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Acute toxicity of copper hydroxide and glyphosate mixture in *Clarias gariepinus*: interaction and prediction using mixture assessment models

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The study aimed to assess the single and joint lethal toxicity, type of interaction and the extent to which simple mathematical model of concentration addition (CA), independent action (IA) and generalized concentration addition (GCA) could predict the joint toxicity of copper hydroxide and glyphosate mixture in Clarias gariepinus. Static bioassay were setup to determine the individual and combined (based on ratio 1:2) lethal concentrations (LCx) of the pesticides. Data from the static bioassays were then fitted into the synergistic ratio (SR), concentration-addition (toxicity unit; TU) and isobologram model to determine the type of interaction between the different classes of pesticides, while the CA, IA and GCA models were used to predicted the observed mixture effects. The estimated 24 h, 48 h, 72 h and 96 h LC50 for copper hydroxide were 198.66 mg/L, 167.51 mg/L, 138.64 mg/L, and 104.82 mg/L; glyphosate were 162.92 mg/L, 103.88 mg/L, 61.95 mg/L, and 52.6l mg/L; while the mixtures were 63.18 mg/L, 59.06 mg/L, 56.42 mg/L, and 50.67 mg/L, respectively. Glyphosate was 2 times more toxic than copper hydroxide to *C. gariepinus* when acting singly. The SR and RTU was <1 indicate that the interaction between the pesticides was synergistic. Synergism was also corroborated by the isobologram model. The interaction of the mixture of copper hydroxide and glyphosate followed the IA model while the CA and GCA model underestimated the observed mixture effects. The study showed that copper hydroxide was practically non-toxic, while glyphosate and the mixture were slightly toxic to *C. gariepinus*

Keywords: Model, pesticides, prediction, catfish, mixture toxicity

INTRODUCTION

Agriculture relies greatly on chemicals including inorganic fertilizers and synthetic pesticides to improve agricultural products and yield and their usage is increasing [1]. Fungicides and herbicides account for 51% of the type of pesticides used globally [2]. In addition to the increasing usage, the concentration of pesticide used and frequency of application of pesticides is increasing [3]. It is also common for farmers to mix pesticides of different chemical class to combat pesticide resistance by pests and improve production efficiency [1, 3].

Surface waters are natural sinks of pesticides from agricultural fields. Pesticides mixtures in surface water consist of different chemicals having either similar or dissimilar modes of action [4] and different possible chemical interaction in living organism. The terms additivity, synergism and antagonism have being used extensively to describe chemical interactions. The toxicological effects of the interaction between components of a pesticide mixture in non-target organisms is complex and depend on the chemical nature of the individual pesticides, mode of action, the concentration and the site of toxic action [5]. It is often difficult to understand and predict the combined effects of mixtures because the assessment of chemical toxicity is usually carried out for each chemicals acting alone, without considering potential joint effects. It is also not feasible to perform toxicity test for mixture to assess joint toxicity due the large number of chemicals [6].

Several mathematical models have being developed and utilized to estimate interaction including the synergistic ratio (SR) and isobologram [7,8]. The concentration addition (CA) developed by Loewe, also called dose addition and the independent action (IA) model developed by Bliss are among the models

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commonly used to predict the effects of mixture based on the previous knowledge of the effects of each of the components of the mixture [6, 9, 10, 11]. Other models such as toxic equivalency factor [12], generalized concentration addition (GCA) model [13], and full logistic model [14] have being developed to address some of the shortcomings of the CA and IA model. CA is better suited for mixtures whose components share similar modes of action, while IA model is apt for mixtures with components with different modes of action [6]. GCA on the other hand is suited for mixture components with different efficacy [15].

CA and GCA model, may be unsuitable models to predict interactions between copper hydroxide and glyphosate based on the assumptions of the models. CA model was however applied because both pesticides share one mechanism of toxicity with is inhibition of antioxidants and oxidative stress. Again the extent to which mechanism of action of components of a mixture may be similar may not be known to effectively apply CA model [15]. GCA was utilized because even though the mixtures were prepared based on equi-toxic ratio (equivalent toxicity), given that concentration of each pesticide in the mixture is different, their efficacy may also be different. Thus while this study aimed to assess the single and joint lethal toxicity, type of interaction of the fungicide and herbicide, it may be useful to see how CA and GCA compares with IA model in predicting the joint toxicity of the pesticides in *Clarias gariepinus* given the characteristics of the pesticide mixture.

MATERIALS AND METHODS

The research consist of two related experiments; the study of acute lethal toxicity of formulated copper hydroxide and glyphosate pesticides to estimate the lethal concentrations (LC) of each pesticide; and acute lethal toxicity study of the pesticide mixture to estimate the LC of the mixture. Data from the two experiments were fitted into different models to determine the type of interaction between the pesticides in the mixture and predict the toxicity of the mixture.

Test Chemical

The pesticides used for this study was sourced from local vendors in Abia State, Nigeria and are among the list of pesticides approved for use in cocoa farms in Nigeria. Formulated pesticides used in the study include;

- Fungicide - Kocide 101, a wettable powder. The active ingredients are 100 g containing copper hydroxide – 77%, inert ingredients - 23%, metallic copper equivalent 50%. The fungicide registered trade mark of Du pont de Nemours (France), represented by Reiss & Co. (Ghana), Ltd.
- Herbicide - Drysate, emulsfiable concentrate. The active ingredients are 360 g glyphosate/l in form of 480 g/L glyphosate-isopropylamine salt. Manufactured by Sichuan Leshan Fuhua Tongda agrochemical technology Co. Ltd., China, represented by Crop care Ltd., Kano State, Nigeria.

Collection and acclimatization of fish samples

C. gariepinus fingerlings (1.5-2 cm in length) were purchased from a private fish farm in Aba, Abia State, Nigeria and transferred to laboratory where they were acclimated for a week. The fishes were kept in a plastic aquarium containing borehole water. All the fishes were fed with commercially available fish feeds and excess feed and faeces was siphoned daily. After the one week of acclimation, the fish was transferred randomly into the plastic aquaria to test the pesticide and the mixture.

Stock and Test solution

The copper hydroxide stock solution was prepared by mixing 1g of the wettable powder in 1 L of bore hole water, while glyphosate stock solution was prepared by making up 1 mL of the pesticide to 1 L. Stock solution of the pesticide mixture was prepared by mixing 2 g of copper hydroxide powder and 1 g (2.083 ml) of glyphosate pesticide and making up the mixture to 1 L. Mixture was prepared based on equi-toxic ratio (2:1 i.e. 2 parts of copper hydroxide and 1 part of glyphosate). Test solutions of the required concentration was prepared by dilution the stock solutions for the range finding and definite test.

Acute Toxicity of Each Pesticide

The range finding test were conducted to establish the range of concentrations used for the definite toxicity test. Twenty *C. gariepinus* fingerlings were exposed in two replicates of ten to nominal copper hydroxide concentrations of 50 mg/l, 75 mg/l, 100 mg/l, 125 mg/l, 150 mg/l and 200 mg/l and glyphosate concentration of 50 mg/l, 75 mg/l, 100 mg/l, 125 mg/l and 150 mg/l in a plastic aquaria containing 1 litre of the test solutions. The fishes were exposed for 96 hours and mortalities were recorded at 24, 48, 72 and 96 hours and lethal concentration at these time interval were estimated.

Acute Toxicity of the Pesticide Mixture

Similarly, range finding test were conducted and ten *C. gariepinus* fingerlings were exposed in duplicates to established nominal pesticide mixture concentrations of 50 mg/L, 55 mg/L, 60 mg/L, 65 mg/L and 70 mg/L. Mortalities were recorded at 24, 48, 72 and 96 h and lethal concentrations at these time intervals were also estimated.

Toxicity factor for relative potency measurements was calculated using equation (1);

$$\text{Toxicity factor} = \frac{LC_{50}\text{ of a chemical at 24 h}}{LC_{50}\text{ of chemical at other h (48,72,96)}} \quad (1)$$

Physicochemical analysis of test solutions

Temperature, pH, dissolved oxygen, electrical conductivity, total dissolved solids of the test solution was monitored during the experiment using portable meters.

Determination of the type of interaction between the pesticides in the mixture.

Two models were used and not three determine the type of interaction between the fungicide and the herbicide.

Model 1: The SR model [7]. SR was calculated using equation (2)

$$SR = \frac{LC_{50}\text{ of a chemical acting alone}}{LC_{50}\text{ of mixture}} \quad (2)$$

Where SR=1 describes additive action, SR<1 describes antagonism action and SR>1 describes synergistic action.

Model 2: The toxic unit (TU) of the mixture CA was calculated using equation (3) while chemical interaction was established based on the assumption of equation (4).

$$TU_1 = \frac{C_1}{LC_{x1}} \qquad TU_2 = \frac{C_2}{LC_{x2}} \quad (3)$$

$$TU_1 + TU_2 = 1 \quad (4)$$

C_1 and C_2 are the individual concentrations of the substances 1 and 2 constituting the mixture. The sum of C_1 and C_2 equals the total concentration of the mixture that caused effect x. ΣTU_x=1 describes additive action, ΣTU_x <1, describes synergism, and ΣTU_x >1 describes antagonism. LC_x denote concentration that causes x% effect. The joint actions between two chemicals in binary mixtures are presented in form of isobolograms (Figure 1) [8]. Isobole I illustrates additive action; Isobole II illustrates synergism; Isobole III illustrates sub-additive action; Isobole IV illustrates antagonism.

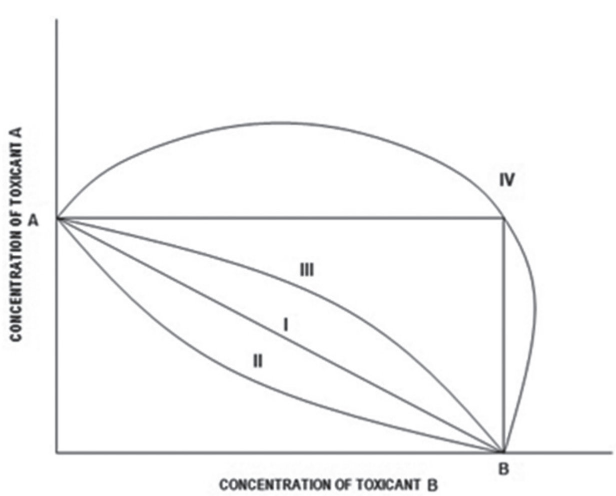

Figure 1. Isoboles depicting the types of interactions between two chemicals A and B

Prediction of Mixture Toxicity

The prediction of mixture toxicity was based on the CA model, the IA model [10] and GCA model [9,13].This estimated concentration response relationship is then compared with the observed concentration response for the experimental mixture.

CA was modelled using equations (5) and (6). Calculations were performed for different given combined effects x and a graph of the concentration effect curve for the mixture was plotted and the LC determined by probit analysis.

$$LC_{x\ mix} = \frac{1}{\frac{P_1}{LC_{x1}} + \frac{P_2}{LC_{x2}}} \quad (5)$$

where LC_x mix is the LC_x of a mixture, LC_{x1} and LC_{x2} are the equivalent LC values of the single pesticides 1 and 2 that alone would produce the same effect x as the mixture, P_1 and P_2 is the proportion of each pesticide 1 and 2 in the mixture calculated from their concentrations as

$$P_i = \frac{C_i}{\sum_i C_i} \quad (6)$$

IA was modelled using equation (7). E_{mix} is the effect of the mixture at a specific concentration; E_1 is the effect of pesticide 1 at that specific concentration and E_2 is the effect of pesticide 2. A range of concentration points were selected and the effects (E) were calculated, and a graph of the concentration effect curve for the mixture was plotted.

$$E_{mix} = 1 - ((1-E_1) \times (1-E_2)) \quad (7)$$

GCA was modelled using equation (8). The equation was adapted from Hadrup et al., [16]

$$E = \frac{\text{max effect levelA } [A]/LC_{50A}\ \text{max effect levelB } [B]/LC_{50B}}{1 + \frac{[A]}{LC_{50A}} + \frac{[B]}{LC_{50B}}} \quad (8)$$

E is the effect of the mixture at a specific concentration. 'max effect level A' is the maximal effect level of pesticide A, [A] is the concentration of A in the mixture at a specific mixture concentration, LC_{50A} is the LC_{50} value of A, while 'max effect level B' is the maximal effect level of pesticide B, [B] is the concentration of B in the mixture at a specific mixture concentration, LC_{50B} is the LC_{50} value of B. Thus for a range of mixture concentrations, effect values (E) were calculated and concentration-mortality curve was plotted.

Compliance between the Predicted and the Observed Mixture Toxicity

In order to quantify the compliance between the predicted and the observed toxicity of the mixtures, the model deviation ratio (MDR) [17] was calculated using equation (9).

$$MDR = \frac{\text{Predicted } LC_{x\ mix}}{\text{Observed } LC_{x\ mix}} \quad (9)$$

where MDR = 1 indicates perfect compliance between predicted and observed toxicity of the mixture, MDR > 1 indicates that the mixture is more toxic than predicted (i.e. an underestimation of mixture toxicity by the models), MDR < 1 indicates that the mixture is less toxic than predicted (i.e. an overestimation of mixture toxicity by the models).

The MDR values were calculated for all effect levels (5%, 50% and 95%) for which the respective estimates for the single substances had been determined.

Statistical Analysis

The concentration-response data of each pesticide was analyzed using the probit method [18]. Similarly, the probit method was also used to analyse experimental and model derived toxicological data for the pesticide mixture. The indices of toxicity measurement derived from the probit analyses were; LC_5 (Lethal concentration that causes 5% mortality of exposed organisms), LC_{50} (Lethal concentration that causes 50% mortality of exposed organisms) and LC_{95} (lethal concentration that causes 95% mortality of exposed organisms). All statistical analysis was computed with SPSS version 22 except the isobole which was plotted with 2013 MS Excel, while the least squares method which uses the Levenberg-Marquardt algorithm was used to fit both the observed and the predicted values to plot the concentration-response graph using Statistica version 10.

RESULTS

Physicochemical parameters of test solutions

All the physicochemical parameters of the of the test solution presented in Table 1 were not different significantly except electrical conductivity and total dissolved solids of copper hydroxide possible due to the presence of copper ions in the copper hydroxide and mixture test solutions.

Mortality Count of exposed *C. gariepinus*

Table 2 shows the mortality pattern of exposed fingerlings.

Lethal Concentrations of Copper hydroxide, Glyphosate and the mixture for *C. gariepinus*. The estimated LC of copper hy-

Table 2. Observed mortality count of *C. gariepinus*

Pesticide	Conc. (mg/L)	Total exposed	Mortality count at 24 h	48 h	72 h	96 h
Copper hydroxide	Control	20	0	0	0	0
	50	20	0	0	2	3
	75	20	0	0	3	5
	100	20	2	2	5	7
	125	20	3	8	9	13
	150	20	7	9	12	14
	200	20	9	11	14	19
Glyphosate	Control	20	0	0	0	0
	50	20	2	3	8	9
	75	20	3	7	12	16
	100	20	4	9	15	18
	125	20	5	11	17	19
	150	20	12	15	20	20
Mixture	Control	20	0	0	0	0
	50	20	4	7	8	9
	55	20	5	8	9	15
	60	20	7	9	10	18
	65	20	10	11	12	19
	70	20	15	16	18	20

Table 1. Physicochemical parameters of test solutions

Pesticide	Conc. (mg/L)	Temperature (°C)	Electrical Conductivity (μs/cm)	Total Dissolved Solids (ppm)	Dissolved Oxygen (mg/L)	pH
Glyphosate	50	27.4 ± 0.82	48.5 ± 1.29	23.5 ± 1.29	4.1 ± 0.08	7.45 ± 0.65
	75	27.5 ± 0.58	57.5 ± 2.08	27.5 ± 0.58	4.35 ± 0.13	7.15 ± 0.13
	100	27.3 ± 0.82	56.5 ± 4.2	27.5 ± 1.29	4.4 ± 0.18	7.2 ± 0.12
	125	27.5 ± 0.58	55 ± 1.15	30.5 ± 0.58	4.4 ± 0.08	7 ± 0.16
	150	27.2 ± 0.82	51.5 ± 1.29	30 ± 0.82	4.3 ± 0.08	7.2 ± 0.16
	Control	27.7 ± 1.15	42 ± 6.63	32 ± 20	4.375 ± 0.39	7.3 ± 0.14
Copper hydroxide	50	27.4 ± 1.66	214 ± 206.44	107 ± 102.88	4.275 ± 0.22	7.475 ± 0.67
	75	27.3 ± 1.53	277 ± 256.57	138.75 ± 128.82	3 ± 1.82	7.55 ± 0.47
	100	27.3 ± 1.47	158.2 ± 131.7	77.75 ± 64.33	3.85 ± 0.47	7.6 ± 0.52
	125	27.7 ± 0.75	264.2 ± 271.22	131.75 ± 135.64	3.475 ± 1.16	7.525 ± 0.49
	150	27.2 ± 1.36	203.5 ± 164.86	96.5 ± 76.98	4.65 ± 2.34	7.425 ± 0.38
	200	27.3 ± 0.97	276.6 ± 231.7	98.7 ± 84.5	4.2 ± 0.78	7.6 ± 0.52
	Control	27.7 ± 1.15	42 ± 6.63	32 ± 20	4.375 ± 0.39	7.3 ± 0.14
Mixture	50	28.3 ± 0.96	147.4 ± 124.96	74 ± 63.01	4.05 ± 0.6	7.525 ± 0.1
	55	27.7 ± 0.96	110.5 ± 71.74	51.75 ± 31.69	4.65 ± 0.98	7.325 ± 0.29
	60	27.7 ± 0.96	136.2 ± 106.57	73 ± 47.24	4.15 ± 0.13	7.55 ± 0.42
	65	28 ± 0.82	156.5 ± 138.77	78 ± 70.19	4.275 ± 0.34	7.45 ± 0.24
	70	28 ± 1.15	133.7 ± 100.12	67.75 ± 50.74	3.475 ± 0.91	7.275 ± 0.39
	Control	28.5 ± 0.58	48.5 ± 1.29	21.5 ± 1.29	4.65 ± 0.13	7.4 ± 0.26

droxide, glyphosate and the mixture that may cause mortality in 5%, 50% and 95% of the exposed catfish after 24, 48, 72 and 96 h is shown in table 3. The toxicity factor indicates that toxicity of the pesticides and the mixture increased as the duration of exposure increased. It also show glyphosate was more toxicity than copper hydroxide and the mixture.

Type of interaction between the Copper hydroxide and Glyphosate

Result of the type of interaction between the copper hydro

Table 3. Estimated LC of Copper hydroxide and Glyphosate for *C. gariepinus*

Pesticide	Duration	LC_5 (95% CI)	LC_{50} (95% CI)	LC_{95} (95% CI)	TF
Copper hydroxide	24 h	90.77 (56.53-109.92)	198.66 (168.21-289.27)	434.78 (295.66-1286.4)	1.00
	48 h	79.68 (52.28-96.65)	167.51 (146.89-208.28)	352.15 (261.03-709.65)	1.19
	72 h	45.47 (22.79-61.87)	138.64 (117.28-176.4)	422.75 (282.33-1075.67)	1.43
	96 h	42.34 (25.30-55.22)	104.82 (90.23-121.20)	259.49 (200.51-426.79)	1.9
Glyphosate	24 h	45.87 (12.24-65.23)	162.92 (126.90-362.75)	578.59 (293.93-9032.91)	1.00
	48 h	32.69 (10.57-48.43)	103.88 (85.70-132.24)	330.09 (213.04-1177.90)	1.57
	72 h	24 (8.64-36.08)	61.95 (44.98-73.71)	159.85 (125.11-281.73)	2.63
	96 h	24.26 (9.66-35.07)	52.61 (37.29-62.63)	114.1 (94.35-170.59)	3.1
Mixture	24 h	43.45 (30.23-49.07)	63.175 (59.52-69.48)	91.86 (78.88 – 146.16)	11.00
	48 h	35.34 (11.85 – 43.10)	59.06 (52.74-65.34)	98.68 (79.74-262.130)	1.07
	72 h	35.81 (16.12-43.66)	56.44 (49.70-60.69)	88.97 (75.42-171.40)	1.12
	96 h	40.67 (31.10-45.12)	50.67 (46.06-53.16)	63.13 (59.69-71.59)	1.25

Table 4. SR1 was calculated using LC50 of a glyphosate acting alone/ LC50 of mixture, while SR 2 was calculated using LC50 of a copper hydroxide acting alone/ LC50 of mixture

Parameters	Duration			
	24 h	48 h	72 h	96 h
SR1	3.14	2.84	2.46	2.07
SR2	2.58	1.76	1.10	1.04
Interpretation	Synergism	Synergism	Synergism	Synergism

Table 5. Type of interaction between the copper hydroxide and glyphosate based on CA

Concentration (mg/L)	Summation of toxic units (ΣTU_x)				Interpretation
	24 h	48 h	72 h	96 h	
50	0.27	0.36	0.51	0.63	Synergism
55	0.30	0.40	0.56	0.70	Synergism
60	0.32	0.43	0.61	0.76	Synergism
65	0.35	0.47	0.66	0.83	Synergism
70	0.38	0.50	0.71	0.89	Synergism

Table 6. Comparison of observed LC_x and predicted LC_x of copper hydroxide and glyphosate mixture

Method	Duration	LC_5 (95% CI)	LC_{50} (95% CI)	LC_{95} (95% CI)
Observed	24 h	43.45 (30.23-49.07)	63.175 (59.52-69.48)	91.86 (78.88 – 146.16)
	48 h	35.34 (11.85 – 43.10)	59.06 (52.74-65.34)	98.68 (79.74-262.130)
	72 h	35.81 (16.12-43.66)	56.44 (49.70-60.69)	88.97 (75.42-171.40)
	96 h	40.67 (31.10-45.12)	50.67 (46.06-53.16)	63.13 (59.69-71.59)
Predicted (CA)	24 h	69.18 (38.82-91.14)	184.4 (155.88-229.95)	491.52 (347.13-104.17)
	48 h	54.3 (31.27-70.67)	138.69 (118.10-171.30)	354.22 (253.99-729.19)
	72 h	35.08 (19.633-46.98)	98.08 (81.96-121.24)	274.2 (195.05-548.77)
	96 h	33.96 (22.01-42.87)	78.72 (67.65-93.67)	182.49 (139.36-305.28)
Predicted (IA)	24 h	52.95 (25.09-67.82)	133.93 (109.12-231.47)	338.8 (207.55-1806.17)
	48 h	32.84 (17.15-44.07)	90.32 (75.98-113.53)	248.44 (172.41-571.22)
	72 h	24.97 (16.99-35.04)	57.29 (47.83-66.53)	131.44 (105.44-195.52)
	96 h	26.23 (17.62-32.68)	49.91 (42.50-57.03)	94.97 (79.72-127.96)
	24 h	12.32	NR	NR
Predicted (GCA)	48 h	11.3 (0.174-26.78)	240.8 (126.10-3881.67)	NR
	72 h	5 (0.036-15.09)	150.64 (84.95-928.23)	NR
	96 h	5.25 (0.31-13.33)	82.97 (52.96-158.88)	NR

NR- not reliable.

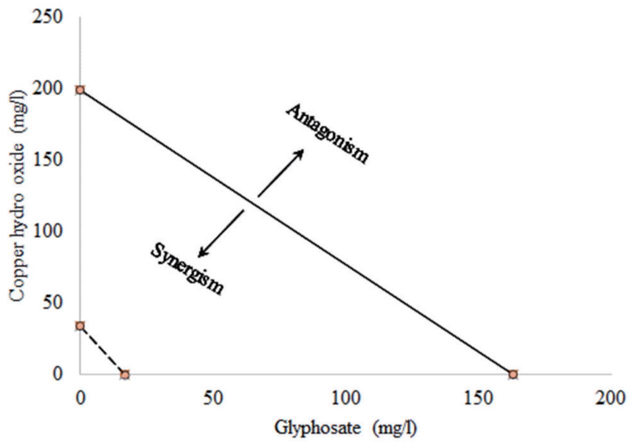

Figure 2. Isobole illustrating the type of interaction between copper hydroxide and glyphosate

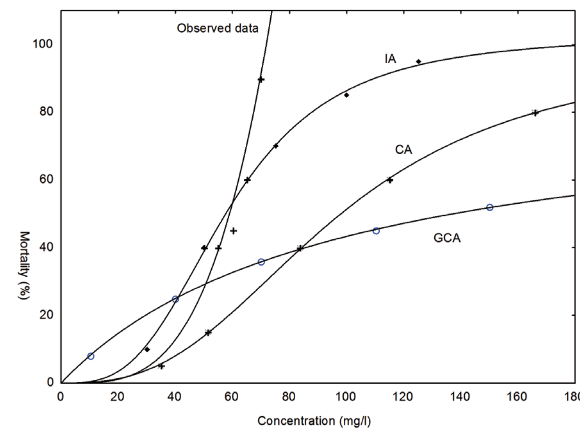

Figure 5. Dose response plot of observed response compared with predicted response at 72 h

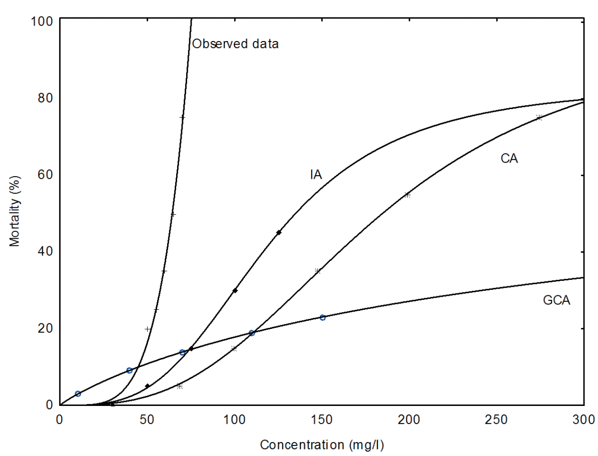

Figure 3. Comparison of the observed concentration-response plots *C. gariepinus* exposed of the pesticide mixtures compared with the predicted response at 24 h

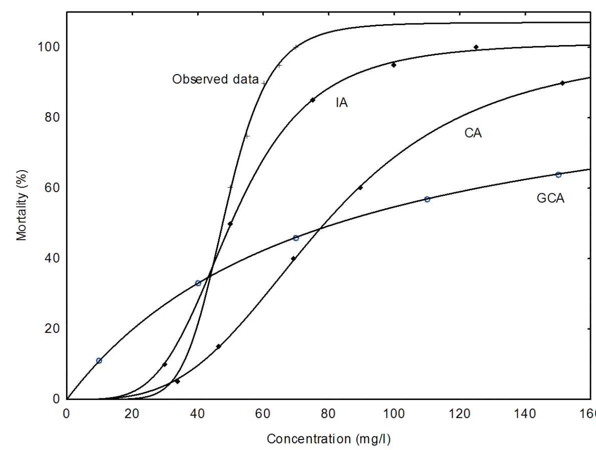

Figure 6. Dose response plot of observed response compared with predicted response at 96 h

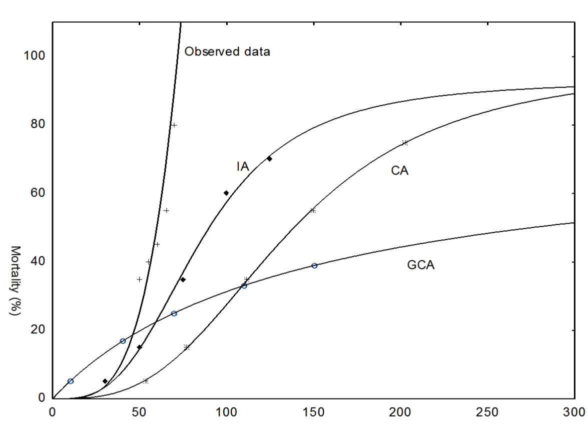

Figure 4. Dose response plot of observed response compared with predicted response at 48 h

oxide and glyphosate as presented in Table 5, 6 and Figure 2 indicate that the interaction between copper hydroxide and glyphosate may be synergistic given that the SR1 and SR2 > 1 (Table 4), ΣTU_x <1 (Table 5) and the isobole of the mixture was below the additivity line (Figure 2).

Figure 2 indicates a synergistic interaction between copper hydroxide and glyphosate in *C. gariepinus*. Although not depicted in the graph, synergism occurred at 24, 48, 72 and 96 h.

Figure 3-6 show that the extent to which the models predicted the observed mixture effects after 24, 48, 72, and 96 h with IA model performing better than the CA and the GCA model.

Prediction of the toxicity of copper hydroxide and glyphosate mixture

Table 6 summarizes the comparison of observed LC_x and predicted LC_x values.

In Table 7, the MDR values indicate that the CA model underestimated the 24, 48, 72 and 96 h LC_5, LC_{50} and LC_{95} endpoints of the copper hydroxide and glyphosate mixture. How-

Table 7. Compliance between observed and predicted toxicity by models

Pesticide	Duration	MDR for 5% effect level	MDR for 50% effect level	MDR for 95% effect level
Predicted (CA)	24 h	1.6[b]	2.9[b]	5.4[b]
	48 h	1.5[b]	2.3[b]	3.6[b]
	72 h	1.0[a]	1.7[b]	3.1[b]
	96 h	0.8[c]	1.6[b]	2.9[b]
Predicted (IA)	24 h	1.2[b]	2.1[b]	3.7[b]
	48 h	0.9[c]	1.5[b]	2.5[b]
	72 h	0.7[c]	1.0[a]	1.5[b]
	96 h	0.6[c]	1.0[a]	1.5[b]
Predicted (GCA)	24 h	0.3[c]	NC	NC
	48 h	0.3[c]	4.1[b]	NC
	72 h	0.1[c]	2.7[b]	NC
	96 h	0.1[c]	1.6[b]	NC

NC- Not calculated, 'a' indicate perfect compliance between predicted and observed toxicity of the mixture, 'b' indicate that the mixture is more toxic than predicted (i.e. an underestimation of mixture toxicity by the models), 'c' indicates that the mixture is less toxic than predicted (i.e. an overestimation of mixture toxicity by the models).

ever, CA model accurately predicted the 72 h LC_5 of the mixture. Similarly, the IA model accurately predicted the 72 and 96 h. LC_{50} of the mixture. Nevertheless it overestimated the 48, 72 and 96 h. LC_5 and underestimated 24, 48 h. LC_{50} and the 24, 48, 72 and 96 h. LC_{95} of the mixture. The GCA model overestimated the 24, 48, 72 and 96 h. LC_5 but underestimated the 48, 72 and 96 h LC_{50} of the mixture.

DISCUSSION

The study evaluated the single and joint lethal toxicity of copper hydroxide and glyphosate, the type of chemical interaction of the mixture in *C. gariepinus* fingerlings, and predicted mixture toxicity using models. All the physico-chemical parameters showed optimal water quality for the survival of the fingerlings during the 96 h acute toxicity tests. Experimentally derived LC_{50} is used to classify the toxicity of a chemical as highly toxic, moderately toxic and slightly toxic if the LC_{50} is between 0.1-1 mg/L, 1-10 mg/L and 10-100 mg/L respectively. LC_{50} value greater than 100 mg/L are considered practically non-toxic [19]. In the current study, the 24, 48, 72, and 96 LC_{50} indicate that formulated copper hydroxide fungicides was practically non-toxic to *C. gariepinus* fingerlings while glyphosate was slightly toxic as the duration of exposure increased. The 96hr LC_{50} of active ingredient copper hydroxide reported in the current study for *C. gariepinus* is lower than the LC_{50} of 180 mg/L reported for *Lepomis macrochirus* (Bluegill sunfish) but higher than the 25 mg/L reported for *Oncorhynchus mykiss* (rainbow trout) [20]. Lower LC_{50} values 34.72, 31.90, 27.40 and 24.60 mg/L for 24, 48, 72 and 96 h of exposure respectively were reported for *C. gariepinus* juveniles exposed to glyphosate (Glycot®) [21].

Pesticides are essentially applied as formulated products thus it is appropriate to evaluate the acute toxicity of commercial pesticides mixtures. In this study, the binary mixture of copper hydroxide and glyphosate was slightly more toxic to *C. gariepinus* fingerlings than each pesticides acting alone as the 24, 48, 72, and 96 h LC_{50} values of the pesticide mixture were lower than those of the pesticides alone. It is likely there was synergistic interaction between glyphosate and copper hydroxide which may have influenced the toxicity of relatively non-toxic copper hydroxide fungicide. Although the underlying mechanisms of the combined action is not clear, it is likely that inhibition of biotransformation enzymes by copper may have decreased the metabolism of glyphosate leading to increase accumulation to LC in the fish. The mechanism of toxicity of glyphosate in fish may involve multiple mechanism including oxidative stress, inhibition of acetylcholinesterase and genotoxicity, while copper is an osmo-regulatory toxicant which can cause inhibition of enzymes including biotransformation enzymes such as cytochrome P450 associated monooxygenase [22, 23]. Toxicants which influence the amount of biotransformation enzymes can influence on the toxicity of other chemicals [24].

The risk of individual chemicals is less than the risk of a mixture of chemicals, thus understanding the effects of pesticide mixture is critical to the ecological risk assessment of pesticides [25]. Predictive models play important role in this regard. In the current study, the IA model provided a better estimation of the observed response compared with the CA and GCA. Although CA performed better than the GCA model. IA predicted the 72 and 96 h LC_{50} of the mixture while CA the 24 h LC_5 of the mixture. Mixtures of toxicants with dissimilar mode of action tend to follow the IA model which is the case in the current study [26,27]. The CA model may have underestimated the observed response because the components of the mixture may have failed to meet one of the criteria of the CA model, which is the similar as the response shape of Tanaka and Tada [11]. In this study, the response curve of copper hydroxide was much steeper than that of glyphosate and this may explain some of the underestimation of the mixture effects by the CA model. Wang et al.[28] showed that deviation of the mixture toxicity predicted by the CA model would occur when the components have significantly different slope from each other. Tanaka and Tada, opined that components with steeper response curve may be more biodegradable and eliminated from the exposed organisms than the other component [11]. Again CA model is limited in that it can only predict up to the maximal effect level of the chemical with the lowest efficacy which in this case is copper hydroxide. The GCA is an extension of the CA model developed to overcome the limitation of the CA model and could not sufficiently predict the mixture effects [13].

This study showed that the commercially formulated glyphosate was two times more toxic than copper hydroxide to *C. gariepinus* and the mixture of both pesticide produced a synergistic effect which was better predicted by the IA model due to the different mode of action of the pesticides.

REFERENCES

1. Wilson C, Tisdell C. Why farmers continue to use pesticides despite environmental health and sustainability costs. Ecol Econ 2001;39(3):449-462.
2. Aktar MW, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2009;2(1):1-12.
3. Robinson DE, Nurse R. Effect of herbicide-fungicide tank-mix combinations on weed control and tomato tolerance. Acta Hortic 2009; 823(823):129-134.
4. Altenburger R, Walter H, Grote M. What contributes to the combined effect of a complex mixture? Environ Sci Technol 2004; 38(23):6353–6362.
5. Hernández AF, Gil F, Lacasaña M. Toxicological interactions of pesticide mixtures: an update. Arch Toxicol 2017; 91(10):3211-3223.
6. Beyer J, Petersen K, Song Y, Ruus A, Grung M, Bakke T, et al. Environmental risk assessment of combined effects in aquatic ecotoxicology: A discussion paper. Mar Environ Res 2014; 96:81-91.
7. Hewlett PS, Plackett RL. A unified theory for quantal responses to mixtures of drugs: non-interactive action. Biometrics 1959;15(4):591-610.
8. Ariens EJ, Simmons AM, Offermeier J. Interaction to general toxicology. 1st ed. Academic Press; 1976, p. 252.
9. Loewe S. The problem of synergism and antagonism of combined drugs. Arzneimittelforschung 1953; 3(6):285–290.
10. Bliss CI. The toxicity of poisons applied jointly. Ann Appl Biol 1939; 26(3):585–615.
11. Tanaka Y, Tada M. Generalized concentration addition approach for predicting mixture toxicity. Environ Toxicol Chem 2017;36(1):265-275.
12. Safe SH. Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. Environ Health Perspect 1998; 106(4):1051–1058.
13. Howard GJ, Webster TF. Generalized concentration addition: a method for examining mixtures containing partial agonists. J Theor Biol 2009;259(3): 469–477.
14. Ezechiáš M, Cajthaml T. Novel full logistic model for estimation of the estrogenic activity of chemical mixtures. Toxicology 2016;359–360:58-70.
15. Watt J, Webster TF, Schlezinger JJ. Generalized concentration addition modeling predicts mixture effects of environmental PPARγ agonists. Toxicol Sci 2016;153(1):18-27.
16. Hadrup N, Taxvig C, Pedersen M, Nellemann C, Hass U, Vinggaard AM, et al. Concentration addition independent action and generalized concentration addition models for mixture effect prediction of sex hormone synthesis in vitro. PLoS ONE 2013;8(8);e70490.
17. Belden JB, Gilliom RJ, Lydy MJ. How well can we predict the toxicity of pesticide mixtures to aquatic life? Int Environ Assess Manage 2007; 3(3):364–372.
18. Finney DJ. Probit Analysis. 3rd ed. Cambridge University Press; 1971 p. 333
19. Choudhury N. Ecotoxicology of Aquatic System: A Review on Fungicide Induced Toxicity in Fishes. Pro Aqua Farm Marine Biol 2018; 1(1):180001
20. Blue Shield® DF Copper Fungicide version 1/AUS 102000017064 Bayer Crop science Pty Ltd Victoria Australia 9th Sep 2016 https://www-cropbayercomau/-/media/bcs-inter/ws_australia/use-our-products/product-import-files/534/9056pdf [accessed on Jan 19, 2019]
21. Ani LC, Nwamba HO, Ejilibe CO, Nwani CD. Acute toxicity of glyphosate-based herbicide glycot on juvenile African cat fish Clarias gariepinus (Burchell 1822). J Fisheries Livest Prod 2017;5(3):252.
22. Kennedy C. Review on glyphosate fate and toxicity to fish with special relevance to salmon and steelhead populations in the Skeena River watershed [cited 2019 Jan 12]. Available from: http://wwwbucksuzuki-org/images/uploads/docs/Glyphosate_report_Final_Nov_21_2017pdf
23. Kim J, Ahn T, Yim S, Yun C. Differential effect of copper (II) on the cytochrome P450 enzymes and NADPH–cytochrome P450 Reductase: Inhibition of Cytochrome P450-Catalyzed Reactions by Copper (II) Ion. Biochemistry 2002;41(30):9438-9447.
24. Nielsen E, Østergaard G, Larsen JC. Toxicological risk assessment of chemicals A practical guide New York: Informa Healthcare 2008; 418 p. Available from: https://booksgooglecomng/books?id=g2jTEOClROQC
25. Kortenkamp A, Backhaus T, Faust M. State of the Art report on Mixture Toxicity. Directorate General for the Environment: Report to the EU Commission 2009 [cited 2019 Jan 12]. Available from: http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf
26. Faust M, Altenburger R, Backhaus T, Boedeker W, Scholze M, Grimme LH. Predictive assessment of the aquatic toxicity of multiple chemical mixtures. J Environ Qual 2000;29(4): 1063-1068.
27. Altenburger R, Nendza M, Schüürmann G. Mixture toxicity and its modeling by quantitative structure-activity relationships. Environ Toxicol Chem 2003;22(8):1900-1915.
28. Wang N, Wang XC, Ma X. Characteristics of concentration inhibition curves of individual chemicals and applicability of the concentration addition model for mixture toxicity prediction. Ecotoxicol Environ Saf 2015;113:176–182.