The efficacy of clove (Eugenia caryophyllata) powder as anaesthesia on African catfishes (Clarias gariepinus and Heterobranchus bidorsalis) fingerlings

Abstract

The efficacy of clove powder as anaesthetic agent on C. gariepinus and H. bidorsalis fingerlings was investigated in this study and the water quality parameters of the test solutions also determined. The time of induction and recovery for various stages and concentrations was recorded in minutes using a stop watch. The concentrations for the anaesthesia bioassay were 60, 70, 80, 100, 120, and 140mg/l. The water quality parameters examined after 30mins did not differ (P<0.05) from those of control hence may not have contributed to the observed behaviour of the test fish. Fingerlings exposed to 60 and 70mg/l had partial anaesthesia and did not achieve stage 4 (deep anaesthesia) after 30mins. Those exposed to concentration above 70mg/l were completely immobilized (deep anaesthesia) within 3–10mins depending on concentration. The mean time under each concentration increased significantly (P<0.05) from stages 4. The various stages of induction and recovery of C. gariepinus were higher than those of H. bidorsalis except induction stages 1 and 3 and recovery stage 3. Increasing concentration proportionally decrease the induction time but increase the time required for full recovery. An ideal anaesthetic must have among others quick induction and slightly longer recovery time to allow for varied manipulation as desirable. In this study clove powder at 120 and 140 mg/l induced deep anaesthesia in less than 5mins with a recovery time of 25–27mins, making it an ideal anaesthetic for African clarrid fingerlings.

Keywords: clove powder, african catfishes, fingerlings, induction, recovery

Introduction

Aquaculture practices frequently exposed fish to a variety of acute stressor that have the potential to negatively affect the normal performance and survival of organism. Anaesthetic abolishes pain in fish and induces a calming effect, loss of equilibrium, mobility and consciousness. In fisheries research and aquacultural operations, anaesthetics are necessary to minimize stress and reduce physical injury of fish during various handling procedures and transportation. Anaesthetics have also been reported to minimize mortality and reduce susceptibility to pathogens and infections. Anaesthetics most commonly used by commercial fish culturist and biologist include metomidate, tricaine methane sulphonate (MS 222), etomidate, benzocaine- hydrochloride, phenoxyethanol and eugenol. Some plant extract such as Derris (rotenone), tephrosia (tephrosin), Erythrophleum (alkaloid and tanin), Pyrus (tannin and saponin) and Tobacco (nicotine) have also been reported to have anaesthetic effects on fish. Clove oil derived from clove plant Eugenia caryophyllata (4- allyl-methoxyphenol) is regarded as the best anaesthetics to various fishes.

The desirable attributes of anaesthetics used for fin fish include, short induction and recovery time, non-toxicity to fish and humans, no lasting physiological effects, rapid clearance from the body, high solubility in fresh and salt water, availability and cost effectiveness. Sudagare et al. stated that although clove oil is adjudged the best anaesthetics for aquaculture organism but not readily soluble in water, not easily available and costly. To solve this problem, biologists and aquaculturists have been searching for alternative anaesthetics that apart from been less toxic, efficacious and safe for humans, should be readily available and cheap.

Clove powder is derived from the dry flower buds and stalks of clove plant E. caryophyllata. The efficacy of this powder has been evaluated on common Iranian fish Roach, Rutilus rutilus. Clove powder is readily available, highly soluble in water and cheap. In Nigeria clove products are sold in most herbal shops and markets use primarily for flavouring food (spice) and supplement for topical treatment of tooth ache, common cold, cough and inflammation of the mouth and throat. In cross river, clove flower buds are commonly known as “zobo” spice sold in most markets across the state.

The African catfishes, Clarias gariepinus and Heterobranchus bidorsalis constitute the largest group of cultured species after carp, salmonoids and tilapia, and they grow well under various culture systems of the world. Their culture is becoming more popular among fish farmers in Cross River, where they are being transported from hatcheries sites to grow out ponds research laboratories. According to Sudagare et al., clove powder is highly soluble in water, cheap and effective anaesthetic to Roach with reversible damage to damage to bloodstream. Similar observation was reported by Okey et al., for hybrid catfish on clove powder. The aim of the study is to determine the efficacy and effective concentration of clove powder on the two species of African catfishes C. gariepinus and H. bidorsalis fingerlings.

Materials and methods

Dry E. caryophyllata flower buds were procured from a herbal shop in watt market Calabar, Calabar South Local Government Area of Cross River State, Nigeria. The materials were identified at the Department of Botany University of Calabar (UNICAL). They were taken to the Fisheries Laboratory Cross River University of Technology (CRUTECH,) Obubra Campus. The flower buds were sundried for 30
The efficacy of clove (Eugenia caryophyllata) powder as anaesthesia on African catfishes (Clarias gariepinus and Heterobranchus bidorsalis) fingerlings

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min and then pulverized with a sterile manual blender and sieved with 100 micron net to obtain a fine powder. The powder was put in an airtight container and stored in a dry place prior to commencement of the experiment. Two hundred each of apparently healthy fingerlings of C. gariepinus (3.26g±2.30SD; 8.34cm±1.82SD) and H. bidorsalis (2.89g±1.80SD; 7.55cm ±1.04SD) were procured from UNICAL fish Farm, Calabar and acclimated for 14 days at the Fisheries Wet Laboratory, Cross River University of Technology (CRUTECH) Obubra, Nigeria. No mortality was recorded during the period of acclimation and feeding was discontinued 12hours before and during the experiment to minimize contamination of the test medium. A stock solution of clove power with a concentration of 200mg/L was prepared by dissolved 2g of clove powder into 10L of borehole water. Exposure concentrations of clove powder were 60, 70, 80, 100, 120 and 140mg/l, respectively. Thirty six glass aquaria were clean and randomly labeled and each filled with water to the 15litres mark for induction test and 20L mark for recovery. The different concentrations were prepared by serial dilution of the stock solution and water added to make up to 20L that gave the desired test concentrations. The mixture was stirred with a glass rod for homogenous mixing. Each concentration was stock with 10 fingerlings each in triplicate and monitored for the onset of induction (anaesthesia) for 30 min as periods greater than this were considered not ideal and impractical for routine fish handling procedures.14,22 The test fish were monitored following the various stages of induction and recovery time using a stop watch (Table 1). Any of the test fish that lost balance and ceased respiratory movements of the opercula (deep anaesthesia, stage 4) was removed immediately and transferred to about 20L of clove powder free water. The time for the fish to enter the desirable anaesthesia level (induction) and that which is required for an anesthetized fish to regain equilibrium and began active swimming (recovery time) were recorded at each stage.

### Results

#### Water quality parameters

The result of the water quality parameters were monitored in the experimental and control tanks: temperature, pH dissolved oxygen, alkalinity, conductivity and hardness. The water temperature was measured using the mercury in glass thermometer and recorded in °C. Conductivity was with conductivity meter (PACM 35 Model) and pH meter (Model 3015 Jenway) for pH. Dissolved oxygen was measured with a digital oxygen meter. Alkalinity was determined by standard method.24 Total hardness was determined by ethylene diamine- tetra acetic acid (EDTA) titration method. Data obtain from the various stages of induction (anaesthesia) and recovery time were subjected to various statistical tools. Descriptive statistic was used to obtain the means and standard error (Mean±S.E.). Differences among mean time for different dosage to achieve various stages of anaesthesia and recovery time on concentration were subjected to one way ANOVA using SPSS 18.0 version. Differences in means were separated using Tukey–Honest Significant Different (T-HSD) test and F-test for significant level at (p<0.05) between the treatments.25,26

#### Induction and recovery of C. gariepinus and H. bidorsalis fingerling from clove powder anaesthetic

The C. gariepinus fingerlings exposed to concentrations below 80mg/l did not reach stage 4 (complete immobilization) but with partial loss of equilibrium (stages 1–3) in 6–13mins. At 80mg/l, C. gariepinus fingerlings lost equilibrium in 6–9 mins and completely immobilized in 12 mins and begins recovery in 7-9mins. At 120 and 140 mg/l fish were completely anaesthetized (stage 4) within 2-5mins and regained equilibrium between 10–27mins. No mortality resulted from anaesthesia within 60-120mg/l of clove powder after 30 mins of exposure.

#### Table 1 Stages of anaesthesia (Induction) and recovery

| Stages | Anaesthesia(induction) | Recovery |
|--------|------------------------|----------|
| I      | Acceleration of the opercular movements, increased respiratory activity, accompanied by uncoordinated locomotion. | Body immobilized but opercular movements just starting and weak, uncoordinated locomotion |
| II     | Sporadic loss of equilibrium, difficulty maintaining position while at rest, high reaction to external stimuli. | Regular opercular movements and gross body movements beginning. |
| III    | Complete loss of equilibrium; inability to regain upright position. | Equilibrium regained, normal swimming and pre-anaesthetic appearance. |
| IV     | No reaction to handling or a sharp prod in the peduncle. | |

Source: Iwama et al.25

During the study, the following water quality parameters were monitored in the experimental and control tanks: temperature, pH dissolved oxygen, alkalinity, conductivity and hardness. The water temperature was measured using the mercury in glass thermometer and recorded in °C. Conductivity was with conductivity meter (PACM 35 Model) and pH meter (Model 3015 Jenway) for pH. Dissolved oxygen was measured with a digital oxygen meter. Alkalinity was determined by standard method.24 Total hardness was determined by ethylene diamine- tetra acetic acid (EDTA) titration method. Data obtain from the various stages of induction (anaesthesia) and recovery time were subjected to various statistical tools. Descriptive statistic was used to obtain the means and standard error (Mean±S.E.). Differences among mean time for different dosage to achieve various stages of anaesthesia and recovery time on concentration were subjected to one way ANOVA using SPSS 18.0 version. Differences in means were separated using Tukey–Honest Significant Different (T-HSD) test and F-test for significant level at (p<0.05) between the treatments.25,26

#### Table 2 Induction and recovery of C. gariepinus and H. bidorsalis fingerlings

| Stages | Induction |
|--------|-----------|
| I      | 6-9 mins |
| II     | 12 mins |
| III    | 1-27 mins |
| IV     | 30 mins |

The result of the water quality parameters as shown in Table 2 indicates that their mean values did not differ (p>0.05) from those of the control. The mean values of the various parameters decreased with increasing concentrations as follows, temperature (28.13-26.88°C), dissolved oxygen (4.74–4.12mg/l), pH(6.78–6.77) and hardness (39.32–38.80mg/l CaCO₃). Conductivity (166.07-167.05μS/cm/l) and alkalinity (37-41-38.51mg/l) had a slight increase above those of control at 140mg/l of clove powder after 30mins of exposure.

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The anaesthesia and recovery time of H. bidorsalis from clove powder were similar to those of C. gariepinus fingerlings in which increasing concentrations of clove powder proportionally decreased the time required for induction. No complete immobilization was achieved between 60–70mg/l of the anaesthetic. The time required to attained partial anaesthesia at stage 3 (19.00 and 10.00mins) was
The efficacy of clove (Eugenia caryophyllata) powder as anaesthesia on African catfishes (Clarias gariepinus and Heterobranchus bidorsalis) fingerlings

significantly higher than that of stage 2 (10.88 and 7.73) for 60 and 70mg/l respectively. At 120–140mg/l, the time to achieved complete immobilization was 4.93 and 2.33mins and completely regained equilibrium in 16.80 and 25.20mins respectively. At 140mg/l stages induction stages 1 and 2 were not observed and the time to achieved stages 3 (1.20min) and 4 (2.33min) were not significant (P<0.05). The recovering time at stages 3 was higher (p<0.05) than those of stages 1 and 2 (Table 4). The ANOVA showed that clove caused highly significant (P<0.001) on the stages of induction and recovery.

Table 2 Water Quality Parameters in Experimental Tanks of African catfishes fingerlings exposed to clove powder for 30 mins

| Conc. (mg/l) | Parameter | Dissolved Oxygen (mg/l) | Temperature (°C) | pH | Conductivity (µS/cm) | Alkalinity (mg/l) | Hardness (mg/lCaCO₃) |
|-------------|-----------|-------------------------|-------------------|----|---------------------|-----------------|---------------------|
| 0           |           | 4.74±0.27              | 28.13±1.07        | 6.7±0.28 | 166.07±2.04         | 37.41±0.45 | 39.32±0.81         |
| 60          |           | 4.29±0.23              | 27.53±1.18        | 6.6±0.29 | 167.17±1.47         | 37.91±0.49 | 38.40±0.36         |
| 70          |           | 4.37±0.35              | 26.87±1.25        | 6.8±0.27 | 166.51±1.31         | 38.18±1.96 | 37.61±0.58         |
| 80          |           | 4.48±0.12              | 27.37±0.78        | 6.7±0.41 | 165.96±1.32         | 40.76±2.69 | 38.33±0.54         |
| 100         |           | 4.38±0.36              | 26.67±1.02        | 6.7±0.16 | 166.79±2.14         | 36.75±0.85 | 38.72±0.83         |
| 120         |           | 4.38±0.38              | 27.74±0.91        | 6.7±0.19 | 167.23±2.50         | 38.67±1.05 | 38.26±0.33         |
| 140         |           | 4.12±0.12              | 26.88±0.19        | 6.7±0.22 | 167.05±2.71         | 38.51±0.47 | 38.80±0.85         |

Means with the same superscript under the same parameter are not significantly different at p<0.05

Table 3 The mean time (min.) of induction and recovery of C. gariepinus fingerlings from clove powder anaesthetic

| Conc. (mg/l) | Stages of induction(min) | Stages of recovery(min) |
|-------------|--------------------------|-------------------------|
|             | I            | II           | III          | IV          | I           | II          | III         |
| 60          | 7.7±0.24      | 14.7±0.47    | 0.0±0.00     | 0.0±0.00    | 0.0±0.00     | 0.0±0.00    | 0.0±0.00    |
| 70          | 6.4±0.29      | 9.13±0.43    | 12.07±1.41   | 0.0±0.00    | 0.0±0.00     | 0.0±0.00    | 0.0±0.00    |
| 80          | 3.6±0.20      | 5.73±0.63    | 8.5±0.63     | 11.20±0.72  | 6.47±0.18    | 5.20±0.73   | 8.47±3.74   |
| 100         | 2.6±0.20      | 4.33±0.13    | 5.73±0.18    | 7.00±0.35   | 8.3±0.24     | 8.6±0.35    | 15.4±0.47   |
| 120         | 1.0±0.00      | 1.67±0.07    | 2.73±0.24    | 4.93±0.13   | 9.90±0.21    | 13.03±0.94  | 17.87±2.2   |
| 140         | 0.0±0.00      | 0.20±0.11    | 0.80±0.12    | 2.13±0.67   | 16.17±0.42   | 19.46±1.18  | 27.12±0.98  |

Means with the same superscript under each of the concentration are not significant (P>0.05)

Table 4 The mean time (min) of induction and recovery of H. bidorsalis fingerlings from clove powder anaesthetic

| Conc. (mg/l) | Stages of induction (min) | Stages of recovery (min) |
|-------------|--------------------------|-------------------------|
|             | I            | II           | III          | IV          | I           | II          | III         |
| 60          | 6.47±0.27     | 10.88±0.27   | 19.00±1.50   | 0.0±0.00    | 0.0±0.00     | 0.0±0.00    | 0.0±0.00    |
| 70          | 4.63±0.09     | 7.73±0.18    | 10.00±0.12   | 0.0±0.00    | 0.0±0.00     | 0.0±0.00    | 0.0±0.00    |
| 80          | 3.20±0.12     | 5.27±0.13    | 7.87±0.13    | 10.73±0.37  | 4.47±0.18    | 8.53±0.18   | 12.17±0.45  |
| 100         | 1.20±0.12     | 2.33±0.24    | 5.00±0.20    | 7.6±0.23    | 5.53±0.27    | 10.60±0.35  | 13.00±0.15  |
| 120         | 0.00±0.00     | 1.27±0.07    | 2.93±0.07    | 4.93±0.47   | 6.57±0.32    | 13.80±0.31  | 16.80±0.35  |
| 140         | 0.00±0.00     | 0.00±0.00    | 1.20±0.12    | 2.33±0.07   | 11.87±0.24   | 18.40±0.49  | 25.20±0.93  |

Means with the same superscript under each of the concentration are not significant (P>0.05)

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The efficacy of clove (Eugenia caryophyllata) powder as anaesthesia on African catfishes (Clarias gariepinus and Heterobranchus bidorsalis) fingerlings

The induction stages of the fingerlings at the various levels of concentrations were not significant (P>0.05) except at 60 and 100mg/l where the time in C. gariepinus was higher (Table 5). The mean values of the various stages of induction and recovery showed that, stages 2, 4 (deep anaesthesia) and recovery stage 1 were significantly higher (P<0.05) in C. gariepinus. The mean time to regain complete equilibrium (recovery 3) in the both species was not significant (P>0.05) (Figure 1). The interaction of species and concentrations also had significant changes (P<0.01) on all the stages of induction and recovery except at induction stage 1 and recovery stage 3, which was not significantly (P>0.05) different.

Table 5 Comparative mean values of various stages of induction and recovery of C. gariepinus and H. bidorsalis fingerlings on the various levels of clove powder

| Conc. (mg/l) | Stage of induction (min) | Stage of recovery (min) |
|-------------|--------------------------|-------------------------|
|             | C.g | H.b | C.g | H.b | C.g | H.b | C.g | H.b | C.g | H.b | C.g | H.b | C.g | H.b |
| 60          | 7.73±0.24 | 6.47±0.27 | 14.73±0.47 | 10.88±0.27 | 0.00±0.00 | 19.00±1.50 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 70          | 6.40±0.29 | 4.63±0.09 | 9.13±0.43 | 7.73±0.18 | 12.07±0.41 | 10.00±1.2 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 80          | 3.60±0.20 | 3.20±0.12 | 5.73±0.63 | 5.27±0.13 | 8.53±0.63 | 7.87±0.72 | 11.20±0.37 | 10.73±0.18 | 6.47±0.18 | 4.47±0.18 | 2.33±0.18 | 8.53±0.47 | 8.47±1.2 | 12.17 |
| 100         | 2.60±0.20 | 1.20±0.12 | 4.33±0.24 | 2.33±0.13 | 5.73±0.24 | 5.00±0.13 | 7.00±0.24 | 7.60±0.20 | 8.33±0.20 | 5.53±0.20 | 3.73±0.20 | 6.47±0.35 | 6.47±1.2 | 10.00 |
| 120         | 1.00±0.00 | 1.67±0.07 | 1.27±0.07 | 2.73±0.24 | 2.93±0.07 | 4.93±0.24 | 4.93±0.13 | 9.90±0.47 | 6.57±0.21 | 13.00±0.32 | 16.00±0.94 | 17.87±0.31 | 19.46±10.88 |
| 140         | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

Means with the same superscript under each of the variables are not significant (P>0.05), C. gariepinus(C. g), H. bidorsalis(H.b)

Figure 1 Comparison of the various stages of induction and recovery time (min.) of C. gariepinus and H. bidorsalis fingerlings exposed to clove powder anaesthetic for 30 min. Bars ±S.D Mean with different letters differs significantly (P<0.05;HSD).

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recommended for some European and Asian fishes. This implies that some water quality especially temperature can affect the rate of biodegradation and excretion of the toxicant from the blood streams via the gills hence affecting the recovery time.

Physiological responses in fish to anaesthetics are different. Kucuk\(^2\) reported that the gills area, body weight, species and metabolic rate have effects on the rate of anaesthetics absorption and induction (anaesthesia). This may have accounted for the slight differences in the induction and recovery time in the various species of clarids recorded in this study. Generally, an ideal anaesthetic ought to induce anesthesia quickly in less than 3 min, permit a fast recovery in 5min or less, produce no poison to fish, cause no hazard to human and be inexpensive.\(^4\) The result from this study indicated that clove powder at the concentrations of 80-140mg/l induced deep anaesthesia between 3-10min. with a recovery time of between 8–27mins making it an ideal anaesthetic for clarids. However, quick induction and a slightly longer recovery time would allow for varied manipulations as desirable, hence clove powder is an ideal anaesthetics for the African clarid fingerlings.

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Conflict of interest

The author declares that there is no conflict of interest.

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