



Evaluation of Anaesthetic Efficacy of *Ocimum gratissimum* on *Clarias gariepinus* Fingerlings during Transport

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

High fish mortality caused by stressors during transportation hurts the aquaculture sector specifically fish farmers. Synthetic anaesthetics made from chemical materials are banned because of safety and residual effects. This study was conducted to determine the effectiveness of *Ocimum gratissimum* (Scent leaf) powder as a natural anaesthetic in transporting *C. gariepinus* fingerlings. A total of 300 *C. gariepinus* fingerlings were exposed to 0.00, 10.00, 20.00, and 30.00 g/6L inclusion levels of *O. gratissimum* leaf powder in 12 gelcans (10litres) filled with 6L of water each. Twenty-five *C. gariepinus* fingerlings were introduced into each treatment and control in a complete randomized design (four trts and three replicates). The experimental fish were transported from Minna to Suleja at 204 km/h (to and fro). Anaesthetized fish exhibit decreased induction time with an increase in

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concentration of the *O. gratissimum* powder while recovery time increased with an increase in concentration. The longest induction and shortest recovery times were recorded in 10 g/6l (T2), while the shortest induction and longest recovery times was recorded in 30 g/6l (T4). The survival rate of fingerlings decreased with an increase in the concentration of the *O. gratissimum*. A high mortality rate was recorded in trt 4 (30 g/6l) due to increased concentration of experimental plants. The result obtained from the analysis of physical and chemical characteristics of water before, during, and after the transportation showed variation in most of the parameters. There was a significant difference ($p < 0.05$) in dissolved oxygen, pH, and sulphate but no significant difference ($p > 0.05$) was observed in Temperature and Ammonia. Due to its effectiveness and availability; *O. gratissimum* is close to an ideal anesthetic for use in aquaculture. Farmers could therefore use 10.00 g/6L concentration of the powder for transportation of *C. gariepinus*.

Keywords: Anesthetic; *O. gratisimum*; induction; recovery; survival; mortality.

1. INTRODUCTION

"Anesthetic is any substance that courses reversible loss of sensation or reduces sensitivity to pain and may cause unconsciousness which could be used to immobilize fish so they can be easily managed during aquaculture practices such as harvesting or capturing, transportation, sorting or grading, tagging, sampling, artificial reproduction procedures and surgery" (Matin, et al., 2009; Neiffer and Stamper, 2009; Javahery and Moradlu, 2012).

"The handling of fish out of their natural environment always creates stress which affects their physiology and anatomy. Fishes cultured undergo a multi-phase of stress from several stressors such as transportation, grading, weighing, and stocking which are unavoidable in aquaculture" (Gabriel and Akinrotimi 2011) "consequently resulting in poor performance, increased vulnerability to disease, and mortality in extreme cases. To minimize the mortality induced by stress, the fish are usually sedated and immobilized by using sedatives/anesthetics before handling" (King et al., 2005; Ross and Ross 2008). "Over the years, a large number of chemicals and agents have been applied to fish in the hopes of inducing anesthesia, with varying degrees of success, however, only a few of them have found in widespread usage sufficient to allow even a basic understanding of their effects and optimal application" (Aliakbar and Hadideh, 2015).

"*O. gratissimum* (Lamiaceae), commonly known as "alfavaca" also known as "Efirin" in Yoruba language is native to Africa" (Bayoub et al., 2010). "In Nigeria, the plant grows virtually in all regions. It could be found in many farms and residential and industrial areas. It grows and survives well in southwest Nigeria and can be

found in backyards where it is not intentionally planted. The plant has been used for many purposes ranging from human consumption to its application in traditional medicine in Nigeria. It has a good aroma and its leaves has become a delicacy and serve as spicy for fish and meat products such as kilishi, dambu, yaji." (El-Hawarry and Correa 2012). "However, many authors have reported that the plant has some chemical compounds with antimicrobial properties such as eugenol, linalol, methyl cinnamate, camphor, and thymol. It has been demonstrated in previous studies that eugenol (75-98%) isolated from *O. gratissimum* is the major antimicrobial component that inhibits and kills gram-positive bacteria and fungi" (Gafaar et al., 2010 and Kumaravel, Sivasubramanian 2010).

"*C. gariepinus* also known as the African sharp-tooth catfish is a species of catfish of the family *clariidae*, the air-breathing catfish. The African catfish is a large eel-like fish, usually dark grey or black coloration at the back, fading to a white belly. The African catfish is second only in size to the Vundu of Zambesian waters". (Froese, Rainer, Pouly, Daniel (eds. 2014). It has an average length of 1.5 meters and weighs up to 60kg. The fish has a flat bony head, a slender body, and a broad terminal mouth with four pairs of barbells (sensory organs). Only the pectoral fins have spines. African sharp-tooth catfish have a scale-less slimy skin and accessory air-breathing organs limps which enables them to stay out of water for many hours provided a good condition prevails.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in two phases. The first phase was the transportation of the

treated fingerling from Minna to Suleja. The second phase (post-transportation phase) was an analysis of physical and chemical parameters of the water

2.2 Experimental Plant

Scent leaf (*O. gratissimum*) leaves was collected from farmlands and confirmed with (Agbaje 2008) plant key and conveyed to the wet and dry laboratory. Upon arrival, it was washed thoroughly to remove dirt, insect or worm. The leaves were shed dried for 72 hours, grinded and sieved into fine particles (<250 µm). Sensitive weighing balance (model 20001) was used to measure (10g, 20 g and 30 g respectively).

2.3 Experimental Fish

Three hundred pieces of *C. gariepinus* fingerlings (a high valued aqua cultural fish species) comprised of both sexes with a mean weight of 3 g were acquired and acclimatized for three days. Before the transportation, the fish were starved for 12 hours. A Complete randomized design comprising of four trts and three replicates were used for the experiment and designated as; control (without anesthetic), 10 g, 20 g, and 30 g of *O. gratissimum*, powder /liter respectively. 25 pieces of *C. gariepinus* fingerlings each were introduced in plastic gallons devoid of the control. The fish were transported to and fro Minna and Suleja at a distance of (204 km). The induction and recovery time were noted.

2.4 Water Quality Parameter

Dissolve oxygen, pH, temperature, sulphate, and ammonia were analyzed before, during, and after transportation using a thermometer, dissolved oxygen meter, pH meter, ammonia using the nitrogen distillation method, and sulphate using the volumetric method.

2.5 Statistical Analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS version 23 and the mean

difference was determined using DUNCAN multiple range test

3. RESULTS

3.1 Induction and Recovery Time of *C. gariepinus* Fingerlings after Administration of the

Table 1 the induction and recovery time of *C. gariepinus* fingerlings exposed to various concentrations of *O. gratissimum* powder (scent leaf) presented in Table 1. The fish exhibit normal behavioral activities such as swimming, balance, opercula movement, and general movement of the tail and fins at 0.00 g/6l concentration (control). The last induction time of 148 minutes was recorded in trt 2 (10 g/6l) concentration, where there was a total loss of movement and the fish sank motionless at the bottom of the gelcan.

The fastest induction time of the anesthetic agent was 25 minutes recorded in trt 4 (30 g/6l) concentration, where there was total loss of movement in the fish, while the induction and recovery time in control was 0.00 and 0.00 respectively. No mortality of the fish species was recorded during the induction time. The recovery time of *C. gariepinus* fingerlings was faster at lower concentrations 10 g/6l using *O. gratissimum* powder at 160 minutes and longer as the concentrations increased.

3.2 Mortality Rate of *C. gariepinus* Fingerlings Exposed to Various Inclusion Levels of *O. gratissimum*

Table 2 depicts the mortality rate of fingerlings subjected to different inclusion levels of *O. gratissimum*. The trends of mortality of the fingerlings exposed to various inclusion levels (0, 10, 20, 30 g) increase with the concentration. At 30 g/6l concentration, the percentage of mortality increased significantly (p<0.05) with an increase in the *O. gratissimum*.

Table 1. Induction time and recovery time in *C. gariepinus* fingerlings treated with varying inclusion levels of *O. gratissimum* powder

Concentration (g/6l)	Overall anaesthesia time (min)	Overall recovery time (min)
0.0	0:00	0:00
10	10:28 am (148 min)	1:08 pm (160 min)
20	9:31 am (91 min)	1:20 pm (229 min)
30	8:25 am (25 min)	1:35 pm (310 min)

Table 2. Mortality rate of *C. gariepinus* Fingerlings exposed to various inclusion level of *O. gratusimum*

Time (mins)	Mortality rate %			
	T1 (0 g)	T2 (10 g)	T3 (20 g)	T4 (30 g)
0	0	0	0	0
30	0	0	0	0
60	0	0	0	0
90	0	0	0	0
120	0	0	0	0
150	0	0	0	0
180	0	0	0	11.67
210	0	0	26.67	16.67
240	0	0	26.67	48.33
270	0	0	41.67	66.67
300	0	0	58.33	66.67
330	6.67	1.67	68.33	73.33
360	8.33	1.67	68.33	100
390	8.33	1.67	68.33	100

3.3 Survival Rate of *C. gariepinus* Fingerlings Exposed to Various Inclusion Level of *O. gratusimum*

Table 3 shows the survival rate of fingerlings treated with different inclusion levels of *O. gratusimum* as an anesthetic. The survival rate of fingerling exposed to various inclusion levels (0, 10, 20, 30 g/L) became high at 10g/6l and the percentage of survival decreased significantly ($p>0.05$) with an increase in the *O. gratusimum* concentration.

3.4 Variation of Physical and chemical Parameter of Water for before, during, and after Transportation with Different Inclusion Levels of *O. gratusimum* (0 g, 10 g, 20 g, 30 g)

Fig. 1 shows the physical and chemical parameters of time variation of water with different inclusion levels of *O. gratusimum* (0 g, 10 g, 20 g, 30 g). There was no significant difference in water temperature ($p>0.05$) across all treatments. However, the highest mean value was recorded during transportation (31.22 ± 0.87) while the lowest was recorded before transportation (30.29 ± 0.52). Significant difference ($p<0.05$) was recorded in pH level among all the treatments during transportation. The highest mean value was recorded before transportation (6.44 ± 0.12) while the lowest was recorded during transportation (5.96 ± 0.29). There was a significant difference ($p<0.05$) in dissolved oxygen (mg/l). However, the highest mean was recorded before transportation

(5.59 ± 1.56) while the lowest was recorded after transportation (2.71 ± 2.34). The Ammonia level also shows a significant difference ($p<0.05$) with the highest recorded during transportation (0.04 ± 0.02) and the lowest after transportation (0.02 ± 0.01). However, the variation differs significantly. For sulphate, the result does not differ significantly ($p>0.05$). However, the highest value was recorded during transportation (14.11 ± 10.27) while the lowest was recorded after transportation (11.08 ± 2.80).

3.5 Physical and chemical Parameters of Water with Different Inclusion Levels of *O. gratusimum*

Table 4 depicts the Mean Value of Physical and chemical characteristics of Water with -Different Inclusion Levels of *O. gratusimum* (0 g, 10 g, 20 g, and 30 g). Throughout the experiment, the water temperature ($^{\circ}\text{C}$), did not show any variation with increased concentration of *O. gratusimum* in all the treatments. However, the highest mean value was recorded in T1 (31.96 ± 1.19) which serves as the control, and the lowest mean value was recorded in T4 (28.75 ± 5.93) with 30 g of *O. gratusimum*. There were significant differences ($p<0.05$) in the values obtained for pH. The highest mean value was recorded in T1 (6.38 ± 0.18) and the lowest in T4 (5.93 ± 0.47). DO decreases as the concentration of *O. gratusimum* increases. The values obtained for dissolved oxygen was significantly higher ($p<0.05$) in T1 (6.55 ± 1.40 mg/l) while the lowest was recorded in T4 (1.62 ± 2.30). Thus, the values differ significantly ($p<0.05$) compared with the control.

Table 3. Survival Rate of *C. gariepinus* Fingerlings Exposed to Various Inclusion level of *O. gratissimum* (0 g, 10 g, 20 g and 30 g)

Time (min)	Survival rate %			
	T1 (0 g)	T2 (10 g)	T3 (20 g)	T4 (30 g)
0	100	100	100	100
30	100	100	100	100
60	100	100	100	100
90	100	100	100	100
120	100	100	100	100
150	100	100	100	100
180	100	100	100	88.33
210	100	100	73.33	88.33
240	100	100	73.33	51.67
270	100	100	58.33	33.33
300	100	100	41.67	33.33
330	93.33	98.33	31.67	26.67
360	91.67	98.33	31.67	0
390	91.67	98.33	31.67	0

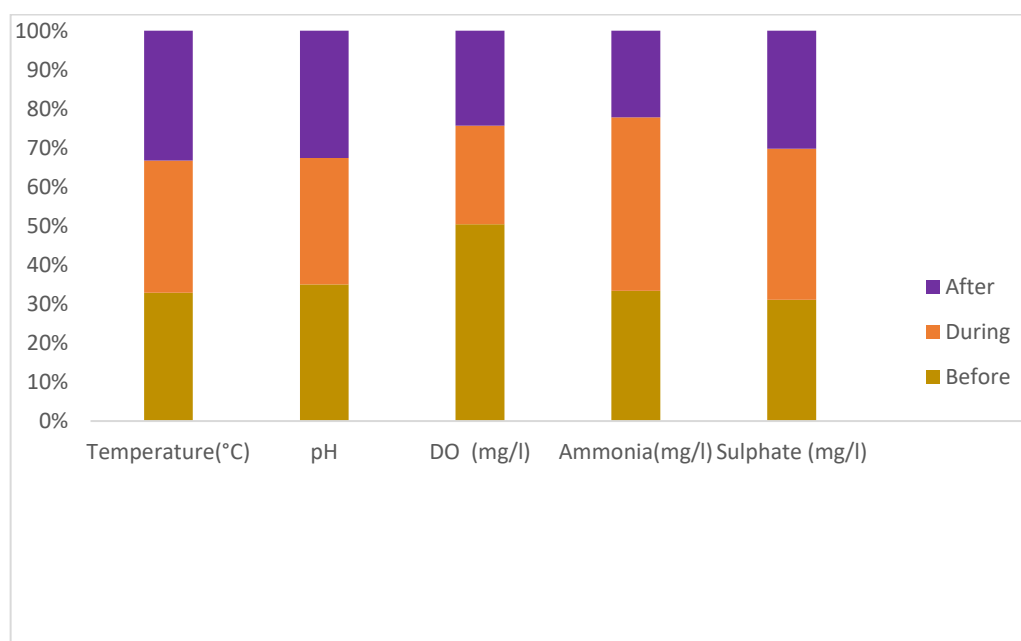


Fig. 1. Variation of physical and chemical parameters of water treated with *Ocimum gratissimum*

Table 4. Mean Value of Physicochemical parameters of Treatment Variation of Water with Different Inclusion Level of *O. gratissimum* (0 g, 10 g, 20 g, and 30 g)

Parameters	T1	T2	T3	T4
Temperature (°C)	31.96±1.19 ^a	31.21±1.03 ^a	30.93±0.80 ^a	28.75±5.93 ^a
pH	6.38±0.18 ^a	6.22±0.20 ^{ab}	6.02±0.34 ^b	5.93±0.47 ^b
D O(mg/L)	6.55±1.40 ^a	4.24±1.03 ^b	2.38±1.90 ^c	1.62±2.30 ^c
Ammonia(mg/L)	0.04±0.03 ^a	0.04±0.01 ^a	0.03±0.01 ^a	0.02±0.01 ^a
Sulphate(mg/L)	6.62±1.05 ^b	12.27±1.95 ^a	16.84±10.78 ^a	12.93±0.77 ^a

Mean with different superscript on the same row are significantly different ($p < 0.05$)

For Ammonia, there was no significant difference ($p>0.05$) in all the treatments, but the highest mean value was recorded in T1 (0.04 ± 0.03) and the lowest mean was recorded in T4 (0.02 ± 0.01). Significant differences were observed in the values of sulphate ($p<0.05$) with the highest mean score recorded in T3 (16.84 ± 10.78) and the lowest recorded in T1 (6.62 ± 1.05).

4. DISCUSSION

"In aquaculture, transporting live fish is a challenge. Many factors influence the success of fish transport among which includes the duration of transport, water parameters, the size, density, and physical condition of the fish and the duration of the depuration before fish transport" (Golombieski et al., 2003; Carneiro et al., 2009; Becker et al., 2012). This study revealed the influence of *O. gratissimum* as an anesthetic on the induction, recovery, survival, and mortality of fish and the physicochemical characteristics of water. Cooke et al. (2004) reported highly sedated fish, lose perception, balance, and normal swimming. This conforms to this study which the fingerlings exhibit loss of balance, opercula beat, and normal swimming in treatments three and four due to an increase in concentration of *O. gratissimum*.

The sedation time decreases with increasing concentration of *O. gratissimum* powder. Also, there was a direct relationship between recovery time and concentrations of *O. gratissimum*. As the concentration increases, the transition time to induction decreases, and recovery time also increases. This result is in line with the findings of Kamble, et al. (2014) on reduction in induction time with increased concentration of clove oil on *Cyprinus carpio*. A similar result was recorded by Agokei & Adebisi (2010) when Nile tilapia (*O. niloticus*) was exposed to "different concentrations of aqueous and alcoholic extract of Tobacco. Results from several studies indicate that the induction times under anesthetics may increase, decrease or remain unchanged with the concentrations of the anesthetics, species and size of exposed fish" (Walsh and Pease, 2002). In a study using juvenile angel fish *Pterophyllum scalare*, Mitjana et al. (2014) observed that at "the highest doses 80mg/l for clove oil and 140 mg/l for MS 222, the induction time remained unchanged. Transportation involves capture, loading, transport, unloading and stocking and so can induce large stress responses that can affect fish over a prolonged

recovery period" (Ashley, 2007; Sneddon et al., 2016; Souza et al., 2018; Cogliati et al., 2019). "The trend between the survival and the mortality rate is directly related. At the initial stage, the survival rate among all treatments was 100%. Mortality was recorded after 150 minutes in T3 and T4. Subsequently, at 330 minutes, mortality was recorded in T1 and T2, High number of mortalities was recorded in T4 while the lowest mortality was recorded in T2. This may be due to the increase in concentration of *O. gratissimum* and metabolic activities that took place during the transportation of fish. This is in consonant with the report of" (Soto and Burhanuddin, 1995; Munday and Wilson, 1997; Iversen et al., 2003; Hisano et al., 2008; Inoue et al., 2011; Javahery et al., 2012). "The survival rates of experimental fish decreased sharply at higher concentrations of anesthetics. In this regard, light sedation is more appropriate for transport procedures because it allows fish to maintain balance, swimming activity, and respiration" (Cooke et al., 2004). Measuring the water quality parameters is vital for the efficiency of fish transportation (Sampaio and Freire, 2016). In this study, the temperature of the water remained within the comfortable value range for the species, according to Silva and Fujimoto (2015). Water temperature was similar across treatments within the 4 hrs transportation periods. But the value was reduced in T4. There is a direct relationship between water-dissolved oxygen and *O. gratissimum*. DO value reduces significantly with an increase in concentration of *O. gratissimum*. The decrease in DO with an increase in concentration of *O. gratissimum* in this study confirms the report of Audu et al. (2013) that DO decreases with an increase in concentration of *C. sativa*. The dissolved oxygen level decreased below the optimum range, the pH value was acidic and sulphate also increased beyond the optimum range, this made the mortality of experimental fish to be high in T3 and T4.

In turn, pH exhibited different behaviors, with pH falling during transportation. At the end of the transportation experiment, pH was significantly higher in the control group as compared to the fish anesthetized with 30 g/l *O. gratissimum*. INOUE et al. (2005) reported that pH decreased after 4 hrs of transportation of juvenile *matrinxã* (*Brycon cephalus*, Gunther) anesthetized with 5 mg L⁻¹ clove oil, probably due to the increase in CO₂ values. The reduction in water pH after transport indicates an increase in CO₂. Ammonia levels decrease significantly with an increase in

concentration of *O. gratissimum*. This result confirms the report of Singh et al. (2004) that "the Indian carp fry *C. catla*, *L. rohita*, and *C. mrigala* exposed to 2-phenoxyethanol (0.09 mg L⁻¹) exhibited decreased NH₃ excretion. The sulphate level in T1 was lower than those in the other treatment. There was a direct relationship between sulphate level and *O. gratissimum*, as the concentration of *O. gratissimum* increases the sulphate level also increases. An increase in deterioration of water quality promotes more stress to fish and, consequently, mortality" (Luz et al., 2013; Moreira et al., 2015). "Anesthetics are beneficial for decreasing fish arousal and avoiding physical injuries during transport. Light sedation is ideal for transport procedures because it allows fish to maintain balance, swimming activity, and respiration" (Cooke et al., 2004).

5. CONCLUSION

The finding in this study revealed that the use of *O. gratissimum* at light concentration has sedation potential on transportation of *C. gariepinus* fingerlings, particularly on short distance. However, high concentration of this plant extract has effects on the survival of *C. gariepinus* fingerlings. Further research should be conducted to evaluate the metabolic, hematology and histology impact of *O. gratissimum* powder on fish transportation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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