Acute Single and Joint Toxicity Effects of Pendimethalin and Fenitrothion on Zebrafish (*Danio rerio*)

Rahma Mohamed Mahmoud[1], Shiqi WEI[1], Xi CHEN[1,2], Mingxiao LI[1], Shunlong MENG[1,2]*

1. Wuxi Fishery College, Nanjing Agricultural University, Wuxi 214081, China; 2. Scientific Observing and Experimental Station of Fishery Resource and Environment in the Lower Reaches of the Changjiang River, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China

Abstract In this study, the single and jiont acute toxicity effects of pendimethalin (herbicide) and fenitrothion (organophosphate insecticide) were investigated on juvenile zebrafish (*Danio rerio*) under semi-static conditions. Mortality was assessed at 24, 48, 72, and 96 h. The study revealed that pendimethalin exhibited higher toxicity than fenitrothion. The 96-h LC_{50} values were 0.477 mg/L for pendimethalin and 2.634 mg/L for fenitrothion. Joint exposure produced enhanced toxicity, with 96-h LC_{50} values of 0.204 mg/L (pendimethalin equivalent) and 1.139 mg/L (fenitrothion equivalent). Regression analysis showed a significant positive correlation ($p < 0.05$) between pesticide concentration and mortality, while toxicity indices confirmed synergistic interactions. These findings underscore the ecological risks posed by pesticide mixtures and highlight the importance of regulating pesticide use to safeguard aquatic organisms and maintain environmental sustainability.

Key words Acute toxicity; Joint toxicity; Pendimethalin; Fenitrothion; Zebrafish (*Danio rerio*)

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Pendimethalin (PND), a dinitroaniline herbicide, is extensively employed in agriculture as a pre-emergence agent for the control of grasses and broad-leaved weeds[1-2]. It acts by inhibiting cell division, thereby preventing seedling establishment, and is effective on roots and coleoptiles but not after emergence[3]. Registered in 1972, pendimethalin has been classified as a moderately persistent, bioaccumulative, and toxic compound, with possible carcinogenic effects[4]. It is highly toxic to aquatic organisms, particularly fish and invertebrates, and its persistence in soils (half-life ~90 d) raises concerns about runoff and contamination of aquatic ecosystems[5].

Fenitrothion (FNT), an organophosphate insecticide, is widely used to manage agricultural pests and in vector control programs. Its toxicity is mediated through inhibition of acetylcholinesterase, resulting in disruption of nerve transmission and eventual paralysis. Although effective, its persistence and frequent detection in water bodies highlight the ecological risks associated with its use.

Despite their extensive application, limited studies have addressed the combined toxicological effects of pendimethalin and fenitrothion on aquatic species. Considering their likely co-occurrence in agricultural runoff, understanding their joint toxicity is essential. Zebrafish (*Danio rerio*), recommended by OECD and ISO guidelines due to its sensitivity and suitability as a model organism, provides an appropriate system for aquatic risk assessment. Therefore, this study was conducted to investigate the acute single and joint toxicity of pendimethalin and fenitrothion on zebrafish, with emphasis on LC_{50} values, concentration-response relationships, and interactive effects.

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Rahma Mohamed Mahmoud (2001-), female, Mauritania, master, devoted to research about aquatic environmental toxicology.

* Corresponding author.

Materials and Methods

Test organisms and acclimation

Healthy juvenile zebrafish (*Danio rerio*) of both sexes were used. For the PND trial, fish averaged (2.9 ± 0.2) cm and (0.38 ± 0.1) g. For the FNT trial, fish averaged (3.5 ± 0.3) cm and (1.0 ± 0.1) g ($n = 30$). Fish were sourced from commercial suppliers and transported with continuous oxygenation. Upon arrival at the FFRC laboratory, the fish were acclimated for 14 d in aerated, de-chlorinated tap water in 200 L tanks following OECD 203 guidelines [OECD, 2019]. Temperature during acclimation was 24 - 26 ℃. The fish were fed commercial pellets once daily and food was withheld 24 h before tests. A semi-static regime was maintained, and the water quality was monitored daily. Batches were to be discarded if mortality exceeded 5%. No mortality occurred.

Test water

De-chlorinated tap water was used throughout. During testing, temperature was maintained at 31 - 32 ℃, pH at 7.7 - 7.9, and dissolved oxygen (DO) in the range of 4.6 - 4.9 mg/L. Solutions were renewed every 24 h (semi-static).

Preliminary test

Concentrations 1.8, 1.4, 10.5 and 0.2 mg/L and 6.0, 4.0, 2.0, and 1.0 mg/L were selected for pendimethalin and fenitrothion, respectively. Zebrafish responses were observed for 24 to 96 h to determine the appropriate concentration range of the test .

Acute toxicity tests

After obtaining the results from the pre-tests, a semi-static experimental design was adopted. Triplicate groups ($n = 10$ fish per group) were exposed to a series of concentrations of pendimethalin (0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 1.00 mg/L) and fenitrothion (1.7, 2.1, 2.6, 3.2, 3.9, 4.8, 5.9 mg/L), respectively. A control group was established using de-chlorinated

water only. Mortality was recorded at 24, 48, 72, and 96 h.

Joint toxicity test

The joint toxicity was done after finishing the single toxicity. Generally, 96 h-LC_{50} was used as a toxicity unit, and joint toxicity test was carried out according to the rule of 1∶1 of toxicity units of the two toxicants. The concentrations for pendimethalin and fenitrothion were 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.5 mg/L and 0.83, 1.10, 1.38, 1.66, 1.93, 2.21 and 2.76 mg/L, respectively. Interactions between the chemicals and zebrafish were evaluated using the additive index (AI). The following formulas were used to conduct the joint toxicity test in a 1∶1 ratio of toxicity units for the two toxicants.

$$\frac{A_m}{A_t}+\frac{B_m}{B_t};\ AI=\frac{1}{s}-1\ (S<1),\ AI=1-S\ (\geqslant 1)$$

Wherein, S is biological activity of chemicals (pendimethalin and fenitrothion 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; and AI is additive index, and it is interpreted as additive if $AI=0$, synergistic if $AI>0$, and antagonistic if $AI<0$.

Results and Analysis

Acute toxicity of pendimethalin and fenitrothion

The experiments demonstrated that pendimethalin and fenitrothion both caused time- and dose-dependent mortality in zebrafish, with deaths increasing as concentration and exposure time rose. For pendimethalin, the LC_{50} values were 2.124 6 mg/L at 24 h, 1.091 6 mg/L at 48 h, 0.544 mg/L at 72 h, and 0.477 mg/L at 96 h, while for fenitrothion, the values were 27.40, 6.96, 3.56, and 2.63 mg/L over the same time intervals. These results indicate that pendimethalin was more acutely toxic than fenitrothion, since its LC_{50} values were consistently lower. Pendimethalin acts by disrupting microtubule assembly and cell division, impairing tissue growth and inducing oxidative stress, whereas fenitrothion, an organophosphate insecticide, inhibits acetylcholinesterase leading to accumulation of acetylcholine, overstimulation of receptors, erratic swimming, paralysis, and eventual death. A significant positive correlation ($P<0.05$) between concentration and mortality was observed at all time points, confirming that toxicity increased proportionally with exposure.

Joint toxicity of pendimethalin and fenitrothion to zebrafish

The combined exposure of zebrafish to pendimethalin (PND) and fenitrothion (FNT) revealed notable synergistic toxicity. The acute 96 h-LC_{50} values within the mixture were 0.203 8 mg/L for PND and 1.139 1 mg/L for FNT, with confidence limits of 0.315 4 – 0.131 6 mg/L and 1.795 0 – 0.722 8 mg/L, respectively, showing a statistically significant positive association ($P<0.05$). The biological activity (S) rose progressively over time, measured at 0.282 8, 0.508 3, 0.794 1, and 0.859 6 at 24, 48, 72, and 96 h, respectively, while the additive index (AI) values declined from 0.717 1 and 0.491 6 at 24 and 48 h to 0.205 8 and 0.140 3 at 72 and 96 h. These findings indicate that the mixture exerted synergistic effects at all exposure times, with the strongest interaction in the early phase (24 – 48 h) that gradually diminished toward additivity by 96 h.

Table 1 Acute toxicity of pendimethalin to zebrafish

Treatment	Death rate//%			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.3	10	10	20	30
0.4	20	20	30	33
0.5	20	23	40	56
0.6	26	33	50	60
0.7	30	30	53	70
0.8	30	43	70	79
1.0	30	46	70	90
Regression equation	$Y=1.246X+4.592\ 2$	$Y=2.027\ 8X+4.922\ 8$	$Y=2.861\ 1X+5.756\ 4$	$Y=3.440\ 5X+6.105\ 7$
Correlation coefficient	0.774 3	0.926 5	0.839 4	0.917 2
LC_{50}	2.124 6	1.091 6	0.544 0	0.477 0
95% Confidence limit	10.734 – 0.420 58	2.953 – 0.403 4	1.101 5 – 0.268 6	0.857 8 – 0.265 37

Correlation coefficients (P-value) at 24 h = 0.008, at 48 h = (0.000 5), at 72 h = (0.003) and at 96 h = (0.000 6).

Table 2 Test results from acute toxicity of fenitrothion

Treatment	Death rate//%			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
1.7	0	10	20	20
2.1	10	16	30	30
2.6	20	20	40	56
3.2	20	30	50	63
3.9	26	30	50	70
4.8	26	40	60	90
5.9	30	40	70	100
Regression equation	$Y=0.961\ 9X+3.617$	$Y=1.940\ 5X+3.364\ 6$	$Y=2.329\ 4X+3.714\ 3$	$Y=4.412\ 7X+3.143\ 6$
Correlation coefficient	0.306 4	0.947 9	0.968 5	0.96
LC_{50}	27.401 8	6.962 6	3.564 0	2.634 4
95% Confidence limit	223.376 2 – 27.40	19.70 – 6.962 617	8.476 9 – 3.564 0	4.162 3 – 2.634 4

Correlation coefficients (P-value) at 24 h = 0.254, at 48 h = (0.000 2), at 72 h = (0.000 06) and at 96 h = (0.000 6).

The positive correlation observed in this study may be attributed to the increased uptake of pendimethalin and fenitrothion through the gills of zebrafish, enhancing internal exposure and accelerating toxic responses. The 96-h LC_{50} for pendimethalin was 0.477 mg/L, which is consistent with values reported for other herbicides of the dinitroaniline group, ranging from 0.32 to 0.68 mg/L in freshwater fish. Similarly, the 96-h LC_{50} for fenitrothion was 2.634 mg/L, falling within the range of 1.5 – 5.0 mg/L previously reported for organophosphate insecticides in cyprinids. These findings indicate that pendimethalin exerts higher acute toxicity compared to fenitrothion. Other studies have also confirmed strong interspecies variation in LC_{50} values. For instance, organophosphates such as fenitrothion have shown LC_{50} values from 1.7 to 7.2 mg/L depending on the test species and water quality

parameters. while herbicides like pendimethalin have been reported as highly toxic to *Channa punctata* and *Oreochromis niloticus*, with LC_{50} values ranging from 0.45 to 0.70 mg/L. Such discrepancies may be attributed to differences in organism physiology, size, acclimation conditions, and sensitivity of test species. Overall, the results of the present study confirm that pendimethalin and fenitrothion are both toxic to zebrafish, with pendimethalin showing greater potency. Considering that both pesticides are widely used in agriculture, their co-occurrence in aquatic ecosystems through runoff and leaching raises serious ecological concerns. Both compounds have been previously detected in surface waters near agricultural fields. and their persistence and bio accumulative properties make them significant threats to non-target aquatic organisms.

Table 3 Death rate of zebrafish under joint effects of pendimethalin and fenitrothion

Concentrations		Death rate//%			
PEN//μg/L	FEN//μg/L	24 h	48 h	72 h	96 h
Control	Control	0	0	0	0
0.15	0.83	0	10	30	36
0.2	1.10	13	36	40	40
0.25	1.38	40	46	50	56
0.3	1.66	43	50	66	76
0.35	1.93	43	50	66	90
0.4	2.21	50	56	80	93
0.5	2.76	50	60	90	96

Table 4 Joint toxicity of pendimethalin and fenitrothion to zebrafish

Pesticide	Parameter	Time			
		24 h	48 h	72 h	96 h
Pendimethalin	Regression Equation	$y=2.467\,6x+5.935\,1$	$y=3.875\,5x+7.061\,1$	$y=3.397x+7.152\,5$	$y=4.612\,9x+8.186$
	Correlation Coefficient	0.693 1	0.852	0.959 6	0.945 4
	LC_{50}	0.417 8	0.293 8	0.232 4	0.203 8
	95% Confidence	0.946 8 – 0.184 4	0.494 7 – 0.174 5	0.421 1 – 0.128 3	0.315 4 – 0.131 6
Fenitrothion	Regression Equation	$y=2.528\,9x+4.056\,5$	$y=3.735\,9x+4.172$	$y=3.295\,1x+4.615\,9$	$y=4.438\,1x+4.748\,9$
	Correlation Coefficient	0.734 1	0.825	0.940 9	0.911 9
	LC_{50}	2.360 9	1.665 8	1.307 8	1.139 1
	95% Confidence Limit	5.244 4 – 1.062 8	2.859 2 – 0.970 5	2.413 1 – 0.708 8	1.795 0 – 0.722 8
Joint Effect	S	0.282 8	0.508 3	0.794 1	0.859 6
	AI	0.717 1	0.491 6	0.205 8	0.140 3
	Conclusion	Synergistic	Synergistic	Synergistic	Synergistic

The synergistic effect observed in the mixture of pendimethalin and fenitrothion (PND + FNT) can best be explained by the interactions between the two pesticides themselves. Toxicokinetics and toxicodynamics are useful concepts to describe how these chemicals interact in combined exposure scenarios. Toxicokinetic interactions occur when one pesticide alters the absorption, distribution, metabolism, or elimination of the other compound, resulting in an increased internal dose of the second pesticide. For example, the persistence and hydrophobicity of pendimethalin may enhance fenitrothion uptake by impairing detoxification pathways in zebrafish. On the other hand, toxicodynamic interactions occur when both compounds act on different but overlapping physiological targets. Pendimethalin interferes with microtubule assembly and cell division, while fenitrothion inhibits acetylcholinesterase, leading to continuous nervous stimulation and paralysis. When acting together, these pathways amplify stress at the cellular and systemic levels, explaining the observed synergistic reduction in LC_{50} values. Such interactions typically result in greater-than-additive mortality, as confirmed by the computed synergistic indices ($S>0$, $AI>0$). The combined exposure thus represents a higher ecological risk than individual applications, highlighting the importance of mixture toxicity studies in aquatic risk assessment.

Conclusions

The present study confirmed that pendimethalin and fenitrothion exhibit significant combined toxicity to zebrafish, with behavioural impairments and mortality responses strongly influenced by both concentration and exposure time. The interaction indices revealed that the pesticides acted most strongly together during early exposure, with effects declining toward additivity over time. These results demonstrate that even when interaction strength decreases, early-phase exposures can exert severe impacts on aquatic organisms. From an ecological and management perspective, the findings underscore the risks associated with simultaneous pesticide use in agricultural systems. Promoting sustainable farming practices that reduce chemical dependency and encourage biological pest control can mitigate these risks.

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