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Acute Toxicity of Formalin on *Clarias gariepinus* Juveniles

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Abstract
The work was carried out to determine the acute toxicity (LC_{50}) of formalin on the juvenile of African catfish, Clarias gariepinus. Juveniles of the African catfish, Clarias gariepinus (average weight of 12.7 g) were exposed to varying concentrations of formalin for 96hrs under laboratory conditions using static bioassays with continuous aeration to determine its acute toxicity. The LC_{50} of exposed juveniles was found to be 112.20 mg/l with lower and upper confidence limits being 95.09 and 132.40 mg/l respectively. Fish showed signs of respiratory distress, loss of balance, erratic swimming and death. The effects of these conditions were directly proportional to the toxicant (formalin) concentration. Precautions in careful choice of formalin concentrations to be used for treatments, non target fish and other organisms should be prevented from coming in contact with formalin or its residues in the use of formalin for control of ectoparasite on fish are strongly recommended.
***Key words**: Formalin, Clarias gariepinus, acute toxicity.*

Introduction

Formalin, {$H_2C\text{-}(OH)_2$} which is a generic term, describes a solution of 37% - 50% formaldehyde gas, ($H_2C{=}O$) dissolves easily in water, alcohol and acetone. Formalin is one of the most effective and widely used chemicals in fish farm management. It is used for bath, flush or flowing treatment methods to control external parasitic infection of fish. It is extremely effective against most protozoan parasites such as *Ichthyophthirius spp*, *Costia spp*,*Epistylis spp*, *Chilodonella spp* and *Dactylogyrus spp* as well as the larger parasites such as monogenetic trematodes e.g *Cleidodiscus spp* (Schnick, 1974; Jung, 2004; Pedersen *et al*., 2008). Formalin is also a frequently used chemical for treating cultured fish with fungal infection (IPCS, 1989). Although, formaldehyde is known to be biologically degradable (Kato *et. al*., 1983; Adroer *et al*., 1990) and undergoes relatively rapid biodegradation in aquatic environments (Kamata, 1966), its continuous inputs to the aquatic environment may cause harmful effect on aquatic organisms. Therefore, acute toxicity tests have been widely used to obtain rapid estimates of concentrations of toxic chemicals that cause direct, irreversible harm to test organisms (Parrish, 1995). The African Catfish, *Clarias gariepinus* is a common member of freshwater aquaculture species (Hetch *et al*., 1996), and is the second most important freshwater fish (after tilapia) in Africa — with the exception of Nigeria where production of African catfish far exceeds that of tilapia and accounts for 70–80 percent of the total freshwater fish production in the country (Ponzoni and Nguyen, 2008). They are endemic to Africa and have wide geographical distribution from the Middle East in the North Orange river in South Africa and Northern Africa (Teugels, 1984). They are hardy and adaptable due the presence of assessory breathing organs, they thrive well in variety of climatic conditions in Europe including Netherlands, Germany and Belgium, (Verreth, 1993). Despite the high potential for production of this species, the industry on the African continent has not been well established owing to lack of good quality seed hence, the choice for this research studies. Owing to the dearth of information on the use of formalin on *C. gariepinus* juvenile in Nigeria, this research was carried out to determine the acute toxicity (LC_{50}) of formalin on *C. gariepinus* juveniles.

Materials and Methods

Juveniles of African catfish, *Clarias gariepinus* of same broodstocks were collected from the University of Agriculture, Makurdi fish farm, Nigeria**.** The fish (of average weight of 12.7g) were acclimatized under laboratory conditions for 14 days in hatchery of the Department of Fisheries and Aquaculture's laboratory, University of Agriculture, Makurdi in plastic bowls of capacity 75liters each with well aerated dechlorinated water from the University's water works.

During the acclimatization period, the fish were fed twice daily (8:00 am and 4pm) at 4% of their body weight with commercial feeds (Coppens).

Mortality was less than 3% during acclimatization as the water was changed every day to remove accumulated feacal material and unconsumed feed. Fish were not feed 24 hours before and during the exposure period, which lasted 96 hours. Methods for acute toxicity test as recommended by UNEP (1989) were employed. Formalin was obtained as 40%formaldehyde.

After acclimatization, test runs were done for 96 hours to determine the concentration used for the experiment. The following concentrations were then administered into each of the twelve bowls: 0.00, 90.00, 100.00, 110.00, 120.00 and 130.00 mg/l. Each concentration had a replicate while 0.00mg/l served as the control experiment. 10 juveniles of the specimen were randomly selected and stocked in each of the 12 plastic basins used for the experiment. A static bioassay was then used to determine the toxicity of formalin for *C. gariepinus* juvenile. Fish were monitored for behavioral changes at the beginning and every 6 hours thereafter.

The solutions were renewed after every 24 hours in each bowl. Physio-chemical parameters (Temperature, pH, Dissolved Oxygen, CO_2, and Alkalinity) of the water were monitored every 24 hours using methods described by APHA *et al*. (1985).

Fish were considered dead when the opercula and tail movements stopped and there was no response to a gentle prodding. Dead fish were removed immediately from test solutions to avoid fouling the test media. The 96 h LC_{50} was determined as a probit analysis using the arithmetic method of percentage mortality. The lower and upper confidence limits of the LC_{50} were determined as described by UNEP (1989). Results obtained were subjected to statistical analysis with Duncan's multiple range F-test to test for significant difference ($P < 0.05$) between the various concentrations of formalin and the control.

Results

The mean mortality values are as presented in Tables 2 and 3. The Logarithmic-probability curve of the mean mortality rates is presented in Figure 1. It was observed that during exposure, fish placed in media devoid of formalin survived the 96 hour exposure period.

Table 2: Mortality record of *Clarias gariepinus* Juveniles exposed to formalin for 96 hours.

Concentration (mg/l)	Log conc.	Total No. of fish	No. Dead 96 hours	Mean mortality	% mortality	Probit
0.00	-	-	-	-	-	-
90	1.95	20	3	1.5(0.71)	15	3.96
100	2.00	20	4	2(0.00)	20	4.16
110	2.04	20	10	5(2.80)	50	5.00
120	2.07	20	12	6(0.00)	60	5.25
130	-	20	20	10(0.00)	100	-

Note: There is no log and probit for 0% or 100%. The values in the parentheses represent the standard deviation

Table 3: Mean Mortality Rates of *Clarias gariepinus* Juveniles Exposed to Acute Concentration of Formalin for 96 hours.

Concentration (mg/l)	Log conc.	%Mean Total Mortality	Mean Probit Value
130	-	100	-
120	2.07	60	5.25
110	2.04	50	5.00
100	2.00	20	4.16
90	1.95	15	3.96
0.00	-	0	-

The LC_{50} of *C. gariepinus* juveniles exposed to various concentration of formalin was 112.20mg/l respectively.

The regression equation of the relationship was calculated to be probit y = 10.838 x - 17.274, log concentration and on R-square value $R^2 = 0.9192$.

This expression i.e. the regression equation, R^2 value indicates that mortality rate of fish increased with increase in concentration of formalin.

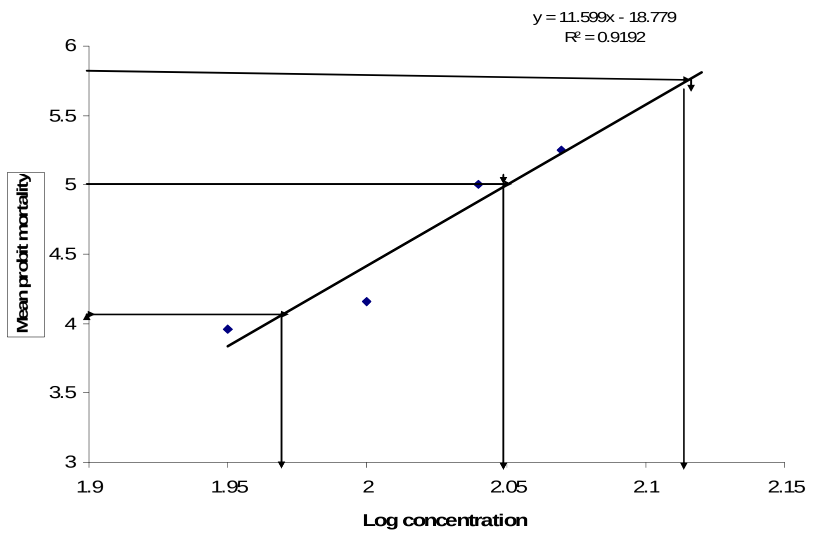

Fig 1: Linear Relationship between mean probit mortality and log concentration of C gariepinus juveniles exposed to formalin for 96 hours

During exposure, the test fish exhibited various behavioural patterns before death occurs; these include restlessness, respiratory distress, loss of balance, gulping for air, vertical movement and excessive accumulation of mucus. The reaction to the toxicant was more pronounced in the plastic basin containing the highest three concentrations of formalin. These fluctuated slightly except for the temperature that remained the same within the treatments. The dissolved oxygen values decreased slightly with increase in concentration of the toxicant. Free carbon dioxide, total alkalinity and ammonia increased slightly at higher concentration of the toxicant compared to the control with the highest values of concentration 130mg/l.

Discussion

The physicochemical parameters of the test solution fluctuated slightly during the bioassays but were not thought to have affected fish mortality since they were within the suggested tolerance range as prescribed by Mackereth (1963).

Table 3. The Mean water quality parameters obtained during exposure of *Clarias gariepinus* fingerling to formalin.

Parameters	Concentration (µl/l)					
	130	120	110	100	90	0
Temp (^oC)	28.07(2.19)	28.08(2.19)	28.03(2.17)	28.08(2.19)	28.10(2.19)	28.33(2.52)
Dissolved Oxygen (mg/l)	6.80(0.09	(6.90(0.05)	7.00(0.00)	7.15(0.06)	7.03(0.05)	7.48(0.05)
Total Alkalinity (mg/l)	34.05(0.05)	32.71(0.61)	32.78(0.63)	33.21(0.21)	32.99(0.67)	32.30(0.48)
Free CO_2 (mg/l)	4.01(0.06)	3.80(0.18)	3.86(0.07)	3.70(0.09)	3.83(0.05)	3.78(0.05)
Ammonia (mg/l)	0.24(0.01)	0.23)0.01)	0.22(0.00)	0.21(0.00)	0.21(0.00)	0.20(0.00)
pH	6.71(0.10)	6.83(0.05)	6.82(0.08)	6.85(0.11)	6.66(0.03)	7.19(0.06)

*Parentheses indicate standard deviation.

The LC_{50} value derived from the toxicity test revealed that *C. gariepinus* juvenile is sensitive to the formalin. The regression equation obtained by Kori-Siakpere (2008) when he exposed C. *gariepinus* fingerlings to potassium permanganate was y = 1.38 + 7.84x R^2 = 0.84, this difers from the one in Fig 1 of the present study. This disparity may be due to the difference in toxicant and weight of the fish though both equations indicated that the mortality rate of *C. gariepinus* juveniles and formalin concentrations were positively correlated increased mortality of the fish with increase in the concentration of toxicants. The 96hour LC_{50} had earlier been reported for the African catfish, *C. gariepinus* by Onusiruka and Ufodike (1994), to be 25.71, 26.92 and 8.3mg/l for the floral parts of Akee Apple (*Blighia sapida),* the bark of sausage plant (*Kigelia African)* and the block of *Blighia sapida* respectively. While Oronsaye and Ogbebo (1995) reported that LC_{50} (96hour) for copper sulpahte to be 0.4mg/l for *C. gariepinus.* Also, Ayuba and Ofojekwu (2002) reported the 96hours LC_{50} of *C. gariepinus* fingerlings exposed to *Datura innoxia* root extracts to be 204.17 mg/L with lower and upper confidence limits being 125.89 and 384.59 mg/L respectively. The difference in the result of the present study and those of these researchers may be due to difference in toxicants, the age of the fish and environmental conditions.

The restlessness, loss of balance, erratic swimming, respiratory distress and vertical movement and death, in this investigation is in agreement with the findings of Wise *et al.* (1987), , Okwuosa and Omoregie (1995) Omoregie *et al.* (1998) and, Ayuba and Ofojekwu (2002) when they exposed fish to acute concentrations of different toxicants.

In this investigation, mucus accumulation was observed on the body surfaces and gill filament of dead fish. This is in consonance with Annune, *et al*. (1994) and Buchmann *et al*. (2004) mucus accumulation results from increase in the activity of mucus cells due to subsequent exposure to pollutants. This results in an increase in the production and accumulation of mucus over the body surface and gills of the fish which reduced respiratory activities of fish as reported by Konar (1970).
The effects of a toxicants on any aquatic organism may be acute (toxic effect produced within a short period of time, usually 96 hours or less); chronic (toxic effect as a result of bioaccumulation over several exposures); sub chronic (toxic effect manifesting few days after short period of exposure) or delay toxicity (toxic effect manifesting several years after exposure). In the present study, the fish has started showing gradual symptom of toxicity through the unusual and erratic swimming behavior in water which eventually led to death.

Conclusion

The 96 hour LC_{50} for the African catfish (*C. gariepinus*) juvenile exposed to formalin has been determined to be 112.20 mg/l with lower and upper confidence limits of 95.09 and 132.40 mg/l respectively. Therefore, fish farmers should use formalin in fish treatment with caution.

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