

Assessment of Mustard Seed Powder as Anaesthetic Agents in Three Sizes of *Clarias gariepinus*

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ABSTRACT

Aim: The aim of this study is to evaluate the effects of anaesthesia with mustard seed extracts in three sizes: fingerlings, juvenile and adult of *C. gariepinus*.

Method and Materials: A total of 180 specimens of *C. gariepinus* comprising of 60 each of fingerlings (mean length 6.87cm±1.54 SD and mean weight 10.23g±123SD); juveniles (mean length 17.78cm±2.88 SD and mean weight 106.99g±4.78SD), and adults (mean length 29.33cm±3.01 SD and mean weight 654.43g±11.89SD), were procured from Production Ponds in African Regional Aquaculture Centre, (ARAC), Aluu, Rivers State of Nigeria. They were exposed to mustard seed extracts at different concentrations of 10.00, 20.00, 30.00, 40.00 and 50.00mg/L.

Results: The results obtained indicated a size related response to mustard seed extracts. The induction time decreased significantly ($P < 0.05$) as the concentrations of the mustard seed extracts increased. The recovery time for all the three sizes stages generally increased as the concentrations of the anaesthetics increased. Also, the recovery times in adult fish were higher at all concentrations; this was closely followed by juvenile fish, while the shortest recovery time was observed in fingerlings in all concentrations of exposure.

Conclusion: The revealed that mustard seed can effectively induce anaesthetics in fish with the optimum dosage of 10.0mg/L; 20mg/l and 30.0mg/L for fingerlings, juveniles and adult sizes respectively.

Keywords: Anaesthetics; Aquaculture; *Clarias gariepinus*; Mustard seed; Plant extracts.

Introduction

Aquaculture in Nigeria is essentially based on catfish culture and the prospects of fish supply in the country depend on its development and culture [1]. *C. Gariepinus* (family Clarridae), is the most commonly cultivated fish species in the country, making Nigeria the largest producers of *C. gariepinus* in Africa and third in the world, after Thailand and Indonesia [2]. The growth of aquaculture in Nigeria at present is largely boosted by a steady rise in the culture of *C. gariepinus*. It is grown by both small and large scale fish farmers in different parts of the country, with a total production of over 70,000 tons annually [3]. The commercial catfish industry in Nigeria has undergone rapid expansion in recent years. Intensive production by farmers to meet ever increasing catfish demand has resulted in different operational strategies to enhance

optimum performance of fish in the culture medium [4]. The intensive nature of fish farming involves manipulation of fish, which entails some management practices such as handling, confinement, transportation and other farm operations from the hatchery to the final commercial stage [5].

Anesthesia is frequently applied in aquaculture because of its valuable benefits in minimizing fish stress and preventing physical injuries to fish while handling them during routine practices such as artificial spawning, measuring or weighing fish, sorting and tagging, administration of vaccines, live transport, sampling for blood or gonadal biopsies and collecting of gametes, including surgical procedures to cite some of the main applications according to [6]. When a farmer chooses anaesthetics, he or she should take into consideration some important factors such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment, nature of the experiment and species of fish [7].

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When the fish is removed from the anesthetic, the recovery should be rapid, the anesthetic should be effective at low doses [8]. Adequate knowledge about the ideal and optimum concentration of anesthetics for various fish species is necessary because in-appropriate concentrations may lead to adverse effects such as stress, therefore access to safe and effective fish sedatives is a critical [9].

Natural products hold the promise of "shifting the demand curve" for synthetic chemicals for aquaculture. Since the beginning of human civilization, herbs have been used by mankind for its therapeutic value. An impressive number of modern drugs have been isolated from natural sources, [10]. Many of these isolations were based on the uses of the agents in traditional medicine. Ethno veterinary medicine is widely practiced in rural areas in parts of Africa, including Nigeria. In small holder livestock systems in developing countries, ethno veterinary medicine is probably the only to maintain and restore the health of animal species. Most rural farmers do not have access or the financial resources to synthetic drugs. These farmers rely on the ancestral indigenous knowledge in order to control various livestock diseases, according to [11].

Mustard seed contains an essential oil (allyl isothiocyanate) which, when applied to the outside of the body, increases the circulation and thus helps the elimination of poisons. This makes it of great value in treating a number of complaints, from a simple chill to rheumatism. Externally, mustard is often applied to ease bronchitis, neuralgia or toothache but it is also available as an ointment [12]. Various genera of this plant, including more than 200 wild and 40 cultivated species are available in the U.S. and Canada. From an economical and practical point of view, three main species are known worldwide, namely yellow mustard, brown mustard and black oriental mustard [13].

Despite the huge activities on *C. gariepinus*, the information on the use of naturally derived anaesthetics to manage both intentional and unintentional stress across its production chain is scarce, hence the need for this study [14]. This study will shed more light on stress management in fish farming using plant extract as anaesthetics. In Nigeria, data concerning the use of mustard seed extracts as anaesthetics on *Clarias gariepinus* species are scarce. Synthetic

anaesthetics are currently used to mitigate stress response, but they are also toxic, expensive and difficult to obtain in some places [15]. Therefore, a safer, cheaper and readily available alternative is preferred in the management procedures in commercial aquaculture to reduce stress and high mortality in fish. Plant extracts are potential sources of new anaesthetic with low environmental and health risks. Information on the use of mustard as an anaesthetic agent in *C. gariepinus* is limited, thus necessitating the need for this work. The aim of this study is to evaluate the effects of anaesthesia with mustard seed extracts in three sizes: fingerlings, juvenile and adult of *C. gariepinus*.

Methods and Materials

Sources and Acclimation of Experimental Fish

A total of 225 specimens of *Clarias gariepinus* of 75 each of fingerlings (mean length 6.85cm \pm 1.54 SD and mean weight 8.23 \pm 115 SD), juveniles (mean length 15.58cm \pm 1.90 SD and mean weight 100.50g \pm 3.68 SD) and adults (mean length 29.33 cm \pm 3.01 SD and mean weight 654-43g \pm 11.89 SD), were procured from production ponds in African Regional Aquaculture Centre (ARAC), Aluu, Rivers State of Nigeria. They were transferred into 50L of Jerry Cans to the Fish Disease Laboratory at the center and were acclimated for a period of 1 week (seven days). During this period they were fed with ARAC feed (35.0% CP) at 3% body weight. The water in the acclimation tanks were renewed every two days.

Preparation of Mustard Seed

Dried Mustard Seed was purchased from fruit garden market at Kaduna Street, D-Line, Port Harcourt in Port Harcourt City Local Government Area of Rivers State. Plant authentication was done using the keys of Agbaje, [16]. The seeds were taken to the laboratory and grounded into powder using a kitchen blender (Model H2, Ken Wood, Japan). The milled mustard seed was sieved using 0.1 micro plastic meshes to obtain the fine powder.

Experimental Design

The design of the experiment was Completely Randomized Design (CRD) having five treatments level each with three replicates for each of the life stages. A total of 45 plastic basins of dimension (52 x 44 x 34 cm³) each were used for the experiments. The 45 basins were labeled based on life stages of the fish, treatment levels

and replicates. Each basin will be stocked with five (5) fish per tank. A total of 225 fish were stocked.

Experimental Procedure

The powder was weighed into different concentrations (10.0, 20.0, 30.0, 40.0 and 50.0mg/L) using weighing balance. It was applied directly in three replicates into the water (10L) in 30L experimental plastic aquaria. The mixtures were stirred vigorously to ensure homogenous mixture. The fish was weighed with 2.0 kg round top weighing scale (Model 1123HK, Digital Scales, Ltd, Beijing, China) which the length was measured with transparent meter rule. They were introduced into prepared experimental aquaria, containing five concentrations of powdered mustard seed (10.00; 20.00; 30.00; 40.00 and 50.00 mg/L) at the rate of five fish per tank in triplicates.

Determination of Induction and Recovery Time

The time for onset of anaesthesia for the exposed fish was measured using a digital stopwatch. Fish behaviour was monitored individually through the induction and recovery stages in each life stage and concentrations. In the induction stage, five different behaviours were observed [8]. After the anaesthesia, fish was removed individually using a scoop net and transferred into a clean water tank. Recovery time which followed the following stages; reappearance of opercula movements, partial recovery of equilibrium, irregular balance, total recovery of equilibrium and lastly, normal swimming was observed [8]. Recovery time was then recorded.

Table 3.1: Stages of Induction and Recovery in Fish

| Stages of Induction | Description |
|---------------------|---------------------------------------|
| I | Decrease in caudal fin strokes |
| II | Decrease in swimming ability |
| III | Loss of equilibrium |
| IV | Cessation in Operculum beat frequency |
| V | Immobilization |
| Stages of Recovery | Description |
| I | Reappearance of Opercula movement |
| II | Fin movement resumes |
| III | Partial Swimming Resumes |
| IV | It regains full equilibrium |
| V | Fish regains full and active swimming |

Source: Coyle *et al.*[8].

Evaluation of Water Quality Parameters

pH

The water pH was determined in situ in each of the aquarium with a pH meter (Hanna Products,

Portugal). This was achieved by dipping the end of the electrode into the test solution and the mode button was selected and readings were taken.

Temperature

The temperature of the water was measured by placing the mercury in glass thermometer in the water and taking a reading after five minutes at 15cm depth.

Nitrite, Ammonia, Dissolved Oxygen and Sulphide

Nitrite, Ammonia, Dissolved Oxygen and sulphide were evaluated using LaMotte fresh water test kit (Model AQ4, Chestown, Maryland, USA).

Statistical Analysis

The data obtained from this study was collated and analyzed using statistics software SPSS version 22. Data was tested first for normality (Kolmogorov-Smirnov test) and homoscedasticity of variance (Bartts test). When these conditions were met with full satisfactions, a two way analysis of variance (ANOVA) was employed to reveal significant differences in measured variables among control and experimental groups. When a different was detected ($P < 0.05$), Tuckey's multiple comparison test was applied to identify which treatment should be significantly different.

Results

Physico-chemical Variables of the Water in the Experimental Tanks during the Study Period

The water quality parameters in experimental tanks in three life stages of *C. gariepinus* exposed to mustard seed extracts are presented in Table 1. The results indicated a significant reduction ($P < 0.05$) in the values of dissolved oxygen which reduced with increasing concentration of the anaesthetics. While other water quality parameters were within the same range with no significant different in relation to the concentration of the anaesthetics ($P > 0.05$).

Induction Time in *C. gariepinus* Exposed to Mustard Seed Extracts

The induction time in various life stages of *C. griepinus* exposed to mustard seed extracts are presented in Tables 2 to 4. The use of mustard seed extracts as anaesthetics resulted in different induction times depending on the dosage and size of the fish. The various stages of induction which include: decrease in caudal fin strokes; decrease in swimming ability; loss of equilibrium; cessation in Operculum beat frequency and

immobilization, decreased with the increase in the concentrations of mustard seed extracts in all the three life stages of the fish. (Tables 2 to 4). Furthermore, the induction times in adult size of *C. gariepinus* were higher than the juveniles which in turn were higher than the fingerlings.

Recovery Time in C. gariepinus Exposed to Mustard Seed Extracts

The results of the recovery time in *C. gariepinus* exposed to mustard seed extracts are presented in Tables 4.5 to 4.7. The result indicated a significant ($P < 0.05$) increased in the recovery time, as the concentrations of clove bud extracts increased, The various stages of recovery in the exposed fish differs significantly at various concentrations.

Comparative Induction time in C. gariepinus Exposed to Mustard Seed Extracts

The comparative induction time (time taken for the fish to be anaesthetized) was observed in *C. gariepinus* exposed to mustard seed extracts. The

highest induction time ($181.51 \pm 11.32s$) was recorded in adult fish at 10.0mL^{-1} . While the lowest ($95.92 \pm 5.42 s$) was recorded in fingerlings at 50.00mL^{-1} (Fig. 1).

Comparative Recovery time in C. gariepinus Exposed to Mustard Seed Extracts

For the recovery time, the longest recovery time ($506.18 \pm 11.23 s$) was observed in the adult fish at 50.00 mg/l and the shortest ($129.34 \pm 1.63(s)$) in fingerlings at 10.00mg/l of the clove extracts. The recovery time for all the life stages generally increased as the concentrations of the anaesthetics increased. Also, the recovery times in adult fish were higher at all concentrations; this was closely followed by juvenile fish. However, a shortest recovery time was observed in fingerlings in all concentrations of exposure (Fig. 2).

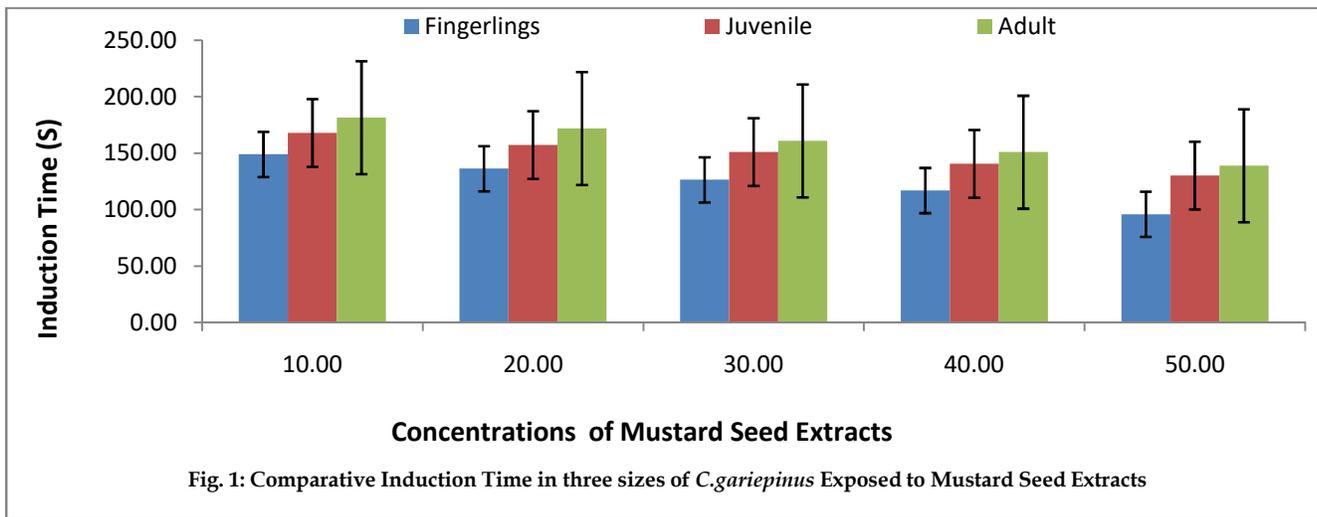


Fig. 1: Comparative Induction Time in three sizes of *C.gariepinus* Exposed to Mustard Seed Extracts

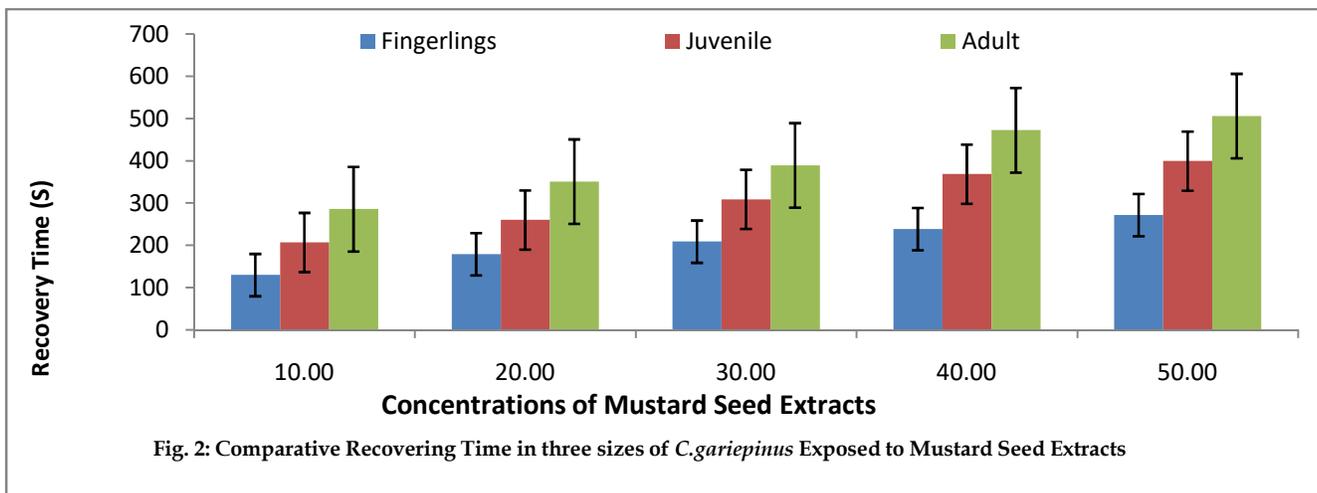


Fig. 2: Comparative Recovering Time in three sizes of *C.gariepinus* Exposed to Mustard Seed Extracts

Table 1: Water Quality Parameters in Experimental Tanks of *C. gariepinus* Exposed to Mustard Seed Extracts (Mean \pm SD)

| Parameters | Concentrations (mg/l) | | | | |
|-----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 |
| DO(mg/l) | 6.01 \pm 0.35 ^a | 6.08 \pm 0.21 ^a | 5.94 \pm 0.46 ^b | 5.89 \pm 0.30 ^b | 5.83 \pm 0.54 ^b |
| Temp. ($^{\circ}$ C) | 27.85 \pm 0.42 ^a | 28.04 \pm 0.55 ^a | 28.11 \pm 0.22 ^a | 28.11 \pm 0.41 ^a | 28.12 \pm 0.46 ^a |
| pH | 6.71 \pm 0.21 ^a | 6.61 \pm 0.01 ^a | 6.60 \pm 0.01 ^a | 6.60 \pm 0.01 ^a | 6.51 \pm 0.15 ^a |
| Nitrite | 0.04 \pm 0.02 ^a | 0.05 \pm 0.00 ^a | 0.06 \pm 0.00 ^a | 0.06 \pm 0.00 ^a | 0.07 \pm 0.00 ^a |
| Sulphide | 0.28 \pm 0.02 ^a | 0.29 \pm 0.02 ^a | 0.30 \pm 0.02 ^a | 0.33 \pm 0.02 ^a | 0.34 \pm 0.01 ^a |
| Ammonia | 0.04 \pm 0.01 ^a | 0.04 \pm 0.00 ^a | 0.05 \pm 0.01 ^a | 0.06 \pm 0.01 ^a | 0.06 \pm 0.01 ^a |

Means within the same roll with different superscripts are significantly different (P<0.05).

Table 2: Induction time (s) in Fingerlings of *C. gariepinus* Exposed to Mustard Seed Extracts (Mean \pm SD)

| Stages of Induction | Concentration (mg/l) | | | | |
|---------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|
| | 10 | 20 | 30 | 40 | 50 |
| I | 51.98 \pm 2.49 ^c | 52.14 \pm 1.35 ^c | 42.89 \pm 1.29 ^b | 40.29 \pm 1.25 ^b | 31.66 \pm 1.10 ^a |
| II | 91.76 \pm 3.87 ^e | 81.19 \pm 1.59 ^d | 75.67 \pm 1.67 ^c | 66.47 \pm 1.39 ^b | 57.23 \pm 1.28 ^a |
| III | 131.44 \pm 4.24 ^e | 112.23 \pm 1.84 ^d | 105.47 \pm 1.66 ^c | 87.64 \pm 1.54 ^b | 68.78 \pm 2.36 ^a |
| IV | 136.23 \pm 2.62 ^c | 133.28 \pm 2.11 ^c | 121.24 \pm 1.84 ^c | 105.82 \pm 1.68 ^b | 84.36 \pm 5.45 ^a |
| V | 148.98 \pm 2.99 ^b | 136.32 \pm 2.43 ^b | 126.43 \pm 2.05 ^b | 116.98 \pm 1.83 ^b | 95.92 \pm 2.54 ^a |

Means within the same roll with different superscripts are significantly different (P<0.05)

KEY: I- Decrease in caudal fin strokes; II- Decrease in swimming ability; III- Loss of equilibrium; IV- Cessation in Operculum beat frequency; V- Immobilization

Table 3: Induction time (s) in Juveniles of *C. gariepinus* Exposed to Mustard Seed Extracts (Mean \pm SD)

| Stages of Induction | Concentration (mg/l) | | | | |
|---------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 |
| I | 75.48 \pm 1.58 ^d | 67.54 \pm 1.43 ^c | 58.77 \pm 1.46 ^b | 48.91 \pm 1.31 ^a | 43.52 \pm 1.26 ^a |
| II | 120.86 \pm 1.98 ^d | 108.96 \pm 1.78 ^c | 99.65 \pm 1.82 ^b | 78.92 \pm 1.51 ^a | 64.93 \pm 1.43 ^a |
| III | 136.23 \pm 2.56 ^c | 133.39 \pm 2.11 ^c | 131.42 \pm 2.17 ^c | 109.81 \pm 1.72 ^b | 98.33 \pm 1.57 ^a |
| IV | 141.61 \pm 3.11 ^c | 138.82 \pm 2.43 ^b | 135.21 \pm 2.52 ^b | 131.72 \pm 1.93 ^b | 112.75 \pm 1.72 ^a |
| V | 167.98 \pm 3.42 ^b | 157.24 \pm 2.87 ^b | 150.98 \pm 2.88 ^a | 140.61 \pm 2.23 ^a | 130.16 \pm 1.87 ^a |

Means within the same roll with different superscripts are significantly different (P<0.05)

KEY: I- Decrease in caudal fin strokes; II- Decrease in swimming ability; III- Loss of equilibrium; IV- Cessation in Operculum beat frequency; V- Immobilization

Table 4: Induction time (s) in Adult of *C. gariepinus* exposed to Mustard Seed Extracts (Mean \pm SD)

| Stages of Induction | Concentration (mg/l) | | | | |
|---------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 |
| I | 108.3 \pm 1.59 ^e | 86.49 \pm 1.43 ^d | 75.67 \pm 1.47 ^c | 67.87 \pm 1.31 ^b | 58.89 \pm 1.25 ^a |
| II | 136.67 \pm 1.98 ^c | 132.87 \pm 1.78 ^c | 131.26 \pm 1.82 ^c | 113.65 \pm 1.52 ^b | 97.64 \pm 1.42 ^a |
| III | 144.96 \pm 2.56 ^b | 139.25 \pm 2.11 ^a | 136.83 \pm 2.18 ^a | 134.43 \pm 1.71 ^a | 131.45 \pm 1.57 ^a |
| IV | 153.24 \pm 2.95 ^c | 145.63 \pm 2.45 ^b | 142.41 \pm 2.51 ^b | 139.11 \pm 1.93 ^a | 135.23 \pm 1.73 ^a |
| V | 181.52 \pm 3.52 ^d | 171.91 \pm 2.78 ^c | 160.98 \pm 2.89 ^b | 150.97 \pm 2.13 ^b | 138.97 \pm 1.89 ^a |

Means within the same roll with different superscripts are significantly different (P<0.05)

KEY: I- Decrease in caudal fin strokes; II- Decrease in swimming ability; III- Loss of equilibrium; IV- Cessation in Operculum beat frequency; V- Immobilization

Table 5: Recovery Time (s) in Fingerlings of *C. gariepinus* Exposed to Mustard Seed Extracts (Mean \pm SD)

| Stages of Recovery | Concentrations (mg/l) | | | | |
|--------------------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 |
| I | 41.18 \pm 1.08 ^a | 55.12 \pm 11.21 ^b | 59.69 \pm 1.28 ^b | 68.23 \pm 1.31 ^c | 74.68 \pm 1.36 ^c |
| II | 62.24 \pm 1.25 ^a | 89.12 \pm 11.34 ^b | 98.27 \pm 1.46 ^c | 109.36 \pm 1.52 ^c | 129.15 \pm 1.58 ^d |
| III | 88.31 \pm 3.32 ^a | 118.13 \pm 11.45 ^b | 129.84 \pm 9.63 ^c | 143.48 \pm 1.72 ^d | 159.84 \pm 6.85 ^d |
| IV | 107.37 \pm 11.39 ^a | 142.14 \pm 5.56 ^b | 164.42 \pm 1.78 ^c | 187.61 \pm 4.93 ^d | 206.41 \pm 1.98 ^e |
| V | 129.94 \pm 11.46 ^a | 179.14 \pm 8.65 ^b | 208.99 \pm 4.98 ^b | 238.74 \pm 9.98 ^c | 271.89 \pm 2.33 ^c |

Means within the same roll with different superscripts are significantly different (P<0.05)

KEY: I- Reappearance of Opercula movement; II- Fin movement resumes; III- Partial Swimming Resumes; IV- Regains full equilibrium; V- Fish regains full and active swimming

Table 6: Recovery Time (s) in Juvenile of *C. gariepinus* Exposed to Mustard Seed Extracts (Mean \pm SD)

| Stages of | Concentration (mg/l) | | | | |
|-----------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 |
| I | 58.63 \pm 1.28 ^a | 65.91 \pm 1.38 ^a | 79.87 \pm 1.42 ^b | 89.91 \pm 1.51 ^c | 99.59 \pm 9.58 ^c |
| II | 99.36 \pm 1.45 ^a | 119.72 \pm 1.65 ^a | 131.65 \pm 1.74 ^b | 156.89 \pm 11.92 ^c | 169.96 \pm 7.95 ^c |
| III | 130.99 \pm 7.42 ^a | 162.52 \pm 1.91 ^b | 195.41 \pm 1.95 ^b | 230.79 \pm 12.32 ^c | 242.54 \pm 8.53 ^c |
| IV | 162.63 \pm 9.79 ^a | 217.34 \pm 2.18 ^a | 257.18 \pm 12.36 ^b | 298.68 \pm 13.73 ^b | 318.95 \pm 6.98 ^b |
| V | 207.26 \pm 7.97 ^a | 260.13 \pm 11.45 ^b | 308.95 \pm 12.67 ^c | 368.57 \pm 20.13 ^c | 399.49 \pm 13.47 ^c |

Means within the same roll with different superscripts are significantly different (P<0.05)

KEY: I- Reappearance of Opercula movement; II- Fin movement resumes; III- Partial Swimming Resumes; IV- Regains full equilibrium; V- Fish regains full and active swimming

Table 7: Recovery Time (s) in Adult of *C. gariepinus* Exposed to Mustard Seed Extracts (Mean \pm SD)

| Stages of Induction | Concentration (mg/l) | | | | |
|---------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 |
| I | 79.23 \pm 5.38 ^a | 89.28 \pm 9.48 ^b | 106.36 \pm 5.48 ^b | 117.59 \pm 11.64 ^c | 129.32 \pm 11.71 ^d |
| II | 121.35 \pm 11.69 ^a | 142.47 \pm 11.85 ^b | 184.62 \pm 6.96 ^c | 185.98 \pm 12.18 ^c | 208.54 \pm 8.32 ^d |
| III | 175.46 \pm 11.98 ^a | 208.65 \pm 13.21 ^a | 251.87 \pm 2.53 ^b | 279.59 \pm 2.70 ^b | 299.75 \pm 2.92 ^b |
| IV | 230.58 \pm 14.29 ^a | 284.83 \pm 2.58 ^b | 319.13 \pm 2.94 ^b | 373.98 \pm 5.25 ^c | 396.97 \pm 3.53 ^c |
| V | 285.69 \pm 12.57 ^a | 351.10 \pm 14.95 ^b | 389.38 \pm 13.48 ^b | 472.57 \pm 13.78 ^c | 506.18 \pm 24.13 ^c |

Means within the same roll with different superscripts are significantly different (P<0.05)

KEY: I- Reappearance of Opercula movement; II- Fin movement resumes; III- Partial Swimming Resumes; IV- Regains full equilibrium; V- Fish regains full and active swimming

Discussion

The water quality parameters in experimental tanks in three life stages of *C. gariepinus* exposed to mustard seed extracts indicated a significant reduction (P<0.05) in the values of dissolved oxygen which reduced with increasing concentration of the anaesthetics. While other water quality parameters were within the same range with no significant different in relation to the concentration of the anaesthetics. This result agrees with the report in which the experimental waters of *C. gariepinus* exposed to clove seed extracts [15].

The induction time in *C. gariepinus* exposed to mustard seed extracts reduced with increasing concentrations of the extracts. These findings are in agreement with other studies in which fish exposed to plant extracts as anaesthetics [17,18,19,20]. When using anaesthetics, it is expected that there will be a strong negative correlation between the applied concentration and the time required to induce anesthesia to the desired stage, as observed previously for several fish species [21]. The recovery time was directly proportional with increasing doses of mustard seed extracts. The longest recovery time was observed at 40.0mg/L and the shortest time to reach total recovery stage was detected at 20.0mg/L. Longer recovery time with the increased anesthetic dosage has been reported in (*Hippocampus kuda*) [22].

Long exposure to anesthetic led to more anesthetic absorption by fish which, in turn, lengthened the recovery time [7]. The statement is not completely trustworthy otherwise this could be proved with present result because if it could be said that longer exposure to low concentration of the anesthetic leads to more anesthetic absorption. It could be said that short exposure to high anesthetic concentration do it as well. On the other hand, comparison with anesthesia duration, anesthetic concentration plays more important role on the recovery time [23]. It is believed that the independence of the recovery time from the anesthesia duration, as a result of that anesthetic, is taken up by the fish through a concentration gradient at the gill interface. Therefore, when equilibrium level established between the gill and anesthetic solution, no further anesthetic will taken up by the fish, and during recovery, the anesthetic agent is leaked through such gradient. Therefore, the recovery time is controlled by the anesthetic concentration but not duration of anesthesia [23].

Earlier reports indicate that size does not have a direct influence on the time required to induce anaesthesia in fish [24]. However, a considerable variation exists both within specie and between species in response to anaesthetics application, in aquaculture [25]. Variations in anaesthetic response in two sizes of yellow perch (*Perca flavescens*) exposed to MS- 222, eugenol and 2 -phenoxyethanol were observed [26]. The induction times were higher in the bigger fish

compared to the smaller ones. Also, increased efficacy and sensitivity with increasing body size have been observed in Atlantic salmon, (*Salmo salar*) and in gold fish, (*Carassius auratus*) anaesthetised with metomidate [27], also, in rainbow trout (*Onchoryhncus mykiss*) anaesthetized with MS-222 [28]. Moreover, larger (60g) of white sea bream, (*Diplodus Sargus*) took a longer time to anaesthetize than smaller (30g) fish when exposed to 2-phenoxy ethanol[29]. Furthermore, the same trend was reported in Nile tilapia (*Oreochromis niloticus*) exposed to Sodium bicarbonate [30]. In the present study, the sedation and anaesthesia induction times were higher in adult size of *C. gariepinus* exposed to mustard seed extracts as anaesthetics than in juveniles and fingerlings. This may be due to the fact that larger fish have a small gill surface area in relation to body weight and consequently a small area for anaesthetics diffusion [31]. In addition, larger African catfish probably have lower metabolic rates [32], which may also contribute to a slower rate of anaesthetic absorption.

Conclusion

The results in this study concluded that applications of mustard seed extracts as anaesthetics did not affect the water quality parameters. Though, mustard seed extracts acted as an anaesthetic agent in sedating three sizes of *C. gariepinus*. It acted by widespread depression of the central nervous system produced by an action on nerve axons, transmitter release or membrane excitability. The induction time recorded in this work, reduced as the dosage of mustard seed extracts increased, while the recovery of anaesthetized fish increased notably with increasing concentrations of the extracts. This trend followed the pattern of typical fish anaesthetics. In terms of induction time and recovery time to anaesthetic, the aqueous extracts of mustard seed met the criterion which for an ideal anaesthetics. An effective concentration of 10.0mg/L; 20mg/l and 30.0mg/L of mustard seed extracts could be used to sedate fingerlings, juveniles and adult sizes respectively.

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