**Efficiency of *Ocimum gratisimum* as an anesthetic in the transportation of *Clarias gariepinus* fingerlings**

**Abstract**

High fish mortality caused by stressors during transportation hurts the aquaculture sector specifically fish farmers. Synthetic anesthetics 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 anesthetic in transporting *Clarias gariepinus* fingerlings. A total of 300 *Clarias gariepinus* fingerlings were exposed to 0.00, 10.00, 20.00, and 30.00g/6L inclusion levels of *Ocimum gratissimum* leaf powder in 12 kegs (10litres) filled with 6L of water each. Twenty-five (25) *C. gariepinus* fingerlings were introduced into each treatment and control in a complete randomized design (four treatments 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 concentration of the *Ocimum gratissimum* powder while recovery time increased with an increase in concentration. The longest induction and shortest recovery times were recorded in 10g/6l (T2), while the shortest induction and longest recovery times were recorded in 30g/6l (T4). The survival rate of fingerlings decreased with an increase in the concentration of the *Ocimum gratissimum*. A high mortality rate was recorded in treatment 4 (30g/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 sulfate but no significant difference (p>0.05) was observed in Temperature and Ammonia. Due to its effectiveness and availability; *Ocimum gratissimum* is close to an ideal anesthetic. Farmers could therefore use 10.00g/6L concentration of the powder for transportation of *Clarias gariepinus*.

Keywords: Anesthetic, *Occimum 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).

*Ocimum 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 use 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, linaol, methyl cinnamate, camphor, and thymol. It has been demonstrated in previous studies that eugenol (75-98%) isolated from *Ocimum gratissimum* is the major antimicrobial component that inhibits and kills gram-positive bacteria and fungi (Gafaar *el al*., 2010 and Kumaravel, Sivasubramanian 2010).

*Clarias 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.

# **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 physicochemical parameters

#  **2.2 Experimental Plant**

Scent leaf (*Ocimum 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 (<250µm). Sensitive weighing balance (model 20001) was used to measure (10g, 20g and 30g respectively).

 **2.3 Experimental Fish**

Three hundred pieces of *Clarias gariepinus* fingerlings (a high valued aqua cultural fish species) comprised of both sexes with a mean weight of 3g were acquired and acclimatized for three days. Before the transportation, the fish were starved for 12 hours. A Complete randomized design comprising of four treatments and three replicates were used for the experiment and designated as; control (without anesthetic), 10g, 20g, and 30g of *Ocimum gratisimmum,* powder /liter respectively. 25 pieces of *Clarias 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 (204km). The induction and recovery time were noted.

# **2.4 Water Quality Parameter**

 DO, pH, temperature, sulfate, and ammonia were analyzed before, during, and after transportation using a thermometer, dissolved oxygen meter, pH meter, ammonia using the nitrogen distillation method, and sulfate 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 *Clarias gariepinus* fingerlings after administration of the**

Table 1 the induction and recovery time of *C. gariepinus* fingerlings exposed to various concentrations of *Ocimum 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 treatment 2 (10g/6l) concentration, where there was a total loss of movement and the fish sank motionless at the bottom of the gallon.

The fastest induction time of the anesthetic agent was 25 minutes recorded in treatment 4 (30g/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 10g/6l using *Ocimum gratissimum* powder at 160 minutes and longer as the concentrations increased

**Table 1: Induction time and recovery time in *Clarias gariepinus* fingerlings treated with varying inclusion levels of *Ocimum gratissimum* powder.**

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

**3.2 Mortality Rate of *Clarias gariepinus* Fingerlings Exposed to Various Inclusion Levels of *Ocimum gratisimum***

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

**Table 2:** **Mortality Rate of *Clarias gariepinus* Fingerlings Exposed to Various Inclusion level of *Ocimum gratisimum***

|  |  |
| --- | --- |
|  **Mortality rate %** |  |
| **Time (mins)** | **T1(0g)** | **T2 (10g)** | **T3 (20g)** | **T4 (30g)** |
| 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 *Clarias gariepinus* Fingerlings Exposed to Various Inclusion level of *Ocimum gratisimum***

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

**Table 3:** **Survival Rate of *Clarias gariepinus* Fingerlings Exposed to Various Inclusion level of *Ocimum gatisimum* (0g, 10g, 20g and 30g)**

|  |
| --- |
|  **Survival rate %** |
| **Time (min)** | **T1(0g)** |  **T2 (10g)** |  **T3 (20g)** | **T4 (30g)** |
| 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 |

#  **3.4 Variation of Physicochemical Parameter of Water for before, during, and after Transportation with Different Inclusion Levels of *Ocimum gratisimum* (0 g, 10 g, 20 g, 30 g)**

Figure 1 shows the physico-chemical parameters of time variation of water with different inclusion levels of *Ocimum gratisimum* (0g, 10g, 20g, 30g). 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 sulfate, 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).

 **3.4 Physico-chemical parameters of Water with Different Inclusion Levels of *Ocimum gratisimum***

Table 4 depicts the Mean Value of Physical and chemical characteristics of Water with -Different Inclusion Levels of *Ocimum gratisimum* (0g, 10g, 20g, and 30g). Throughout the experiment, the water temperature (°C), did not show any variation with increased concentration of *Ocimum gratisimum* 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 30g of *Ocimum gratisimum*. 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 *Ocimum gratisimum* 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. 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 was 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).

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

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

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

# **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 *Ocimum gratisimum* 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 *Ocimum gratisimum*.

The sedation time decreases with increasing concentration of *Ocimum gratisimum* powder. Also, there was a direct relationship between recovery time and concentrations of *Ocimum gratisimum*. 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 140mg/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 mortality was recorded in T4 while the lowest mortality was recorded in T2. This may be due to the increase in concentration of *Ocimum gratisimum* 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. 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). 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 4hrs transportation periods. But the value was reduced in T4. There is a direct relationship between water-dissolved oxygen and *Ocimum gratisimum*. DO value reduces significantly with an increase in concentration of *Ocimum gratisimum.* The decrease in DO with an increase in concentration of *Ocimum gratisimum* in this study confirms the report of Audu *et al*. (2013) that DO increases with an increase in concentration of *C. sativa*. The dissolved oxygen level decreased below the optimum range, the PH value was acidic and sulfate 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 *Ocimum gratisimum*. INOUE *et al.* (2005) reported that pH decreased after 4 h 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 *Ocimum gratisimum*. 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 sulfate level in T1 was lower than those in the other treatment. There is a direct relationship between sulfate level and *Ocimum gratissimum*, as the concentration of *Ocimum gratissimum* increases the sulfate 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 more appropriate 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 *Ocimum gratissimum* at light concentration has sedation potential on transportation of *Clarias gariepinus* fingerlings, particularly on short distance However, high concentration of this plant extract has effects on the survival of *Clarias gariepinus* fingerlings. Further research should be conducted to evaluate the metabolic, hematology and histology impact of *Ocimum gratissimum* powder on fish transportation

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