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Comparative Effects of the Acute Toxicity of Clove (*Eugenia Aromatica*) Powder to *Clarias Gariepinus* and *Heterobranchus Bidorsalis* Fingerlings

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Abstract: *The study was carried out to determine the effects of acute toxicity of clove powder to African catfish species commonly reared in Nigeria. Two fifty fingerlings each of C. gariepinus (mean weight 3.26± 2.30g and length 8.34 ±1.82cm), and H. bidorsadis (mean weight 2.96± 1.80g and length 6.55 ±2.84cm) were exposed to various concentrations (0, 50, 60, 70, 80, 100 and 120) for 96hours. The sequential behavioral responses (Opercular and tail beat frequencies) and cumulative mortality were recorded at the various intervals of 12, 24, 48, 76 and 96hours. Data obtained were subjected to a two way analysis of variance(ANOVA) and mean separation using Duncan multiple range test DMRT with statistical package for social science (SPSS). The responses of the OBF and TBF were similar in the both species, they were decreasing with increase in concentration and time. The mean values were slightly higher in C. gariepinus than H. bidorsalis. Mortality increased with increase in concentration and time for the both species. The 96hrLC50 for C. gariepinus (54.33mg/l) was slightly higher than that of H. bidorsalis (52.97mg/l). The MLT50 values of the decreased with increased in concentration and slightly higher in C. gariepinus than H. bidorsalis. The higher LC50 values of clove powder is an indication that it is less toxic to clariids, hence can be used to completely immobilized (anaesthetized) the fish in concentrations lower than 60mg/l without causing mortality.*

Keywords: *comparative toxicity, clove powder, behavioral responses, mortality and African catfishes.*

1. INTRODUCTION

Plants part have been shown to cause death of fish and changes in behavioural, haematological, biochemical responses and histopathological effects on clarids [1] [2][3]. Change in water quality by the presence of contaminants makes it potentially harmful to life, instead of sustaining them [4]. The accumulation of toxicants in an aquatic environment can result in reduced reproductive capacities; alter growth rates and reduced ability to with stand variation in pH, temperature and dissolved oxygen [5]. Plants are inexhaustible sources of biological active substances and more than 60,000 species have been reportedly used all over the world for various purposes [6]. According to Wang and Huffman [7] ichthyotoxins plants contains different active ingredient such as nicotine, pyrethrum, rotenone, resins, tannins, alkaloids, flavenoids and saponins. Studies by several authors have shown that herbal plant and their products have been used as natural alternatives for treatment and management of various diseases including dental decay [8], pesticide [9], molluscicide [10], Anaethetic [11] [12] and Piscicide [13] [14]. The piscicidal and medicinal activities of some plant extract have been reported by various researchers [5] [15] [3] [16]. Piscicidal plants such as *Blighia sapida*, *Kegelia biglobosa*, *Lepidagathis alopercuriodes* and *Agave americana* are commonly used by fisher folks to harvest fishes [17] [18] [19]. Yadav [20] reported that *Agave americana* has been by fishermen in catching fish while Obumanu *et al* (2007) reported the use of *L. alopercuroides* for the quick kill of hardy mudskipper fish. Ichthiotoxins have also been reportedly used when estimating fish stock [21] [16]. Assessment of the effects of these biocides on fish indicates that exposed fish demonstrated altered behavior, disruption of the internal biochemical and physiological processes which may lead to death [17] [2] [3]. Behavioural responses (Opercula and Tail beat frequencies) and mortality have been used to assess the potential hazard of a number of environmental toxicants to the clarids and have been proven reliable indicators of stress [22] [1] [3].

Clove are use in Indian ayurveodic medicine, Chinese and western herbalism. It has been use as carminative to increase hydrochloric acid in the stomach, natural anthelmintic and applied to decay tooth cavity [23]. It is regarded as one of the world best spice and anaesthetics. Phytochemical screening of *E. aromatic* shows the presence of saponin, alkaloid, tannin, flavenoids and glycoside [24]. In spite of it medicinal and culinary importance, cytotoxicity and mortality have been reported in some organisms due to the presence of alkaloids, saponin and tannin [25]. This study was conducted to compare toxicological effects of *E. aromatica* to two important African cultured fish species, *C. gariepinus* and *H. bidorsalis* fingerlings.

2. Materials and Methods

Dry flower bud of *E. aromatica* were procured from a herbal shop in Watt market Calabar, Calabar South Local Government Area of Cross River Nigeria. The material was identified at the Department of Botany University of Calabar. The clove buds were sundried for 30 minutes and then pulverized with a sterile manual blender, sieved with 100micron net to obtain a fine powder and stored in an airtight container prior to the experiment. Two hundred and fifty apparently healthy fingerlings each of *C. gariepinus* and *H. bidorsalis* were obtained from University of Calabar Fish Farm, Calabar. There were transported in jerry cans to Fisheries Labouratory, Cross River University of Technology (CRUTECH) Obubra campus. The fish were acclimated to labouratory conditions for 7 days in rectangular plastic aquaria (30 x 25 x 25cm^3) with 20L of River water. The fish were not fed 24hrs before the commencement of the treatment following the procedure in APHA [26]. The mean body weight and length for *C. gariepinus* (mean weight 3.26± 2.30g and length 8.34 ±1.82cm), and *H. bidorsadis* (mean weight 2.96± 1.80g and length 6.55 ±2.84cm) were taken using weighing balance and measuring board respectively. The water quality parameters were determined following the methods of APHA, [26] and recorded as follows: Temperature (28.13±1.70^{0}C), Dissolved Oxygen (4.74±0.27mg/l), pH (6.78±0.28), alkalinity (37.41± 045mg/l) and hardness (39.32±0.81mg/l). Twenty one (21) aquaria were used each holding 10 fingerlings. The fishes were exposed to graded concentrations (50, 60, 70, 80, 100 and 120mg/l) of clove powder solution prepared from solution of 200mg/l. after introduction of the desired concentrations in the aquarium, it was properly mixed to avoid hot spots. Ten fish were randomly added to the aquarium containing 20L of water and the substance. The solution and the control were renewed daily and the experiment lasted for 96 hours. Behavioral changes (Opercula and Tail beat frequencies) and cumulative mortality at 12, 24, 48, 72 and 96 hour were recorded. Any fish that refused to respond to prodding is consider dead and was removed immediately to avoid contamination of the test solutions.

Statistical analysis: data (OBF, TBF and Mortality) obtained were analyzed with a two – way analysis of variance (ANOVA) using SPSS version 25. The differences among means with in the species were separated at 5% significant level (P< 0.05) by honest significant difference (HSD). Mean comparism of the species on the various parameters was done using T – test. The lethal concentrations (LCs) of the clove powder and the median lethal times (MLT) from the exposure time of the fish were determined by probit analysis [27] [28].

3. Results

The water quality parameters recorded at the control did not differ (P > 0.05) from those of the test solutions, although the slightly decreased with increase in concentrations. The behavioral responses and mortalities of both species were similar and showed a declined with increase in exposure duration and concentrations. The responses of the OBF and TBF declined with increase in concentration of clove powder and duration of exposure in the both species (Tables 1 and 2). The mean values of the OBF in *C. gariepinus* were higher (P < 0.05) than those of *H. bidorsalis* from 70 – 120mg/l of clove powder. However, the TBF did not differ (P> 0.05) in the species on the various concentration of clove. Mortality in the both species at the various concentrations of clove powder did not differ (P> 0.05), except from 100mg/l which was higher (P<0.05) in *C. gariepinus* than *H. bidorsalis*. The responses of the OBF and TBF against time showed and inverse relationship in the both species. The mean value decreases with increase in time of exposure. The values in *C. gariepinus* were higher (p <0.05), except at the 12th and 96th hours. The TBF though higher in *C. gariepinus* were not at (P< 0.05) and cumulative mortality of both species on the various interval were not significant (p < 0.05) except at 72nd and 96th hours.

Table1. *The mean values of behavioural responses (OBF and TBF/min) and mortality of C. gariepinus and H. bidorsalis fingerlings on the various levels of Clove*

Conc. (mg/l)	Behavioural Responses and Mortality (%)					
	OBF		TBF		Cum. Mort.	
	C.gariepinus	*H.bidorsalis*	*C.gariepinus*	*H.bidorsalis*	*C.gariepinus*	*H.bidorsalis*
00	114.32±1.84^a	107.16±1.69^a	33.96±0.97^a	36.42±1.02^a	0.00±0.00^a	0.00±0.00^a
50	110.04±1.84^a	100.04±1.61^a	34.22±4.05^a	31.42±1.02^a	0.00±0.00^a	2.00±1.38^a
60	109.92±1.87^a	101.69±1.97^a	32.60±4.55^a	33.20±1.05^a	10.00±1.63^a	9.33±1.38^a
70	101.40±1.83^a	85.61±1.83^b	33.55±4.24^a	27.29±1.21^b	24.67±1.63^a	18.67±1.63^a
80	97.76±1.83^a	88.99±1.61^b	31.01±1.27^a	27.47±4.14^a	44.67±1.63^a	38.33±1.55^a
100	99.44±2.37^a	90.96±2.18^b	31.44±1.06^a	29.04±1.31^a	56.67±1.23^a	42.22±1.73^a
120	107.95±2.90^a	90.72±2.27^b	31.55±1.52^a	30.56±1.61^a	66.67±2.58^a	50.00±2.19^b

Means with the same superscript under each of the variables are not significantly different (p>0.05). OBF-opercular beat frequency, TBF-tail beat frequency, Cum. mort. - Cumulative mortality

Table2. *The mean values of behavioural responses (OBF and TBF/min) and mortality of C. gariepinus and H. bidorsalis fingerlings on the durations*

Time (hr)	Behavioural Responses (min) and Mortality (%)					
	OBF		TBF		Cum. Mort.	
	C.gariepinus	*H.bidorsalis*	*C.gariepinus*	*H.bidorsalis*	*C.gariepinus*	*H.bidorsalis*
12	113.35±1.55^a	102.67±1.43^a	34.25±0.84^a	37.11±0.86^a	10.0±1.38^a	10.48±1.17^a
24	107.76±2.55^a	97.11±1.43^b	34.21±0.84^a	31.46±0.86^a	23.33±1.38^a	19.05±1.17^a
48	104.37±1.68^a	91.44±1.54^b	33.10±2.20^a	28.98±0.93^a	25.56±1.49^a	22.78±1.39^a
72	105.29±1.84^a	93.89±1.69^b	31.65± 0.97^a	27.93±1.02^a	28.67±1.63^a	20.67±1.38^b
96	95.97±1.84^a	92.39± 1.89^a	29.34± 0.97^a	26.56±1.14^a	36.00±1.14^a	26.00±1.14^b

Means with the same superscript under each of the variables are not significantly different (p>0.05). OBF-opercular beat frequency, TBF-tail beat frequency, Cum. mort. - cumulative mortality.

The overall interactions of the behavioural changes (OBF and TBF) and percentage mortality of the species after 96hours revealed that only OBF of *C. gariepinus* was higher (P< 0.05), TBF and cumulative mortality did not differ (P> 0.05) in the both species (Figure 1)

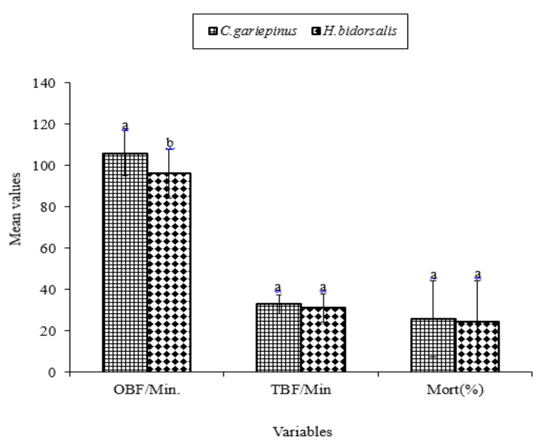

Figure1. *Comparison of opercular beat frequency, tail beat frequency and cumulative mortality (%) of C. gariepinus and H. bidosarlis fingerlings exposed to Clove powder for 96hrs. Bars represent mean ± S.D. Mean with different letters differ significantly (P < 0.05; HSD test).*

The predicted linear equations of the relationship between the dependent variables (OBF, TBF and Cumulative mortality) and independent variables (concentration and Time) showed that all the species were responsive to the toxicant. The pattern of relationship was similar in the both species and equations of the OBF and TBF had negative (-ve) 'b' values while cumulative mortality (%) had a positive (+ve) 'b' value. However, *C. gariepinus* had a higher R^2 value which is an indication of better relationship on the variables than *H. bidorsalis* and OBF had a higher R^2 value indicating a better tool for assessing behavioral responses.

Table3. *Predicted equations for the relationship between the independent (Concentration and Time) and dependent variables (OBF and TBF min^{-1})*

Variables		Species	Prediction Equation (Y = a + bX)	R^2
Independent	Dependent			
Concentration	OBF	*C. gariepinus*	Y = 115.21 – 0.158X	0.788
	OBF	*H. bidorsalis*	Y = 105.94 – 0.159X	0.597
	TBF	*C. gariepinus*	Y = 32.40 – 0.026X	0.578
	TBF	*H. bidorsalis*	Y = 34.85 – 0.059X	0.488
	Mort	*C. gariepinus*	Y = -9.83 + 0.478X	0.809
	Mort	*H. bidorsalis*	Y = -11.37 + 0.500X	0.789
Time	OBF	*C. gariepinus*	Y = 113.86 – 0.169X	0.848
	OBF	*H. bidorsalis*	Y = 100.67 – 0.103X	0.601
	TBF	*C. gariepinus*	Y = 35.45 – 0.059X	0.947
	TBF	*H. bidorsalis*	Y = 35.85 – 0.108X	0.800
	Mort	*C. gariepinus*	Y = -11.91+ 0.254X	0.841
	Mort	*H. bidorsalis*	Y = -12.62 + 0.142X	0.706

Y= dependent variable, X = independent variable, a = intercept, b = slope, OBF= opercula beat frequency, TBF= Tail beat frequency, Mort.= mortality

The LC_{50}, safe concentrations and toxicity factor (T.F) of Clove powder for *C. gariepinus* and *H. bidorsalis* fingerlings were relatively closed. The values in *C. gariepinus* were slightly higher than those of *H. bidorsalis* at the various intervals (Table 4). The 12hrLC_{50} of *C. gariepinus* (96.53mg/l) is 1.09 times higher than that of *H. bidorsalis* (88.27mg/l) but reduces to 1.03 times at the 96hr$LC_{50.}$ The relative toxicity factor of the both species showed a similar pattern, decreasing with increase in exposure time and concentration respectively. The 96hrLC_{50} (54.33mg/l) was 1.78 times more toxic than at 12hrsLC_{50} (96.56mg/l) for *C. gariepinus*. The ratio of 96hrLC_{50} (88.27mg/l) to 12hrLC_{50} (52.97mg/l) in *H. bidorsalis* (1.67times) was slightly lower than that of *C. gariepinus*. The 12hr LC_{50} toxicity factor was 1.84 and 1.60 times less than the 96hr LC_{50} for *C. gariepinus* and *H. bidorsalis* fingerlings respectively.

The time required for 50mg/l to kill 50% of *C. gariepinus* fingerlings in 96hrs (96hr MLT_{50}) was 101.55hrs which, was lower than that in *H. bidorsalis* (96hr MLT_{50}) 116.90hrs at the same concentration. The MLTs and relative toxicity time (R.T) of *C. gariepinus* were lower than those of *H. bidorsalis* (Table 5). The MLT_{50} at 120mg/l was 8.38 and 6.66 hours less than the time required to kill 50% of the fish at 50mg/l of clove powder for *C. gariepinus* and *H. bidorsalis* respectively. The MLT_{50} values of *C. gariepinus* were lower than that of *H. bidorsalis* in all the concentrations of clove powder.

Table4. *Comparative LC_{50}, associated 95% confidence limits, safe concentration and toxicity factor of Clove powder to C. gariepinus and H. bidorsalis fingerlings for 96hr exposure.*

Time (hr)	***C. gariepinus***			***H. bidorsalis***		
	LC_{50} (mg/l)	Safe Conc. (mg/l)	T F	LC_{50} (mg/l)	Safe conc. (mg/l)	T.F
12	96.56 (92.07–98.49)	9.65	1	88.27 (84.94–92.05)	8.83	1
24	78.29 (74.23– 75.36)	7.83	1.23	75.18 (69.55– 86.76)	7.52	1.17
48	75.36 (69.78– 161.32)	7.54	1.28	68.46 (64.99– 72.16)	6.85	1.29
72	58.57 (41.16– 61.40)	5.86	1.34	53.16 (60.04– 54.97)	6.32	1.40
96	54.33 (50.93– 55.33)	5.23	1.84	52.97 (50.80– 59.11)	5.50	1.60

LC= Lethal Concentration, conc. = concentration, T.F= Toxicity factor =LC_{50} value at 12hrs ÷ LC_{50} value of any other periods

Table5. *Comparative Mean Lethal Time, associated 95% confidence limits and relative toxicity of C. gariepinus and H. bidorsalis fingerlings exposed to Clove powder for 96hrs*

Conc. (mg/l)	***C. gariepinus***			***H. bidorsalis***		
	MLT_{50}	MLT_{95}	RT	MLT_{50}	MLT_{95}	RT
50	101.55(97.80-109.36)	122.51 (113.13-146.72)	1	116.90 (98.15-226.35)	167.36 (128.10-531.43)	1
60	69.17 (0.00-0.00)	79.66 (0.00-0.00)	1.47	73.15 (60.71-91.83)	126.36 (103.32-184.22)	1.60
70	50.63 (0.00-0.00)	60.66 (0.00-0.00)	2.01	52.40 (43.77-66.46)	80.02 (66.15-121.92)	2.23
80	22.08 (0.00-0.00)	26.21 (0.00-0.00)	4.60	17.27 (0.00-0.00)	47.90 (0.00-0.00)	6.77
100	12.11 (1.78-19.64)	15.89 (0.00-0.00)	8.39	9.28 (0.00-0.00)	32.87 (0.00-0.00)	12.60
120	8.38 (121-139)	12.17 (78-96)	12.12	6.66 (0.00-0.00)	17.67 (0.00-0.00)	17.55

MLT=Mean Lethal Time, R.T=Relative Time=MLT_{50} values at 60mg/l ÷ MLT_{50} value at any other concentrations

4. DISCUSSION

The water quality parameters of the various test media did not vary significantly ($P < 0.05$) from those of the control. Since the parameters examined were within acceptable ranges [29] [26] hence, they may not have acted synergistically with the toxicant to affect the behaviour as well as mortalities recorded in the various fish species. Similar observation were recorded by Onusiriuka and Ufodike [30] in the acute test solutions of Akee apple, *Blighia sapida* and sausage plant, *Kigelia africana* extracts to which *C. gariepinus* was exposed and Okey, [31] in acute test solutions of *L. alopecuroides* exposed to *C. gariepinus*, *H. bidorsalis* and their hybrid fingerlings. *Clarias gariepinus* and *H. bidorsalis* fingerlings at the various concentrations of the Clove powder were stressed progressively with time before death. The stressful behaviour included loss of balance, erratic swimming, air gulping, sudden quick movement, secretion of mucus, rolling movement and hypersensitivity. Several workers who studied a number of fish species including *C. gariepinus*, *H. bidorsalis* and their hybrids [32] [33] [31] [3] have reported similar findings. The stressful behaviour of clariids which tend to show the toxic effect have also been reported in *C. gariepinus*, exposed to aqueous extracts of *Blighia sapida* and *Kigelia africana* [31]. The various concentrations of Clove powder caused changes in the behavoiurs (Opercular and Tail beat frequencies) of the exposed fish species. The abnormal behavioural responses of these clariids in this study were similar to that earlier reported by Omoniyi *et al*. [34], Gabriel and Okey, [31] and Okey *et al*. [3] on various clariids exposed to plant derived toxicants. These behavioural changes may be attempts to escape or avoid the toxicants. The stressful behavioural responses of the fish may also be because of respiratory impairment due to the effect of the component of the extracts such as saponins, flavoniod, tannins and alkaloids on the gills, skin and general metabolism of the exposed fishes. These observations compared favourably with that works of previous authors who assessed the acute effects of plant extract on fishes; *Thevetia peruviana* on *C. gariepinus* [35], *Azadirachtha indica* on *Hyperopsis bebe occindentalis* [36], Cassava leaf extract on *C. angularis* [37] and *Carica papaya* (Pawpaw) seed powder on *Clarias gariepinus* [38]. The initial increased in OBF and TBF might be an attempt by the fish to increase gaseous exchange across the gills to enhance internal metabolic rate. This can also lead to increase energy supply due to internal hypnotic condition created by the toxicant [39]. However, with continued depletion in available oxygen and store energy in the organism, the beats gradually decline. Similar observations were recorded by several workers on African catfishes exposed to various xenobiotics [1] [2] [3]. Literatures have also reported that increase utilization of energy substances (carbohydrate, protein and lipids when fish is under xenobiotic leads to stress [40] [41]. Fatigue due to exhaustion may have accounted for the death recorded in the exposed fish. The mortality rate was concentration and time dependent, suggesting that the degree of exhaustion due to depleted energy sources may have been raised by increasing the concentration and exposure durations.

The positive "b" (slope) value for the predicted linear equations of the species on concentration and time on mortality is an indication that mortality increased with increased in concentration and time.

This is in agreement with other researchers who reported a positive "b" value for mortality and negative "b" value for OBF and TBF against concentration and time [2] [14] [39]. The R^2 value of the various parameters shows a higher values in *C. gariepinus* than in *H. bidorsalis* except the "a" (intercept) which was higher in *H. bidorsalis*. This showed that *C. gariepinus* was more responsive and less susceptible to the toxicant. According to Giorgi *et al*. [42], differential responses of organism to toxicant may be genetically mediated. Dutt *et al*., [43] reported that concentration, species, size and specific environmental conditions can effect fish responses to toxicants, which is in accordance with the observations in these study.

The study also showed a relatively similar LC_{50} values for the both species but *H. bidorsalis* suggest to be slightly susceptible to clove powder at 96hr LC_{50}, *C. gariepinus* (71.25mg/l) and *H. bidorsalis* (70.06mg/l). The trends shows a decrease in the LC values of as the duration of exposure increases. This agrees with the reports of several workers who have exposed fishes to toxicants [44] [45] [1] [3] [39]. The toxicity factor for 12hrLC_{50} (88.56mg/l) was 1.63 times larger than 96hr LC_{50} (54.33mg/l) for *C. gariepinus* while in *H. bidorsalis* 12hr LC_{50} (96.56mg/l) was 1.84 times larger than 96hr LC_{50} (52.33mg/l). The smaller the concentration the more toxic the substance. This implies that 96hr LC_{50} of clove on the species was more toxic than the 12hr LC_{50}. The 96hr LC_{50} reported for these species was higher than 0.65mg/l and 0.59mg/l reported for *C. gariepinus* and *H. bidorsalis* repectively on *L. alopercuriodes* [2]. Velisek *et al.*, [46] reported a lower 96hrLC_{50} value of 18.4mg/l for European catfish, Silurus glandis exposed to clove oil. Other researchers have also reported a lower 96hrLC50 value on African catfishes exposed to various toxicants [33] [30] [34]. This shows that clove is less toxic to African catfishes compared to *L. alopercuriodes*, *Kigela africana* and cassava effluent. The trend of the Mean Lethal time (MLT) values showed that increased concentration decreases the time at 50% mortality. However the at 120mg/l, the MLT_{50} was 8.38 and 7.06 hours for *C. gariepinus* and *H. bidoraslis* respectively. The higher the time at 50% mortality the more hardy the fish to the toxicant. The MLT_{50} at 120mg/l for *C. gariepinus* is about 1.27 hours higher than that of *H. bidorsalis* but about 12.12 and 17.55 hours less than the MLT_{50} at 50mg/l. Similar differential tolerance of fish species to toxicant have been reported by Keremah *et al*. [2] and Gabriel and Edori [39] on African clariids.

Death of exposed fish may have resulted from the interference of the toxicant with normal functioning of the nervous system which impaired the normal muscular activity and coordination [47]. Death may also be due to disruption and failure in gill functions hence, reducing availability of surface for gaseous and ionic exchange [48]. Clove powder is less toxic compared to some biocides and could be safely applied to immobilize the fish without causing death in small quantity. Hence, reason for it's used as anaesthetics for various fishes especially its oil (Eugenol) extracted from clove plant.

5. CONCLUSION

The study showed similar behavioural pattern and mortality on the clove powder concentration and duration of exposure. The *C. gariepinus* was more responsive to the toxicant with higher OBF, TBF and mortality. The examined parameters showed inverse relationship with concentration and time of exposure. The values of the 96hrLC_{50} for the species showed they are not significantly different ($p>0.05$) and the 96hrMLT_{50} at the 160mg/l was slightly higher in *H. bidorsalis*. The higher LC values recorded for this plant implies it can be used as anaesthetic to completely immobilized fishes during some aquacultural operations.

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