

Sedation and Survival Levels in Various Life Stages of *Tilapia guineensis* Exposed to Mustard Seed Powder as Anaesthetics

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Abstract: Immobilization and survival rates at different life stages in *Tilapia guineensis* exposed to mustard seed powder as anaesthetics agent was evaluated. A total of 450 *T. guineensis* comprised of 150 each of fingerlings (average length 3.87 ± 1.44 cm, average weight 30.03 ± 2.88 g), juveniles (average length 6.45 ± 1.23 cm, average weight 66.44 ± 2.02 g) and adult (average length $13.77 (\pm 2.66)$ cm, average weight 150.99 ± 9.77 g) were collected from the pond at low down ebb of the creek. The fish were conveyed to the laboratory in six exposed 50 L synthetic basins and allowed to acclimate for 7 days. Fish were exposed to a 0.00 control; 10.00; 20.00; 200 mg/l 30.00 mg/l and 40.00 mg/l anaesthetic arranged from a standard solution of mustard seeds. Three replicates of 10 fish per tank were used for each life stage. Time to reach and recover from various stages of anaesthesia was recorded after exposures. In addition, mortality was also recorded at each stage of life. Several physical and chemical characteristics of water in the investigational containers were assessed. The crush substances of mustard seed was noted to have induced sedation depending on the concentration and size of the treated fish. The time to attain profound sedation reduced with rising concentration at the entire life phases of the fish, but was prolonged in adults than in fingerlings and juveniles. The faster you reach deep anaesthesia, the longer it takes to fully recover from the effects of the anaesthetics. The association between the time needed to achieve deep sedation and to complete recovery is greatly reliant on mustard seed powder concentration ($P < 0.05$), with over-all recovery time being contrariwise relational to recovery time. Survival rates of 90.00, 95.00 and 100.00% have been measured in fingerlings, juveniles and adult fish under heavy anaesthetics influence. This study found that mustard seeds can be effectively used as an anaesthetic for *T. guineensis* at an ideal dosage of 30.00 mg/L.

Keywords: Anaesthetics, *Tilapia*, Stress, Aquaculture, Mustard seed.

INTRODUCTION

Anesthetics minimize stress on fish and reduce physical stress on fish when performing routine tasks such as artificial spawning, measuring and weighing fish, grading and tagging, administering vaccines, and collecting blood and blood. It is widely used in aquaculture due to its valuable advantage of preventing damage. Main uses include gonadal biopsies, including surgical procedures, and gamete collection. (Akinrotimi, 2014). In addition to efficiency, price, obtainability and accessibility, aquaculture farmers consider several important factors such as harmfulness to fish, individuals, and the surroundings, type of experiment, and fish species elements must be considered. (Akinrotimi *et al.*, 2013a). When fish are released from anesthesia, recovery ought to be quick and anesthesia would be applicable at low dosage (Akar, 2011).

Information on standard and most preferred concentrations of anesthetics for many fish species is essential. This is because an

incorrect concentration may resulted in some deleterious consequences which include stress. Right to use to harmless and applicable fish tranquilizers is therefore an urgent need for fisheries scientists, administrators and farmers (Akinrotimi *et al.*, 2016). The choice of anesthetic agent is generally driven by many factors, including availability, economics, realism of utilization, efficiency, user protection, and chemicals approved by regulatory authorities for individual ingestion in animals (Ross and Ross, 2008). In general, beneficial properties of fin anesthetics include fast induction and recovery times, nontoxicity to fish and individuals, non-sustained functional consequences, quick ejection from the fish system, in both freshwater and seawater. Great solubility in water and relatively cheaper are also some of the characteristics that an anaesthetics must have before it can be used in aquaculture activities. Recovery ought to be achieved within few minutes so as to put a stop to stress and adverse consequences on the fish and reduce mortality during the stunning process (Cooke *et al.*, 2004).

Recently, organic farming has shown alternatives to the chemicals currently used in aquaculture, and anesthetics are one of those key products. Teixeira *et al.* (2017) reported that natural products have the potential to alter the propensity for artificial aquaculture compounds. From the time when human civilization starts, herbs have been utilized by humanity for their beneficial values. Quite an amount of contemporary medications have been sequestered from organic origins (Feng *et al.*, 2011). In small-scale animal husbandry organizations in emerging nations around the world, Ethno-veterinary medicine is perhaps one of the first means of preserving and restoring animal species health conditions. Majority of the fish farmers operating in remote regions does not have access nor financial means to purchase synthetic medications (Akinrotimi *et al.*, 2014). Mustard seeds contain an essential oil that, when applied topically to the body, stimulates blood flow and aids in the removal of toxins. Therefore, it is very useful in treating various ailments, from simple colds to rheumatic conditions. Mustard seed is frequently being utilized to relieve pains from bronchitis and toothache, however, it can also be used as a gel (Lambooji *et al.*, 2009). This study is necessary because information on the use of mustard seeds in *T.guineensis* is limited. Therefore, in this study, we investigated sedation and survival rates for various life phases of *Tilapia guineensis* exposed to mustard seed powder.

MATERIALS AND METHODS

Test Site and Fish

The trial was conducted at the African Regional Aquaculture Center, a subsidiary of the Nigerian Institute of Marine Research, Buguma, Rivers State, Nigeria. Overall, 450 *T. guineensis*, comprised of 150 each of fingerlings, juveniles and adult, were collected from the pond at low down ebb of the creek. The fish were conveyed to the laboratory in six exposed 50 L synthetic basins and allowed to acclimate for 7 days.

Preparation of mustard seeds

Dried mustard seeds were purchased at Orchard's Market, Port Harcourt, D-Line, Kaduna Street, Port Harcourt City Municipality, Rivers. State, Nigeria. Botanical certification was performed by the key of Agbaje (2008). The seeds were brought back to the test site and ground into powder via a food processor (model H2, Ken Wood, Japan). The ground mustard seeds were sifted through a 0.1 minute synthetic sieve to achieve a fine powder.

Experimental Plan

A fully randomized design (CRD) was used in the trial, with 3 replicates for each life stage and 5 treatment levels. The experiment used a total of 45 plastic bowls, each measuring 52 x 44 x 34 cm³. Based on the fish life stage, the level of treatment, and the replication, thirty tanks were labeled. Ten fish were housed in each tank. There were 300 fish used in all.

Experimental Procedures

The powder was weighed into several concentrations (10.0, 20.0, 30.0, and 40.0 mg/L) using a balance. It was poured directly into three copies of a 30 L plastic laboratory water tank filled with water (10 L). To produce a uniform mixture, the mixture was briskly agitated. A 2.0 kg round scale (model 1123HK, Digital Scales, Ltd., Beijing, China) was used to weigh the fish, and a

transparent tape measure was used to measure their length. These were dispensed into five concentrations of powdered mustard seeds (0.00, 10.00, 20.00, 30.00 and 40.00 mg/L) in prepared laboratory aquariums at a rate of ten per aquarium. These were performed three times in a row.

Determination of Induction Time and Recovery Time

Using a digital timer, the duration of anesthesia in exposed fish was timed. Individual fish behavior patterns were observed during the induction and recovery phases at various anesthetic doses and life stages. Following these phases, there is a recovery period: recurring operculum movements, partial balance recovery, unstable balance, complete balance recovery, and finally, when normal swimming is noted [8]. The obtained recovery times were then recorded.

Assessment of Water Quality Parameters

Each aquarium's water pH was checked there on-site with a pH meter (Hanna Products, Portugal). In order to do this, the electrode's tip was dipped into the test solution, the mode button was pressed, and measurements were then taken. A mercury thermometer was lowered into the water and read at a depth of 15 cm after 5 minutes to determine the water's temperature. A LaMotte Freshwater Test Kit (Model AQ4, Chestown, MD, USA) was used to measure dissolved oxygen while nitrite and ammonia were determined using a colorimetric method (APHA, 1998).

Statistical Analysis

SPSS version 22 statistical software was used to compile and analyze the study's data. The Kolmogorov-Smirnov test and the Bartz test were used to check the data's normality and variance equality. A two-way analysis of variance (ANOVA) was employed to identify significant differences in measured variables between the control and experimental groups once these requirements had been fully satisfied. Tuckey's multiple comparison test was used to evaluate which treatments should be significantly different if a difference was seen (P 0.05).

RESULTS

Table 1 lists the water quality data in *T. guineensis* experiment tanks that were subjected to mustard seed extracts. The findings showed that the values of dissolved oxygen decreased significantly (P 0.05) with increasing anesthetic concentration. Other measures of water quality, however, were within the same range and did not significantly alter in response to the anesthetics' concentration (P>0.05). Tables 2 to 4 show the survival rates of *T. guineensis* at various life stages (fingerlings, juveniles, and adults) after exposure to mustard seed extracts. According to the findings, varying induction times were experienced when using mustard seed extracts as anesthetics, depending on the dosage and life stage of the fish. Fish of adult size had the largest induction times, whereas fingerlings had the lowest. According to the results of the recovery time in *T. guineensis* exposed to mustard seed extracts, the recovery time rose significantly (P 0.05) as the extract concentrations increased. Between mustard seed extract doses of 0.00 and 30.00 mg/l, a 100% survival rate was observed in all sizes. However, 95.00% of the exposed fish's fingerling and adult sizes were seen at a dosage of 40.00 mg/l.

Table 1: Measurements of water quality in *T. guineensis* experimental tanks exposed to mustard seed extracts (Mean $\pm$ SD)

Parameter	Concentrations (mgL ⁻¹)				
	0.00	10.00	20.00	30.00	40.00
Temperature (°C)	28.84 $\pm$ 0.77 ^a	28.89 $\pm$ 0.97 ^a	29.78 $\pm$ 1.74 ^a	28.99 $\pm$ 0.98 ^a	29.88 $\pm$ 0.32 ^a
pH	6.52 $\pm$ 0.99 ^a	6.78 $\pm$ 0.99 ^a	6.63 $\pm$ 0.99 ^a	6.88 $\pm$ 0.99 ^a	6.59 $\pm$ 0.66 ^a
DO(mgL ⁻¹)	6. 62 $\pm$ 0.49 ^c	6.54 $\pm$ 0.84 ^c	5.98 $\pm$ 0.99 ^b	5.55 $\pm$ 0.818 ^b	4.54 $\pm$ 0.71 ^a
Nitrite (mgL ⁻¹)	0.0088 $\pm$ 0.01 ^a	0.050 $\pm$ 0.02 ^a	0.055 $\pm$ 0.02 ^a	0.058 $\pm$ 0.02 ^a	0.067 $\pm$ 0.02 ^a
Ammonia (mgL ⁻¹)	0.20 $\pm$ 0.01 ^a	0.20 $\pm$ 0.01 ^a	0.20 $\pm$ 0.02 ^a	0.20 $\pm$ 0.03 ^b	0.21 $\pm$ 0.01 ^b

Mean within the row with different superscripts are significant (P<0.05)

Table 2: Fingerling sizes of *T. guineensis* Treated with Mustard Seed Extracts for Induction, Recovery and Survival (Mean $\pm$ SD)

Parameters	Concentrations (mgL ⁻¹)				
	0.00	10.00	20.00	30.00	40.00
IT	0.00 $\pm$ 0.00 ^a	1229.99 $\pm$ 25.75 ^d	777.66 $\pm$ 45.88 ^c	394.88 $\pm$ 37.66 ^b	192.02 $\pm$ 20.88 ^a
RT	0.00 $\pm$ 0.00 ^a	199.66 $\pm$ 39.88 ^b	299.65 $\pm$ 42.05 ^c	399.09 $\pm$ 35.99 ^d	499.88 $\pm$ 65.92 ^a
Surv.	100.0 $\pm$ 0.00 ^a	100.0 $\pm$ 8.65 ^a	100.00 $\pm$ 9.77 ^a	100.0 $\pm$ 0.80 ^a	90.00 $\pm$ 17.99 ^a

Key: IT- Induction time (s); RT-Recovery time (s); Surv- Survival (%)

Mean within the row with different superscripts are significant (P<0.05)

Table 3: Juvenile Sizes of *T. guineensis* Treated with Mustard Seed Extracts for Induction, Recovery and Survival (Mean $\pm$ SD)

Parameters	Concentrations (mgL ⁻¹)				
	0.00	10.00	20.00	30.00	40.00
IT	0.00 $\pm$ 0.00 ^a	1260.88 $\pm$ 28.39 ^d	800.66 $\pm$ 45.21 ^c	399.65 $\pm$ 37.04 ^b	199.02 $\pm$ 20.43 ^a
RT	0.00 $\pm$ 0.00 ^a	199.66 $\pm$ 39.42 ^b	268.77 $\pm$ 42.05 ^c	375.00 $\pm$ 30.00 ^d	480.33 $\pm$ 43.66 ^a
Surv.	100.0 $\pm$ 0.00 ^a	100.0 $\pm$ 0.66 ^a	100.00 $\pm$ 0.08 ^a	100.0 $\pm$ 0.09 ^a	95.0 $\pm$ 11.89 ^a

Key: IT- Induction time (s); RT-Recovery time (s); Surv- Survival (%)

Mean within the row with different superscripts are significant (P<0.05)

Table 4: Adult Sizes of *T. guineensis* Treated with Mustard Seed Extracts for Induction, Recovery and Survival (Mean $\pm$ SD)

Parameters	Concentrations (mgL ⁻¹)				
	0.00	10.00	20.00	30.00	40.00
IT	0.00 $\pm$ 0.00 ^a	1889.00 $\pm$ 17.99 ^d	1490.00 $\pm$ 11.88 ^c	985.00 $\pm$ 9.99 ^b	550.09 $\pm$ 23.77 ^a
RT	0.00 $\pm$ 0.00 ^a	300.00 $\pm$ 10.99 ^b	459.00 $\pm$ 18.77 ^{ab}	588.10 $\pm$ 4.91 ^c	788.09 $\pm$ 18.77 ^d
Surv.	100.0 $\pm$ 0.00 ^a	100.00 $\pm$ 0.10 ^a	100.00 $\pm$ 0.00 ^a	100.00 $\pm$ 0.10 ^a	100.00 $\pm$ 0.01 ^a

Key: IT- Induction time (s); RT-Recovery time (s); Surv- Survival (%)

Mean within the row with different superscripts are significant (P<0.05)

DISCUSSION

When *T. guineensis* was exposed to mustard seed extracts during its three life phases, the water quality metrics in the exposure tanks revealed a substantial decrease ($P < 0.05$) in the values of dissolved oxygen levels, which decreased with increasing anaesthetics content. When compared to anaesthetics concentrations, other water quality measures were within the same range and did not significantly differ from one another. This outcome is in line with the findings of Akinrotimi et al. (2018) in the *T. guineensis* experimental waters exposed to clove seed extracts. In this investigation, the concentration of the extracts caused a decrease in the induction time of *T. guineensis* exposed to them. The findings of this investigation and those conducted by Akinrotimi et al. (2015) on *Clarias gariepinus* treated to clove seed extracts are in agreement. On the other hand, the values of the exposed fish's recovery time found in this study were inversely correlated with increasing doses of mustard seed extract. The lowest time to complete recovery was seen at 10.0 mg/L, whereas the greatest recovery time was observed at 40.0 mg/L. This finding is consistent with Wilfred-Ekpikpo's study from 2021 on the treatment of mustard seed extract in black jaw tilapia, *Sarotherodon melanotheron*. Additionally, longer recovery durations for largemouth bass have been linked to higher anesthetic doses (Small, 2004).

Moreover, the sequence of changes in different stages of anesthesia and recovery identified in this experiment was consistent with duo of Akinrotimi and Aiyelaja (2018) reports on *T. guineensis* exposed to nutmeg as an anesthetic. In addition, the report of this study also confirms the submission of Agokei and Adebisi (2010) on tobacco-exposed *C. gariepinus* as an anesthetic. Ross and Ross (1999) assert that the amount of anesthetic in an exposed organism's central nervous system (CNS) directly affects the degree of anesthesia. In this study, compared to juveniles and adult fish, fingerlings showed shorter induction periods and longer recovery times. This means that a small size fish has the capacity to absorb mustard seed powder (anesthetic) faster than the juveniles and adults. This is consistent with the observations of Akinrotimi (2014), in *C. gariepinus* exposed to four different plant extracts. Also with the submission by Akinrotimi et al. (2016) in juveniles and adults of *Tilapia ginensis* treated with clove seed extract. The 100% viability and survival of exposed fish is a clear evidence that mustard is an ideal anaesthetic that can be used to effectively sedate the specie *T. guineensis* during aquaculture operations. Wifred-Ekpeikpo (2021) reported similar trends in survival rates for *S. melanotheron* juveniles and those exposed to high concentrations of mustard seed powder.

CONCLUSION

According to the study's findings, dry mustard seed powder is a potent treatment that is also highly water soluble, generally safe, especially in small dosages, environmentally benign, and economically viable. The findings of this investigation also demonstrated that the anesthetic properties of mustard seed extracts were unaffected. In this investigation, it was discovered that mustard seed extracts functioned as an anesthetic to calm *T. guineensis*. Based on the findings of this study, fingerlings, juveniles, and adult sizes of *T. guineensis* fish can be sedated with mustard seed extract at an effective dose of 30.0 mg/L. For the purpose of the aquaculture industry's sustainability, aquaculture health professionals should promote the use of mustard seed

powder rather than pricy synthetic anesthetics.

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