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Research Article

Anaesthetic Effect of Clove (*Eugenia Aromaticum)* Seed Extract on *Clarias Gariepinus* (Burchell 1822) Broodstock

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Abstract: The efficacy of clove seed extract as anaesthesis on *Clarias gariepinus* broodstock were investigated. Fifty two gravid *Clarias gariepinus*, males and females (658.12±4.83 to 1136.70±321.99 g and 42.34±4.97 to 50.70±6.29 cm) were subject to various of concentrations (1.5, 2.0, 3.0, 3.5 and 4.0 g/L, respectively) of clove seed extract in concrete tank (1.8×1.2×1.0 m deep) in replicates indoors until they were immobilised. The result show that the induction time of the broodstock decreased as the concentration of the clove seed extract increases. However, the recovery time increased with an increase in concentration of the extract. Induction time was shorter (4.26±0.81, 4.51±0.31, 4.75±0.37 and 5.84±0.06, min) in fish treated with higher (2.5, 3.5, 3.0 and 4.0 g/L, respectively) dosages of the extract. Fish recovered faster (1.51±0.13, 1.87±0.26 and 2.10 min) in fish exposed to lower dosages (1.5, 2.0 and 2.5 g/L, respectively). Clove seed extract at 2.5-4.0 g/L was able sedate *C. gariepinus* broodstock within 4.0-6.0 min and recovered within 6.0 min. Clove seed extract possess the desirable properties of affordable, accessible and suitable anaesthetic agent for handling, restraint and immobilisation of broodstock during breeding.

Keywords: Anaesthesia, broodstock, *clarias gariepinus*, clove seed extract

INTRODUCTION

The African catfish *Clarias gariepinus* is widely cultured in Africa, Europe and some parts of Asia due to its hardy nature. It has been the suitable candidate for aquaculture owing to high prolificacy and thriving under simple culture conditions, the existence of arborescent air breathing organ, omnivorous feeding habit, fast growth rate and improve feed utilisation (Britz and Pienaar, 1992; Hecht *et al*., 1996). Thus, *C. gariepinus* is in great demand because of its striking attributes and enriched taste of its fleshy tissue (Sogbesan and Ugwumba, 2008).

To reduce mortality and stress during fish handling, anaesthetics are commonly used to sedate and immobilize fish (Ross and Ross, 2008). Anaesthetics used for fish must have the following desirable characteristics like high solubility in fresh and marine water, rapid induction and recovery time, non-poisonous or injurious to fish and humans, short physiological effects, rapidly wear off of effects and excretion from the body, availability and affordability (Marking and Meyer 1985). Common anaesthetics such as 2-phenoxyethanol, quinaldine, tricaine methane sulphonate (MS-222), eugenol and benzocaine (Velisek *et al*., 2005) are expensive and scarce especially in Nigeria.

Anaesthetic induction is often accompanied by initial excitement (hyperactivity), a momentary 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 min, with total loss of balance and muscle tone, allowing an uneventful and rapid (<10 min) recovery with low tissue drug residues after recovery, thus being safe (not toxic) to users and consumers. The anaesthetic should be inexpensive and easy to use (Gilderhus and Marking, 1987; Klimanková *et al*., 2008) and soluble in aqueous solvent (Gunn, 2000).

Commonly used chemicals as fish anaesthetics are carbon dioxide (CO_2) gas, quinaldine and 3-aminobenzoic acid ethyl ester methanosulphonate (MS-222), which are considered safe for both fish and human (Griffiths, 2002; Woody *et al*., 2002). The use of Carbon dioxide (CO_2) has been reported to be slow acting, often results in only light sedation and is difficult to administer, as well as toxic to many fish species (Gilderhus and Marking, 1987).

Recently special attention has been paid to clove oil as an anaesthetic substance in the aquaculture as an alternative to MS-222 (Anderson *et al*., 1997). Coyle

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et al. (2004) reported induction time with MS-222 of as little as 15 sec. They further maintained that, Salmonids were quickly anaesthetized after immersed in 25 to 50 mg/L. Channel catfish *Ictalurus punctatus*) require 100 to 250 mg/L for full anaesthesia, with a 3-min induction time, while up to 100 mg/L is required for some species, including tilapia (Coyle *et al*., 2004).

The Clove oil, a natural product, has been in use for a long time in medicine, cosmetics and food industry as a food aromatizer. It has been used in human medicine as a mild anaesthetic from ancient times (Ross and Ross, 2008; Taylor and Roberts, 1999). It has an antibiotic, antiseptic, antimicotic and antibacterial effect (Hamackova *et al*., 2006). Eugeunol has been used as topical anaesthesia in dentistry (Soto and Burhanuddin, 1995) and successfully as an anaesthetic in rabbit fish (Soto and Burhanuddin, 1995), gold fish, Crucian carp (Endo *et al*., 1972) and Indian major carps (Farid, 1999). Eugenol is the active component of clove oil and it represents 70-90% of its weight (Zaikov *et al*., 2008).

Peake (1998) reported that a concentration of 60 $mL.^{-1}$ (approximately, 0.057 $mL.^{-1}$) of clove oil as efficient anaesthesia for Pike, Hamackova *et al*. (2006) recommended the concentration of 0.025-0.033 mL^{-1} of clove seed as anaesthesia for the same fish species. Zaikov *et al*. (2008) reported complete immobilization of Pike (*Esox lucius L.*), after application 0.04 mL of clove oil per litre of water in 5.50 to 9.50 min. Clove oil at concentrations between 0.25 to 0.50 mL was reported to be an effective anaesthesia on four hardy freshwater fish species (*Anabus testudineus*, *Mystus vittatus, Channa punctatus* and *Channa orientalis*) in Rajshahi, Bangladesh (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 frisii kutum*.

Simoes *et al*. (2011) reported that the most appropriate clove oil concentration to L^{-1} induce surgical anaesthesia was 90 mg/L of water, while for biometry studies or other brief handling, the recommended concentration is 50-60 mg/L as it provides fast recovery. Accordingly the maximum anaesthesia time should be 10 min. The 10 min 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) that induction of anaesthesia and recovery was the best at 0.02% clove oil while at 0.03% concentration produced shorter induction and longer recovery period.

Akinrotimi *et al*. (2013) reported size related responses, with the induction and recovery time in the juvenile consistently higher than the fingerlings after in two species of *L. facipinnis* and *L. grandisquamis* exposed to clove seed extracts. They reported faster induction period of 55.81 sec and highest recovery time (350.11 sec) 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/L^{10} of water as anaesthetic agent on *Clarias gariepinus* fingerlings up to 53-65 min. Solomon *et al*. (2014) reported that, the most effective concentration of freeze-dried back extract of *Tephrosia. vogelii* was 0.06 g/L with an induction time of 32 sec and a recovery time of 182 min.

Ayuba and Ofojekwu (2005) reported shorter sedative period of 1.00 min at 0.50 g/L of pure unseparated extract of *Datura innoxia* and 58.50 min at 3.00 g/L concentrated after using 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 on morphometric and meristic characters, etc. These procedures can lead to stress, trauma, or even death. In large sized fish, handling tends to be difficult especially egg striping during induced breeding. The use of anaesthesia to immobilize the fish, will decreases the stress, easy handling and guarantees health of the fish. Although Tricaine (MS-222) is most widely anaesthesia on fish it is relatively expensive, not easily accessible and regarded as potentially carcinogen. Additionally, food fish anesthetized with MS222 require a minimum of 21 days withdrawal period (Kennedy *et al*., 2007). Little or no information has been documented on the potentiality of clove seed extract as an anaesthesia in African catfish *C. gariepinus* broodstock except for C. *gariepinus* fingerlings (Diyaware *et al*., 2014). The objective of this study therefore is to assess the efficacy of clove seed extract as anaesthesia in *Clarias gariepinus*.

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° 11^{1} 42" and latitude 12.0° 48^{1} 37''. Maiduguri has two distinct seasons: rainy season which commences in late May and ends in September with its peak in August, while the dry season starts in October to early May.

Collection and processing of clove seed powder: Clove (*Eugenia aromaticum*) seed (bud) were procured from a local market in Maiduguri, North east Nigeria. The clove seed were ground into fine/coarse powder using a hammer miller and packed in air tight bottle until required.

Experimental fish: Gravid *C. gariepinus* broodstock male and female were procured from a fish farm in

Maiduguri. The brood fish were placed in a makeshift 50 L capacity plastic container (dimensions) filled to two-third level with fresh tap water. The brood fish were then transported to the Fish hatchery and conditioned in 2 m^2 concrete fish tank for 7 days at 6 fish per tank.

Experimental design: Fifty two gravid *Clarias gariepinus* broodstock males and females (658.12±4.83 to 1136.70±321.99 g and 42.34±4.97 to 50.70 ± 6.29 cm length) were subject to various concentrations (1.5, 2.0, 3.0, 3.5 and 4.0 g/L) of the clove seed extract in concrete tank (1.8×1.2×1.0 m deep) in replicates indoors until they were immobilized. The stock solutions of the clove seed extracts were prepared in 2 L capacity plastic buckets and later transferred into the concrete. Each of the clove seed concentration were tested for the anaesthetic effect using 6 broodstock (3 each male and female).

The anaesthetised fish following treatment with clove seed extract were transferred into another 1.8×1.2×1.0 m deep recovery concrete tank half-filled with fresh tap water. The recovery times were recorded as soon as they were removed from the sedation tank as well as after 24 h post treatment. The time for onset of induction, induction time and recovery time for each treatment were recorded digital stopwatch.

Data analysis: Data obtained from the experiment were subjected to one way Analysis of Variances (ANOVA). Means were separated using fisher's LSD with means of Statistix 8.0.

RESULTS

The complete induction of the *C. gariepinus* broodstock with clove seed extract was characterized by calmness, sedation and immobilization. At the end of induction period as well as 24 h post induction periods, no mortality was recorded in all the treatments. At the onset of induction, *C. gariepinus* broodstock showed erratic, excitative, swirling, upside and disorientation (movement) in all the induction tanks. Table 1 show the anesthetic effect of Clove seed extract on *C. gariepinus* broodstock. The fish responded to the clove seed extract faster with increase in the concentration of clove seed extract. The onset of induction were faster (1.46±0.05, 1.95±0.25 and 2.65±0.39 min) in fish treated with higher (4.0, 3.50 and 3.0 g/L, respectively) dosages of the clove seed extracts. On the other hand, it took longer time (6.87±0.85, 3.68±0.28 and 3.19±0.82 min) for the fish treated with lower amounts of 1.5, 2.0 and 2.5 g/L, respectively) dosages of clove seed extract. Significant variation ($p<0.05$) were observed in the period of onset of induction in fish treated with 1.5 g/L of the clove seed extract compared to the entire treatments.

The complete induction of the broodstock with clove seed extract was characterized by calmness, loss of equilibrium and suppression of ability to respond to external stimuli. There was a decrease induction time as the concentration of the clove seed extract increases. Induction time was shorter (5.84±0.06, 4.51±0.31 and 4.75±0.37 min) in fish treated with higher (4.0, 3.5 and 3.0 g/L) dosage of the extract. There was no significant differences ($p<0.05$) in the induction time among the fish treated with 3.0, 3.5 and 4.0 g/L. However, significant variations ($p>0.05$) were observed between the induction of fish treated with 4.0 compared to those treated with 1.5 and 2.5.

The response to recovery from sedation was observed to be significantly slower (2.88±0.05) in *C. gariepinus* broodstock treated with higher (4.0 g/L) concentration of clove seed extract. However, fish treated with lower dosage (1.5, 2.0, 2.5 and 3.0 g/L) responded to recovery faster (1.30±0.06, 0.49±0.02, 0.44±0.15 and 1.83±0.17), respectively.

Complete recovery from the sedation was slower as the clove seed extract concentration increased. Slower recovery times (6.21±0.42, 6.34±0.91 and 6.41±0.10 min) were significantly observed in fish exposed to higher concentration (3.0, 3.5 and 4.0 g/L, respectively. No significant variations ($p>0.05$) were observed in the recovery time of fish treated with lower (1.5, 2.0 and 2.5 g/L) concentrations of clove seed extract.

The water quality parameters observed in both the induction tanks and recovery tanks are shown in Table 2. Temperatures ranged between 28 to 29.43°C, while pH and dissolved oxygen were between 7 to 7.27 and 4.66 to 5.03 g/L). The water quality parameters observed during this study are with the range for fish culture as suggested by Viveen *et al*. (1985).

Table 1: Mean (±SEM) Effect of Clove seed extract as anesthesia on *Clarias gariepinus* broodstock

	Clove seed extract concentration levels (g/L)						
Parameters	0.0	1.5	2.0	2.5	3.0	3.5	4.0
FW(g)	1106.50±21.10^{a}	1136.70±321.99^{a}	803.33±83.73^{ab}	658.12±4.83^{b}	711.10±11.10^{b}	754.56±9.87^{ab}	731.11±17.88^{ab}
SL (cm)	48.70±5.21^{a}	50.70±6.29^{a}	46.03±1.97^{a}	44.62±0.72^{a}	42.34±4.97^{a}	48.14±0.50^{a}	48.45±1.12^{a}
OI (mins.)	00.00	6.87±0.85^{a}	3.68±0.28^{b}	3.19±0.82^{bc}	2.65±0.39^{bcd}	1.95±0.25^{cd}	1.46±0.05^{d}
IT (mins.)	00.00	8.76 ± 0.21^{a}	5.56±0.43^{bc}	4.26±0.81^{c}	4.75±0.37^{bc}	4.51±0.31^{bc}	5.84±0.06^{b}
OR (mins.)	00.00	1.30±0.06^{c}	0.49±0.02^{d}	0.44±0.15^{d}	1.83±0.17^{bc}	2.00±0.50^{b}	2.88±0.05^{a}
RT (mins.)	00.00	1.87±0.25^{b}	2.10±0.60^{b}	1.51±0.13^{b}	6.21±0.42^{a}	6.34±0.91^{a}	6.41±0.10^{a}
S (%)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Mean within the same row having similar superscript are not significantly different ($p>0.05$); FW = Fish weight; SL = Standard weight; OI = Onset of induction; IT = Induction time; OR = Onset of Recovery; RT = Recovery Time; S = Survival

Table 2: Mean (±SEM) water quality parameter in the induction tanks

Clove seed inclusion level (g/L)	Temperature (°C)	pH	Dissolved oxygen (mg/L)
1.5	28.43±0.35^{bc}	7.05±0.12^{b}	4.86±0.39^{a}
2.0	28.37±0.35^{bc}	7.19±0.16^{b}	4.93±0.19^{a}
2.5	28.37±0.32^{bc}	7.57±0.03^{a}	4.90±0.15^{a}
3.0	28.10±0.21^{c}	7.08±0.14^{b}	4.66±0.35^{a}
3.5	29.40±0.17^{a}	7.27±0.02^{ab}	5.03±0.32^{a}
4.0	29.13±0.12^{ab}	7.19±0.06^{b}	4.99±0.06^{a}

DISCUSSION

The induction time of *C. gariepinus* observed in this study decreased as the concentration of the clove seed increased, while the recovery time increased with increasing concentration of the extract. Similar trend has been reported by Akinrotimi *et al*. (2013) in two species of Grey Mullet (*Liza facipinnus* and *Liza grandisquamis*) fingerlings treated with clove seed extract. The range 4-4.5 min at 2.5 to 3.5 g/L induction time recorded in this study is within the recommended induction time (3-5 min) for ideal anaesthesia for fish as suggested by Marking and Meyer (1985). The 2.5 g/L clove seed extract that induced sedation within 5 min in this study was faster than 53-65 min of 2.5 g/L[10] of ethanoic extract of tobacco (*Nicotianna tobaccum*) reported by Jegede (2014) for *Clarias gariepinus* fingerlings and 58.50 min induction time at 3.00 g/L concentrated after using crude extract of *Datura innoxia* (Ayuba and Ofojekwu, 2005) on *C. gariepinus* fingerlings. However, Ayuba and Ofojekwu (2005) also reported faster induction time of 1.00 min at 0.50 g/L of pure unseparated extract of same plant and on *C. gariepinus* fingerlings. The reason for such variations could be due to the differences in the variety of plant used as anaesthesia and the fish size.

The increase in the recovery time with increase in the concentration of clove seed extracted may be dedicated to the large amount of the sedative deposited on the gills that might have taken longer period to dissolved.

The 100% survival in this study is in line with the findings of Agokei and Adebisi (2010) in Oreochromis niloticus fingerlings exposed to tobacco leave extract and Akinrotimi *et al*. (2013) in two species of Grey Mullet (*Liza facipinnus* and *Liza grandisquamis*) fingerlings treated with clove seed extract.

Clove seed extract have meet the criterion for ideal anaesthesia for fish for being cheaply available, easy to use, safe to users and consumers (non-toxic), uneventful and rapid recovery (less than 10 min) and low tissue residues after recovery.

CONCLUSION

Clove seed extract at of 2.5-4.0 g/L was able to sedate *C. gariepinus* broodstock within 4-6 min and recover from sedation within 6.0-6.5 min. Clove seed extract possess the desirable properties of affordable, accessible and suitable anaesthetic agent for handling, restraint and immobilisation of Clariid broodstock during breeding.

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