrin toxicity (in a 1 : 1 binary mixture) on zebrafish. The additive index (AI) was employed for this purpose. The outcome will give a basis for further toxicity tests. Simultaneously, it will provide necessary information for the management and control of these kinds of pollutants thus formulation of relevant water quality standards and tracking aquatic pollution scenarios. Zebrafish were chosen as the test organisms for water quality assessment due to their accessibility, small size, short test cycle, ease of maintenance, and suitability as an OECD and ISO standard test organism[7].

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Materials and Methods

Test organisms and acclamation

The present study used healthy adult Zebrafish with an average weight of (1 ±0.1) g and a length of (3.5 ±0.35) cm. The fish were acclimatized for 2 weeks in the laboratory and healthy fish were selected for the test. Feeding was stopped 24 h before the experiment and a semi-static environment was maintained during the test. No dead fish were observed during the acclimation period.

Test water

Test water was de-chlorinated for 1 week and the temperature was maintained at (20 ±1) ℃, DO 9.08-9.15 mg/L, and pH 7.64-7.72, respectively.

Preliminary test

Concentrations 7, 10, 15, 20 mg/L and 300, 400, 500, and 600 mg/L were selected for deltamethrin and lambda-cyhalothrin, respectively. Zebrafish responses were observed for 24 and 48 h to determine the appropriate concentration range for the test.

Acute toxicity tests

After obtaining results from the pre-tests, actual concentrations were made. Single acute toxicity of deltamethrin and lambda-cyhalothrin was done with concentrations 25.12, 31.62, 39.812, 50.12, 63.10, 79.43, 100 and 5.01, 7.08, 10.00, 14.13, 19.95, 28.18, 39.81 μg/L respectively. Observations for each concentration were done at 24, 48, and 96 h. Each group was replicated 3 times.

Joint toxicity test

The individual acute toxicity testing of the two pyrethroids provided the basis for determining the joint toxicity. The concentrations for deltamethrin and lambda-cyhalothrin were 1.77, 2.51, 3.54, 5.00, 7.06, 9.98, and 14.09 μg/L and 4.15, 5.88, 8.30, 11.73, 16.57, 23.40, and 33.06 μg/L, respectively. Interactions between the chemicals and zebrafish were evaluated using the additive index (AI)[8]. The following formulas were used to conduct the joint toxicity test in a 1 ∶ 1 ratio of toxicity units for the two toxicants:

$$\frac{A_m}{A_i}+\frac{B_m}{B_i}$$

$$AI=\frac{1}{s}-1\ (S<1),\ AI=1-S\ (\geqslant 1)$$

Wherein, S is the biological activity of chemicals (lambda-cyhalothrin and deltamethrin mixture); A and B are toxicants; A_i and B_i are LC_{50} (mg/L) of A and B respectively in single toxicity test; A_m and B_m are LC_{50} (mg/L) of A and B respectively in joint toxicity test; AI is additive index; and $AI=0$ means additive effect, $AI>0$ means synergistic effect, and $AI<0$ means antagonistic effect.

Results and Analysis

Acute toxicity of deltamethrin and lambda-cyhalothrin

Deltamethrin and lambda-cyhalothrin single toxicity were tested on zebrafish. The findings of both experiments revealed that the number of dead fish in each concentration group increased gradually from low concentration to high concentration. The 24 h-, 48 h-, and 96 h- LC_{50} values for deltamethrin and lambda-cyhalothrin were 60.83, 45.10, 27.21 and 39.19, 21.24, 11.6 μg/L. The mortality rate was time and dose-dependent. A significant positive correlation ($P<0.05$) was found between logarithmic concentrations and all-time intervals, indicating that the time taken for the fish to die was directly proportional to the concentration of both chemicals. The results of the experiments however demonstrated that the tested chemicals were super toxic to zebrafish[9]. Both chemicals usually attack the nervous system of fish when it comes into contact in water. The neurotoxic effects are attributed to the blocking of sodium channels and inhibiting the (GABA) receptors in the nervous filament which results in an excessive stimulation of the central nervous system and brain hypoxia leading to deaths[10]. The correlation coefficients, regression equations, LC_{50}, and their 95% confidence limits calculated by linear regression are shown in Table 1 and Table 2.

Joint toxicity of lambda cyhalothrin and deltamethrin (LC + DM) to zebrafish

The combined toxic effects of exposing zebrafish to an LC + DM mixture are outlined in Table 3.

The acute 96 h-LC_{50} values (95% confidence limit) for deltamethrin and lambda-cyhalothrin within the LC + DM mixture were determined to be 1.304 and 3.059 μg/L, respectively, with corresponding confidence limits of 0.046 – 0.228 μg/L and 0.077 – 0.351 μg/L. Notably, a statistically significant positive association was observed between these values ($P<0.05$). Additionally, the biological activity (S) at 24, 48, and 96 h was found to be 0.331, 0.296, and 0.313, respectively. Furthermore, the additive index (AI) at 24, 48, and 96 h was determined to be 2.02, 2.378, and 2.199, respectively. It is important to highlight that a synergistic effect was observed, as indicated by the computed AI values exceeding zero.

Table 1 Acute toxicity of deltamethrin to zebrafish

Concentration//μg/L	Death rate//%		
	24 h	48 h	96 h
Control	0	0	0
25.12	11.1	22.7	44.4
31.62	5.6	22.7	55.6
39.81	5.6	27.8	72.2
50.12	27.8	44.4	83.3
63.10	44.4	72.2	83.3
79.43	72.2	94.4	100
100.00	94.4	94.4	100
Regression equation	$y=5.0107x-3.9396$	$y=4.5536x-2.5325$	$y=3x+0.696$
Correlation coefficient	0.853	0.907	0.941
LC_{50}	60.827	45.101	27.206
95% confidence limit	2.423 – 2.584	2.922 – 3.188	3.072 – 3.549

Correlation coefficients (P-value) at 24 h = 0.002, at 48 h = 0.000 9, at 72 h = 0.005 and at 96 h = 0.006.

Table 2 Acute toxicity of lambda-cyhalothrin

Concentration//μg/L	Death rate//%			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
5.01	22.2	27.8	33.3	33.3
7.08	5.6	16.7	22.2	22.2
10.00	5.6	11.1	27.8	44.4
14.13	16.7	22.22	38.9	61.1
19.95	50	77.8	77.8	77.8
28.18	38.9	50	61.1	61.1
39.81	55.6	72.2	83.3	88.9
Regression equation	$Y=1.5667x+2.505$	$Y=1.7571x+2.6679$	$Y=1.7714x+2.9657$	$Y=1.869x+3.0106$
Correlation coefficient	0.533 8	0.541 0	0.747 5	0.807 1
LC_{50}	39.188	21.244	10.073	11.599
95% confidence limit	5.940 – 6.566	4.007 – 4.822	2.791 – 3.683	2.578 – 3.627

Correlation coefficients (P-value) at 24 h = 0.062, at 48 h = 0.059, at 72 h = 0.001 2 and at 96 h = 0.005.

Table 3 Death rate of zebrafish under joint effects of lambda-cyhalothrin and deltamethrin

Concentrations		Death rate // %		
Lambda-cyhalothrin μg/L	Deltamethrin μg/L	24 h	48 h	96 h
Control	Control	0	0	0
4.15	1.77	6	33	67
5.88	2.51	28	61	72
8.30	3.54	56	78	89
11.73	5.00	61	72	94
16.57	7.06	61	89	100
23.40	9.98	89	100	100
33.06	14.09	94	100	100

The results of the acute toxicity test demonstrated a clear relationship between the concentration of the pesticide and the mortality rate of the fish. This finding is consistent with previous reports, including one conducted by Salako *et al.* [9]. The positive correlation shown in Table 4 may be attributed to an increased uptake of the pesticide through the fish's gills. The study determined that the 96-h LC_{50} value for lambda-cyhalothrin was 3.059 μg/L, which falls within the range of the 96-h LC_{50} value of 2.7 μg/L reported by Farag *et al.* [11]. The 96-h LC_{50} values for deltamethrin were 1.304 μg/L, indicating that both pesticides were super toxic to zebrafish compared to previous studies. Wang *et al.* [12] reported different LC_{50} values for the two pyrethroids, ranging from 0.875 to 7.55 μg/L, respectively. However, the variations in findings could be attributed to several factors, including the organisms' slightly different responses to toxicants, which may explain the discrepancies among different studies. These pyrethroids have a significant impact on aquatic organisms due to their limited metabolism within these organisms[13].

Table 4 Joint toxicity of lambda-cyhalothrin and deltamethrin to zebrafish

Pesticide	Parameter	24 h	48 h	96 h
Lambda-Cyhalothrin	Regression equation	$y = 3.111x + 1.867$	$y = 2.4218x + 3.2583$	$y = 2.6529x + 3.7119$
	Correlation coefficient	0.926 *	0.859 *	0.949 *
	LC_{50}	10.164	5.238	3.059
	95% confidence limit	0.443 – 0.654	0.247 – 0.543	0.077 – 0.351
Deltamethrin	Regression equation	$y = 3.1116x + 3.0189$	$y = 2.4218x + 4.1551$	$y = 2.6529x + 4.6943$
	Correlation coefficient	0.926 *	0.859 *	0.949 *
	LC_{50}	4.332	2.233	1.304
	95% confidence limit	0.128 – 0.339	0.021 – 0.316	0.046 – 0.228
Joint effect	S	0.331	0.296	0.313
	AI	2.025	2.378	2.199
	Conclusion	Synergistic	Synergistic	Synergistic

The synergistic effect observed in the mixture of lambda-cyhalothrin and deltamethrin (LC + DM) can be best explained by the interactions between the pesticides themselves. The terms toxicokinetics and toxicodynamics are used to describe how the pesticides interact in a combined toxicity scenario[14]. Toxicokinetics refers to the interactions that occur when one chemical influences the absorption, distribution, metabolism, or elimination of another compound, leading to an increased internal dose of the latter chemical. On the other hand, toxicodynamic interactions occur at specific target sites within the same toxicity pathway. Interaction at the same receptor location typically results in antagonism. Toxicodynamic interactions are defined by Gundert-Remy & Sonich-Mullin[15] as interactions between the biological reactions to a chemical and the effects of the chemical on receptors, cellular targets, or organs. These interactions are influenced by various factors such as biological targets, duration and timing of exposure to the combination, dose levels, and the route of exposure.

Conclusions

Exposure to chemicals like deltamethrin and lambda-cyhalothrin in the aquatic environment can subject organisms to predation, starvation, and potential extinction. To conserve these resources, risk assessments such as this study should continue to be conducted to establish safety margins for non-target aquatic organisms. In the pursuit of sustainable development, it is crucial to prioritize chemicals that minimize human health risks and reduce environmental pollution. Assessing, monitoring, and conserving ecological environments play pivotal roles in this endeavor. Therefore, regulatory authorities should consider prohibiting the use of these pesticides, while scientists should concentrate on the research and development of new, beneficial, and environmentally friendly alternatives. The findings from this study can contribute to strengthening the existing legislation governing pesticide usage and ensuring the safety of both the environment and human populations.

References

[1] SHARMA A, KUMAR V, SHAHZAD B, *et al.* Worldwide pesticide usage and its impacts on ecosystem[J]. SN Applied Sciences, 2019, 1(11): 1446.
[2] AHAMAD A, KUMAR J. Pyrethroid pesticides: An overview on classification, toxicological assessment and monitoring[J]. Journal of Hazardous Materials Advances, 2023, 10: 100284
[3] SALAKO AF, AMAEZE NH, SHOBAJO HM, *et al.* Comparative acute toxicity of three pyrethroids (deltamethrin, cypermethrin and lambda-cyhalothrin) on guppy fish (*Poecilia reticulata* peters, 1859)[J]. Scientific African, 2020, 9: e00504.

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China Agriculture Press, 2013. (in Chinese).
[2] TIAN Kg. Human and animal comorbidity[M]. Beijing: China Agricultural Press, 2013. (in Chinese)
[3] LI Jd, MENG Xl, ZHU Xl. Study on recombinant goatpox virus vaccines vectored with H or F gene of peste des petits ruminants virus[J]. Chinese Veterinary Science, 2015,45(2): 111 –117.
[4] BOUSSINI H, CHITSUNGO E, BODJO SC. First report and characterization of peste des petits ruminants virus in Liberia, West Africa[J]. Tropical Animal Health and Production, 2016, 6: 17.
[5] TAYLOR W. The global eradication of peste des petits ruminants (PPR) within 15 years-is this a pipe dream[J]. Tropical Animal Health and Production, 2016,48(3): 559 –567.
[6] BARON MD, DIALLO A, LANCELOT R. Peste des Petits Ruminants Virus[J]. Advances in Virus Research, 2016, 95(3): 1 –42.
[7] WANG H, WU X-D, BAO JY. Epidemic analysis and control strategies of peste des petits ruminants in China[J]. Advances in Virus Research, 2015, 6: 142 –155. (in Chinese).
[8] AZIZ UR, ABUBAKAR M, RASOOL MH. Evaluation of risk factors for peste des petits ruminants virus in sheep and goats at the wildlife-livestock interface in Punjab Province, Pakistan[J]. Biomed Research International, 2016, 15(5): 245.
[9] HOLZER B, HODGSON S, LOGAN N. Protection of cattle against rinderpest by vaccination with wild-type but not attenuated strains of peste des petits ruminants virus[J]. Journal of Virology, 2016, 90(10): 5152 –5162.
[10] LIU YH, XU ZZ, HUA QY. Advance in molecular biology of peste des petits ruminants virus[J]. Progress in Veterinary Medicine, 2006, 27 (12): 1 –6. (in Chinese).
[11] LI L, WU X, LIU F. Rapid detection of lineage IV peste des petits ruminants virus by real-time RT-PCR[J]. Journal of Virological Methods, 2016, 148(1): 131 –133.
[12] SHARMA KK, KSHIRSAGAR DP, KALYANI IH. Diagnosis of peste des petits ruminants infection in small ruminants through in-house developed Indirect ELISA: Practical considerations[J]. Veterinary World, 2015, 8(4): 443 –448.
[13] WU X, LIU F, LI L. Diagnosis of peste des petits ruminants infection in small ruminants through in-house developed Indirect ELISA: Practical considerations[J]. Virus Genes, 2016, 52(3): 422 –427.

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[4] MEHLER WT, DU J, LYDY MJ, *et al*. Joint toxicity of a pyrethroid insecticide, cypermethrin, and a heavy metal, lead, to the benthic invertebrate *Chironomus dilutus*[J]. Environmental Toxicology and Chemistry, 2011, 30(12): 2838 –2845.
[5] HOŁYŃSKA-IWAN I, SZEWCZYK-GOLEC K. Pyrethroids: How they affect human and animal health[J]. Medicina, 2020, 56(11): 582.
[6] VAN DEN BERG H, DA SILVA BEZERRA H S, AL-ERYANI S, *et al*. Recent trends in global insecticide use for disease vector control and potential implications for resistance management[J]. Sci Rep, 2021, 11 (1): 23867.
[7] KOKKALI V, VAN DELFT W. Overview of commercially available bioassays for assessing chemical toxicity in aqueous samples[J]. TrAC Trends in Analytical Chemistry, 2014, 61: 133 –155.
[8] SVARTZ GV, ARONZON CM, PÉREZ COLL CS. Combined endosulfan and cypermethrin-induced toxicity to embryo-larval development of Rhinella arenarum[J]. Journal of Toxicology and Environmental Health, Part A, 2016, 79(5): 197 –209.
[9] SALAKO AF, AMAEZE NH, SHOBAJO HM, *et al*. Comparative acute toxicity of three pyrethroids (deltamethrin, cypermethrin and lambda-cyhalothrin) on guppy fish (*Poecilia reticulata* peters, 1859)[J]. Scientific African, 2020, 9: e00504.
[10] VELMURUGAN B, SELVANAYAGAM M, CENGIZ EI, *et al*. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of Cirrhinus mrigala[J]. Environmental Toxicology and Pharmacology, 2007, 24(3): 286 –291.
[11] FARAG MR, ALAGAWANY M, BILAL RM, *et al*. An overview on the potential hazards of pyrethroid insecticides in fish, with special emphasis on cypermethrin toxicity[J]. Animals, 2021, 11(7): 1880.
[12] WANG W, CAI D J, SHAN Z J, *et al*. Comparison of the acute toxicity for gamma-cyhalothrin and lambda-cyhalothrin to zebra fish and shrimp [J]. Regulatory Toxicology and Pharmacology, 2007, 47(2): 184 – 188.
[13] BLOCH D, DIEL P, EPE B, *et al*. Basic concepts of mixture toxicity and relevance for risk evaluation and regulation[J]. Archives of Toxicology, 2023, 97(11): 3005 –3017.
[14] ASHAUER R, ESCHER BI. Advantages of toxicokinetic and toxicodynamic modelling in aquatic ecotoxicology and risk assessment[J]. Journal of Environmental Monitoring, 2010, 12(11): 2056.
[15] GUNDERT-REMY U, SONICH-MULLIN C. The use of toxicokinetic and toxicodynamic data in risk assessment: An international perspective [J]. Science of The Total Environment, 2002, 288(1 –2): 3 –11.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU