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Research Article

Ameliorative Potential of *Moringa oleifera* Leaf Extract on Acute Toxicity induced by Cassava Wastewater Exposure in Fingerlings *Clarias gariepinus*

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ABSTRACT

The study investigated the acute toxicity of the lethal doses and the use of *Moringa*, *Moringa oleifera* leaf extract MLE to ameliorate the toxicity of cassava, *Manihot esculenta* waste water CWW 0.01 mg/L, 0.02 mg/L, 0.03 mg/L, 0.04 mg/L, 0.05 mg/L and 0.00 mg/L of clean water exposed to 360 fingerlings of *Clarias gariepinus* 2.00 ± 0.02 g, 2.50 ± 0.10 cm in a static fisheries laboratory bioassay, Enugu lat. 7.4 N; 80 7'5 and long 60 8'E. 70 6' W for 96 hours. The acute concentrations were ameliorated with 2.00 mg/L MLE and replicated thrice in 18 plastic bowls. Exposed fish indicated stress characteristics such as erratic movements, air gulping and immobilization, loss of balance and period of quiescence before death. Dose dependent ventilation rate and tail beat frequency were indicated at the onset of the experiment but declined towards the end of the exposure period. The 96 hours LC_{50} was determined to be 0.02 mg/L but was ameliorated to be 0.045 mg/L when MLE was added. The test toxicant not exceeding the safety value of 0.0002 mg/L should not be allowed into the aquatic environment meant for the culture of fingerlings of the test fish except where 2.00 mg/L MLE is applied to reduce the toxicity to 0.00045 mg/L of CWW. The characterization and quantification of the coagulant properties of MLE are suggested in further studies.

Keywords: Acute toxicity; Amelioration; Cassava wastewater; *Moringa* leaf extract

INTRODUCTION

Cassava is known to be one of the most important energy food sources in developing countries and is also presently found to be important in advanced societies owing to its raw material advantage in industries, and The increased patronage makes it inevitable to be taken out of the environment and especially in the aquatic sector where its effluent wastewater is often channelled into the receiving bodies of water with little or no pretreatment to reduce the lethal effects on fish and other aquatic organisms [1-3]. Several workers have reported the acute toxicity of cassava wastewater but the amelioration or remediation using natural means appears to be scarce [4-7]. The use of *Moringa* seed extract MSE has been reported on amelioration of various pharmaceutical and other chemical effects on fish but its use of the leaf to remediate or reduce lethal toxicity of cassava wastewater on exposed fish is underreported [8-10].

The objective of the present study is therefore to investigate the 96 h LC_{50}, the safety level and the use of MLE to ameliorate the acute toxicity level of CWW on fingerlings of *Clarias gariepinus* exposed for 96 hours. Most fish farms and hatcheries in rural African communities are located near slow-flowing/controlled flowing bodies of shallow water/channeled to the farms due to the low availability of quality water [11]. Several activities, especially cassava fermentation, go unchallenged upstream, creating a need to purify such water before it is put into use in the fish culture/hatchery. The scenario is feasible by controlled floatation and purification of the contaminated river water with MLE before releasing it to the fish farm/hatchery [12]. It is possible to treat cassava-contaminated river water by controlled floatation/holding a known amount with sandbags and treating the same with 2 mg/L MLE for 4 days to achieve a 100% reduction of toxicity of the CWW before releasing the wastewater to the farm.

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MATERIALS AND METHODS

Experimental fish and CWW

A total of three hundred and sixty (360) fingerlings of African catfish (mean weight 2.00 ± 0.02 g, mean length 2.50 ± 0.10 cm) were obtained from the local outskirts in Enugu Nigeria and transported to the fisheries wet laboratory of the department of animal/fisheries science and management, Enugu State University of science and Technology ESUT lat. 7.4 N; 80 7'5 and long 60 8'E. 70 6' W, Enugu Nigeria. They were held in four Fibre-Reinforced Plastic (FRP) tanks, containing 500 L of de-chlorinated tap water. Aeration was provided to all tanks round the clock to maintain dissolved oxygen contents [13-16]. Before the commencement of the study, the fish were acclimatized for two weeks and were fed a commercial fish diet composed of 40% crude protein. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Cassava wastewater was collected from a local 'garri' factory while the leaf extract of *Moringa* was prepared according to standard procedure and was dissolved in distilled water to make a stock solution that was used in the study. Ethical clearance from the Enugu state university of science and technology committee on experimental animal care was obtained and followed [17].

Acute toxicity test

The toxicity of CWW to *C. gariepinus* was carried out according to the OECD guideline for testing chemicals no. 203 in a semi-static renewal system by using 200 L capacity glass aquaria. Five different concentrations (0.01, 0.02, 0.03, 0.04, 0.05) and control 0.00 mgL^{-1} were selected and prepared in triplicates for definitive exposures after a range-finding test and ten (10) fish were exposed to each replicate. One group was exposed to clean freshwater which served as control. Feed was not offered to the fish for 96 h of the test period [18-20]. Dead fish were immediately removed to prevent deterioration of water quality. The exposure solution was renewed each day and was also conducted under the natural photoperiod of 12:12 light-dark cycle and the physicochemical parameters of the test water were analyzed daily, using standard methods 11 and were recorded (dissolved oxygen 4.48 ± 0.22-7.40 ± 0.45 mg L^{-1} temperature 27.70°C ± 0.5°C, pH 4.7 ± 0.3-7.8 ± 0.13 and free carbon dioxide 4.24 ± 0.6 mg L^{-1}). The test fish were sampled on hours 24, 48, 72 and 96 in each replicate to determine the toxic effects of CWW on the fish. 2 mg/L of MLE was added to each lethal dose and exposed for the same period to estimate its possible use to ameliorate CWW acute toxicity level on test fish. The behavioural response in exposed and control fish were observed and recorded daily. The LC_{50} was determined by probit analysis. The safe level was estimated by applying the safety Application Factor (AF).

Statistical analysis

Data were expressed as mean ± standard error and were analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in the test concentrations and control were subjected to a one-way Analysis of Variance (ANOVA), followed by Duncan range tests to determine significant mean differences.

RESULTS

The behavioural changes of the test fish exposed to acute doses of CWW are shown in Table 1, while Tables 2 and 3 represent the mortality rates followed by their probit 96 h LC_{50} values during acute and amelioration levels in Figures 1 and 2 respectively.

Table 1: Behavioral changes of fingerling of *C. gariepinus* exposed to CWW.

Parameters	Concentration Mg/L	Period (hours)			
		24	48	72	96
Erratic movements	0.01	-	-	+	+
	0.02	-	-	+	+
	0.03	-	-	+	+
	0.04	-	-	++	+++
	0.05	-	-	++	+++
Air gulping and immobilization	0.01	-	-	+	+
	0.02	-	-	+	+
	0.03	-	-	+	+
	0.04	-	-	++	+++

	0.05	-	-	++	+++
Loss of balance and period of quiescence	0.01	-	-	+	+
	0.02	-	-	+	+
	0.03	-	-	+	+
	0.04	-	-	++	+++
	0.05	-	-	++	+++
Opercular ventilation	0.01	+	-	-	-
	0.02	++	+	-	-
	0.03	++	++	+	-
	0.04	+++	++	++	+
	0.05	+++	++	++	+
Tail beat frequency	0.01	+	+	-	-
	0.02	+	+	-	-
	0.03	++	+	-	-
	0.04	+++	++	++	+
	0.05	+++	++	++	+
Parameters, erratic movements, air gulping and immobilization, opercular ventilation loss of balance and period of quiescence, tail beat frequency.	0	-	-	-	-
	0	-	-	-	-
	0	-	-	-	-

Key: - none; + mild; ++ moderate; +++strong; ++++ very strong

Behavioural changes

Exposed fish to acute concentrations of CWW for 96 h indicated a varying degree of the behavioural disorder before death such as exposed fish indicated dose and time-dependent stress such as erratic movements, air gulping and immobilization, loss of balance and period of quiescence before death (Table 1), hyperventilation and increased tail fin frequency (Table 2). The opercular ventilation and tail beat frequency however declined with period and indicated dose-dependent response but no visible abnormal behavioural disorder was observed in the control group of fish during the study.

Table 2: Mortality rate of *Clarias gariepinus* fingerlings exposed to CIWW.

Concentration Mg/L	24 h	48 h	72 h	96 h
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0.01	1	1	1	1
0.01	1	1	1	1
0.01	1	1	1	2

0.02	1	1	1	1
0.02	1	1	1	2
0.02	1	1	2	2
0.03	1	1	1	2
0.03	1	1	2	2
0.03	1	1	2	3
0.04	1	1	2	2
0.04	1	1	2	2
0.04	2	2	2	2
0.05	1	2	2	2
0.05	1	2	2	2
0.05	1	3	1	2

Concentration mg/L	Log concentration	Replicate 1	Replicate 2	Replicate 3	Cumulative mortality
0.01	-2	3	4	5	12
0.02	-1.69	4	5	6	15
0.03	-1.52	5	6	7	16
0.04	-1.39	6	6	8	20
0.05	-1.3	7	7	7	21

Table 2: Mortality rate of Clarias gariepinus fingerlings exposed to CIWW.

Median lethal concentration and safety level amelioration of lethal with MLE

The LC_{50} value was the 96 hours LC_{50} was determined to be 0.02 mg/L (y=1.1546 ×+7.0323, R^2=0.9753) Figure 1 but was ameliorated to be 0.045 mg/L (y=7.3888+15.36, R^2=0.9161) when MLE was added (Figure 2).

0.00+2mg/L MLE	0	0	0	0
0.00+2mg/L MLE	0	0	0	0

0.00+2mg/L MLE	0	0	0	0		
0.01+2mg/L MLE	0	0	0	0		
0.01+2mg/L MLE	0	0	0	0		
0.01+2mg/L MLE	0	0	0	0		
0.02+2mg/L MLE	0	0	0	1		
0.02+2mg/L MLE	0	0	0	1		
0.02+2mg/L MLE	0	0	0	1		
0.03+2mg/L MLE	0	0	1	2		
0.03+2mg/L MLE	0	0	1	2		
0.03+2mg/L MLE	0	0	2	2		
0.04+2mg/L MLE	0	1	2	2		
0.04+2mg/L MLE	0	1	1	2		
0.04+2mg/L MLE	0	2	1	2		
0.05+2mg/L MLE	0	2	2	2		
0.05+2mg/L MLE	0	2	2	2		
0.05+2mg/L MLE	0	3	1	2		
Concentration mg/L	Log concentration	Replicate 1	Replicate 2	Replicate 3	cumulative Mortality	% mortality
0.01	-2	0	0	0	0	0
0.02	-1.69	1	1	1	3	10
0.03	-1.52	3	3	4	10	33.33
0.04	-1.39	5	4	5	14	46.66
0.05	-1.3	6	6	6	18	60

Table 3: Mortality rate of *Clarias gariepinus* fingerlings exposed to CWW ameliorated with aqueous leaf extract of *Moringa* MLE.

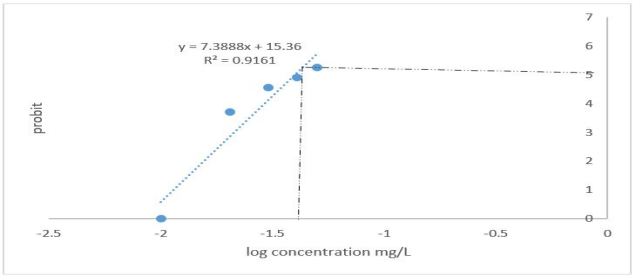

Figure 1: Logarithmic probit line of 96 h LC_{50} of CWW ameliorated with *Moringa* before exposure to fingerlings of *C. gariepinus* gave a value of 0.045 mg/L.

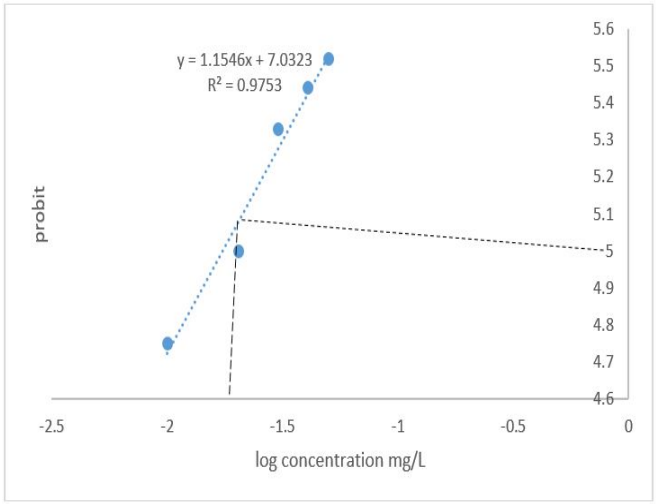

Figure 2: Logarithmic probit line of 96 h LC_{50} of CWW ameliorated with *Moringa* before exposure to fingerlings of *C. gariepinus* gave a value of 0.045 mg/L.

The introduction of CWW into the water environment of the exposed fish initiated a dose-dependent dexterity for attempted escape from the holding container. These responses indicated that the CWW caused some stress to the fish since the control group did not make any attempt to escape. The various behavioural responses observed in this study corroborate with earlier observations. The observed dose and time-dependent trends in opercular ventilation and tail beat frequency are indications that the respiration and energy level of the fish was hampered by the toxicant. The likely water quality effect on pH and DO could be due to the toxicant bioavailability in water which is in tune with other reports. The dose-dependent mortality rate of the exposed fish might be due to abnormal alterations in water quality parameters as well as the nervous disorderliness of the fish's biological system. This is in agreement with previous studies which suggested that alterations in the phospholipid composition of the plasma membrane may have contributed to neural injury associated with cyanide-induced hypoxia. The 96 h LC_{50} value in this study is somewhat in agreement with who reported a value of 0.024 mg/L but it is lower than the value of 9.7 mg/L and 4.28 mg/L reported by Izah SC, et al. The differences in reported 96 h median lethal concentrations values and the present may be due to respective age and species variation.

Moringa seed was earlier suggested to be suitable as a natural component of water treatment. It is a good bio-coagulant and efficient water purifier owing to its water-soluble proteins that play the role of effective coagulation and water treatment and even for the removal of bacteria from wastewater. The reported amelioration of toxicity and mortality by extracts of *Moringa* leaf in the present study may be due to the coagulation action which therefore moderated the water quality to a less toxic level, more amenable to the fish. The leaf extract of *Moringa* is a good source of phenolic compounds and beta carotenes 30 creating an electronic charge that causes the CWW particles to cluster by flocculation-coagulation and adsorption mechanisms respectively to bring solid, liquid and molecular ions of the pollutants to the surface, thus leaving a less toxic and filtered proportion beneath to the exposed fish, absorb the cyanide and other toxic components of cassava and lead to its decreased toxicity to fish, although the mechanism of action is multifaceted. This important observation is suggested for use in the tropics where most of the locals use the same body of controlled slow-flowing river/stream water for drinking, fisheries and the fermentation of cassava tubers. The need however to quantify and characterize the coagulants, flocculants and adsorbents available in the leaf is desirable in future studies.

CONCLUSION

The high toxicity of Cassava wastewater to the fingerlings of *Clarias gariepinus* can be reduced from 0.02 mg/L-0.45 mg/L CWW by the application of 2 mg/L MLE/L of CWW for 4 days. The need to characterize and quantify the coagulant properties and mechanism of action of the MLE is suggested in future studies.

CONFLICT OF INTEREST

The authors declared no potential conflict of interest concerning the research, authorship and or publication of the article.

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REFERENCES

1. Nitayavardhana S, Shrestha P, Rasmussen ML, Lamsal BP, van Leeuwen JH, Khanal SK. Ultrasound improved ethanol fermentation from cassava chips in cassava-based ethanol plants. Bioresour Technol. 2010;101(8):2741-2747.
2. Prachumchai R. Cherdthong A. Wanapat M, So S, Polyorach S. Fresh cassava root replacing cassava chip could enhance milk production of lactating dairy cows fed diets based on high sulfur-containing pellet. Sci Rep. 2022;12(1):1-9.
3. Oghenejoboh KM. Effects of cassava wastewater on the quality of receiving water body intended for fish farming. Br J Appl Sci. 2015;6(2):164.
4. Adewoye SO, Fawole OO, Owolabi OD, Omotosho JS. Ethiop J Health Sci. 2005;28(2):189-194.
5. Asogwa CN, Ezenwajiaku FO, Chidinma Adanna OKOLO, Ekeh FN, Nwibo DD, Chukwuka CO. Behavioural and biochemical responses of juvenile catfish (*Clarias gariepinus*) exposed to graded concentrations of cassava wastewater1. Anim Res Int. 2015;12(1): 2136-2142.
6. Izah SC, Enaregha EB, Episode JO. Changes in in-situ water characteristics of cassava wastewater due to the activities of indigenous microorganisms. MOJ Toxicol 2019; 5(2):78-81.
7. Scheierling SM, Bartone C, Mara DD, Drechsel P. Improving wastewater use in agriculture: An emerging priority. World Bank policy Res Work Pap. 2010(5412).
8. Bijina B, Chellappan S, Krishna JG, Basheer SM, Elyas KK, Bahkali AH,. Protease inhibitor from *Moringa oleifera* with potential for use as therapeutic drug and as seafood preservative. Saudi J Biol Sci. 2011;18(3):273-281.

9. Suhartini S, Hidayat N, Rosaliana E. Influence of powdered *Moringa oleifera* seeds and natural filter media on the characteristics of tapioca starch wastewater. Int J Recycl Org Waste Agric. 2013;2:1-1.
10. Ugwu SN, Umuokoro AF, Echiegu EA, Ugwuishiwu BO, Enweremadu CC. Comparative study of the use of natural and artificial coagulants for the treatment of sullage (domestic wastewater). Cogent Eng. 2017;4(1):1365676.
11. Izah SC, Bassey SE, Ohimain EI. Impacts of cassava mill effluents in Nigeria. J Anim Ecol. 2018;1(1):14.
12. Wade JW, Omoregie E, Ezenwaka I. *Toxicity of cassava(Manihot esculenta Crantz) effluent on the Nile tilapia, Oreochromis niloticus (L.) under laboratory conditions*. J Aquat Sci. 2002;17(2):89-94.
13. Oseni KA. Respiratory surveillance And caplus-ATPase enzyme activity studies of *Clarias gariepinus* exposed to acute toxicity of cyanide. Int Lett Nat Sci. 2016(50).
14. Airaodion AI, Ene AC, Ogbuagu EO, Okoroukwu VN, Ekenjoku JA, Ogbuagu U. Biochemical changes associated with consumption (by rats) of "garri" processed by traditional and instant mechanical methods. Asian J Biochem. 2019;2(4):1-1.
15. Adeyemo OK. Haematological and histopathological effects of cassava mill effluent in Clarias gariepinus. Afr J Biomed Res. 2005;8(3):179-183.
16. Awotwi A, Bediako MA, Harris E, Forkuo EK. Water quality changes associated with cassava production: Case study of white Volta basin. Heliyon. 2016;2(8):e00149.
17. Adekunle IM. Arowolo TA. Omoniyi IT. Olubambi OT. Risk assessment in Nile tilapia (*Oreochromis niloticus*) and African mud catfish (*Clarias gariepinus*) exposed to cassava effluent. Chem Ecol. 2007;23(5):383-392.
18. Olayinka AS. Assessment of toxic potentials of cassava effluent on *Clarias gariepinus*. Int J Agric Sci. 2013;3(3):157.