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Immobilization and survival rate of the life stages of African catfish (*Clarias gariepinus*) exposed to clove (*Eugenia caryophyllata*) powder

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Abstract
This study investigated the anaesthetic effects of clove (*Eugenia caryophylatta*) powder on immobilization and survival in different life stages of *Clarias gariepinus*. Four hundred and fifty (450) fish comprising of 150 each of mean weight: fingerlings (6.54 ± 3.25g), juveniles (15.06 ± 2.50g) and adults (32.10 ± 4.30g) were used for the study. The fish were exposed to 100, 120, 140, 160 and 180mg/l of the anaesthetic prepared from a stock solution of 200mg/l of the clove powder. Ten (10) fish each were use per tank in triplicate for each life stage. The time to attain various stages of anaesthesia and recovery was noted and recorded. Mortality for each life stage was also recorded. Some water quality parameters were monitored and recorded. The powder was observed to cause anaesthesia which was dependent on the concentration and sizes of the exposed fish. The time to attain deep anaesthesia (stage A3) decreased with increasing concentration in all the life stages but longer in adults than juveniles and fingerlings. The shorter the time to achieve stage A3 the longer the time to be fully recovered. The relationship of the time taken to achieve deep anaesthesia and regained full recovery is significantly (P<0.05) dependent on the concentration of clove, with total recovery time inversely proportional to the total time to attained deep anaesthesia. The survival rates of 86.67 and 96.67% were recorded for fingerlings and juveniles respectively in higher concentrations of the anaesthetic while in the adults was 100%. The study revealed that clove bud can effectively be ultilized as anaesthetics agent in *C. gariepinus* at optimal dose of 160mg/l.

Keywords: anaesthesia, recovery, mortality, African catfish and clove buds

Introduction
Anaesthetics are used in aquaculture and fisheries to facilitate various routine procedures that can often cause physical injury and induce physiological stress [1]. They are also use to immobilized fish so they can be handled more easily during harvesting, sampling and spawning procedures. Husen and Sharma [2] stated that sedatives and anaesthetics are very useful in aquaculture as they reduce fish activity, limit oxygen consumption and facilitate routine handling and veterinary procedures. Anaesthesia is a biological reversible state induced by an external agent, which results in the partial or complete loss of sensation or loss of voluntary neuromotor control, through chemical or non-chemical means [3]. Anesthesia is frequently applied in aquaculture being a valuable tool that helps to minimize fish stress and to prevent physical injuries to fish. According to Maricchiolo and Genovese [4], anaesthesia is required for measuring or weighing fish, sorting and tagging, administrating vaccines, live transport, sampling for blood or gonadal biopsies and collecting of gametes, surgical procedures, to cite some of the main applications.
Knowledge about the ideal and optimum concentration of anesthetics for various fish species is necessary because inappropriate concentrations may lead to adverse effects such as stress; therefore, access to safe and effective fish sedatives is a critical need of fisheries researchers, managers, and culturists [5]. The choice of anaesthetics is usually related to several factors such as availability, economic viability, practicality of use, efficacy, user safety and chemical substances approved by regulatory agencies in animals for human consumption [6, 7]. However, most desirable attributes of anaesthetics used for fin fish include short induction and recovery time, non-toxicity to fish and humans, no lasting physiological effects, rapid clearance from the body, high solubility in fresh and salt water and cost effectiveness [8]. Recovery should be attained within few minutes to prevent stress and harmful effects on the fish [9].

According to [8] the most desirable recovery is set to be retained within 5 minutes'. After anaesthetics procedure the fish is recommended to be under closer observation for 24 – 72 hours as death can occur [1]. Mortality of fish exposed to anaesthetic depend on a number of factors which include duration of exposed, concentration of the substance, species and size of the exposed fish.

Varieties of anaesthetics (natural and synthetics) are widely used in the laboratories, aquaculture activities and veterinary procedures to mitigate stress. Most commonly used are metomidate, benzocaine, etomidate, MS. 222 (Tricaine methane sulphonate), 2- phenoxyethanol, quinaldin and eugenol [1, 10, 11]. Synthetics anaesthetics such as metomidate, benzocaine, etomidate, 2 – phenoxyethanol and MS- 222 used in aquacultural operations have some challenges such as poor solubility, long induction and recovery time, highly toxic and low dose, health risk to humans and high cost of the chemicals [12, 13]. In recent times organic farming provided alternatives for those chemicals that are currently being used in aquaculture and anaesthetics are one of such important input, hence different natural anaesthetics were investigated to compare their effectiveness with chemical products [14]. Plant extracts from *Deris* (rotenone), *pyrus*, tobacco, mustard seed, clove, *Ocimum*, and mentha have been reported to induce anaesthesia with little or no side effects on fishes [15, 16, 17, 18, 19]. Clove products (leaves, stems, flower and buds) contains mainly eugenol (4- ally- methoxyphenol) and (4- propenyl- 2 – methoxyphenol) have shown to be potential and effective anaesthetic for food fishes [20, 21, 22, 23]. Studies have shown sizes, body weight, species, environmental conditions and pharmacokinetics of the anaesthetic agent influence its efficacy [24, 25, 17].

African catfishes constitutes the largest group of cultured species after carp, salmonids, tilapias and they grow well under various culture systems of the world [26]. Their culture is becoming more popular among retirees civil and public servants in Cross River, where they are transported from hatcheries sites to grow out ponds and research laboratories [27]. Previous studies on the use of clove flower buds powder as anaesthetic on *Clarias gariepinus* and *Heterobranchus bidorsalis* juveniles and fingerlings on different concentrations have been reported [28, 29]. Information on the anaesthesia and survival rate of the different life stages (fingerling, juveniles and adults) of *C. gariepinus* exposed to the same concentration of clove powder anaesthestics is still scanty. Therefore this study is aimed to determine the optimal dosage for induction, recovery and survival rate of the various life stages of *C. gariepinus* on clove powder anaesthetic.

Materials and Methods

Study Location and Experimental Fish

The study was conducted at the Department of Fisheries and Aquatic Science, Faculty of Agriculture, Cross River University of Technology, Obubra campus. Four hundred and fifty (450) comprising of 150 each of mean weight and length: fingerlings (6.54 ± 3.25g), juveniles (15.06 ± 2.50g) and adults (32.10 ± 4.30g). Were procured from Ezema fish farm in Ikom, Cross River State. They were acclimated at Fisheries wet laboratory for 14 days during which they were fed with Coppens feed at 5% body weight and discontinuous 24 hours prior to the commencement of the bioassay.

Collection and preparation of Clove powder: Dry flower buds of clove was purchased from a herbal shop in watt market Calabar Cross River, Nigeria. The product was authenticated at the Department of Forestry and Wildlife Faculty of Agriculture and Forestry CRUTECH, Obubra campus. The buds were sundried for 30 minutes and then pulverized with a manual blended and sieved with 100 micron net to obtain a fine powder. The powder was stored in an airtight container prior to the commencement of the experiment.

Experimental procedure: Two (2g) grams of the powder was weighed and dissolved in 10 litres of water to prepare a stock solution of 200mg/l. Exposure concentrations of 100, 120, 140, 160 and 180mg/l was prepared from serial dilution of the stock solution in triplicates to 40L plastic aquaria. The mixtures were stirred thoroughly to ensure homogeneity of the test solution. Ten (10) fish of the same life stage were randomly selected into the test aquaria and monitored for the onset of the various stages of induction and recovery and recorded for 30 minutes according [30]. Three (3) behavioral stages each were identified for induction and recovery according to [31].

Table 1: Stages of Anaesthesia and Recovery of the various life stages of *C. gariepinus* to clove powder anaesthetic

Stages	Condition	Behaviour/ Response
Anaesthesia A)		
A1	Light sedation	Partial loss of muscle tone and erratic swimming
A2	Light anaesthesia	Complete loss of balance, slow ventilation or opercula rate
A3	Deep anaesthesia	Total loss of muscle tone and reaction to handling or external stimulus.
Recovery (R)		
R1	Initial recovery	Commencement of opercula movement.
R2	Partial recovery	Return of erratic swimming, irregular balance and partial return to equilibrium
R3	Full recovery	Normalization of equilibrium, swimming and responses to tactile stimulation.

Source: Coyle *et al.* [31], Modified by Gressler *et al.*, [32]

Survival Rate

The number of death fish during the period of anaesthesia, recovery and 24 hours after the completion of the anaesthesia bioassay were noted and recorded according to Ross and Ross (2008). This was used to compute the survival rate (%) according to Adikwu, [33]

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the final}}{\text{Initial number of fish}} \times 100$$

Water Quality Parameters

The temperature of the media was taken using a mercury in glass thermometer, pH values were determined using pH meter, Dissolved Oxygen was determine using Dissolved oxygen meter inserted into the sample glass tanks after standardization in three different buffers. Conductivity was measured with conductivity meter (PACM 35 model) and total hardness by ethylene diamine tetra acetic acid titration method.

Statistical Analysis

The data collated was subjected to two way analysis of variance using a statistical software SPSS version 25 to compute for the significant differences among the variables of clove powder, stages of anaethessia, recovery and life stages of the experimental fish. The differences among the means were compared using Turkey's multiple comparison test at 5% significance level according to [43]. Regression analysis was computed to determine the linear relationship between independent variable (concentration) and dependent variables (deep anaesthesia and full recovery time) according to [35]. Linear equations was predicted for time to achieve deep anaesthesia and regain full recovery from clove anaesthetic.

Results

Water Quality

The result of the water quality parameters (Table 2) indicates that the mean values did not differ significantly (p>0.05) from those of the control in the various life stages of the exposed fish. However, dissolved oxygen, temperature, pH and hardness all decreased slightly with increasing concentration. Conductivity increased slightly from 136.07± 2.04 in the control to 137.05± 2.71 at 180mg/l tank whereas the mean value of pH (6.78± 0.28 to 6.57± 0.22) was relatively closed.

Table 2: Water quality parameters of the test solutions for *C. gariepinus* exposed to Clove powder anaesthetic for 30 minutes (mead ± SD)

Conc. (mg/l)	Parameter					
	DO (mg/l)	Tempt. (ºC)	pH	Cond. (µS/cm)	Alk. (mg/l)	Hardness (mg/lCaCO$_3$)
0	4.74±0.27^a	28.13±1.07^a	6.78±0.28^a	136.07±2.04^a	37.41±0.45^a	39.32±0.81^a
100	4.37±0.35^a	26.87±1.25^a	6.73±0.27^a	136.51±1.31^a	38.18±1.96^a	37.61±0.58^a
120	4.48±0.12^a	27.37±0.78^a	6.69±0.41^a	135.96±1.32^a	40.76±2.69^a	38.33±0.54^a
140	4.38±0.36^a	26.67±1.02^a	6.69±0.16^a	136.79±2.14^a	36.75±0.85^a	38.72±0.83^a
160	4.38±0.38^a	27.74±0.91^a	6.67±0.19^a	137.23±2.50^a	38.67±1.05^a	38.26±0.33^a
180	4.12±0.12^a	26.88±0.19^a	6.57±0.22^a	137.05±2.71^a	38.51±0.47^a	38.80±0.85^a

Mean with the same superscript in the same column are not different (P>0.05, DO = dissolved oxygen, Tempt. = Temperature, Cond. =Conductivity, Alk. =alkalinity.

Anaesthesia and Recovery

The result of the mean time for the various stages of anaesthesia and recovery on the life stages of *C. gariepinus* exposed to clove powder anaesthetics is presented in tables 3 – 5. The result showed that clove powder resulted in different stages of anaesthesia and recovery times depending on the dosage and sizes of the exposed fish. The lower the dosage the longer the time in the various stages of anaesthesia (induction) and recovery. Fingerlings exposed to 180mg/l took only about 0.51 mins to be completely immobilized (A3, deep anaesthesia) and 30. 45 mins to fully recover (table 3). In juveniles 180mg/l caused deep anaesthesia (A3) in 2.25 mins and fully recovers at about 15.55mins (table 4) while adults 6.43 mins was required to attain complete immobilization and fully recovers in 8.52 mins (table 5). The shorter the time required for the onset of anaesthesia the longer the time to attained full recovery. In fingerlings the time to attain each stage of anaesthesia reduced significantly (p<0.05) with increasing concentration whereas that of recovery increased significantly (p<0.05) from 100 – 180mg/l. similar trend was obtained in juveniles and adult *C. gariepinus* exposed to clove anaesthetic.

Table 3: The mean time (mins) for anaesthesia and recovery of *C. gariepinus* fingerlings exposed to clove powder for 30 mins

Conc. (mg/l)	Stages Anaesthesia (A)			Stages of Recovery (R)		
	Sedation (AI)	Light Anaethesia (A2)	Deep Anaesthesia (A3)	Initial Recovery (R1)	Partial Recovery (R2)	Full Recovery (R3)
100	4.33± 0.09^a	5.76± 0.27^a	7.06± 1.50^a	1.65± 0.18^d	2.03± 0.45^e	4.25± 1.34^d
120	2.56± 0.18^b	3.15± 0.12^b	4.83± 0.75^b	2.86± 0.54^d	4.07± 1.04^d	5.87± 0.65^d
140	1.07± 0.54^c	1.86± 0.15^c	3.05± 0.45^b	5.02± 0.25^c	10.01± 1.02^c	14.50± 0.84^c
160	0.20± 0.25^c	0.30± 0.05^d	1.15± 0.20^c	11.55± 1.05^b	17.83± 2.04^b	27.52± 2.15^b
180	0.00± 0.00	0.00± 0.00	0.51± 0.02^d	16.55± 1.18^a	23.58± 2.05^a	30.45± 2.74^a
Means with the same superscript under the same columns are not significant at 5%						

Table 4: The mean time (mins) for anaesthesia and recovery of *C. gariepinus* juveniles exposed to clove powder for 30 mins

Conc. (mg/l)	Stages Anaesthesia (A)			Stages of Recovery (R)		
	Sedation (AI)	Light Anaethesia (A2)	Deep Anaesthesia (A3)	Initial Recovery (R1)	Partial Recovery (R2)	Full Recovery (R3)
100	15.14± 1.25^a	20.51± 2.06^a	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
120	7.33± 1.07^b	9.06± 0.45^b	11.48± 0.15^a	1.85± 0.74^c	2.46± 0.22^d	4.40± 0.24^d
140	5.62± 0.65^c	7.44± 0.56c	9.03± 0.08^b	2.64± 0.06^c	4.05± 0.35^c	7.23± 0.47^c
160	2.02± 0.55^d	3.58± 0.35^d	5.48± 0.55^c	4.13± 0.75^b	7.08± 0.98^b	13.58± 1.18^b
180	1.08± 0.35^d	1.95± 0.93^e	2.25± 0.47^d	6.24± 0.24^a	9.11± 0.11^a	15.55± 0.56^a
Means with the same superscript under the same columns are not significant at 5%						

Table 5: The mean time (mins) for anaesthesia and recovery of *C. gariepinus* adults exposed to clove powder for 30 mins

Conc. (mg/l)	Stages Anaesthesia (A)			Stages of Recovery (R)		
	Sedation (AI)	Light Anaethesia (A2)	Deep Anaesthesia (A3)	Initial Recovery (R1)	Partial Recovery (R2)	Full Recovery (R3)
100	18.34± 0.24^{a}	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
120	10.58± 0.05^{b}	19.08± 0.18^{a}	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
140	7.73± 1.02^{c}	10.22± 0.54^{b}	13.43± 0.72^{a}	1.34± 0.02^{b}	2.68± 0.10^{b}	3.04± 0.22^{c}
160	5.28± 0.55^{d}	7.58± 0.47^{c}	9.52± 0.36^{b}	2.56± 0.70^{b}	4.08± 0.18^{a}	6.35± 0.67^{b}
180	4.08± 0.56^{d}	5.57± 0.35^{d}	6.43± 1.18^{c}	4.42± 0.92^{a}	5.56± 0.22^{a}	8.52± 0.65^{a}
Means with the same superscript under the same columns are not significant at 5%						

The time to achieved deep anaesthesia in fingerlings were shorter but with longer full recovery time when compared to those required by juveniles and adults respectively (Figure 1).

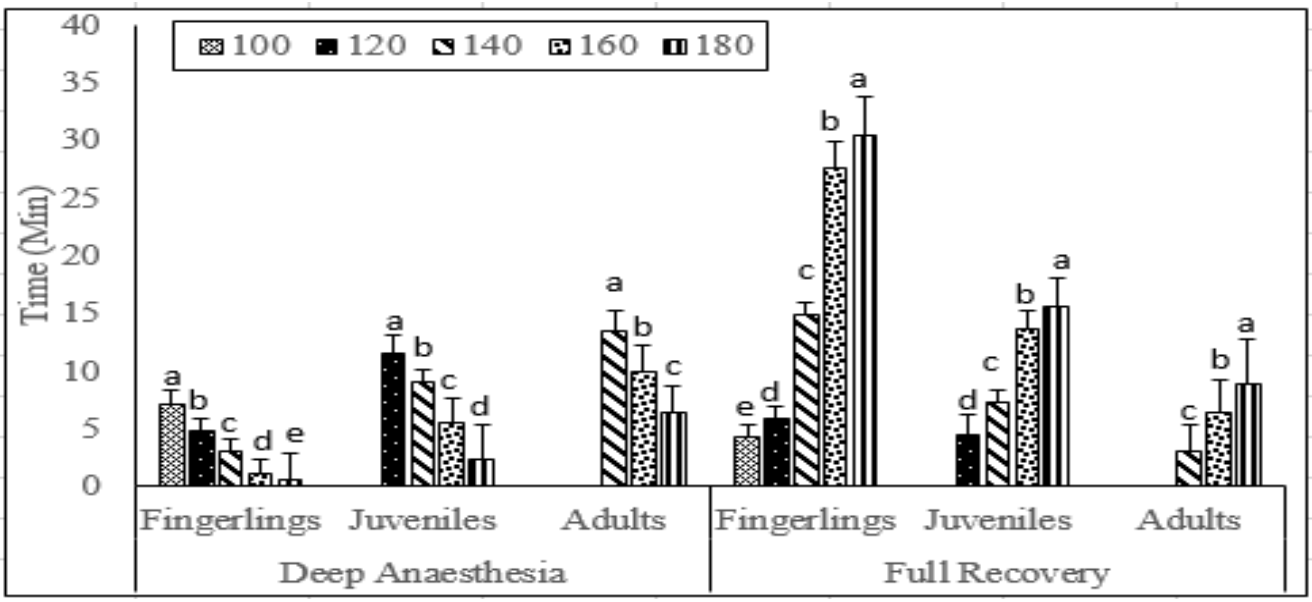

Fig 1: Deep anaesthesia and full recovery time in the life stages of *C. gariepinus* exposed to clove powder anaesthetic for 30 mins. Bar with different letter differ significantly at 5% (HSD)

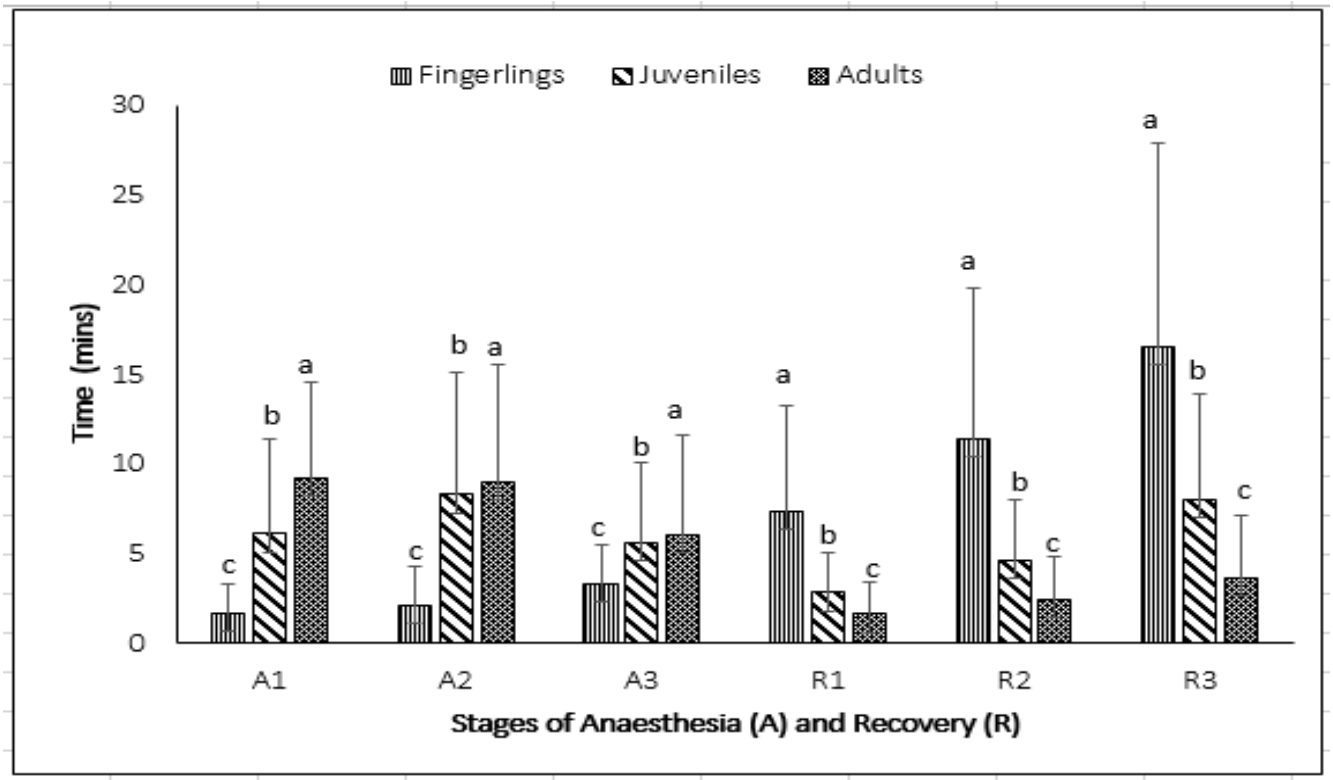

Fig 2: Comparison of the variuos stages of anaesthesia and recovery time (min) of the life stages of *C. gariepinus* exposed to clove powder anaesthetic for 30 mins. Bar with different letter differ significantly at 5% (HSD)

The predicted linear equations for relationship between independent (concentration of clove powder) and dependent (deep anaesthesia and full recovery time) variables for the various life stages of *C. gariepinus* is presented in Table 6. The result showed that the estimated time to attain deep anaesthesia and full recovery in all the life stages were significantly (p<0.001) dependent on the concentrations with higher R^2 values ranging from 0.94 to 1. The values of the unit increase in concentration will decrease the time to attain deep anaesthesia by 0.175, 0.156 and 0.084 min in adults, juveniles and fingerlings respectively. The predicted time to attain deep anaesthesia at zero concentration (without clove powder) was higher in adults (37.93 min), than in juveniles (30.49 min) and fingerlings (15.07 min). Similar pattern was observed in the time to regain full recovery from deep anaesthasia at zero concentration was higher in adults (-17.42 min) than juveniles (-19. 71 min) and fingerlings (-35.31 min). The unit increase in concentration resulted in an increase in the time to regain full recovery by 0.147, 0.200 and 0.371 for adults, juveniles and fingerlings respectively.

Table 6: Predicted equations for the relationship between independent variable (Concentration) and dependent variables (deep anaesthesia and full recovery time)

Variable		Life stage	Predicted equation (Y = a + bX)	R^2
Independent (X)	**Dependent (Y)**			
Concentration	Deep Anaesthesia	Adult	Y = 37.93 – 0.175X	1.00
		Juvenile	Y = 30.49 – 0.156X	0.99
		Fingerling	Y = 15.07 – 0.084X	0.97
	Full Recovery	Adult	Y = -17.42 + 0.147X	0.99
		Juvenile	Y = -19.71 + 0.200X	0.95
		Fingerling	Y = -35. 31 + 0.371X	0.94

The survival rates of exposed fish is presented in Figure 3. The result revealed that survival of the life stages were dependent on the concentration and sizes of the fish. The survival rates of 100, 96.67 and 86.67% were recorded for adults, juveniles and fingerlings respectively exposed to the various concentration (100 – 180mg/l) under investigation. The mortality rate recorded for juveniles was not significantly different (p>0.05) from those of the adults.

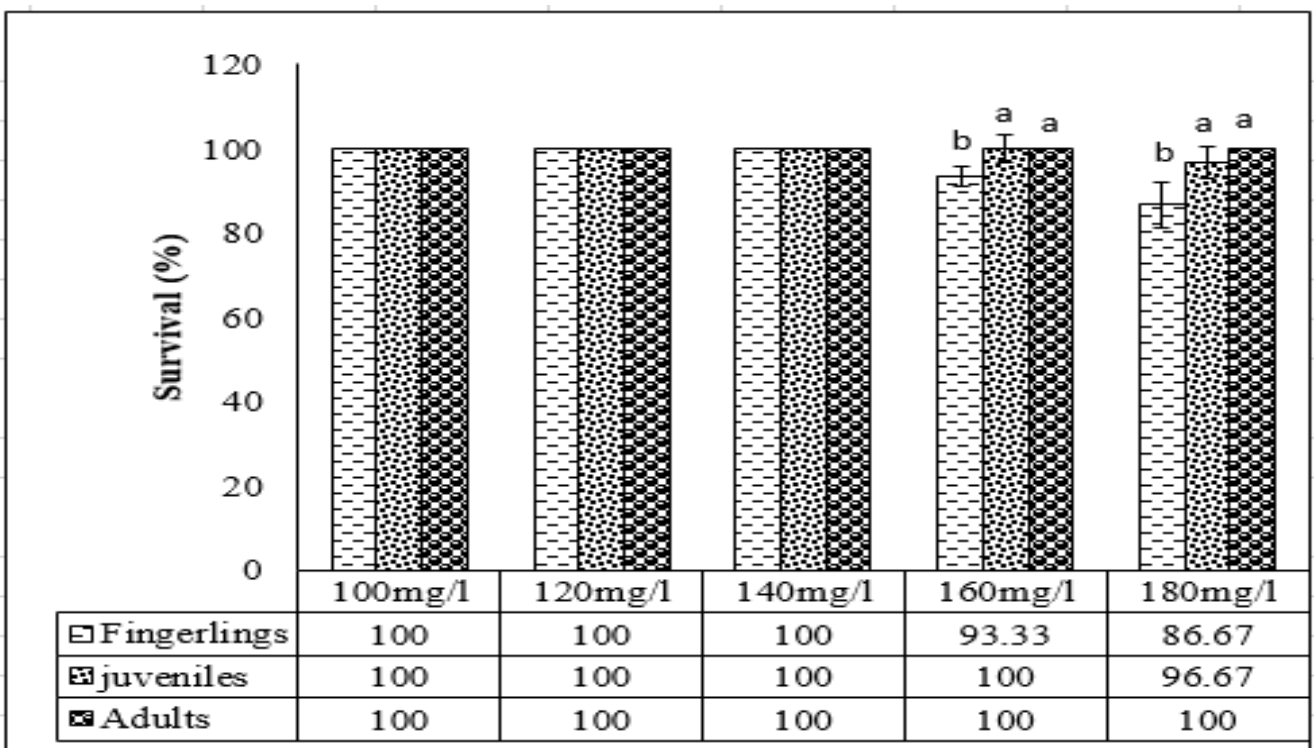

Fig 3: Survival rate (%) in the life stages of *C. gariepinus* exposed to clove powder anaesthetic for 30 minutes

Discussions

Water Quality Parameters

The water quality parameter of the test media did not differ (p>0.05) from those of the control tanks. The slight changes in some of the parameter were still within the acceptable and tolerable range for culture of African catfishes. This therefore may not have acted synergistically with the anaesthetic (clove) to cause the behavioural changes and culminated in anaesthesia and euthanasia in higher concentrations in this study. This findings agrees with [28, 36] on *C. gariepinus* juveniles exposed to clove seed and flower buds respectively.

Anaesthesia, Recovery and Survival

Anaesthetics are needed for easy handling, sorting, transporting and surgical procedures in aquaculture and fisheries [37]. Ideal anaesthetic ought to induce anaesthesia quickly in less than 6 mins at low concentration, recovers faster in less than 10 mins, less toxic to the exposed fish, not hazardous to human and be inexpensive [38]. The observed behavioural changes under the various stages of anaesthesia and recovery in this study appears to be concentration dependent. Shorter immobilization time was achieved at higher concentrations of the clove powder as was reported in

plant based anaesthetics used by other workers [40, 39, 36, 28, 21]. The progression through these various stages of anaesthesia and recovery was consistent with the descriptions by [42, 41, 39, 30]. According to [43, 3], the degree of anaesthesia is influenced by the concentration of anaesthetic in in the central nervous system (CNS) of the exposed organism. In this study fingerlings had lower induction and higher recovery times than juveniles and adults. This implies that the rate of absorption of clove powder (anasethetic) is faster in fingerlings than juveniles and adults. This agrees with the findings of [19] in *S. melanotheron* exposed to mustard seed and Okey [44] in fingerlings and juveniles of *C. gariepinus* exposed to different ranged of concentrations of clove powder. In this study rapid deep anaesthesia of less than 4 mins for fingerlings and juveniles was induce with higher concentrations 140 – 180mg/l. Sudagara *et al.* [20] found that 350mg/l of clove powder solution was required to completely immobilized *Rutilus rutilus* in less than 4mins while [6] reported a similar time with 80mg/l of clove oil on European catfish (*Silurus glandis* L) juveniles an described clove anaesthetic as effective and safe. several workers have also reported induction time of 2 – 8 mins using clove oil of 30 – 100mg/l on *Oncorhynchus mykiss* [41], *Anguilla reinliditii* [45], *Esox luctus* [42] and *Brycon cephalus* [46]. These range were lower than the effective dosage of 140 – 180mg/l of clove powder solution recorded for *C. gariepinus* in this study. However a much higher range have been reported by some researchers using plant extracts to induce anaesthesia in 2 – 6 mins with *Pyrus communis* on *C. gariepinus* [16], *Lippia alba* on silver catfish, *Rhamdia quelen* [47] and extracts from *Mentha piperita* on *Colossoma macropomum* [48]. The differences in the effective dosages can be attributed to the specific properties of the species and pharmacokinetics of the anaesthetic agents [25].

The relationship of the time taken to achieve deep anaesthesia and regained full recovery is significantly (P<0.05) dependent on the concentration of clove, with total recovery time inversely proportional to the total time to attained deep anaesthesia in this study. This finding is in agreement with previous works [49, 35, 17]. However, a lower R^2 value of 0.86 was predicted for *Bryzon amazonicus* juveliles with essential oil of *O. gratissimum* at 20 to 80mg/l [17] than the range of 0.94 to 1.00 predicted for the various life stages of *C. gariepinus* in this study. The predicted time to attain deep anaesthesia and full recovery were all higher in adults > juveniles > fingerlings. This implies that size played a prominent role in the absorption and assimilation of the anaethetic. Kucuk [24] reported that the gill area, body weight, species and metabolic rate have effects on the rate of absorption and induction of anaesthetics. Lower concentration of anaesthetics should be encouraged to provide a greater margin of safety for that animal to avoid expenses and waste of anaesthetic solution [40]. Some authors have also suggested that the criteria for determining anaesthetic efficacy include: anaesthesia in less than 6 mins, fast recovery of not more than 15 mins except for surgical operations, easy to handle, cheap, highly soluble in water and without mortality [11, 35]. Based on these criteria clove powder can effectively be use at 140 – 160mg/l for the life stages since the fish were completely immobilized in less than 2 – 9 mins and no significant mortality recorded for fingerlings at the higher concentration. Recovery time increases as induction time reduces with increase in concentration of clove powder in all the life stages in this study. The recovery time reported in this study was comparable to those reported by other researchers using various plant extracts on African catfishes [50, 29, 21, 16]. Soto and Burhanuddin [51] and Anderson *et al* [52] both used 120mg/l of clove oil and reported different recovery time of 150 sec in *Siganus lineatus* and 190 sec for rainbow trout respectively. The effective concentrations for fingerlings differ from those of juveniles and adults which could be attributed to the sizes of the life stages. This was in line with the findings of some workers who research on different life stages of fishes [28, 19, 6]. Celik and Yilmaz [53] also stated that the pharmacokinetics of the anaesthetics may cause differences among the duration of anaesthesia and recovery in animals.

After anaesthetic procedure the fish is recommended to be under closer observation for 24 – 72 hours as death can occur [1]. The zero mortality recorded for adults and 3% and 13% for juveniles and fingerling at higher concentrations is a proved the clove is an ideal anaesthetic for *C. gariepinus*. Similar trend in the survival rates was recorded for fingerlings and juveniles of *S. melanotheron* exposed to higher concentrations of mustard seed powder [19]. Death can be attributed to the fact that, at higher concentrations the fish was unable to counter the effects of the increase and self- mechanism against cell destruction.

Conclusion

Dry clove bud powder is effective, highly soluble in water, relatively safe especially at low dosage, economically sustainable and ecological friendly. This study proved that clove powder solution can effectively be ultilized to induce anaesthesia in all the life stages of *C. gariepinus* and very short time depending on the dosage. Fingerling and juveniles should not be exposed concentrations about 180mg/l to avoid significant mortality. Concentration of 140mg/l of clove powder is effective to completely immobilized al the life stages under investigation though at different intervals without mortality recorded. The use of clove powder should be encourage in fisheries and aquaculture in preference of the synthetic anaesthetics which and expensive, scares, and difficult to handle for sustainability of the industry.

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