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ANESTHETIC EFFECTS OF CLOVE (*EUGENIA AROMATICUM*) SEED EXTRACT ON *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS UNDER SEMI-ARID CONDITIONS

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Abstract: The anesthetic effects of clove (*Eugenia aromaticum)* seed extract on *Clarias gariepinus* fingerlings were investigated under semi-arid conditions. Various concentrations of the clove seed extract at 25.0, 50.0, 75.0, 100.0, 125.0 and 150.0mg per liter of water were used for the experiment. Each concentration was tested on a group of 10 *Clarias gariepinus* fingerlings (24.13–25.30g in weight and 5.97–7.00 cm in length) in glass aquariums. There was a decrease in induction time as the concentration of the clove seed extract increased. Fingerlings treated with 150mg/l of the extract produced the shortest induction time (2.28±0.15 minutes), followed by fish treated with 100 and 125mg/l (3.31±0.55 and 3.07±0.07 minutes, respectively). The longest induction time (10.60±0.98, 7.52±0.25 and 5.96±1.17 minutes) was observed in fingerlings sedated with 50, 25 and 75mg/l, respectively. Recovery was significantly faster (2–3.92 minutes) in fish treated with lower dosages (25 to 125mg/l) of the clove seed extract. Mortality rates after 24 hours of recovery were higher (4.79±0.25 and 3.10±0.54%) in fish anesthetized with higher (150 and 125mg/l) concentrations of the clove seed extract, respectively.

Key words: *Eugenia aromaticum* (clove), seed extract, anesthesia, *Clarias gariepinus*, fingerlings.

Introduction

The African catfish *Clarias gariepinus* is widely cultured in Africa, Europe and some parts of Asia for its hardy nature. It has been the suitable candidate for aquaculture because of the following characteristics viz: high prolificacy and simplicity of culture, the existence of an arborescent air breathing organ, omnivorous, feeding habits, enhanced/rapid growth rate and improved feed

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conversion rate (Britz and Pienaar, 1992; Hecht et al., 1996). *Clarias gariepinus* is in great demand because of its striking attributes and palatability (Sogbesan and Ugwumba, 2008).

Stress in fish caused by physical disturbances encountered during fish farming activities, such as handling and transport, instigates a variety of responses that may be adaptive or detrimental (Barton and Iwama, 1991). Fish undergo a series of biochemical and physiological changes in an attempt to compensate for the challenge imposed upon them by stress (Sharma and Dash, 1991). According to Sharma and Dash (1991), handling stress is the most common factor that causes scale loss and skin damage, which subsequently leads to pathogen invasion. Handling stress physically removes mucus resulting in decreased chemical protection, osmoregulatory function and lubrication, hence, causing the fish to use more energy. This facilitates pathogens to invade.

Anaesthetics are commonly used to reduce mortality and stress during fish handling (King et al., 2005; Ross and Ross, 2008). Anaesthetics used for fish handling should have the following characteristics viz: highly soluble in fresh and marine water, rapid induction and recovery time, non-toxic to fish and humans, short physiological effects, rapid wear elimination from the body, readily available and inexpensive (Marking and Meyer, 1985). The anaesthetic should be inexpensive and easy to use (Gilderhus and Marking 1987; Klimankova et al., 2008).

Anaesthetics such as 2-phenoxyethanol, quinaldine, tricaine methane sulphonate (MS-222), eugenol, and benzocaine are very expensive and scarce, especially in Nigeria. The commonly used chemical fish anaesthetics including carbon dioxide (CO_2) gas, quinaldine and 3-aminobenzoic acid ethyl ester methanosulphonate (MS–222) are comparatively safe for both fish and humans (Griffiths 2000; Woody et al., 2002). Carbon dioxide (CO_2) has been reported to be slow acting, often resulting in only light sedation, and difficult to administer, as well as toxic to many fish species (Gilderhus and Marking, 1987). Anaesthetic induction is often accompanied by hyperactivity, usually a response of only a few seconds to the sensation or slightly irritant properties of the drug. In general, induction should be rapid and without marked hyperactivity (Ross and Ross, 2008).

An ideal anaesthetic for fish should induce anaesthesia in 3 to 5 minutes, with total loss of balance and muscle tone, allowing an uneventful and rapid (i.e. less than 10 minutes) recovery with low tissue residues after recovery, thus being safe for users and consumers.

Anderson and McKinley (1997) have reported that special attention has been recently paid to clove oil as an anaesthetic substance in the aquaculture to replace MS-222. The clove oil is a natural product, used (for a long time) in medicine, cosmetics and food industry as a food aromatizer. According to Taylor and Roberts (1999), clove has been used in human medicine as a mild anaesthetic from ancient

times. Clove has an antibiotic, antiseptic, antimitotic and antibacterial effect (Hamackova et al., 2006). Eugeunol, which is found in clove, has been used as topical anaesthesia in dentistry (Soto and Burhanuddin, 1995). Farid (1999) has reported that eugenol has been successfully used as an anaesthetic in rabbit and fish, as well as in Indian major carps.

Zaikov et al. (2008) have opined that eugenol is the active component of clove oil, representing 70–90% of its weight. They also suggest that clove seed may also contain eugenol acetate and caryophyllene just like the oil, which has been reported to contain more than 17 and 12%, respectively. Zaikov et al. (2008) have further reported complete immobilization of pike (*Esox lucius L.*), after application of 0.04 ml of clove oil/litre of water for 5.50 to 9.50 minutes. Clove oil at concentrations between 0.25 and 0.50 ml/liter was reported to be effective anaesthesia on four hardy freshwater fish species (*Anabus testudineus, Mystus vittatus, Channa punctatus* and *Channa orientalis*) in Rajshahi, Bangladesh by Alam et al. (2012). Javahery et al. (2012) reported that the minimum concentration of clove oil that gave desirable anaesthetic effects was 0.044 mg/l for 2–13 g of *Rutilus frisiikutum*.

Simoes et al. (2011) reported that the most appropriate clove oil concentration to induce surgical anaesthesia in fish was 90 mg/l of water, while for biometric studies or other brief handling, they recommended concentration of 50□60 mg/l as it provided fast recovery. According to them, the maximum anaesthesia time should be 10 minutes. The 10-minute exposure to clove oil at a concentration of 30 mg/l caused a significant increase in the concentration of glucose (GLU) and inorganic phosphate (PHOS) immediately after anaesthesia (Velisek et al., 2005). Matin et al. (2009) have reported that induction of anaesthesia and recovery are best at 0.02% clove oil, while at 0.03% concentration it produces shorter induction and longer recovery period. The most appropriate clove oil concentration to induce surgical anaesthesia in *Oreochromis niloticus* according to Simoes et al. (2011) is 90 mg/L^{-1}, while for biometric or other brief handling, 50–60 $mg/L^{-1}$is better because it provides fast recovery with maximum anaesthesia time of 10 minutes. Martin et al. (2009) recommended 25mg/l of clove oil for inducing anaesthesia to *Oncorhynchus tshawytscha*, *O. kisutch* and *O. mykiss*. Waterstrat (1999) gave information for an effective concentration of 100 $mg1^{-1}$ for *Ictalurus punctatus*. Woody et al. (2002) recommended a concentration of 50 $mg1^{-1}$ for inducing anaesthesia to *Oncorhynchus nerka*. Akinrotimi et al. (2013) reported size related responses among juvenile and fingerlings of *L. facipinnis* and *L. grandisquamis* in terms of induction and recovery time when exposed to clove seed extract. They reported consistently faster induction and recovery time in fingerlings than that of juvenile. They reported a faster induction period of 55.81 seconds and the highest recovery time (350.11 seconds) in *Liza falcipinnis* exposed to clove seed at 25 mg/L.

Jegede (2014) suggested the use of ethanol extract of tobacco (*Nicotianna tobaccum*) at 2.5 g/10L of water as an anaesthetic agent in *Clarias gariepinus*

fingerlings for 53–65 minutes. Solomon et al. (2014) reported that the most effective concentration of the freeze-dried bark extract of *Tephrosia vogelii* was 0.06g/l with an induction time of 32 seconds and a recovery time of 182 minutes. Ayuba and Ofojekwu (2005) documented a shorter sedative period of 1.00 minutes at 0.50g/l of the pure unseparated extract of Toloache (*Datura innoxia*) and 58.50 minutes at a concentration of 3.00g/l after using a crude extract of the same plant on *Clarias gariepinus*.

In aquaculture, different manipulations are done with the fish during induced breeding, transportation, blood sampling, surgical operations, data collection of morphometric and meristic characters, etc., which can lead to stress, trauma, or even death. Anaesthesia has been commonly used on European sea bass *Dicentrarchus labrax* juveniles during evaluating the skeleton deformities (Ayuba and Ofojeckwu, 2005; Koumoundours et al., 1997).

The use of anaesthesia to immobilize fish will decrease the stress, allowing easy handling and guaranteeing the health of the fish. The most widely used anaesthetic on fish is tricaine (MS-222). MS-222 is relatively expensive and not commonly available. It has been classified as a carcinogen and food fish if anesthetized with MS222 require a minimum of 21 days of a withdrawal period (Kennedy et al., 2007). Transportation, biometric studies, egg striping during induced breeding, semen collection from Clariid and other fish handlings cause stress and even heavy mortality in fish and these activities should be avoided in fish culture. According to Simoes et al. (2011), transportation of *O. niloticus* fingerlings using clove oil as an anaesthetic may increase the level of Na^+ and K^+ disorders in the fish. Little or no information has been documented on the utilization of clove seed extract as anaesthesia on fish. The objective of this study therefore is to assess the efficacy of clove seed extract as an anaesthetic agent on *Clarias gariepinus* fingerlings.

Materials and Methods

Study area

The study was conducted in the Fish hatchery unit of the Department of Fisheries, University of Maiduguri located at longitude 13.0° 111 42” and latitude 12.0° 481 37’’. Maiduguri has two distinct seasons: the rainy season, which commences in late May and ends in September with its peak in August, while the dry season starts in October and lasts to early May.

Collection and processing of clove seed powder

Clove (*Eugenia aromaticum*) seed (dry buds) was procured in the local market in Maiduguri. The buds were ground into in an airtight plastic bottle until required.

Experimental fish

Clarias gariepinus (24.13±0.19–25.30±0.35g and 5.97±0.03–7.00±0.56 cm length) fingerlings were obtained from a fish hatchery of the Department of Fisheries, University of Maiduguri, Nigeria. The fish were acclimatized in an indoor concrete tank (2.0 x 1.3 x 1.0m) three days before the commencement of the experiment.

Experimental design

Various concentrations of clove seed granules (25.0, 50.0, 75.0, 100.0, 125.0 mg) were soaked for 24 hours in one liter of distilled water. A stock solution was prepared in the first instance. The soaked clove seed granules were sieved through a 0.2-µm mesh sieve. The stock solution was transferred into 40-liter capacity glass aquariums containing 5 liters of water. Twenty (20) fingerlings were placed into the different concentrations (treatment groups) for anaesthetic trials in triplicates. Each treatment was tried separately. At the end of induction, the fish were transferred into fresh water (20 liters) in a glass aquarium for recovery. The time at which the fingerling responded to anaesthesia, the time of induction, response time to recovery and immediate post sedation mortality were recorded. Mortality after twenty-four (24) hours after sedation was also recorded using a digital stopwatch.

Data analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA). Differences of means were determined using Tukey's HSD with the aid of Statistics 8.0.

Results and Discussion

Table 1 shows the mean anesthetic effects of clove seed extract on *Clarias gariepinus* fingerlings. The time of complete induction also decreased with an increase in the concentration of the clove seed extract. The decrease in the induction time with an increase in the concentration of the clove seed extract observed in this study is congruent with the findings of Akinrotimi et al. (2013), who reported a similar trend in the effect of clove seed extracts on two species of grey mullets (*Liza facipinnnis* and *L. grandisuamis*). Kennedy et al. (2007) documented the use of clove oil in *Heteropneustes fossili*, and Matin et al. (2009) reported the use of clove oil in two sizes of *Rutilis frisii* Kutum (Javaher et al., 2012). The time of response to induction decreased with an increase in the concentration of the clove seed extract.

Fish exposed to a 25 to 50mg/l extract produced a slower anaesthesia effect compared to those treated with higher dosages (75 to 150mg/l). Fingerlings immersed in 150mg/l of clove seed extract responded faster (0.35±0.06 minutes) to the anaesthesia. No significant variation was observed between the responses to anaesthesia by fish exposed to 100, 125 and 150mg/l (Table 1). Response to the anaesthesia was accompanied by uncoordinated, erratic and twisted movements.

Table 1. Mean (±SEM) anesthetic effect of clove seed extract on *Clarias gariepinus* fingerlings.

Parameters	Clove seed levels (mg/L^{-1})					
	25	50	75	100	125	150
RI (minutes)	3.72 ± 0.35^{a}	3.54 ± 0.04^{a}	1.93 ± 0.45^{b}	1.22 ± 0.15^{bc}	0.41 ± 0.06^{c}	0.35 ± 0.06^{c}
IT(minutes)	7.52 ± 0.25^{ab}	10.60 ± 0.98^{a}	5.96 ± 1.17^{bc}	3.31 ± 0.55^{cd}	3.07 ± 0.07^{cd}	2.28 ± 0.15^{c}
RR (minutes)	1.23 ± 0.03^{b}	1.21 ± 0.05^{b}	1.44 ± 0.04^{b}	1.99 ± 0.51^{b}	3.32 ± 0.09^{a}	3.98 ± 0.25^{a}
RT (minutes)	2.48 ± 0.09^{c}	3.16 ± 0.05^{bc}	3.26 ± 0.13^{bc}	3.62 ± 0.14^{bc}	3.92 ± 0.32^{ab}	5.05 ± 0.5^{a}
PRM (%)	0.00	0.00	2.00 ± 0.58^{ab}	1.00 ± 0.58^{b}	3.67 ± 0.88^{a}	3.67 ± 0.33^{a}
PRM (%)	2.71 ± 0.15^{bc}	2.22 ± 0.51^{a}	3.56 ± 0.24^{abc}	2.22 ± 0.11^{c}	3.10 ± 0.54^{abc}	4.79 ± 0.25^{a}

Means in the same column having the same superscript are not significantly different ($p>0.05$);

Key: RI = Response to induction, IT = Induction time, RR = Response to recovery, RT = Recovery time, PRM = Post recovery mortality.

Complete induction was noticed by the loss of equilibrium and lack of response to external stimuli. The shortest mean induction time was 2.28±0.15 minutes in fingerlings exposed to 150mg/l of clove seed extract, while the longest induction time of 10.60±0.93 minutes was observed in fish exposed to 50mg/l. There was no significant difference between ($p>0.05$) the mean induction times of fish exposed to 75, 100 and 125mg/l of clove seed extract compared to those of fish exposed to 150mg/l. However, the induction time of fish sedated with 25 and 50mg/l differed significantly from those treated with 100, 125 and 150mg/l. The shortest and the longest induction times (2.28 and 10.60 minutes) at the dosages of 150mg/l and 50mg/l, respectively, were slower (55.61 and 210.61 seconds) than the shortest and longest induction times (25mg/l and 5mg/l), respectively reported by Akinrotimi et al. (2013) for fingerlings and juveniles of *Liza facipinnnis* and *L. grandisuamis* anaesthetized with clove seed extract. The variation in the induction time could be due to the higher concentration of clove seed extracts used and the variation in the species of fish used for the experiment. Jegede (2014) reported longer induction (63–109 minutes) and recovery periods (44–85 minutes) in *C. gariepinus* fingerlings anaesthetized using tobacco extract.

Recovery commenced with rapid swimming with the head up as well as erratic movements and finally, normal swimming and followed by response to external stimuli. The response to recovery was produced significantly earlier (1.23±0.03,

1.21±0.05, 1.44±0.04 and 1.99±0.51 minutes) in fish treated with lower concentrations (25, 50, 75 and 100mg/l, respectively) of the clove seed extract. However, the longest duration of recovery was observed in fingerlings treated with 150 and 125mg/l (3.98±0.25 and 3.32±0.09 minutes, respectively).

Recovery time increased with an increase in the concentration of the clove seed per litre of water. The longest recovery time of 5.05±0.05 minutes was observed in *C. gariepinus* fingerlings anaesthetized with 150mg/l, while the shortest recovery time was recorded in fish treated with 25mg/l (Table 1). No statistical variation ($p>0.05$) was observed between the recovery time of fish treated with 150mg/l of clove seed extract compared to those treated with 125mg/l. Similarly, there was no significant difference ($p>0.05$) between the recovery times of fish treated with 125, 100, 75 and 25mg/l. The longer recovery time at higher concentrations of clove seed extract observed during this study agrees with the findings of Martins et al. (2009) and Javahery et al. (2002), who reported similar cases for *Hepteropneustes fossilis* and *Channa punctatus* and two sizes of *Rutilis frisii* Kutum, respectively anaesthetized with clove oil. The shorter recovery time (2.48 minutes) using 25mg/l of clove seed extract recorded in this study was faster than 7.90 minutes as documented by Matin et al. (2009) for *Hepteropneustes fossilis* and *Channa punctatus* and 5.50 minutes for pike (*Esox lucius*) at 0.04ml/L of clove oil (Zaikov and Hubenova, 2008). However, the recovery time was longer than 172.66 and 246.91 seconds as reported by Akinrotimi (2013) for fingerlings and juveniles, respectively, of *Liza falcipinus* after induction with 5mg/l of clove seed extract, although they used a higher concentration of the clove seed extract.

No mortality occurred in fish treated with 25 and 50mg/l of clove seed extract. They recovered normally. However, fish treated with 75 to 150mg/l showed mortality immediately after recovery. Mortality at 24 hours after recovery was observed in all the treatment groups. The highest (4.79±0.25%) mortality rate after 24 hours was recorded in fingerlings treated with 150mg/l, followed by 75 and 125 mg/l of clove seed extract. There were no significant differences ($p>0.05$) between the mortality values after 24 hours, after recovery of fingerlings sedated with 150, 75 and 126 mg/l. The 24-hour post-recovery mortality reported in this study was not in tandem with the findings of Akinrotimi et al. (2015), who did not record any mortality during their study. The mortality rates of 2□4.70% recorded immediately after recovery as well as 24 hours after recovery during this study were higher than those reported by Kennedy et al. (2007). However, Jegede (2014) reported 20, 45 and 60% mortality after sedating *Clarias gariepinus* fingerlings with 3.75, 5.00 and 6.25g/10, respectively per liter of water.

Water quality parameters

The mean water quality parameters recorded in sedation tanks are shown in Table 2. The water quality parameters recorded in the anaesthesia tanks during this

study were within the recommended range for fish culture recommended by Viveen et al. (1986).

Table 2. Mean (±SEM) water quality parameters in the sedation tanks.

Parameters	Clove seed level (mg/liter)					
	25	50	75	100	125	150
Temperature (^{o}C)	28.27±0.17^{a}	28.53±0.29^{a}	28.40±0.42^{a}	28.53±0.33^{a}	27.83±0.46^{a}	28.53±0.33^{a}
Dissolved oxygen (mg/l)	4.29±0.23^{a}	4.57±0.18^{a}	4.50±0.25^{a}	4.33±0.33^{a}	4.03±0.24^{a}	4.13±0.34^{a}
pH	7.34±0.03^{a}	7.50±0.15^{a}	7.47±0.13^{a}	5.53±0.18^{a}	7.40±0.15^{a}	4.87±2.43^{a}

Means in the same column having the same superscript are not significantly different ($p>0.05$).

Conclusion

This study reveals that *Clarias gariepinus* fingerlings responded to the anaesthetic effect of the clove seed extract at all the tested concentrations. The safe and effective clove seed concentration range for anaesthesia in *Clarias gariepinus* fingerlings was 100–150 mg/l. The clove seed extract is the effective anesthesia for Clarias fingerlings, therefore, could be utilized for transportation/handling of fish fingerlings and biometric study to reduce stress and mortality. There is need to investigate the longest period the fingerlings can withstand the anesthetic effect to ascertain the distance and duration of the transportation and handlings in aquaculture husbandry.

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ANESTETIČKO DEJSTVO EKSTRAKTA SEMENA KARANFILIĆA (*EUGENIA AROMATICUM*) NA MLAĐ *CLARIAS GARIEPINUS* (BURCHELL, 1822) U SEMIARIDNIM USLOVIMA

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R e z i m e

Ispitivana su anestetička dejstva ekstrakta semena karanfilića (*Eugenia aromaticum)* na mlađ *Clarias gariepinus* u semiaridnim uslovima. Za eksperiment su korišćene različite koncentracije ekstrakta semena 25,0, 50,0, 75,0, 100,0, 125,0 i 150,0mg po litru vode. Svaka koncentracija je testirana u grupi mlađi *Clarias gariepinus* (težine 24,13–25,30g i dužine 5,97–7,00cm) u staklenim akvarijumima. Došlo je do smanjenja vremena indukcije kako se koncentracija ekstrakta semena karanfilića povećavala. Mlađ tretirana sa 150mg/l ekstrakta pokazala je najkraće vreme indukcije (2,28±0,15 minuta), a zatim su sledile ribe tretirane koncentracijama sedativa od 100 i 125mg/l (3,31±0.55 odnosno 3,07±0,07 minuta). Najduže vreme indukcije (10,60±0,98, 7,52±0,25 i 5,96±1,17 minuta) zabeleženo je kod mlađi gde je tretman uključivao 50, 25 odnosno 75mg/l sedativa. Oporavak je bio znatno brži (2–3,92 minuta) kod riba tretiranih nižim dozama (25 do 125mg/l) ekstrakta semena karanfilića. Stope smrtnosti posle 24 časa oporavka bile su više (4,79±0,25 odnosno 3,10±0,54%) kod riba koje su anesteziranе višim (150 odnosno 125mg/l) koncentracijama ekstrakta semena karanfilića.

Ključne reči: *Eugenia aromaticum* (karanfilić), ekstrakt semena, anestezija, *Clarias gariepinus*, mlađ.

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