International Journal of Fisheries and Aquaculture Research

Vol.7, No.2, pp.1-19, 2021

ISSN: ISSN 2397-7507,

Online ISSN: ISSN 2397-776

EVALUATION OF ACUTE TOXICITY AND ANAESTHETIC EFFICACY OF SCENT LEAF (*OCIMUM GRATISSIMUM*) IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*) JUVENILES

Okey, I. B[1]. and Igiri, M. R[2].

1. Department of Fisheries and Aquatic Science, CRUTECH Obubra Campus, Cross River, Nigeria.
2. Department of Forestry and Wildlife Management, CRUTECH Obubra Campus.

ABSTRACT: *This study assessed the acute toxicity and anaesthetic efficacy of scent leaf (Ocimum gratissimum) powder on Clarias gariepinus juveniles. Four hundred and fifty juveniles of C. gariepinus of average weight and length, 24.25g ± 4.25SD and 27.60cm ± 3.75SD were used for the study. Toxicity bioassay was conducted with 100, 150, 200, 250mg/l and 0.00mg/l as control at the rate of 10 fish per tank in triplicates for 96 hours. Anaesthetic bioassay was conducted with 100, 120, 140, 160 and 180mg/l of scent leaf with10 fish in each tank in triplicates for 30 min. fish mortality data was collected against the durations in the acute toxicity test while data on time of anaesthesia and recovery at various stages were also noted and recorded. The Trimmed Spearman's Karber method was used to estimate the lethal concentration (LC) and mean lethal concentration (MLT) while data on anaesthesia and recovery time were subjected to analysis of variance followed by Turkeys test for significant differences at 5%. The LC_{50} and maximum allowable toxicants concentration values were observed to decrease from 12 to 96 hour. The 96hrLC_{50} value was estimated as 203.02mg/l which was observed as high. The MLT_{50} values decrease for 164.36 hours in 100mg/l to 126.34 hours in 250mg/l. water quality parameters did not vary significantly ($P > 0.05$) from those of control. Deep anaesthesia (A3) was achieve in all the concentrations except in 100mg/l which only achieve sedation (stage A1) and with no mortality observed during the experiment. The shortest time for deep anaesthesia (2.25 min) was observed at the concentration of 180mg/l. the shortest time (4.08 min) to regain equilibrium and swim normally was observed in 120mg/l. Both anaesthesia and recovery time were observed to be dependent on concentration. Scent leaf was effective to anaesthetize C. gariepinus juveniles in routine management procedures. This study therefore recommend the use of scent leaf at 160mg/l as effective dose when considering optimal induction and recovery time for sustainable aquaculture..*

KEYWORDS: lethal concentration, immobilization, recovery, scent leaf and African catfish.

INTRODUCTION

The toxicity and effects of anaesthetics are of special interest since they are frequently used in research and routine aquaculture procedure to immobilized fish and minimize their stress responses (King *et al.*, 2005). Toxicity refers to the degree at which a substance is being harmful, destructive or

poisonous to life. The lethal concentration (LC_{50}) refers to the dose of a toxicant which kills 50% of the sample population and is useful in predicting the effects of a potential toxin and the maximum allowable toxicant concentrations (MATC) in aquaculture system (Johnson and Finley 1980; Boyd 2005). In order to determine the relative toxicity of a new chemical for use as anaesthetic to aquatic animals, an acute toxicity test must be conducted to estimate the 50% lethal concentration (LC_{50}) and mean lethal time (MLT_{50}) values (Ross and Rose 1999). To evaluate the efficacy of a given substance as an anaesthetic to fish, it is necessary to know its toxicity level. Mortality is used to estimate the lethal concentrations (LCs) and median lethal time (MLTs) of the toxicant on the exposed fish. The relevant duration of short term toxicity testing is probably 24 to 96 hours as the case for many toxicants (Lewis and Morris, 1986). The 96hrsLC_{50} value was estimated for the following clove oil 0.24mg/l, cinnamon oil 0.57mg/l, *Zingiber cassumunar* 1.47mg/l and tobacco 6.51mg/l on *Eutroplus suratensis* (Sindhu 2015). Velisek *et al* (2005) and (2006) reported a 96hrsLC_{50} value of 14.10 and 18.40mg/l of clove oil on *Oncorhynchus mykiss* and *Silurus glandis* respectively. The 96hrsLC_{50} values of clove buds powder for *C. gariepinus* and *Heterobranchus bidorsalis* was reported as 71.25 and 70.06mg/l respectively (Okey, 2014; and Okey *et al* 2017). The mean lethal time (MLT) provides information on the pattern of death fishes with duration of exposure under acute toxicity of toxicant (Gabriel and Okey, 2009). The 96hrsMLT_{50} of the lowest (60mg/l) and highest (160mg/l) of clove powder on *C. gariepinus* juveniles were 54.49 and 8.09 hours respectively (Okey *et al* 2018). The higher the concentration the lower the time to achieve 50% mortality. The smaller the LC_{50} value the more toxic the substance and less suitable for use as anaesthetic. Okey (2006) reported a 96hrLC_{50} value of 0.65 and 0.59mg/l for *C. gariepinus* and *H. bidosarlis* respectively exposed to *Lepidagathis alopercuroides* leaves extracts and stated that it was highly toxic hence, not suitable for use as anaesthetic to African catfishes. Knowledge on the toxicity and anaesthesia (ideal and optimum concentrations) of plant extracts use as anaesthetic for *C. gariepinus* and other fish species will be necessary because inappropriate concentrations may lead to adverse effects on tissues and organs leading to stress and mortality (Hoseini *et al* 2011; Hoseini and Ghelichpour 2013).

Tremendous increase in aquaculture in recent years worldwide have subjected farmed fish to a number of stressors due to handling procedures and transportation from hatcheries to production sites (Husen and Sharma 2014; Weimin *et al* 2012). These stressors have often been responsible for high mortalities recorded hence, reducing profit margin, hindering growth and development of Fisheries and Aquaculture Subsector in Nigeria. Anaesthetics are used in aquaculture and fisheries to facilitate various routine procedures that can often cause physical injury and induce physiological stress (Ross and Ross 2008). They are also use to immobilized fish so they can be handled more easily during harvesting, sampling and spawning procedures. Sadatives and anaesthetics are very useful in aquaculture as they reduce fish activity, limit oxygen consumption and facilitate routine handling and veterinary procedures (Husen *et al* 2014). Anesthesia 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 (Summerfelt and Smith,

1990). Anesthestics are frequently applied in aquaculture being a valuable tool that helps to minimize fish stress and to prevent physical injuries to fish while handling them during routine practices. Anaesthetics are also use in aquaculture, fisheries and biological researches, as a way to reduce hypermotility, stress and mortality during handling and transportation (King *et al* 2005; Ross and Ross 2008; Okey *et al* 2013). 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 (Maricchiolo and Genovese, 2011). Knowledge about the ideal and optimum concentration of anaesthetics for various fish species is necessary because inappropriate concentrations may lead to stress therefore, access to safe and effective fish anaesthetic is a critical need of Fisheries researchers, Managers, and Culturists (Trushenski *et al*., 2013).

Conventional anaesthetics such as tricaine methane sulphonate (MS-222), benzocaine, metomidate, etomidate and quinaldine are hazardous, expensive, not very effective and readily available in developing countries (Munday and Wilson, 1996). Poor solubility in water, long induction and recovery time, strong odour and long withdrawal period before human consumption are the major drawbacks for some of these chemical anaesthetics (Yanar and Kumlu, 2000). Adewale *et al* (2017) stated that synthetic anaesthetics are discouraged because of their residual effects in the tissues of fish hence the increasing advocacy for organic aquaculture. Alternative anaesthetics for use on food fishes, which are effective and have a short depuration period and zero withdrawal period, would fill a priority need in Fisheries Science (Gilderhus and Marking, 1987). The use of natural plant extracts as anaesthetics should be encourage because they are cheaper, safer and highly biodegradable. Low doses of some extracts of plant material used as anaesthetics for fish have been experimented and found useful but with some drawback effects on the fish (Adebayo *et al.*, 2010; Agokei and Adebisi 2010). Keene *et al.*(1998) reported that Clove oil induced anaesthesia faster and at lower concentrations than MS -222, although efficacy of anaesthetics can be affected by species, body size, the density of fish in the bath as well as water quality (Woody *et al.*, 2002). Powder produced from clove plant (*Eugenia spp*) have also been used for short-term immobilization of fish, *Rutilus rutilus* (Roach) in Iran (Sudagara *et al.*2009) and clove powder on *C. gariepinus* in Nigeria (Akinrotimi *et al* 2014; Okey *et al* 2017). Previous studies have been reported on the use of certain plant materials to anaesthetized *C. gariepinus* (Agokei and Adebisi, 2010; Olufayo and Ojo 2018; Adebayo and Olufayo 2017) but with some reported degree of side effects on the exposed fish. Some other plant material reported to have anaesthetic effects on fish include *Datura innoxia ugenia aniophylun, Clotalaria sp, Derriss candens, Barringtonia raecemosa, Eryagau npoetidany, Anamirla cucculus, Caryphaambra culifera* (Ramanayaka and Atapatu, 2006), *Nicotiana tobaccum* (Agokei and Adebisi, 2010; Jegede, 2014), *Acorus calamus* (Agokei and Adebisi, 2010) and *Ocimum gratissumum* oil (Adewale *et al* 2017). The essential oil of *Ocimum gratissimum* have also been reported to cause anaesthesia on silver catfish juveniles (Silva *et al* 2012).

https://doi.org/10.37745/ijfar.15

The choice of anaesthetic is usually determined by several factors, such availability, economic viability, practicality of use, efficacy, user safety and chemical substances approved by regulatory agencies in animal for human consumption (Cho and Heath, 2000; Carniero *et al* 2019). Eugenol has been adjudged the best and recommended worldwide as anaesthetic on food fishes because of its zero withdrawal, short induction and longer recovery time ((Ucar and Atamanlp, 2010; Velisek*et al.*, 2005). Clove powder containing eugenol has been reported to be a very effective anaesthetic to fishes (Sudagara *et al* 2009; Okey *et al* 2013; 2018). Some essential oil containing certain quantities of eugenol obtained from various plant such as *Lippia alba* (Cunha *et al*., 2010), *Cinnamomum camphora* (Pedrazzani and Neto, 2016), *Mentha piperita* (Mazandarani and Hoseini, 2017) and *Syzygium aromaticum* (Javahery *et al*., 2012) have shown to cause anaesthesia to various fishes. Essential oil of *O. gratissimum* has been reported to contain about 60 – 70% of eugenol comparable to that of clove oil and caused anaesthesia on some test fishes (Silva *et al.*2012; Boijink *et al* 2016; Benovit *et al* 2016; Adewale *et al* 2017).

Ocimum gratsissimum (Lamiaciae), commonly known as scent leaf or clove basil is found in many tropical countries including Nigeria (Mbakwem-Amebo *et al* 2012). In Nigeria, the plant grows virtually in all regions, found in many farms, residential and industrial areas (Effraim *et al* 2000). The plant has been used for many purposes ranging from human consumption to its application in traditional medicine in Nigeria. It is used as condiment and as sedative for the treatment of stress, headache and other diseases including diarrhea, conjunctivitis, skin diseases and pneumonia (IIori *et al* 1996). Several studies have also shown various effects of *Ocimum* species to include bactericidal, ant-inflammatory, anti-fungal, anti- oxidative, antiulcer, hypoglycemic, nervous stimulation, chemo-preventive and radiation protection. In Cross River State, it is mostly used to prepare "pepper soup" pottage plantain and yam in various ceremonies. Many authors have reported phytochemical properties to include eugenol, methy cinnamate, camphor flavonoid, saponins and thymol (Nahak *et al* 2011; Kumar *et al* 2011).

African Catfish, *Clarias gariepinus* is widely cultured in Africa, Europe and some parts of Asia for it's hardly nature. It has been a suitable candidates for aquaculture because of its high prolificacy, simplicity of culture, possession of arborescent air breathing organ, omnivorous feeding habit, repaid growth rate and high feed conversion rate (Hecht *et al* 1996). *Clarias graiepinus* is in great demand in Nigeria because of its striking attributes and palatability (Sogbesam and Ugumba, 2008). In spite of the enormous uses of *O. gratissimum* there is paucity of information on its acute toxicity and anaesthetic potential on commonly cultured fishes in Nigeria. This study is aimed at investigating the acute toxicity and anaesthetic efficacy of *O. gratissimum* on *C. gariepinus* juveniles. The findings will provide the baseline information for safe and effective fish sedating critically needed by Fisheries researchers, managers and culturists for sustainability of aquaculture industry in Nigeria.

MATERIALS AND METHODS

Location of Study: This research was carried out at the Wet Laboratory, Department of Fisheries and Aquatic Science, Cross River University of Science and Technology (CRUTECH), Obubra Campus.

Collection and Preparation of Scent leaves

Fresh leaves of *O. gratissimum* was sourced within the premises of the University campus and its environs, identified in Forestry Department and air dried for 5 days. It was then pulverized with a sterile manual blender and sieved with 100 micron net to obtain a fine powder. The powder will be put in an airtight container and stored in a dry place prior to the commencement of the experiment.

Experimental Fish and Acclimation

Five hundred (500) apparently healthy juveniles of *Clarias gariepinus* (24.50± 4.25g and 27.60± 3.75cm) were procured from University of Calabar (UNICAL) Fish Farm, Calabar and transported in 50 litres jerry-cans by car to the Fisheries Wet Laboratory, CRUTECH, Obubra Campus. The fish were acclimated for two weeks in groups of 10 fish per rectangular glass aquaria in 30 litres River water. The water in the aquaria was renewed daily and fish fed twice daily with a commercial feed (Coppen) with 40% crude protein at 1% body weight.

Acute Toxicity Bioassay

Experimental Procedure

A range finding test was first conducted to determine the toxic levels of Scent leaf powder using standard procedure following the methods of APHA (1999). Using the appropriate formula the following concentrations was prepared 100, 150, 200, 250, 300 and 350mg/l. Ten (10) fish each are randomly put into the aquarium containing the test solution and the mortality was observed hourly for 12 hours. This was to determine the concentrations to be use in the definitive bioassay.Definitive test was conducted based on the result from range finding test with four (4) graded concentrations and control. Fifteen (15) rectangular glass aquaria (30 x 30 x 60cm) were cleaned, randomly labelled and each filled with the river water 25L mark. The different concentrations of 100, 150, 200 and 250mg/l were prepared by serial dilution from the stock solution 200mg/l to the respective aquaria and water added to make up to 30L. The mixture was stirred thoroughly for homogenous mixing and 10 fish each were randomly stocked. The tanks were not aerated but the test solutions were renewed daily according to UNEP (1989). Mortality was monitored and recorded at the respective time of 12, 24, 48, 72 and 96 hours. Dead fish were removed immediately to avoid contamination of the test solution. The values was used to estimate the lethal concentration and mean lethal time with their associated 95% confidence limit of scent leaf powder using probit according Trimmed Spearman Karber method (Hilmiton, 1977).

Anaesthesia (Induction and Recovery) Bioassay

Range findings test was conducted to determine induction time (Anaesthesia) of Scent leaf powder the was using in the bioassay following the method of (King *et al.*, 2005). A stock solution of scent leaf powder with a concentration of 200mg/L was prepared by dissolved 2g of the powder into 10 litres of river water. Exposure concentrations 100, 120, 140, 160 and 180mg/l for the bioassay were obtained by serial dilution of the stock solution. Thirty (30) glass aquaria were cleaned and randomly labelled and each filled with water to the 25 litres mark for induction test and 30 litres mark for recovery in each of the experiment. Three stages of anaesthesia (A) and three of recovery (R) time were considered and recorded using a stop watch (Table 1). Each aquarium was stocked with 10 fish each in triplicate and monitored for the onset of induction (anaesthesia, A1, A2 and A3) for 30 minutes as periods greater than this were considered impractical for routine fish handling procedures (Agokei and Adebisi, 2010). Any test fish that loss balance and no longer responding to external stimulus (A3) was removed immediately and transferred to 30 litres of scent leaf powder free water (Recovery, R) tanks. The induction and the recovery time were noted for the various stages. The behavioural changes of the fish in response to the effects of the scent leaf observed according to Gressler *et al*., (2017). None of the revived fish were re-used for further experimentation but were kept in another glass aquaria and plastic buckets to monitor post experimental mortality.

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	Sedation	Partial loss of muscle tone, erratic swimming and loss of equilibrium
A2	Light anaesthesia	Loss of gross body movement but with slow ventilation or opercula movement.
A3	Deep anaesthesia	Cessation of opercula movement and loss of tactile stimulation (no reaction to handling or external stimulus).
Recovery (R)		
R1	Initial recovery	Body immobilized but reappearance of opercula movement..
R2	Partial recovery	Commencement of gross body and regular opercula movements.
R3	Full recovery	Equilibrium regained, responses to tactile stimulation and preanaesthetic appearance.

Source: Coyle *et al* (2004), Modified by Gressler *et al*., 2017

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

Statistical Data Analysis
The data obtained for mortality at various interval was use to estimate the lethal concentration LC) and mean lethal time (MLT) with lower and upper 95% confidence intervals using the Trimmed Spearman Karber method (Hamilton *et al*., 1977). Data obtained from the water quality, time of anaesthesia and recovery in minutes, were analysed with a one – way analysis of variance (ANOVA) using SPSS version 25. The differences among the means were compared using Turkey's multiple comparison test at 5% significance level (Bhujel 2008).

RESULTS

Water quality parameters
The water quality parameters of the test solutions is presented in Table 2. The results obtained indicated that, the mean values of the various parameters were changing although not significant ($p < 0.05$). The mean values of all the parameters were decreasing except conductivity which was observed to be increasing with concentration of the scent leaf powder. Dissolved oxygen (DO; 5.84mg/l) was higher in the control than in the highest concentration (180mg/l). Temperature is observed to be fluctuating but with the control having the highest of 28.42^0C while the highest concentration had the least.

Lethal concentration (LC)
The result of the lethal concentrations (LCs) that killed 10% (LC_{10}), 50% (LC_{50}) and 99% (LC_{99}) of the test fish and their 95% confidence interval is presented in Table 3. The concentration required to kill a certain percentage of the fish increased within the same interval but decreases as the duration increases. The concentrations that killed 10, 50 and 99% of the test fish at 12^{th} hour were 189.77, 269.32 and 461.41mg/l while 96^{th} hour had 89.98, 203. 02 and 357.42mg/l respectively. The LC_{50} values of scent leaf was observed to decrease from 269.33, 249.19, 241.01, 220.31 and 203.02mg/l in 12 to 96 hour respectively. The 96hrsLC_{50} value estimated for scent leaf on *C. gariepinus* juveniles is 203.02mg/l. The maximum allowable toxicant concentration (MATC) decreased from 26.39 to 20.30mg/l in 12 to 96 hour while the relative toxicity factor (RTF) were increasing with duration of exposure. The values of the RTF indicates that, the concentration that will kill 50% of the

test at 12 hours is 1.33 higher and less toxic than that of 96 hours. The lower that concentration the more toxic the substance less suitable for use as anaesthetics to fish.

Mean Lethal Time (MLT)

The result of the time required to kill 50% (MLT_{50}) and 99% (MLT_{99}) and their relative toxicity time (RTT) is shown in table 4. The result revealed that the time taken for 50% (164.36 hours of the test fish exposed to 100mg/l to die is shorter than that required 99% (328.41 hours). The 96hrsMLT_{50} decreased from 164.36 to 126.45 hours as the concentration increased from 100 to 250mg/l of the scent leaf powder.

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. (°C)	pH	Cond. (µS/cm)	Alk. (mg/l)	Hardness (mg/l$CaCO_3$)
0	5.84±0.83[a]	28.42±1.27[a]	6.86±0.35[a]	125.27±1.24[a]	39.05±1.55[a]	29.40±0.85[a]
100	5.67±0.45[a]	28.07±1.70[a]	6.74±1.07[a]	126.45±1.08[a]	38.32±1.30[a]	28.67±0.75[a]
120	5.58±1.15[a]	27.84±0.45[a]	6.70±1.41[a]	126.06±1.02[a]	38.18±2.45[a]	27.34±0.84[a]
140	5.46±0.76[a]	27.60±2.04[a]	6.68±1.15[a]	127.09±2.04[a]	37.75±0.88[a]	27.22±0.35[a]
160	5.42±0.88[a]	27.64±1.51[a]	6.65±0.90[a]	128.42±1.56[a]	38.02±1.55[a]	28.06±0.40[a]
180	4.88±1.02[ab]	26.89±2.09[a]	6.61±0.25[a]	128.05±2.70[a]	38.15±1.36[a]	27.20±1.05[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

Table 3: Lethal concentration (LC), safe concentration and relative toxicity factor (RTF) of scent leaf powder on *C. gariepinus* juveniles

Time (hour)	Lethal Concentration (LC) with 95% Confidence interval in parenthesis.			MATC	RTF
	LC_{10}	LC_{50}	LC_{99}	(LC_{50} x 0.1)	
12	189.77 (173.30 – 300.24)	269.32 (225.21 – 387.19)	461.41 (356.98 – 765.58)	26.93	1
24	161.75 (143.07 – 189.26)	249.19 (211.70 – 367.33)	460.02 (373.07 – 643.26)	24.92	1.08
48	120.37 (99.62 – 136.66)	241.01 (207.73 – 218.75)	425.49 (135.29 – 561.69)	24.10	1.12
72	107.29 (86.40 – 122.17)	220.31 (196.0 – 263.83)	408.21 (345.58 – 519.62)	22.03	1.22
96	89.98 (67.76 (105.15)	203.02 (182.91 – 235.91)	357.42 (271.10 – 432.22)	20.30	1.33

LC_{50} = 50%, LC_{99} = 99% mortalities, RTF = LC_{50} at 12th hour÷ LC_{50} of other durations., MATC = Maximum allowable toxicant concentration

https://doi.org/10.37745/ijfar.15

Table 4: Mean lethal time (MLT) and associated 95% confidence limits and relative toxicity time for *C. gariepinus* juveniles exposed to various concentration of scent leaf powder.

Concentration (mg/l)	Mean Lethal time (MLT) with 95% Confidence interval in parenthesis		RTT
	MLT_{50}	MLT_{99}	
100	164.36 (131.63 – 253.63)	328.41 (251.94 – 501.15)	1
150	132.46 (112.91 – 290.76)	280.33 (244. 34 – 394.90)	1.24
200	127.34 (109.04 – 163.81)	192.55 (147.22 – 275.25)	1.29
250	126.46 (104.76 – 172.98)	128.52 (144.36 – 261.45)	1.30

MLT_{50} = 50% , MLT_{99} = 99% mortalities, RTT = MLT_{50} of 100mg/l ÷ MLT_{50} of other concentrations.

Anaesthesia and Recovery

The mean time (min) of the various stages of anaesthesia and recovery of exposed fish to scent leaf power is presented in Table 5. There was no mortality observed in any of the exposure levels during period of this experiment. The exposed fish showed some behavioral responses that culminated in loss of reflex and tactile stimulation. Fish treated with 100mg/l only showed partial loss of muscle tone and erratic swimming (stage A1) at about 25.41mins. Those exposed to concentration above 100mg/l reached stages A2 and A3 at various time intervals. The time to achieve sedation (A1) decrease significantly ($P<0.05$) from 7.45 to 0.35mins as the concentration increased from 120 to 180mg/l. the time for the commencement of the opercula (initial recovery, R1) after complete immobilization significantly increased from 2.50 to 7.24 mins, at ($P<0.05$) in 120 to 180mg/l indicating that it increases with concentration. The time to achieve complete immobilization (stage A3) decreases while that to attain full recovery (stage R3) increased as concentration of the powder solution increased (Figure 1). At the least concentration of 120mg/l exposed fish were completely immobilized (A3) in 11.08 mins and regained full recovery (R3) in just 4.08 mins while fish exposed to 180mg/l were completely anaesthetized in just 2.25 mins and took a longer time of 18.50 mins to fully recovered and swim normally. Whereas the time to achieve deep anesthesia decreased from 11.08 > 8.03 > 5.48 > and 2.25 mins, that to attain full recovery increase from 4.08 < 8.20 < 13. 28 < and 18.50 mins as the concentration increased from 120 t0 180mg/l. The result revealed that the time of recovery is inversely proportional to that of induction (anaesthesia) in all the stages and are all dependent on concentration.

https://doi.org/10.37745/ijfar.15

Table 5: 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)
0	-	-	-	-	-	-
100	25.14 ± 1.25^{a}	-	-	-	-	-
120	7.45 ± 1.27^{b}	10.06 ± 2.50^{b}	11.08 ± 3.55^{a}	2.50 ± 1.64^{c}	3.46 ± 0.25^{d}	4.08 ± 1.20^{d}
140	4.06 ± 1.60^{c}	6.50 ± 0.16^{c}	8.03 ± 0.28^{b}	4.05 ± 0.65^{c}	5.25 ± 0.28^{c}	8.20 ± 0.47^{c}
160	1.22 ± 0.50^{d}	3.58 ± 0.35^{d}	5.48 ± 0.55^{c}	5.33 ± 0.75^{b}	7.08 ± 0.98^{b}	13.28 ± 1.18^{b}
180	$0.35\pm 0.35_{d}$	1.25 ± 0.30^{e}	2.25 ± 0.47^{d}	7.24 ± 0.24^{a}	10.20 ± 0.11^{a}	18.50 ± 1.61^{a}

Means with the same superscript under the same columns are not significant at 5%

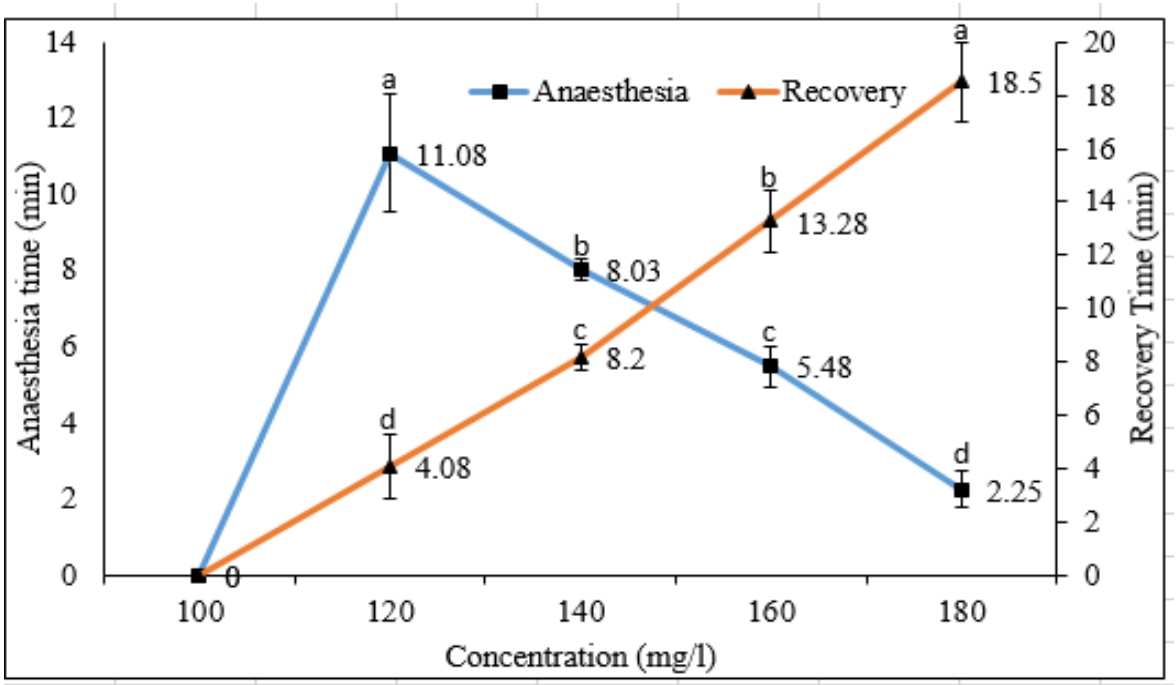

Figure 1: Deep anaesthesia and full recovery time (min) of *C. gariepinus* juveniles exposed to scent leaf powder anaesthetic for 30 mins. Bar with different letter differ significantly at 5% (HSD)

https://doi.org/10.37745/ijfar.15

DISCUSSIONS

Acute toxicity of Anaesthetics

Toxicity effects of anaesthestics on fish are of special interest since they are frequently used in research and routine aquaculture to immobilized and minimize stress responses (King *et al* 2005). Some researchers have studied the acute toxicity effects of anaesthetics used on food fishes. Grush *et al* (2004) studied the anaesthetics effects and acute toxicity of clove oil in one month old zebra fish. Velisek *et al* (2005) and (2006) reported on the acute toxicity effects of clove oil anaesthetic in common carp and European catfish respectively. Recent studies have also been conducted on the acute toxicity of some plant extracts use as alternative to synthetic anaesthetics to food fishes. Sindhu and Ramachandran (2013) studied the acute toxicity of clove oil anaesthetic to *Barilus bakeri*. The toxicity effects of the extracts of cinnamon, ginger, tobacco leaf and clove oil have been reported by Sindhu (2015). Studies on the acute toxicity of tobacco leaf and clove, *Eugenia caryophylatta* powder have been reported for African catfishes (Agokei and Adebisi, 2010; Okey *et al* 2018). Acute toxicity of a substance is determined by it lethal concentration value most estimated at 50% mortality. The smaller the LC_{50} value the more toxic the substance and suitable for use as anaesthetic.in tis study the range of 203.02 to 269.32mg/l, compared to the range of 54.33 to 96.56mg/l reported for clove powder on *C. gariepinus* juveniles (Okey *et al* 2018) was observed as high. The trend of decrease in the LC_{50} values from the 12 to 96 hours agrees with the reports of several workers who exposed fish to acute concentration to various toxicants (Gabriel and Okey 2009; Okey *et al* 2018; Omogoriola and Ayoola, 2018). Lower 96hrsLC_{50} values were estimated for clove oil (0.24mg/l), cinnamon oil (0.57mg/l), zingiber extracts (1.47mg/l) and tobacco (6.51mg/l) used as anaesthetics in *Eutroplus suratensis* (Sindhu, 2015). Velisek *et al* (2006) had earlier reported a 96hrLC_{50} value of 18.4mg/l in European catfish, *Silurus glandis* exposed to clove oil. In this study the 96hrsLC_{50} value for scent leaf (203.02mg/l) was higher than that of clove powder (74.25mg/l) earlier reported on *C. gariepinus* juveniles by Okey (2014). This implies that scent leaf is less toxic than clove dry flower buds.

The mean lethal concentration (MLT) provide information on the pattern of mortality in fish against duration of exposure on a given concentration under acute toxicity (Gabriel *et al*2009). The trend of 96hrsMLT_{50} values in this study showed that increase concentration reduces the time at 50% mortality. This observation is in agreement with Keremah *et al* (2010), Gabriel and Okey (2009) and Okey *et al* (2018; 2021). The 96hrMLT_{50} at the lower concentration (164.36 hours) and highest concentration (126.45) of scent leaf were higher than 54.59 and 8.09 hour reported for *C. gariepinus* juveniles anaesthetized with clove powder (Okey 2014). The differences in both the LC_{50} and MLT_{50} values for the anaesthetics could be attributed to the variations of species biological and environmental factors. This may have influence the efficacy of the botanicals used anaesthetic. This finding agrees with Akinrotimi *et al* (2014) who reported higher mortality rate with high concentrations of of aqueous extracts of Indian almond tree than clove seed on *C. gariepinus*. Fish mortality was observed to increase with concentration of scent leaf powder in this study. This may

be due increase interference of the active ingredient on the normal functioning of the systems in the test fish. According to Gbem *et al* (1990) death of fish exposed to toxicants may results from the interference with the normal functioning of the nervous system which impaired with the normal muscular activity and coordination. Death may also be due to disruption and failure in gill functions, hence reducing availability of surface for gaseous and ionic exchange (Ayuba and Ofojekwu, 2002). Since scent leaf powder appears to be less toxic based on the high LC values compared to some biocides, it can safely be utilized to immobilize *C. gariepinus* and other fish species in aquacultural procedures.

Water Quality

Knowledge of how water quality influences anaesthesia and sedation helps to limit complication (Neiffer and Stamper, 2009). It is also critical to monitor water quality in order to reduce anaesthetic morbidity and mortality (Harms, 1999). The influence of other environmental conditions (temperature and pH) on the toxicity of anaesthetics has also been investigated (Park *et al*., 2008; Zahl *et al*., 2009). In this study the physicochemical properties of the test medium did not vary significantly ($P > 0.05$) from the control (0mg/l). It was within the acceptable ranges for toxicity test tolerance level (APHA, 2009; Boyd, 1981), hence may not have acted in synergy with the anaesthetics behavior observed in the exposed fish. Similar observation was recorded by Okey *et al* (2013), Akinrotimi *et al.*(2015), Olufayo and Ojo (2018), Iheanacho and Nworu (2017) using, clove flower bud powder, clove seed, clove oil and *Chloromolaena odorata* as anaesthetic agents to African catfish species respectively. However, leaf extracts of *Datura stramonium* according to Adebayo and Olufayo (2017) caused significant changes in temperature, pH and dissolved oxygen in test solution of *H. bidorsalis* juveniles. According to Adeyemo (2005) and Heath (1991) bad quality water can result to physiological changes which will be reflected in the values of one or more of the swimming and anaesthetics activity of fish.

Anaesthesia and Recovery

Anaesthetics are essential component in aquaculture activities needed to facilitate handling of fishes to minimize their stress. According to Serezli *et al* (2012) anaesthetics are often use by fish managers, researchers and professionals to measure, weigh, mark, apply antibiotics, vaccines and draw blood from the without stress. Since stress responses vary widely between species, it became expedient to find the appropriate and effective dose for each culture species. This study revealed that scent leaf powder caused some degree of anaesthesia on *C. gariepinus* juveniles various concentrations used without mortality. The anaesthesia and the recovery time were observed to be dependent on the concentration of the scent leaf powder. The higher the concentration the shorter the time to achieve a particular stage of anaesthesia and the longer the time to recover. This finding was in line with those of Grush *et al* (2004) with clove oil on Zebra fish, Akinrotimi *et al* (2018) with clove seed on *C. gariepinus*, Okey (2019) with clove powder on *C. gariepinus* and Adewale *et al* (2017) with scent leaf extract on *Oreochromis niloticus*. Wilfred – Ekpirikpo (2021) have also reported similar trend of concentration dependent anaesthesia and recovery time on mustard seed

powder on the life stages of *Serotherodon melanotheron*. Researchers using plants extracts as anaesthetics have reported that faster sedation and tranquilization is achieved with higher concentration of the solution (Akinrotimi *et al* 2013; Akbulut *et al* 2010; Hajek *et al* 2006). Fish exposed to 100mg/l only show partial loss of muscle tone and loss of equilibrium (stage A1) at about 25.14min while those exposed to 120 to 180mg/l got completely immobilized at various time of 11.08, 8.03, 5.48 and 2.25min respectively. The higher the concentration the shorter the time to achieve deep anesthesia (A3) and the longer the time to regain full recovery. Similar observation was reported by Akinrotimi *et al* (2014), Okey, (2019), Adebayo and Olufayo (2017) using various plant anaesthetics on African catfishes. In this study 120mg/l of scent leaf powder was required to completely immobilize *C. gariepinus* in 11.08min which is shorter than the 22.32 min required of 120mg/l of clove anaesthetized the same species of fish (Okey, 2014). This implies that the rate at which scent leaf powder is absorbed into the central nervous system is faster than clove powder. According to Feng *et al* (2011) the degree of anaesthesia is influenced by the concentration of the anaesthetic in the central nervous system (CNS) of the organism. Summerfelt and Smith (1990) reported that anaesthetics act on the CNS and induces calming effects, loss of equilibrium, mobility, consciousness and loss of reflex actions.

According to Ross and Ross (2008) an ideal anaesthetic is that which anaesthetizes the fish in 3 min, recovers in 10 min and does not cause mortality during the period of use. However Samoes *et al* (2011) stated that a much longer full recovery time of >15 min is required for surgical anaesthesia where the exposed fish need to the hell for longer aquacultural operations. This study revealed that the recovery time is highly dependent on the time of anaesthesia that is, the shorter the time of anaesthesia the longer the time taken to recover fully. Fish exposed to 180mg/l and where completely immobilized (A3) in just 2.25 min too as longer as 18.50 min to full regain equilibrium and swim normally while in 120mg/l fish took < 5 min to regain full recovery and as long as 11.08 min to attain deep anesthesia. This observation followed the same pattern as those described for clove oil by Hamackova *et al* (2006) on perch, Velisek *et al*(2005) on common carp and Olufayo and Ojo (2018) on *H. bidorsalis* juveniles. The < 5 min and < 20 min of recovery time recorder in this study is shorter than that reported for clove powder (Okey, 2014) but however falls within the range reported for ideal anaesthetics indicating that scent leaf may is ideal anaesthetics for *C. gariepinus*. Soto and Burhanuddin (1995) and Anderson *et al* (1997) 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 recovery time reported in this study was comparable to those reported by other researchers using various plant extracts on African catfishes (Adebayo *et al*2010; Okey *et al*2013; Akinrotimi *et al* 2014; Olufafo and Ojo 2018; Okey, 2019).

Lower concentration of anaesthetics should be considered in order to provide a greater margin of safety for animals, avoid unnecessary expenses and waste of anaesthetics (Teixeira *et al* 2017). The range of 140 to 160mg/l of scent leaf in this study was considered effective and safe since it induce anaesthesia and full recovery without any mortality and unnecessary waste of the powder hence

recommended. Similar range of 120 – 160mg/l was recommended for clove powder on *H. bidorsalis* juveniles. Adebayo *et al* (2010) recommended 190mg/l aqueous extracts of avocado pear leaf as effective anaesthesia for *C. gariepinus* brood stock. Small (2003) reported 100mg/l of clove oil as a safe concentration for anaesthesia of the channel catfish (*Ictalarus punctatus*), adding that exposures to higher concentration will prolong the recovery time and may cause mortality. Higher effective concentration within 250 – 350mg/l of clove powder was recommended for Roach, *Rutilus rutilus* (Sudagara *et al* 2009). Some researchers have reported relatively lower effective concentrations with plants extracts in fishes (Inoue *et al* 2005; Walsh and Pease 2002; Keene *et al*2005; Velisek *et al* 2005; 2006). The variations in the effective concentration in this study compared to those of other researchers may be due to the specific properties of each species and their physiological responses to anaesthetics. Kucuk (2010) reported that the gills area, body weight species and metabolic rate have effects on the rate of anaesthetics absorption and induction. Celik and Yilmaz (2007) also stated that the pharmacokinetics of the anaesthetics may cause differences among the duration of anaesthesia and recovery in animals. This may have accounted for the slight differences in anaesthesia and recovery time in *C. gariepinus* on scent leaf powder and clove powder earlier reported by Okey (2014).

CONCLUSION

Scent leaf is effective, relatively safe, economically sustainable, environmentally friendly, medicinally important and less persistence in aquatic environment. The high 96hrsLC_{50} value estimated for scent leaf revealed that it is less toxic to *C. gariepinus* and can be utilized as anaesthetic. The powder proved to be effective anaesthetics on the exposed fish without causing mortality with 120 – 180mg/l. based on the result of this study and in comparable with those of other researchers 140 – 160mg/l was recommended as effective for immobilization of *C. gariepinus*. The use of scent leaf should be encouraged in preference to synthetic for sustainability of aquaculture industry in Nigeria.

Acknowledgement

We are express our sincere thanks to Tertiary Education Fund (TetFund) for providing the grant and the University for giving me the privilege to access the grant for the accomplishment of this research.

References

Agokei, O.E., and Adebisi, A.A. (2010) Tobacco as an anesthetic for fish handling procedures. J. Medicinal Plants Res. 4(14): 1396-1399

Akbulut, B., Cakmak, E., Aksungure, N. and Cavdar, Y. (2011) Effect of Exposure Duration on Time to Recovery from Anaesthesia of Clove Oil in Juvenile of Russian Sturgeon. Turkish Journal of Fisheries and Aquatic Sciences 11: 463- 467.

Akinrotimi, O. A., Edun, O. M., and Ukwe, O. I. K., (2018). Effects of anaesthetics on metabolic enzyme activities in African catfish, clarias gariepinus (Burchell, 1822). Journal of FisheriesSciences.com vol. 12, pp. 022-028.

Akinrotimi, O. A., Gabriel, U. U., and Deekae, S. N., (2014). Investigations on the potential of Indian almond free (*Terminalia catapa*) leaf extracts as anesthetic agents in African catfish (*Clarias gariepinus*). Journal of Aquatic Sciences, vol. 29, pp. 223-231.

Akinrotimi, O.A., Gabriel, U.U. and Edun, O.M. (2015). The efficacy of clove seed extracts as an anaesthetic agent and its effect on haematological parameters of African catfish (*Clarias gariepinus*). International Journal Aquaculture Fishery Science, 1(2): 042-047.

APHA, AWWA, WEF (1998) Standard methods for the Examination of Water and Wastewaters, 20th Edition. American Public Health Association, Washington, DC

Ayuba, V.O. and Ofojekwu, P.C. (2002). Acute toxicity of the root extract of Jimson's weeds *Datura innoxia* to the African catfish, *C. gariepinus* fingerlings. Journal of Aquatic Sciences, 17(2), 131- 136.

Bhujel, RC (2008). Statistics for Aquaculture. John Wiley & Sons.

Boyd, C. E. (1982) Water quality management for pond fish culture, 317 p, Elsevier, Amsterdam

Boyd, O. (1981). Water Quality in Warm fish ponds. Auburn university, Agricultural experimentation Station, Auburn, Alabama craft master printers Inc. Opelika

Carneiro, W. F., Melo, N., Da Silva, K.C.D., Ferreira, I.S., Paula, D.A.J., Ferrante, M. and Murgas, L.D.S. (2019). Clove oil as anaesthetic for tambacu juveniles (♂*Piaractus mesopotamicus* × ♀ *Colossoma macropomum*). Adv. Anim. Vet. Sci. 7(11): 969-976.

Celik, S. and Yilmaz, E., (2007). Gonad development and sex ratio of sharp tooth catfish (Clarias gariepinus) cultured under laboratory conditions. Turkish Journal of Zoology, vol. 31, pp. 35-46.

Cho, G.K. and Heath, D.D. (2000) Comparison of Tricaine Methanesulphonate (MS 222) and Oil Clove Anaesthesia Effects on The Physiology of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) (Walbaum). Aquaculture Research 31:537–546.

Coyle, S..D., Durborow, R. M. and Tidwell, J.H. (2004). Anesthetics in aquaculture. Southern Regional Aquaculture Center Publication No. 3900.

Cunha, M. A., Barros, F.M.C., Garcia, L.O., Veeck, A.P.L., Heinzmann, B.M., Loro, V.L., Emanuelli, T. and Baldisserotto, B. (2010). Essential Oil of Lippia Alba: A New Anesthetic For Silver Catfish, *Rhamdia Quelen*. Aquaculture 306:403–406.

Effraim, K.D., Salami, H.A. and Osewa, T. S. (2000). The effect of aqueous leaf extract of *Ocimum gratissimum* on haematological and biochemical parameters in rabbits. Afr. J. Biomed. Res., 3(3): 175-179.

Feng, G., Zhoang, P., Zhang, L., Kynand, B., Liu, J., and Huang, X. (2011). Effects of anaesthetics MS-222 and clove oil on blood biochemical parameters of juvenile Siberian sturgeon, (*Acipenser baerii*).Journal of Applied Ichthyology, 27: 595-599.

Gabriel, U.U. and Okey, I. B. (2009). Effect of aqueous leaf extracts of *Lepidagathis alopecuroides* on the behaviours and mortality of hybrid catfish (*Heterobranchus bidorsalis* ♀ x *Clarias*

gariepinus♂) fingerlings. Research Journal of Applied Sciences, Engineering and Technology, 1(3), 116-120.

Gilderhus, P. A. and Marking, L.L. (1987) Comparative Efficacy of 16 Anaesthetic Chemicals in Rainbow Trout. North American Journal of Fisheries Management 7: 288-292.

Gressler, L.T., Silva, L. L. and Heinzmann, B. M. (2017). Anestésicos em animais aquáticos. In: Baldisserotto, B., Gomes, L.C., Heinzmann, B.M., Cunha, M.A. (Eds.), Farmacologia aplicada à aquicultura. UFSM, Santa Maria. 1: 467-537.

Grush, J., Noakes, D. L. and Moccia, R. D. (2004). The efficacy of clove oil as an anesthetic for the zebrafish *Danio rerio* (Hamilton). Zebrafish. 1: 46–53

Hajek, G.J., Klyszejko, B. & Dziaman, R. (2006). The anaesthetic effect of clove oil on common carp, (*Cyprinus carpio* L.). Acta Ichthyologica et Piscatoria, 36: 93–97.

Hamackova J, Kouril J, Kozak P, Stupka Z (2006) Clove Oil As An Anaesthetic For Different Freshwater Fish Species. Bulgarian Journal of Agriculture Science 12: 185-194. 35.

Hamilton, M.A., Russo, R.C., and Thurston, R.V. (1977) Trimmed spearman karber method for estimating media lethal concentration in toxicity bioassays. Environ. Sci. Technol. 11: 714–718

Harms, C.A. (1999) Anesthesia in fish. In: Zoo and Wild Animal Medicine, Current Therapy 4 (Fowler, M.E. and Miller, R.E., Eds), pp 158-163, Philadelphia, WB Saunders

Heath, A.G. (1991). Water pollution and fish physiology. Lewis Publishers, Boca, Ranton, Florida. U.S.A.

Hecht, T., Uys, W. and Britz, P.J. (1988). The culture of sharptooth catfish, *Clarias gariepinus* in southern Africa. South African National Scientific Programmes Report No. 153, 133 pp. Pretoria, Council for Scientific and Industrial Research.

Hoseini, S.M. (2011). Efficacy of clove powder solution on stress mitigation in juvenile common carps, (*Cyprinus carpio* L.). Comparative Clinical Pathology, 20(4): 359–362.

Hoseini, S.M. and Ghelichpour, M. (2011). Efficacy of clove solution on blood sampling and hematological study in Beluga *Huso huso* (L.) Fish. Physiol. Biochem. DOI 10.1007/s10695-011-9529-5

Iheanacho, S. and Nworu S. (2017). Behavioral and Haematological Response of *Clarias gariepinus* juvenile exposed to acute concentrations of Aqueous Extract of Siam Weed (*Chloromolaena odorata*) leaf as Anaesthetic agent. doi: http:// dx.doi.org/10.1101/181164, CC-BY-NC-ND 4.0 International license 1 – 20 pp.

Inoue, L., Afonso, L., Iwama, G.K. and Moares, G. (2005). Effects of clove oil on the stress response of matrinxa (*Brycon cephalus*) subjected to transport. Acta Amazonica, 35(2):289-295

Javahery, S., Haji, H., Moradlu, A. and Ghornani, R. (2012) Efficacy of Clove Oil as an Anaesthetic on two size of *Rutilus Frisii* Kutum. Global Veterinaria 9: 319-322.

Keene, J. L., Noakes, D. G., Moccia, R. D., and Soto, C. G. (1998). The efficacy of clove oil as an anesthetic for rainbow trout, *Onchorhynchus mykiss* (Walbaum). Aquac. Res., 29: 89-101.

Keremah, R.I., Okey, I.B. and Gabriel, U.U. (2010). Relative toxicity of aqueous leaf extracts of *Lepidagathis alopecuroides* (Vahl) R. Br. ex Griseb to the clariids, *Clarias gariepinus* and

Heterobranchus bidorsalis fingerlings. Agriculture and Biological Journal of North America, 1(5), 834 – 840.

King, V.W., Hooper, B., Hillsgrove, S., Benton, C. and Berlinsky, D. L. (2005). The use of clove oil, metomidate, tricaine methanesulphonate and 2- phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquaculture Research, 36(14): 1442–1449.

Küçük, S. (2010). Efficacy of tricaine on *Peocilia latipinna* at different temperatures and concentrations. *African Journal of Biotechnology*, 9(5), 755-759.

Lewis, W.M. and Morris, D.P. (1986) Toxicity of nitrite to fish: A Review. Trans. Am. Fish. Soc. 115: 183–195

Maricchiolo, G. and Genovese, L. (2011). Some contributions to knowledge of stress response in innovative species with particular focus on the use of the anaesthetics. The Open Marine Biology Journal, 5(1): 24–33.

Mazandarani, M. and Hoseini, S.M. (2017). Menthol and 1,8-cineole as new anaesthetics in common carp, *Cyprinus carpio* (Linnaeus, 1758). Aquac. Res. 48(6): 3041–3051. https:// doi.org/10.1111/are.13136

Mitjana, O., Bonastre, C., Insua, D., Falceto, M. V., Esteban, J., Josa, A., and Espinosa, E., (2014). The efficacy and effect of repeated exposure to 2-phenoxyethanol, clove oil and tricaine methane sulphonate as anesthetic agents on juvenile Angelfish (*Pterophyllum scalare*). Aquaculture (Amsterdam, Netherlands), vol. 433, pp. 491-495.

Munday, P.L. and Wilson, S.K. (1997) Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinesnsis*, a coral reef fish. J. of Fish Bio. 51: 931-938

Mylonas CC, Cardinaletti G, Sigelaki I, Polzonetti-Magni A (2005) Comparative Efficacy of Clove Oil and 2-Phenoxyethanol as Anesthetics in the Aquaculture of European Sea Bass (*Dicentrarchus labrax*) and Gilthead Sea Bream (*Sparus aurata*) at Different Temperatures. Aquaculture 246: 467-481

Neiffer, D.L. and Stamper, M.A. (2009). Fish sedation, analgesia, anesthesia, and euthanasia: considerations, methods, and types of drugs. ILAR Journal National Research Council, Institute of Laboratory Animal Resources, 50(4): 343–360.

Okey 1B, Keremah RI, and Ofem BO, (2013). Effect of Clove (*Eugenia aromatica*) powder anaesthetic on some haematological parameters in hybrid catfish (*Heterobranchus bidorsalis* ♀ x *Clarias gariepinus* ♂) juveniles. International Journal of Fisheries and Aquaculture, 5(7), 184-192.

Okey IB, Keremah RI, and Gabriel UU, (2018). The efficacy of clove (*Eugenia caryophyllata*) powder as anaesthesia on African catfishes (*Clarias gariepinus* and *Heterobranchus bidorsalis*) fingerlings. J Aquac Mar Biol. 7(4):182–188.

Okey, I. B. (2006). Toxicity of *Lepidagthis alopecuroides* to *Heterobranchus bidorsalis*, *Clarias gariepinus* and hybrid (*Heterobranchus bidorsalis* ♂ x *Clarias gariepinus* ♀) fingerlings. (Unpublished Masters of Science Thesis).University of Science and Technology, Port Harccourt, Rivers State.

Okey, I. B., Ayotunde, E. O. and Patrick, B. U. (2021). Behavioural Responses and Mortality of Clarias gariepinus Juveniles Exposed to Acute Concentrations of Paraquat. Sumerianz Journal of Agriculture and Veterinary, 4(2):49-54.

Okey, I. B., Gabriel, U. U., and Deekae, S. N. (2018). Comparative effects of the acute toxicity of clove (*Eugenia aromatica*) powder to *clarias gariepinus* and *Heterobranchus bidorsalis* fingerlings. Int. J. Inn. Stud. Aqu. Bio. Fish.. 4: 19-26

Okey, IB (2019). Anaesthetic Effects of clove (*Eugenia caryophylatta*) on Some Haematological and Biochemical Parameters of *Heterobranchus bidorsalis* Juveniles. Journal of Fisheries and Aquaculture Research, 4(1): 016-027.

Olufayo M. O. and Ojo O. M. (2018). Anaesthetic effects and haematological responses of heterobranchus bidorsalis juveniles exposed to clove oil. International Journal of Fisheries and Aquaculture, Vol. 10(9), pp. 116-121

Omogoriola, H. O. and Ayoola, S..O. (2018). Acute Toxicity of Some Nigerian Crude Oils on Black Jaw Tilapia (*Sarotherodon melanotheron*) Juveniles. Nigerian Journal of Fisheries, 15(1): 1349 – 1357.

Park, M.O., Hur, W.J., Im, S.Y., Seol, D.W., Lee, J. and Park, I.S. (2008). Anaesthetic efficacy and physiological responses to clove oil anaesthetized kelp grouper (*Epinephelus bruneus*). Aquaculture Research, 39(8): 877–884.

Ramanayaka, J.C. and Atapatu, N.S.B.M. (2006). Fish anaesthetic properties of some local plant material. Tropical Agricultural Research & Extension9.

Ross, L.G. and Ross, B. (2008). Anaesthetic and sedative techniques for aquatic animals. 3rd Edition. Blackwell publishing.

Serezli R, Basaran F, Muhtaroglu CG, Basaran AK (2012). Effects of 2-phenoxyethanol anaesthesia on juvenile meagre (*Argyrosomus regius*). J. Appl. Ichthyol. 28(1): 87–90. https:// doi.org/10.1111/j.1439-0426.2011.01771.x

Silva, L.L, Parodi, T.V, Reckziegel, P., Garcia, V.O., Burger, M.E., Baldisserotto, B., Malmann, C.A., Pereira A.M.S. and Heinzmann, B.M. (2012). Essential Oil of *Ocimum gratissimum* L.: Anesthetic Effect, Mechanism of Action and Tolerance in Silver Catfish. *Rhamdia quelen* Aquaculture 350–353:91–97. 23.

Sindhu, M. C (2014). Evaluation of Stress Reducing Capacity of Selected Anaesthetics for the Live Transportation of Green Chromide *Etroplus suratensis*: Ph.D. Thesis University of Science and Technology, Kochi – Kerala, India. 463p

Sindhu, M.C. (2009) Clove oil as an anesthetic for transportation of blue hill trout Barilius bakeri (Day) M.Phil Thesis, Cochin University of Science and Technology, Kerala, India, 362 p

Sindhu, M.C. and Ramachandran, A. (2013). Acute toxicity and optimal dose of clove oil as anaesthetic for blue hill trout *Barilius bakeri* (Day). Fish.Technol. 50: 280-283

Small, B. (2003). Anesthetic efficacy of methomidate and comparison of plasma cortisol responces to tricain methansulfonate, quinaldine and clove oil anesthetized channel catfish (*Ictalurus punctatus*). Aquaculture, 218: 177- 185.

Sogbesan, A. O. and Ugwumba, A. A. A. (2008). Nutritional values of some non-conventional animal protein feed stuffs used as fishmeal supplement in aquaculture practices in Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences*, 8: 159-164

Sudagra, M., Mohammadizarejabada, A., Mazandarania, R. and Pooralimotlagh. (2009). The effect of clove powder as an anesthetic and its effects on hematological parameters on roach (Rutilus rutilus). Journal of aquaculture feed science and nutrition, 1 (1): 1-5.

Summerfield, R.C. and Smith, L.S. (1990). Anesthesia, surgery and related tecniques. In: Schreck CB, Moyle PB, Eds. Methods in Fish Biology. Bethesda. American Fisheries Society, pp. 213-272.

Teixeira RR, Souza RC, Sena AC, Baldisserotto B, Heinzmann B.M, Couto RD, Copatti CE (2017). Essential oil of *Aloysia triphylla* in Nile tilapia: anaesthesia, stress parameters and sensory evaluation of fillets. Aquac. Res. 48(7): 3383–3392.

Trushenski, J. T., Bowker, J. D., Cooke, S. J., Erdahl, D., Bell, T., MacMillan, J. R., Yanong, R. P., Hill, J. E., Fabrizio, M. C., Garvey, J. E. and Sharon, S. (2013). Issues regarding the use of sedatives in fisheries and the need for immediate-release options. Transactions of the American Fisheries Society, 142(2): 156-170.

Ucar, A. and Atamanalp, M. (2010). The effects of natural (Clove Oil) and synthetical (2-phenoxyethanol) anesthesia substances on hematology parameters of Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo truttafario*). Journal of Animal and Veterinary Advances, 9(14): 1925-1933.

UNEP (United Nations Environmental Programme) (1989) Estimation of the lethal toxicity of pollutants in marine fish and invertebrates. Ref. Methods Mar. Poll. Studies. 43: 27

Velisek J, Svobodova Z, Piackova V, Groch L. and Nepejchalova L (2005) Effects of Clove Oil Anaesthesia on Common Carp (*Cyprinus Carpio* L.). Veterinary Medicine Czech 50:269–275.

Velíšek, J., Svobodová, Z. and Piačková, V. (2005a). Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta. Veterinarni Brno., 74: 139-146.

Velíšek, J., Wlasov, T., Gomulka, P., Svobodová, Z., Novotny, L. and Ziomek, E. (2006). Effects of clove oil anesthesia on European catfish (*Silurus glanis*). Acta Veterinarni Brno, 75: 99- 106

Walsh, C.T. and Pease, B.C. (2002). The use of clove oil as an anaesthetics for the longfinned ell, (*Anguilla reinhardtii* Steindachner). Aquaculture Research, 33(8): 627-635.

Wilfred–Ekprikpo P. C. (2021). Evaluation of Mustard Seed (*Brassica nigra*) Powder as Anaesthetic Agents in Different Life Stages of Black Jaw Tilapia (*Sarotherodon melanotheron*). Sumerianz Journal of Biotechnology, 4 (2) 57-62.

Woody, C., Nelson, G. and Ramstad, K. (2002). Clove oil as an anaesthetic for adult sockeye salmon: field trials. Journal Fish Biology, 60: 340-347.

Zahl, I.H., Kiessling, A., Samuelsen, O.B. and Hansen, M.K. (2009). Anaesthesia of Atlantic cod (*Gadus morhua*) - effect of pre-anaesthetic sedation, and importance of body weight, temperature and stress. Aquaculture, 295(1- 2): 52–59.