Kanu *et al.*
The Journal of Basic and Applied Zoology (2022) 83:12
https://doi.org/10.1186/s41936-022-00278-7

The Journal of Basic and Applied Zoology

RESEARCH

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Survival analysis of African catfish and Nile tilapia briefly exposed to complex pesticide mixtures

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Abstract

Background: Pulse exposures are the consequences of the intermittent release of pollutants in the environment. Brief exposure of aquatic organisms to high concentrations of pesticides simultaneously occurs, particularly in small watercourses during high flows. The effects of pulse exposure often include effects occurring during and after the exposure. Despite this, routine toxicity tests procedures often ignore brief exposure scenarios and the role of time in toxicity. We conducted a pulse toxicity test by briefly exposing African catfish and Nile tilapia fingerlings to pesticide mixtures of atrazine, mancozeb, chlorpyrifos, and lambda-cyhalothrin. The study aimed to estimate pesticide mixture interaction in pulse-exposed fish and elucidate the influence of species differences on the response of fish to the pesticide mixture.

Results: Despite the similarity in fingerlings weight, African catfish had a significantly higher survival probability than Nile tilapia after exposure to atrazine-mancozeb mixture. However, the survival probability of African catfish and Nile tilapia fingerlings were similar after exposure to atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, chlorpyrifos-lambda cyhalothrin, and quaternary mixture ($p > 0.05$). The survival probability of exposed fingerlings was significantly lower for continuous than pulse exposure to the mixtures ($p < 0.01$). Nevertheless, the survival probability of 60 min of pulse exposure to 13.49 mg/L mancozeb-lambda cyhalothrin was similar to continuous exposure for 96 h. Atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, and the quaternary pesticide mixture were antagonists in African catfish but not in Nile tilapia. At the same time, chlorpyrifos-lambda-cyhalothrin was antagonistic in Nile tilapia but not African catfish.

Conclusions: Pesticide mixture interaction was antagonist but specie-dependent. Innate intrinsic and extrinsic deterministic factors and, to a limited extent, stochastic processes may have influenced the survival probability of African catfish, and Nile tilapia pulsed exposed to complex pesticide mixtures. Pulse toxicity assessment using survival analysis is relevant in ecotoxicology as it enables the study of factors that can influence pulse toxicity.

Keywords: Survival analysis, Pulse-exposure, Pesticide mixture, Hazard ratio, African catfish, Tilapia

Background

Pulse exposure involves one isolated, brief exposure. Repeated pulse exposure involves more than one sole short exposure while fluctuating exposure is continuous exposure to varying toxicant concentrations (Dennis et al., 2012).

Surface waters are natural sinks for pesticide mixtures. Pesticides enter the aquatic ecosystem through

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direct application, spray drift, runoff from agricultural fields, dry and wet deposition, and urban and industrial discharges (Sumon et al., 2018). Entrance of pesticides into the aquatic environment often occurs intermittently (Andersen et al., 2006) with aquatic organisms, including fish, exposed in pulse or repeated pulse to the pesticides. Pulse exposure time could vary from a few minutes to several hours depending on the properties of the pesticide and the characteristics of the water body (Cold & Forbes, 2004). Pesticides in streams typically reach high levels for short periods and decrease to very low or undetectable levels. Although maximum concentrations are present for only a short time before dilution, aquatic life is exposed to high pesticide concentrations during peak periods capable of inducing toxic effects and death in early life stages (Ewing, 1999; Peterson et al., 2001).

Traditionally, ecotoxicology assessed the toxicity of one or a mixture of pesticide compounds using continuous exposure setups. The constant exposure approach is at variance with the pulse or repeated pulse exposure (Dennis et al., 2012). Environmental pesticide exposure is non-constant, particularly for freshwater compartments (Chèvre & Vallotton, 2013). Furthermore, other than concentration and exposure time, other factors could also influence pesticide toxicity. These factors include the presence of other chemicals (mixture), species difference, size, and sex of the test organism. The effect of a covariate on the toxicity of a chemical is expressed using different indices. The median time to death (LT_{50}), the concentration at which 50% mortality occurs (LC_{50}), or the hazard function (the instantaneous probability of dying) are examples of such indices. A change in any one of these, not just the difference in LC_{50} that is typically used, can express the effect of a covariate (Dixon & Newman, 1991).

Atrazine, mancozeb, chlorpyrifos, and lambda-cyhalothrin are commonly used agricultural pesticides (WAAPP, 2013; Wang et al., 2016). Atrazine is a pre and post-emergence herbicide used to control annual broad-leaved weeds and annual grass weeds in yam, maize, sorghum, and sugarcane (Tomlin, 1994). The half-life of atrazine in the surface water is greater than 100 days at 20 °C. Photolysis, microbial degradation via *N*-dealkylation, and hydrolysis of the chloro substituent are pathways for atrazine degradation (WHO, 2003). Mancozeb is a fungicide used to control fungi affecting potato, tomato, citrus, banana, onions, corn, wheat, and mangoes (Kumar et al., 2018). Mancozeb half-life in water is less than 2 days in which it breaks down to release ethylene bis isothiocyanate sulfide (EBIS), which is subsequently converted into ethylene bis isothiocyanate (EBI) by the action of UV light (Raghavendra et al., 2020). Chlorpyrifos is an organophosphate insecticide, which adsorbs onto suspended solids and sediment. Its solubility in water varies between 0.39 and 1.4 mg/L at a temperature range of 19.5 to 25 °C (NCBI, 2021). The hydrolysis half-lives at 25 °C in aqueous buffers at pH 5, pH 7, and pH 9 were 72, 72, and 16 days. The reported half-life in natural water was 24.5 days (NCBI, 2021). Chlorpyrifos is neurotoxic. It inhibits the breakdown of acetylcholine (ACh), a neurotransmitter, by binding to the enzyme acetylcholinesterase. Lambda-cyhalothrin (alpha-cyano-3-phenoxybenzyl (Z)-(1RS)-cis 3-(2-chloro-3,3,3-trifluoro propenyl)-2,2-dimethyl cyclopropane carboxylate) is a pyrethroid insecticide. Its solubility in purified water is 5×10^{-3} mg/L, while it is 4×10^{-3} mg/L in buffered water. Lambda-cyhalothrin is neurotoxic, causing swift paralysis and death to an insect by preventing the closure of the voltage-gated sodium channels in axonal membranes.

Survival analysis is a suitable technique utilized to study pulse exposure and factors that modify the toxicity of pesticides (Kanu et al., 2021a) and other pollutants (Dixon & Newman, 1991; Newman & Aplin, 1992; Newman & McCloskey, 1996). Survival analysis starts with the times individuals die. In contrast, standard toxicity testing analysis begins with data on the percentage of individuals that die after a specific period (e.g., 24, 48, 72, or 96 h) (Dixon & Newman, 1991). Limited research has applied survival analysis to study the pulse toxicity of pesticide mixtures. We had previously used survival analysis to study the influence of pulse length, fish weight, length, and condition factor on the toxicity of atrazine, mancozeb, chlorpyrifos, and lambda-cyhalothrin in African catfish and Nile tilapia (Kanu et al., 2021a). This study aimed to estimate pesticide mixture interaction in pulse-exposed fish and elucidate the influence of species difference and size on the response of fish to pesticide mixture pulse exposure.

Methods

Study design

Fish survival was studied after exposing fingerlings to high concentrations of complex pesticide mixtures for short time intervals. Survival analysis was used to study the effect of pesticide mixture, species difference, fingerling weight, length, and condition factor on the survival of the fingerling was after brief exposure to agricultural pesticide mixtures.

Collection and acclimatization of fingerlings

African catfish, *Clarias gariepinus* and Nile tilapia, *Oreochromis niloticus* fingerlings are commonly cultivated in fish farms and widely distributed in the natural waters of Nigeria. We obtained the fingerlings used in the study from a private fish farm and transported them to the laboratory in the morning. As reported in our previous study, the fish were handled and acclimatized to laboratory conditions (Kanu et al., 2021a).

Test pesticides

In Nigeria, farmers commonly use the pesticides utilized in the study (WAAPP, 2013). They include; Atraz 50FW, a herbicide containing 500 g/L atrazine active ingredient; Z-force, a fungicide containing 80% mancozeb active ingredients; Attacke, a pyrethroid insecticide containing 2.5% lambda-cyhalothrin active ingredient; and Chloview, an organophosphate insecticide containing 40% chlorpyrifos active ingredient.

Pesticides mixture

Stock solutions of atrazine, chlorpyrifos, and lambda-cyhalothrin were prepared by making up one millilitre (1 ml) of the pesticides to one litre (1 L). Mancozeb stock solution was prepared by mixing one gram (1 g) of the pesticide powder with one litre (1L) of water.

Stock solutions of the complex mixtures were prepared based on the ratio of the 96 h LC_{50} of the single pesticides (established in an earlier study as atrazine-17.463 mg/L, mancozeb-24.383 mg/L, chlorpyrifos 0.515 mg/L, and lambda-cyhalothrin (0.434 μg/L). Stock concentration and the ratio of the active ingredients in the mixture are;

Atrazine: Mancozeb	2.39 g/L	(1:1.39)
Atrazine: Chlorpyrifos	3.495 g/L	(33.95:1)
Mancozeb: Chlorpyrifos	4.84 g/L	(47.35:1)
Atrazine: Lambda cyhalothrin	4.0301 g/L	(40.28:0.001)
Mancozeb: Lambda cyhalothrin	5.6201 g/L	(56.18:0.001)
Lambda cyhalothrin: Chlorpyrifos	0.1188 g/L	(0.001:1.18)
Atrazine: Mancozeb: Chlorpyrifos: Lambda cyhalothrin:	9.7651 g/L	(1.299:0.360:0.0132:0.001)

The nominal test concentrations used in the study were,

Atrazine: Mancozeb	14.34 mg/L
Atrazine: Chlorpyrifos	12.58 mg/L
Atrazine: Lambda cyhalothrin	29.02 mg/L
Mancozeb: Chlorpyrifos	7.74 mg/L
Mancozeb: Lambda cyhalothrin	13.48 mg/L
Chlorpyrifos: Lambda cyhalothrin	0.57 mg/L
Atrazine: Mancozeb: Chlorpyrifos: Lambda cyhalothrin	11.72 mg/L

These concentrations were the maximal concentration used in a previous acute toxicity study of the pesticides using *C. gariepinus,* which caused maximal effects after continuous exposure for 96 h. They were the maximum worst-case concentration (Kanu et al., 2021a).

Pulse toxicity test

All toxicity tests were performed following established procedure (APHA, 1985), except in the manner of exposure. In separate experiments, ten *C. gariepinus* and *O. niloticus* fingerlings were exposed in duplicates to the pesticide mixtures for 15, 30, 45, and 60 min, and continuously for 96 h (similar to standard acute toxicity test). After the brief exposure, the fish were rinsed, transferred to clean water, and monitored for 96 h. Mortality and time-to-death were checked and recorded every hour for both the pulsed and continuously exposed fish. The absence of movement and lack of response to gentle prodding established mortality. The weight and length of dead fishes were recorded, and fingerlings still alive after 96-h post-observation period were censored while their length and weight were also measured. Condition factor (CF) was computed using the equation below (Busacker et al., 1990):

$$\mathrm{CF} = \frac{W}{L^3} \times 100$$

W and L are the wet fish weight (g) and fish length (cm), respectively.

Estimation of complex pesticide mixture interaction using survival graph

We used the survival graph to estimate pesticide mixture interaction. The survival probability of fish pulse exposed to a single pesticide was compared with fish pulse exposed to a pesticide mixture containing the single pesticide. Thus, we compared the survival probability of fingerlings pulse exposed to 24 mg/L atrazine with 14.34 mg/L atrazine-mancozeb mixture; 12.28 mg/L atrazine-chlorpyrifos mixture; 11.72 mg/L quaternary mixture; and 29.02 mg/L atrazine-lambda cyhalothrin mixture. 1 mg/L chlorpyrifos was compared to 0.57 mg/L chlorpyrifos-lambda cyhalothrin mixture, while 42 mg/L mancozeb with 7.74 mg/L mancozeb-chlorpyrifos, and 13.48 mg/L mancozeb-lambda cyhalothrin mixture. Our previous study provides details of the survival probabilities of the single pesticides used in this study for comparison with pesticide mixtures (Kanu et al., 2021a).

Statistical analysis

Student T-test was used to test the similarity of the weight, length, and condition factor of Nile tilapia and African catfish exposed to the pesticides. Student T-test was performed using SPSS version 22. The equality of survival distribution of fish exposed continuous and briefly for 15, 30, 45, and 60 min to each pesticide was tested using the log-rank test. The Cox PH model was used to estimate the effect of species, pulse length, weight, fish length, and condition factor on fish survival. Survival analysis was performed using SAS version 9.1.3.

Results

Mean weight, length, and condition factor of fish species

Table 1 shows the average weight, length, and condition factor of fingerlings. The weight, length, and condition factor of African catfish fingerlings exposed to the pesticides were significantly different ($p < 0.05$) from Nile tilapia, except those indicated by the letter "a" were statistically similar ($p > 0.05$).

Survival analysis

Effect on species difference on the survival of exposed fingerlings

In Fig. 1a, with all covariates held constant, African catfish had a significantly higher ($p < 0.05$) survival probability than Nile tilapia after exposure to atrazine-mancozeb mixture even though both species had similar (Table 1). On the other hand, Fig. 1b–g, show that the survival probability of African catfish exposed to atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, chlorpyrifos-lambda cyhalothrin, and the quaternary mixture was similar to Nile tilapia fingerlings ($p > 0.05$). The survival probability of both species was identical despite the dissimilarity in weight of the fingerlings exposed to atrazine-lambda-cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, and quaternary mixture (Table 1).

Effect on exposure duration on the survival of exposed fingerlings

Figure 2 shows the effect of exposure duration on the survival probability of African catfish and Nile tilapia fingerlings.

For both species, the survival probability was significantly lower for fingerlings continuously exposed to pesticide mixtures than those exposed by a pulse ($p < 0.01$). Nevertheless, the survival probability of tilapia exposed continuously to mancozeb-lambda cyhalothrin mixture was similar to 60 min pulse exposure ($p > 0.05$), suggesting the toxicity of 60 min pulse exposure was identical to continuous exposure for 96 h.

Furthermore, the survival probability of Nile tilapia fingerlings exposed for 15, 30, 45 min to atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-lambda cyhalothrin, and chlorpyrifos-lambda cyhalothrin differed significantly ($p < 0.05$) compared to 60 min, indicating survival probability decreased as pulse duration increased.

Effect of pesticide mixture on survival of fingerlings

Figure 3 shows the survival graph of single fish exposed to single pesticides versus pesticide mixture. When the survival line of a pesticide mixture is significantly ($p < 0.05$) above that of a single pesticide in the graph, it indicates that fish survival probability was higher for the pesticide mixture than the single pesticide, which suggests an antagonistic interaction of the mixture components. On the other hand, when the survival line of a pesticide mixture is significantly ($p < 0.05$) below that of a single pesticide, it indicates the interaction of the mixture component was synergistic. Table 4 shows statistical estimates to buttress Fig. 3.

COX proportional hazard analysis

Effect on species difference on the survival of exposed fingerlings for different pulse exposure duration

In Table 2, the survival probability of Nile tilapia decreased by a factor of 6.154, 2.639, 3.282, and 2.703 compared with African catfish fingerlings exposed to atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, and mancozeb-lambda cyhalothrin for 60 min and chlorpyrifos-lambda cyhalothrin for 45 min, respectively. The survival probability of both species was similar after 15, 30, and 45 min of pulse exposure ($p > 0.05$).

Effect of pulse duration on the hazard of pesticide mixture

In Table 3, the survival probability of African catfish exposed to atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, and Nile tilapia exposed to atrazine-mancozeb, mancozeb-chlorpyrifos, and quaternary mixture for 15, 30, 45 min, and 60 min was not significantly different ($p > 0.05$). However, the survival probability of Nile tilapia fingerlings exposed to atrazine-chlorpyrifos for 15, 30, 45 min was 0.145 (86%), 0.199 (80%), and 0.289 (71%) higher than those exposed for 60 min, respectively. In addition, Nile tilapia exposed to atrazine-lambda cyhalothrin for 15 and 30 min were 0.102 (90%) and 0.210 (79%) times less likely to die than those exposed for 60 min. Tilapia fingerlings exposed to mancozeb-Lambda cyhalothrin 15, 30, 45 min were 0.099 (90%), 0.212 (79%), and 0.323 (68%) times less likely to die compared to those exposed for 60 min. African catfish exposed to chlorpyrifos-lambda cyhalothrin and quaternary mixture for 15 min were 0.319 (68%) and 0.336 (66%) times less likely to die, respectively, relative to those exposed for 60 min. In contrast, the probability of survival of Nile tilapia exposed to chlorpyrifos-lambda cyhalothrin for 15 min was higher by 0.117 (88%) than those exposed for 60 min.

Table 1 Fingerlings mean weight, length, and condition factor

Exposure duration	Variables	Fish	Pesticide mixture						
			Atrazine: Mancozeb	**Atrazine: Chlorpyrifos**	**Atrazine: Lambda cyhalothrin**	**Mancozeb: Chlorpyrifos**	**Mancozeb: Lambda cyhalothrin**	**Lambda cyhalothrin: Chlorpyrifos**	**Quaternary**
Continuous	Weight	African catfish	0.48 ± 0.03[a]	0.4 ± 0.02[a]	0.44 ± 0.02	0.44 ± 0.03	0.41 ± 0.03	0.42 ± 0.02[a]	0.55 ± 0.03
		Tilapia	0.43 ± 0.1[a]	0.53 ± 0.11[a]	0.23 ± 0.02	0.83 ± 0.08	1.44 ± 0.21	0.57 ± 0.09[a]	0.4 ± 0.05
	Length	African catfish	3.91 ± 0.07	3.99 ± 0.07	3.92 ± 0.08	3.8 ± 0.09	3.73 ± 0.05[a]	3.6 ± 0.07	4.06 ± 0.09
		Tilapia	2.71 ± 0.16	3.01 ± 0.2	2.52 ± 0.14	3.23 ± 0.15	3.94 ± 0.24[a]	2.98 ± 0.18	2.8 ± 0.15
	Condition Factor	African catfish	0.8 ± 0.05	0.63 ± 0.03	0.76 ± 0.04	0.81 ± 0.06	0.79 ± 0.06	0.96 ± 0.07	0.83 ± 0.04
		Tilapia	1.95 ± 0.12	1.64 ± 0.14	1.61 ± 0.16	2.34 ± 0.09	1.98 ± 0.05	1.98 ± 0.11	1.82 ± 0.12
15 Min	Weight	African catfish	0.57 ± 0.01[a]	0.62 ± 0.01	0.42 ± 0.01	0.44 ± 0.08[a]	0.38 ± 0.02	0.31 ± 0.01[a]	0.52 ± 0.03 [a]
		Tilapia	0.52 ± 0.09[a]	0.38 ± 0.04	0.27 ± 0.02	0.41 ± 0.04[a]	0.29 ± 0.03	0.27 ± 0.02[a]	0.55 ± 0.05 [a]
	Length	African catfish	4.38 ± 0.12	4.34 ± 0.06	3.88 ± 0.11	3.85 ± 0.1[a]	3.7 ± 0.09	3.19 ± 0.08	4.34 ± 0.05
		Tilapia	2.95 ± 0.18	2.85 ± 0.08	2.47 ± 0.06	3.58 ± 0.27[a]	2.43 ± 0.1	2.51 ± 0.09	4 ± 0.1
	Condition Factor	African catfish	0.72 ± 0.05	0.78 ± 0.05	0.77 ± 0.06	0.78 ± 0.13[a]	0.75 ± 0.04	0.96 ± 0.05	0.65 ± 0.03
		Tilapia	2.07 ± 0.15	1.62 ± 0.08	1.85 ± 0.12	1.23 ± 0.2[a]	2.04 ± 0.12	1.73 ± 0.13	0.91 ± 0.09
30 Min	Weight	African catfish	0.52 ± 0.01	0.88 ± 0.05	0.43 ± 0.0 [a]	0.36 ± 0.01[a]	0.5 ± 0.03	0.35 ± 0.02	0.72 ± 0.07
		Tilapia	0.33 ± 0.03	0.43 ± 0.04	0.4 ± 0.06[a]	0.42 ± 0.05[a]	0.8 ± 0.1	0.61 ± 0.16	1.07 ± 0.14
	Length	African catfish	4.04 ± 0.08	4.88 ± 0.14	3.74 ± 0.08	3.54 ± 0.05[a]	4.08 ± 0.11	3.38 ± 0.08	5.72 ± 0.26
		Tilapia	2.48 ± 0.07	2.99 ± 0.12	2.74 ± 0.14	3.71 ± 0.28[a]	3.52 ± 0.19	2.97 ± 0.18	3.89 ± 0.2
	Condition Factor	African catfish	0.8 ± 0.05	0.79 ± 0.06	0.87 ± 0.05	0.81 ± 0.02[a]	0.75 ± 0.04	0.9 ± 0.04	0.39 ± 0.03
		Tilapia	2.23 ± 0.17	1.57 ± 0.05	2.05 ± 0.28	1.06 ± 0.15[a]	1.75 ± 0.1	1.91 ± 0.12	1.7 ± 0.1
45 Min	Weight	African catfish	0.47 ± 0.01 [a]	0.84 ± 0.04	0.44 ± 0.01[a]	0.39 ± 0.02	0.52 ± 0.03[a]	0.35 ± 0.02	0.59 ± 0.05
		Tilapia	0.59 ± 0.07 [a]	0.44 ± 0.05	0.42 ± 0.08[a]	0.96 ± 0.17	0.47 ± 0.05[a]	0.96 ± 0.1	0.44 ± 0.03
	Length	African catfish	3.93 ± 0.08	4.81 ± 0.12	3.9 ± 0.09	3.85 ± 0.1[a]	4.06 ± 0.14	3.47 ± 0.07[a]	4.93 ± 0.16
		Tilapia	3.13 ± 0.17	2.99 ± 0.15	2.68 ± 0.18	4.03 ± 0.32[a]	2.98 ± 0.15	3.53 ± 0.16[a]	3.3 ± 0.13
	Condition Factor	African catfish	0.81 ± 0.05	0.79 ± 0.04	0.77 ± 0.05	0.68 ± 0.02	0.82 ± 0.06	0.84 ± 0.03	0.49 ± 0.03
		Tilapia	1.94 ± 0.15	1.64 ± 0.09	1.95 ± 0.1	1.72 ± 0.32	1.7 ± 0.08	2.01 ± 0.08	1.28 ± 0.11
60 Min	Weight	African catfish	0.53 ± 0.01	0.75 ± 0.05	0.44 ± 0.01[a]	0.37 ± 0.02	0.61 ± 0.01	0.37 ± 0.02[a]	0.6 ± 0.06 [a]
		Tilapia	0.41 ± 0.05	0.51 ± 0.07	0.4 ± 0.05[a]	0.75 ± 0.17	0.96 ± 0.18	0.43 ± 0.08[a]	0.57 ± 0.05[a]
	Length	African catfish	4.21 ± 0.07	4.6 ± 0.15	3.69 ± 0.07	3.59 ± 0.08[a]	4.44 ± 0.12	3.56 ± 0.09	5 ± 0.22
		Tilapia	2.79 ± 0.19	2.94 ± 0.13	2.92 ± 0.18	3.31 ± 0.25[a]	3.28 ± 0.24	2.86 ± 0.17	3.43 ± 0.18
	Condition Factor	African catfish	0.72 ± 0.03	0.83 ± 0.08	0.91 ± 0.05	0.81 ± 0.03	0.74 ± 0.05	0.8 ± 0.03	0.5 ± 0.03
		Tilapia	2.07 ± 0.17	2.06 ± 0.41	1.82 ± 0.28	1.76 ± 0.15	2.28 ± 0.08	1.69 ± 0.12	1.55 ± 0.16

Values indicate mean ± standard error, $N = 20$. Parameters of African catfish were significantly different from Nile tilapia ($p < 0.05$), except those marked with the letter "a" ($p > 0.05$)

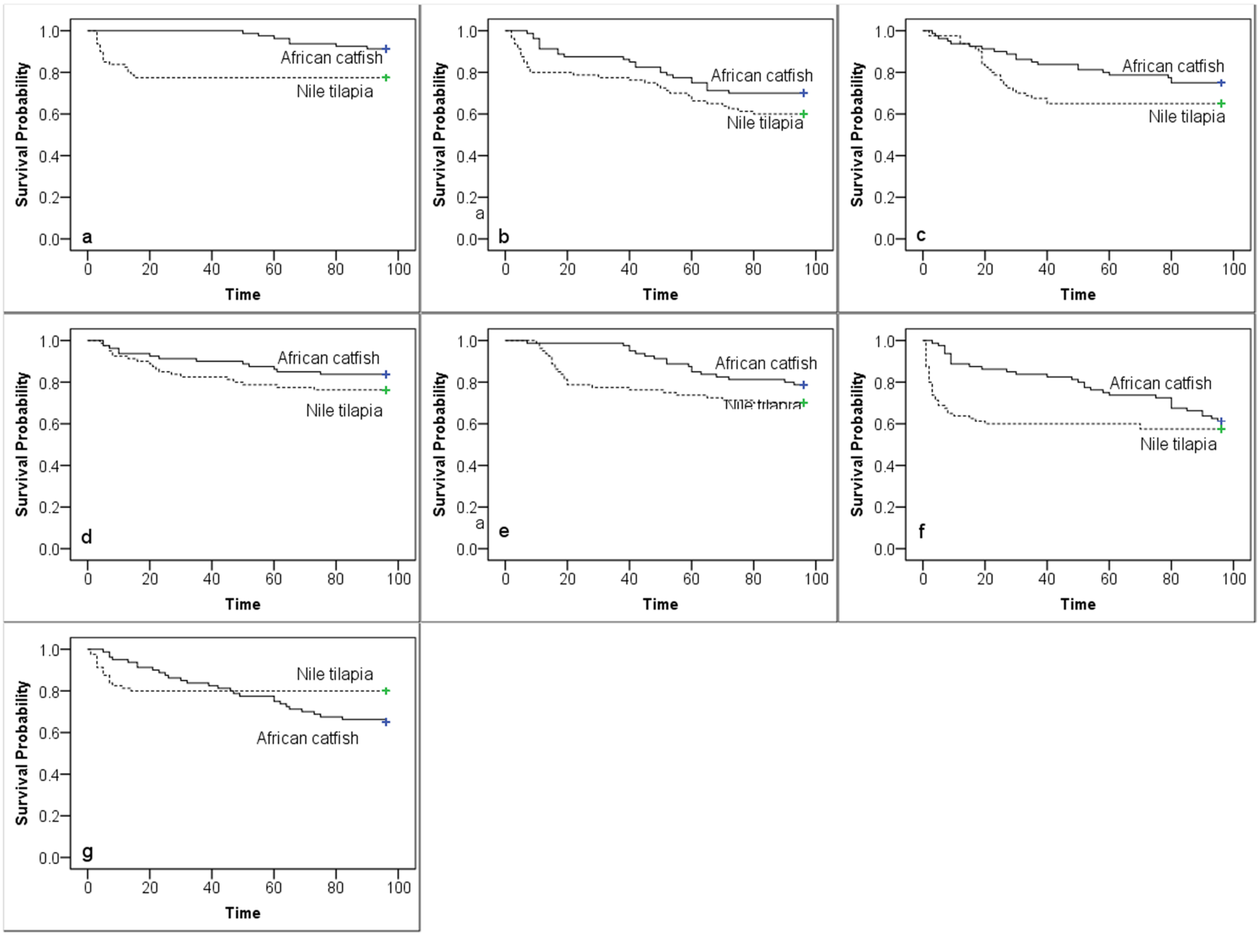

Fig. 1 Survival probability of Fingerings exposed to pesticide mixtures. **a**, **b**, **c**, **d**, **e**, **f**, **g** Atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, chlorpyrifos-lambda cyhalothrin, and quaternary mixture

Pesticide mixture effect on survival of fingerlings

Table 4 shows that overall, the survival probability of Nile tilapia fingerlings pulse exposed to atrazine was not significantly different ($p > 0.05$) from those exposed to atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, and the quaternary pesticide mixture. Similarly, Nile tilapia fingerlings pulsed exposed to mancozeb was not significantly different ($p > 0.05$) from those exposed to mancozeb-chlorpyrifos and mancozeb-lambda cyhalothrin mixture, which suggests that pulse exposure to the pesticide mixture containing binary or quaternary active ingredients was not more toxic to tilapia than pesticides with one active ingredient. It also indicates no interaction of mixture components. However, survival probability was significantly higher ($p < 0.05$) for Nile tilapia fingerlings' pulse exposed to chlorpyrifos-lambda cyhalothrin than chlorpyrifos, which indicates that the mixture was less toxic than the single pesticide, suggesting an antagonistic interaction between chlorpyrifos and lambda-cyhalothrin tilapia fingerlings. The reverse occurred for African catfish fish fingerlings.

The survival probability of African catfish fingerling's pulse exposed to atrazine was significantly lower ($p < 0.05$) than atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, and the quaternary pesticide mixture by a factor of 7.83, 1.88, 2.29, and 1.59, respectively. Likewise, fingerlings pulsed exposed to mancozeb was significantly lower ($p < 0.05$) than fingerlings exposed to mancozeb-chlorpyrifos and mancozeb-lambda cyhalothrin mixture by a factor of 3.15 and 2.51, respectively. Lower pesticide mixture toxicity suggests an antagonistic effect of the pesticide mixture in African catfish. Nevertheless, survival probability was not significantly different ($p > 0.05$) for African catfish fingerling's pulse exposed to chlorpyrifos-lambda cyhalothrin compared with chlorpyrifos.

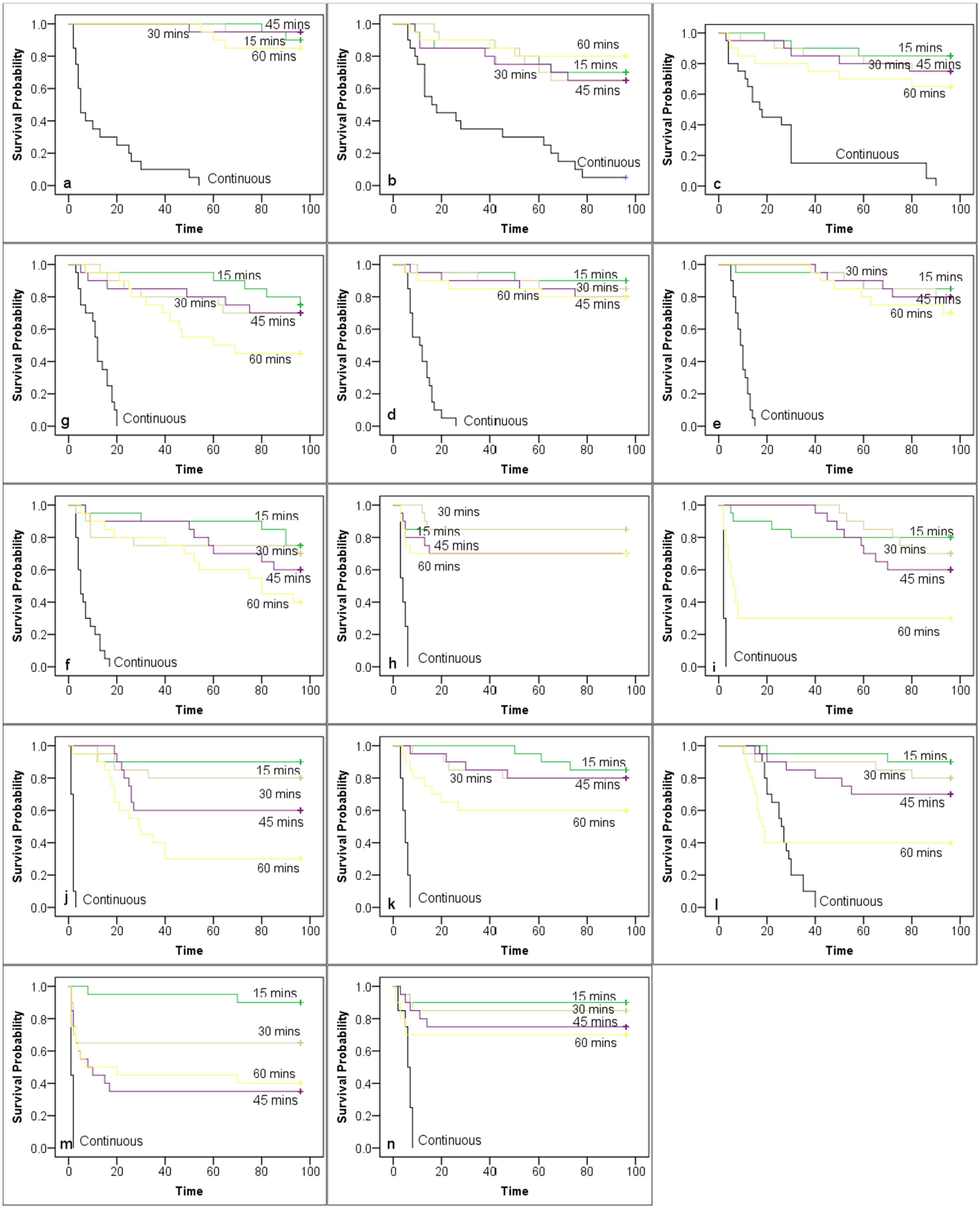

Fig. 2 Influence of pulse duration on African catfish and Nile tilapia survival. Letters **a**, **b**, **c**, **d**, **e**, **f**, **g**—African catfish exposed to atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, chlorpyrifos-lambda cyhalothrin, and quaternary mixture, respectively, while **h**, **i**, **j**, **k**, **l**, **m**, **n**—Nile tilapia exposed to atrazine-mancozeb, atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos, mancozeb-lambda cyhalothrin, chlorpyrifos-lambda cyhalothrin, and quaternary mixture, respectively

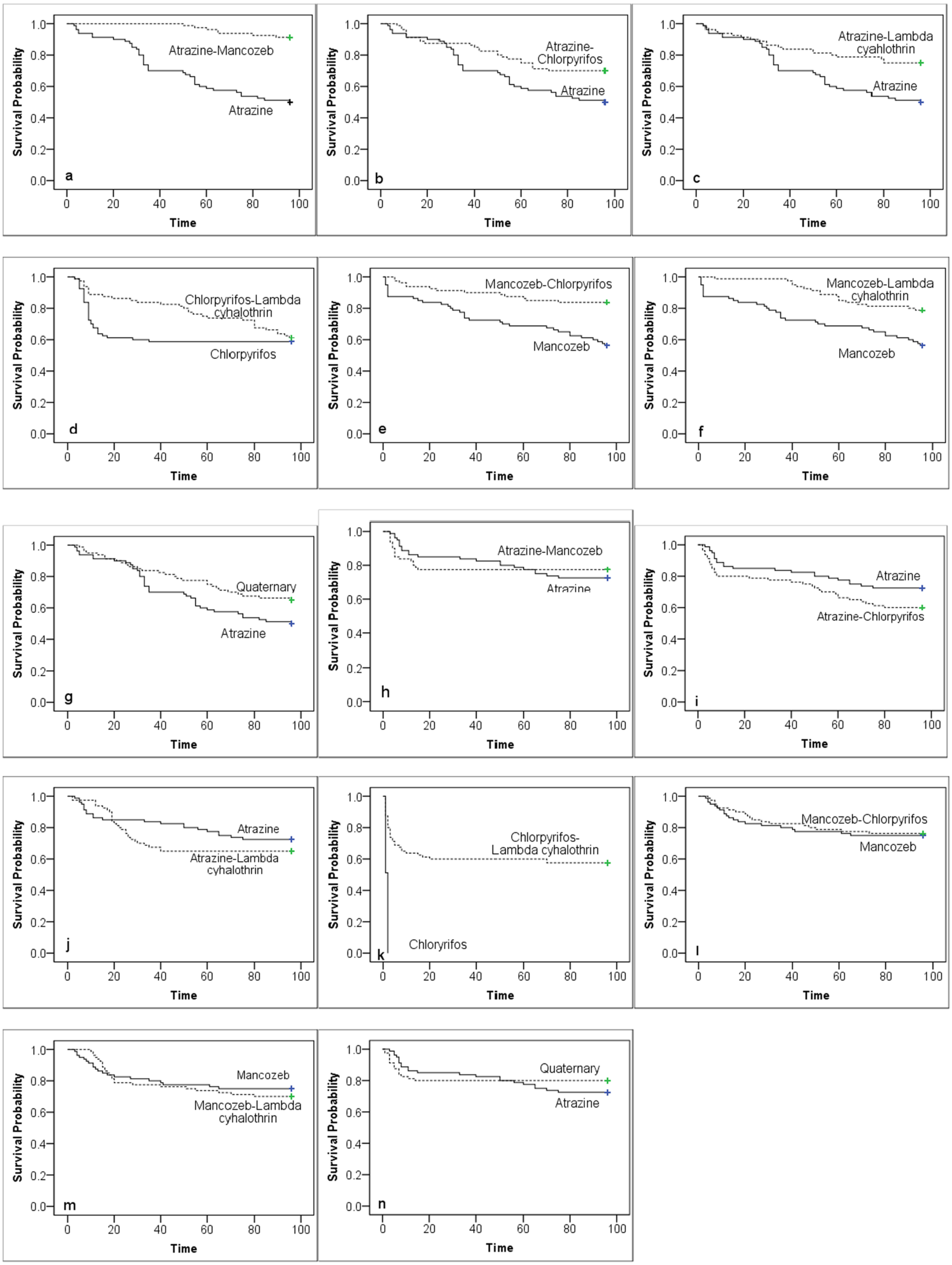

Fig. 3 Survival graph of fish exposed to single pesticides versus pesticide mixture. Letters **a**, **b**, **c**, **d**, **e**, **f**, and *g* are survival graphs of African catfish, **h**, **i**, **j**, **k**, **l**, **m** and **n** are survival graphs for tilapia

Table 2 Cox PH model summary of species effect on survival of fingerlings at different pulse exposure duration

Pesticide	Exposure duration (mins)	*df*	Parameter estimate	SE	Chi-square	Pr > ChiSq	Hazard ratio
Atrazine-Mancozeb	15*	1	.482	.913	.278	.598	1.619
	30*	1	1.179	1.155	1.042	.307	3.252
	45*	1	1.958	1.081	3.281	.070	7.088
	60*	1	.877	.708	1.533	.216	2.404
Atrazine-Chlorpyrifos	15*	1	−.391	.646	.366	.545	.677
	30*	1	−.300	.557	.291	.590	.741
	45*	1	.074	.518	.020	.887	1.077
	60*	1	1.817	.574	10.027	.002	6.154
Atrazine-Lambda cyhalothrin	15*	1	−.363	.913	.158	.691	.695
	30*	1	−.190	.671	.081	.777	.827
	45*	1	.670	.572	1.372	.241	1.954
	60*	1	.970	.469	4.285	.038	2.639
Mancozeb-Chlorpyrifos	15*	1	.374	.913	.168	.682	1.454
	30*	1	.344	.764	.202	.653	1.410
	45*	1	.023	.707	.001	.974	1.023
	60*	1	.856	.613	1.950	.163	2.354
Mancozeb-Lambda cyhalothrin	15*	1	−.449	.913	.242	.623	.638
	30*	1	.027	.707	.001	.970	1.027
	45*	1	.546	.646	.714	.398	1.726
	60*	1	1.188	.505	5.547	.019	3.282
Chlorpyrifos-Lambda cyhalothrin	15*	1	−.948	.837	1.283	.257	.388
	30*	1	.348	.558	.390	.532	1.417
	45*	1	.994	.454	4.800	.028	2.703
	60*	1	.340	.410	.686	.408	1.405
Quaternary	15*	1	−.920	.837	1.207	.272	.399
	30*	1	−.661	.708	.872	.350	.516
	45*	1	−.108	.606	.032	.858	.897
	60*	1	−.543	.510	1.134	.287	.581

* Hazard ratio constant with time; Pr > ChiSq indicates statistical significance if < 0.05

The combined effect of species, pulse duration, weight, length, and condition factor *on the survival of fingerlings*

Table 5, taking all predictors into account, pulse toxicity of atrazine-mancozeb was not significantly associated with any covariate ($p > 0.05$). The survival probability of Nile tilapia and African catfish fingerling exposed to 14.34 mg/L atrazine-mancozeb mixture was similar. Pulse length, fish weight & length, and condition factor did not influence the survival of both species even though African catfish's fish length and condition factor were significantly longer and smaller, respectively, than Nile tilapia fingerlings (Table 1).

Pulse toxicity of 12.58 mg/L mancozeb-chlorpyrifos was associated with 15 min pulse length and fish weight ($p < 0.05$). The likelihood of survival after 15 min exposure increased by a factor of 0.312 compared to 60 min ($p < 0.05$), while survival likelihood increased by 99.1% in fishes weighing 0.1 g more than other fish. The toxicity of pulse exposure to 12.58 mg/L for 30 and 45 min was similar to 60 min irrespective of the species, length, and condition factor difference (Table 1).

Pulse toxicity of 29.02 mg/L atrazine-lambda cyhalothrin was associated with pulse length ($p < 0.05$) alone. The likelihood of survival after 15, 30, and 45 min exposure increased by a factor of 0.120 (88%), 0.275 (72.5%), 0.380 (62%), respectively, compared to 60 min pulse exposure ($p < 0.05$) irrespective of the difference in specie, weight, length and condition factor (Table 1).

Pulse toxicity of 7.74 mg/L mancozeb-chlorpyrifos was associated with 15 pulse length and fish weight ($p < 0.05$). The likelihood of survival after 15 min of exposure increased by a factor of 0.313 compared to 60 ($p < 0.05$). The toxicity of pulse exposure to 7.74 mg/L mancozeb-chlorpyrifos for 30 and 45 min was similar to 60 min pulse exposure ($p < 0.05$) irrespective of the difference in species, weight, length, and condition factor (Table 1).

Table 3 Cox PH model summary of the effect of pulse duration on the hazard of pesticide mixture

Pesticide	Species		*df*	Parameter estimate	SE	Chi-square	Hazard ratio
Atrazine-Mancozeb	African catfish*	15 min	1	−.482[b]	.913	.279	.618[b]
		30 min	1	−1.168[b]	1.155	1.022	.311[b]
		45 min	1	−1.138[b]	1.155	.972	.320[b]
		60 min	3			1.596	
	Tilapia*	15 min	1	−.754[b]	.707	1.137	.470[b]
		30 min	1	−.843[b]	.708	1.418	.431[b]
		45 min	1	−.054[b]	.577	.009	.947[b]
		60 min	3			2.397	
Atrazine-Chlorpyrifos	African catfish*	15 min	1	.423[b]	.646	.429	1.526[b]
		30 min	1	.559[b]	.627	.795	1.749[b]
		45 min	1	.610[b]	.627	.947	1.840[b]
		60 min	3			1.076	
	Tilapia	15 min	1	−1.931[a]	.571	11.455	.145[a]
		30 min	1	−1.612[a]	.493	10.675	.199[a]
		45 min	1	−1.242[a]	.448	7.679	.289[a]
		60 min	3			18.875	
Atrazine-Lambda cyhalothrin	African catfish*	15 min	1	−.998[b]	.690	2.092	.369[b]
		30 min	1	−.453[b]	.586	.599	.636[b]
		45 min	1	−.431[b]	.586	.540	.650[b]
		60 min	3			2.194	
	Tilapia	15 min	1	−2.279[a]	.758	9.054	.102[a]
		30 min	1	−1.561[a]	.568	7.550	.210[a]
		45 min	1	−.793[b]	.444	3.196	.452[b]
		60 min	3			14.563	
Mancozeb-Chlorpyrifos	African catfish*	15 min	1	−.741[b]	.866	.732	.477[b]
		30 min	1	−.334[b]	.764	.191	.716[b]
		45 min	1	−.026[b]	.707	.001	.974[b]
		60 min	3			.921	
	Tilapia	15 min	1	−1.303[b]	.678	3.694	.272[b]
		30 min	1	−.925[b]	.613	2.279	.396[b]
		45 min	1	−.932[b]	.613	2.314	.394[b]
		60 min	3			5.206	
Mancozeb-Lambda cyhalothrin	African catfish*	15 min	1	−.760[b]	.707	1.154	.468[b]
		30 min	1	−.477[b]	.646	.547	.620[b]
		45 min	1	−.471[b]	.646	.533	.624[b]
		60 min	3			1.361	
	Tilapia	15 min	1	−2.315[a]	.767	9.116	.099[a]
		30 min	1	−1.549[a]	.581	7.111	.212[a]
		45 min	1	−1.131[a]	.503	5.051	.323[a]
		60 min	3			14.770	
Chlorpyrifos-Lambda cyhalothrin	African catfish*	15 min	1	−1.144[a]	.534	4.591	.319[a]
		30 min	1	−.834[b]	.502	2.768	.434[b]
		45 min	1	−.557[b]	.457	1.487	.573[b]
		60 min	3			5.718	
	Tilapia*	15 min	1	−2.146[a]	.765	7.868	.117[a]
		30 min	1	−.644[b]	.476	1.828	.525[b]
		45 min	1	.084[b]	.401	.044	1.087[b]
		60 min	3			10.384	

Table 3 (continued)

Pesticide	Species		*df*	Parameter estimate	SE	Chi-square	Hazard ratio
Quaternary	African catfish*	15 min	1	− 1.090[a]	.542	4.051	.336[a]
		30 min	1	− .784[b]	.509	2.374	.457[b]
		45 min	1	− .797[b]	.509	2.446	.451[b]
		60 min	3			5.407	
	Tilapia*	15 min	1	− 1.268[b]	.817	2.409	.281[b]
		30 min	1	− .855[b]	.707	1.460	.425[b]
		45 min	1	− .326[b]	.606	.290	.722[b]
		60 min	3			3.104	

* Hazard ratio constant with time; a- indicates statistical significance ($p < 0.05$); b- indicates not significant ($p > 0.05$)

Table 4 Cox PH model summary of pesticide mixture effect on survival of fingerlings

Fish species	Compared pesticides	*df*	Parameter estimate	SE	Chi-square	Pr > ChiSq	Hazard ratio
African catfish	Atrazine vs. Atrazine-Mancozeb	1	2.059	.411	25.142	.000	7.834
	Mancozeb vs. Mancozeb-Chlorpyrifos	1	1.148	.325	12.472	.000	3.152
	Chlorpyrifos vs. Chlorpyrifos-Lambda cyhalothrin Mixture	1	.225	.251	.809	.368	1.253
	Atrazine vs. Atrazine-Lambda-cyhalothrin	1	.828	.274	9.109	.003	2.289
	Mancozeb vs. Mancozeb-Lambda cyhalothrin	1	.919	.296	9.646	.002	2.507
	Atrazine vs. Atrazine-Chlorpyrifos	1	.634	.258	6.009	.014	1.884
	Atrazine vs. Quaternary mixture	1	.463	.247	3.516	.061	1.588
Tilapia	Atrazine vs. Atrazine-Mancozeb	1	.146	.318	.210	.647	1.157
	Mancozeb vs. Mancozeb-Chlorpyrifos	1	.085	.320	.070	.792	1.088
	Chlorpyrifos vs. Chlorpyrifos-Lambda cyhalothrin Mixture	1	1.895	.275	47.421	.000	6.653
	Atrazine vs. Atrazine-Lambda-cyhalothrin	1	− .290	.285	1.031	.310	.748
	Mancozeb vs. Mancozeb-Lambda cyhalothrin	1	− .161	.303	.284	.594	.851
	Atrazine vs. Atrazine-Chlorpyrifos	1	− .473	.277	2.918	.088	.623
	Atrazine vs. Quaternary mixture	1	.274	.329	.696	.404	1.315

* Hazard ratio constant with time; Pr > ChiSq indicates statistical significance if < 0.05

Pulse toxicity of 13.48 mg/L mancozeb-lambda cyhalothrin was associated with pulse length and fish weight. The likelihood of survival after 15, 30, and 45 min exposure increased by a factor of 0.069 (93.1%), 0.261 (73.9%), 0.311 (68.9%), respectively compared to 60 min ($p < 0.05$), while survival likelihood increased by (99.95%) in fishes weighing 0.1 g more than another fish.

Pulse toxicity of 0.57 mg/L chlorpyrifos-lambda cyhalothrin was associated with species, 15, and 30 min pulse and fish weight & length ($p < 0.05$). The survival probability of African catfish was 5.563 times higher than Nile tilapia. The likelihood of survival after 15 and 30 min exposure increased by a factor of 0.067 (93.3%) and 0.270 (73%), respectively, compared to 60 min of pulse exposure ($p < 0.05$). Survival probability increased by a factor of 25.627 and 0.059 in fishes weighing 0.1 g more than another fish and having condition factor higher by 0.1, respectively.

Pulse toxicity of 11.72 mg/L quaternary mixture was associated with species, 15, and 30 min pulse, length, and condition factor ($p < 0.05$). The likelihood of death after 15 and 30 min exposure decreased by a factor of 0.371 (62.9%) and 0.273 (72.7%), respectively, compared to 60 min of pulse exposure ($p < 0.05$). The likelihood of survival was 2.05 and 30.75 times lower for fish longer than another fish by 1 cm and condition factor higher by 0.1.

Discussion

Concentration and time are important factors that influence toxicity. The high pesticide concentrations used in this study are comparable to the maximum worst-case concentration typically assumed in the tier-one stage of exposure assessment. The use of high concentration in

Table 5 Cox PH model summary of all predictors on survival of fingerlings

Pesticide		Parameter estimate	SE	Chi-square	*df*	Pr > ChiSq	Hazard ratio	95.0% CI for the hazard ratio	
								Lower	Upper
Atrazine-Mancozeb	Species	.245	.978	.063	1	.802	1.278[a*]	.188	8.685
	15 min	− .483	.572	.713	1	.398	.617	.201	1.894
	30 min	− 1.102	.613	3.228	1	.072	.332	.100	1.105
	45 min	.156	.522	.089	1	.766	1.168	.420	3.250
	60 min			4.509	3	.212			
	Weight	− 5.220	2.737	3.636	1	.057	.005	.000	1.157
	Length	1.132	1.011	1.254	1	.263	3.103	.427	22.521
	Condition factor	1.670	.708	5.560	1	.018	5.312	1.326	21.284
Atrazine-Chlorpyrifos	Species	.129	.574	.051	1	.822	1.138[a+]	.369	3.504
	15 min	− 1.165	.410	8.083	1	.004	.312	.140	.696
	30 min	− .696	.385	3.267	1	.071	.499	.234	1.061
	45 min	− .530	.365	2.101	1	.147	.589	.288	1.205
	60 min			8.531	3	.036			
	Weight	− 4.661	1.844	6.387	1	.011	.009	.000	.351
	Length	1.063	.658	2.611	1	.106	2.895	.797	10.507
	Condition factor	1.007	.329	9.375	1	.002	2.737	1.437	5.213
Atrazine-Lambda cyhalothrin	Species	.554	.610	.825	1	.364	1.740[a+]	.526	5.751
	15 min	− 2.117	.504	17.650	1	.000	.120	.045	.323
	30 min	− 1.290	.403	10.265	1	.001	.275	.125	.606
	45 min	− .967	.365	7.039	1	.008	.380	.186	.777
	60 min			23.436	3	.000			
	Weight	− 6.316	3.407	3.436	1	.064	.002	.000	1.436
	Length	− .210	.770	.074	1	.785	.811	.179	3.668
	Condition factor	− .021	.413	.003	1	.960	.979	.436	2.200
Mancozeb-Chlorpyrifos	Species	− .566	.540	1.098	1	.295	.568[b+]	.197	1.637
	15 min	− 1.163	.552	4.430	1	.035	.313	.106	.923
	30 min	− .938	.493	3.625	1	.057	.391	.149	1.028
	45 min	− .099	.460	.047	1	.829	.906	.368	2.229
	60 min			6.799	3	.079			
	Weight	− 1.823	4.821	.143	1	.705	.162	.000	2050.017
	Length	− 1.790	1.242	2.079	1	.149	.167	.015	1.903
	Condition factor	− 1.165	.895	1.694	1	.193	.312	.054	1.802
Mancozeb-Lambda cyhalothrin	Species	.537	.701	.587	1	.444	1.711[a+]	.433	6.760
	15 min	− 2.674	.573	21.746	1	.000	.069	.022	.212
	30 min	− 1.342	.456	8.657	1	.003	.261	.107	.639
	45 min	− 1.167	.451	6.682	1	.010	.311	.129	.754
	60 min			23.575	3	.000			
	Weight	− 2.991	1.331	5.048	1	.025	.050	.004	.683
	Length	.541	.651	.691	1	.406	1.718	.480	6.150
	Condition factor	1.692	.649	6.785	1	.009	5.428	1.520	19.385

Table 5 (continued)

Pesticide		Parameter estimate	SE	Chi-square	*df*	Pr > ChiSq	Hazard ratio	95.0% CI for the hazard ratio	
								Lower	Upper
Chlorpyrifos-Lambda cyhalothrin	Species	1.716	.488	12.379	1	.000	5.563[a#]	2.139	14.472
	15 min	− 2.696	.475	32.190	1	.000	.067	.027	.171
	30 min	− 1.308	.364	12.924	1	.000	.270	.132	.552
	45 min	− .187	.337	.309	1	.579	.829	.428	1.606
	60 min			38.745	3	.000			
	Weight	3.244	.779	17.326	1	.000	25.627	5.564	118.033
	Length	− 2.825	.591	22.856	1	.000	.059	.019	.189
	Condition factor	.302	.483	.392	1	.531	1.353	.525	3.485
Quaternary	Species	2.779	.691	16.162	1	.000	16.099[a#]	4.154	62.396
	15 min	− .990	.478	4.284	1	.038	.371	.145	.949
	30 min	− 1.298	.462	7.884	1	.005	.273	.110	.676
	45 min	− .378	.394	.921	1	.337	.685	.316	1.483
	60 min			10.245	3	.017			
	Weight	− 1.280	.946	1.831	1	.176	.278	.044	1.775
	Length	.719	.389	3.418	1	.064	2.053	.958	4.402
	Condition factor	3.426	.619	30.678	1	.000	30.748	9.148	103.347

[a] Indicates increase in hazard ratio, while [b]indicates a decrease in hazard ratio compared with when other predictors were held constant; *indicates significant when other predators were held constant but no longer significant; [+]indicates not significant when other predators were held constant and not significant now; [#]indicates not significant when other predators were held constant but now significant

pulse assessment adequately characterizes toxic effects and provides a basis for estimating impacts at the predicted environmental concentration (Peterson et al., 2001). Furthermore, aquatic organisms may be exposed to a toxicant for only a short time before a critical threshold is achieved, resulting in an adverse effect (Naddy et al., 2000). The current study results show that the negative impact of brief exposure to pesticides may be comparable to continuous exposure. The probability of tilapia surviving 60 min of short exposure to mancozeb-lambda cyhalothrin mixture was similar to constant exposure for 96 h. This is consistent with an earlier study, which reported that pulse exposure to pesticides might be more hazardous than continuous exposure (Tucker & Burton, 1999).

The classic graphical method used to study chemical interaction is the isobologram (Huang et al., 2019). However, a survival graph can show the interaction between components of a mixture (Kanu et al., 2021b). Chemical interaction could enhance the toxicity of a mixture more than the single chemical and thus decrease the survival probability of organisms exposed to the mixture. Conversely, chemical interaction could reduce mixture toxicity, thus increasing the survival probability of organisms exposed to the mixture. In the current study, the antagonistic interaction of the pesticide mixture in African catfish may explain why the survival probability of Nile tilapia exposed to atrazine-chlorpyrifos, atrazine-lambda cyhalothrin, mancozeb-lambda cyhalothrin, and chlorpyrifos-lambda cyhalothrin was lower than the African catfish. This study also shows that pesticide mixture interaction or lack off was species-dependent. Not all pesticide mixtures studied showed interaction of mixture components in Nile tilapia as chlorpyrifos-lambda cyhalothrin mixture was antagonistic. The same pesticide mixtures were antagonistic in African catfish except for chlorpyrifos-lambda cyhalothrin, which may not have interacted. Finfish vary in their ability to metabolize xenobiotics, as some are efficient metabolizers than others (González et al., 2009). As such, the toxico-dynamics difference in the fish species may explain the antagonism or lack of interaction observed in this study.

We expected the survival probability of African catfish to be higher than Nile tilapia, given that African catfish are supposedly more tolerant to stressors than Nile tilapia (see Kanu et al., 2021a). However, the results of this study negate our prediction of the responses of both species after brief exposure to the pesticide mixture. Our earlier study showed that Nile tilapia had a significantly higher survival probability than African catfish after 60 min of exposure to atrazine, mancozeb, and lambda-cyhalothrin (Kanu et al., 2021a). In the current study, the survival probability of African catfish was approximately 6 and 16 times lower than Nile tilapia after exposure to chlorpyrifos-lambda cyhalothrin and quaternary mixture. On the other hand, survival likelihoods after pulse exposure to other pesticide mixtures were similar. Peterson et al., (2001) reported that *Calineuria californica* was approximately 1,000-fold less sensitive to carbaryl than *Cinygma* sp. only after pulsed exposure but similar after 96 h of continuous exposure. Differential response in this study may be linked to the physicochemical characteristics of pesticide and differences in uptake rate, time to equilibration, fish morphology, and pesticide metabolism, as alluded in our previous report (Kanu et al., 2021a).

A decrease in survival probability of exposed fingerlings as pulse-exposure length increased is consistent with previous reports. Peterson et al., (2001) observed that the survival of *C. californica* and *Cinygma* sp. decreased as pulse exposure time to carbaryl increased. However, in this study, survival probability was similar irrespective of pulse length for African catfish and Nile tilapia exposed to some pesticide mixtures. Low pesticides uptake during pulse exposure may be the reason for this. Peterson et al., (2001) observed low mortality (50% mortality was not reached) for *Calineuria californica* pulsed exposed to three concentrations of carbaryl—17.3, 173, and 1730 mg/L carbaryl for 15, 30, and 60 min. Fifty percent (50%) mortality occurred only after exposure to 1730 mg/L carbaryl for 60 min. The authors attributed the low mortality to low rates of uptake of carbaryl by *C. californica* despite the high concentrations.

Bigger fingerlings had more tolerance for stress than smaller fingerlings and the same for fish with higher condition factors, as shown by this study. Fish condition factor depicts the health status of the fish; as such healthier fish usually have high condition factor (Kanu & Idowu, 2017). Newman and McCloskey (2000) reported that smaller fish were more sensitive to benzocaine than bigger fish. An increase in survival probability with increasing size and condition factor supports the individual tolerance concept (Ashauer & Escher, 2010). However, this study also shows that pesticide pulse toxicity may be independent of fingerling size and condition factor. Fish weight and length did not influence the mortality pattern of African catfish and Nile tilapia mortality following exposure to atrazine-lambda cyhalothrin, mancozeb-chlorpyrifos mixture, and African catfish exposed to the quaternary mixture. Likewise, the likelihood of survival after pulse exposure to some pesticide mixture was independent of fish condition factor as fingerlings with lower condition factors had a higher probability of survival. As such, mortality occurred independently of fish size and condition factor, indicating stochastic process may have influenced fingerling survival.

In this study, pesticide mixture interaction was species-dependent. Innate intrinsic and extrinsic deterministic factors and, to a limited extent, stochastic processes may have influenced the survival probability of African catfish, and Nile tilapia pulsed exposed to complex pesticide mixtures. These findings have general significance relative to conducting exposure assessment for realistic risk assessment of pesticides. Selection of test species for regulatory testing and exposure assessment is critical for accurate ecological risk assessment of pesticides. Test species chosen based on sensitivity established by continuous toxicity tests may underestimate or overestimate pesticide pulse toxicity and undermine their role to provide broad protection to other non-target organisms within the ecosystem. There may be a need for a pulse toxicity test to rank the relative susceptibility of aquatic biota to pulse exposure. Environmental toxicologists could take advantage of the statistical power of survival analysis if the time to death or any other event (the endpoint of interest) is recorded during the exposure assessment.

Conclusions

The disparity in the influence of species difference, size, pulse length, and condition factor on the survival of African catfish and Nile tilapia pulse-exposed to pesticide mixtures depict scenarios that may occur in the environment. Given that ecotoxicology's goal is to predict the toxicity of pollutants in the environment, pulse and repeated pulse exposure toxicity tests should be incorporated in exposure assessment to make pesticides exposure assessment more realistic.

Abbreviations

PH: Proportional hazard; SPSS: Statistical package for social sciences; SAS: Statistical Analysis System; LT: Lethal time; LC: Lethal concentration.

Acknowledgements

Not applicable

Authors' contributions

KCK conceptualized, performed the experiment and statistical analysis, and wrote the original draft. AAO supervised, reviewed, and edited the initial draft, while NHA provided resources, supervised, and edited. All authors read and approved the final manuscript.

Funding

This research received no specific funding.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Approval was obtained from the Ethical Committee at the College of Medicine, the University of Lagos, to use the fish species for pesticide toxicity studies (CMUL/ACUREC/10/20/827). All the fishes were handled humanely in compliance with Directive 2010/63/EU on protecting animals used for scientific purposes and the International Council for Laboratory Animal Science (ICLAS) ethical guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 29 June 2021 Accepted: 17 February 2022
Published online: 07 March 2022

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