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International Journal of Oceanography & Aquaculture
ISSN: 2577-4050

Efficacy of Rauwolfia Vomitoria Root Extracts as Anaesthetic Agents in African Catfish (*Clarias Gariepinus*)

Akinrotimi OA[1]*, Ikeogu CF[2] and Anyaobu-Cookey IK[3]

[1]African Regional Aquaculture Center/Nigerian Institute for Oceanography and Marine Research, Nigeria

[2]Departments of Fisheries and Aquaculture Management, Nnamdi Azikiwe University, Nigeria

[3]Department of Aquaculture, Nigerian Institute for Oceanography and Marine Research, Nigeria

Research Article

Volume 7 Issue 2

Received Date: April 10, 2023

Published Date: April 24, 2023

DOI: 10.23880/ijoac-16000235

***Corresponding author:** Akinrotimi Ojo Andrew, African Regional Aquaculture Center/ Nigerian Institute for Oceanography and Marine Research, Nigeria, Email: ojoakinrotimi@gmail.com

Abstract

The efficacy of Rauwolfia Vomitoria root extracts as Anaesthetic agent in juveniles and adult sizes of Clarias Gariepinus were investigated. The time of induction (time taken for the fish to get anaesthetized) and recovery (time taken for the fish to recover from the effects of the anesthetics) for various concentrations were recorded in seconds using a stop watch. The concentrations used for the anesthesia bioassay were: 0.00-control, 50.00, 100.00, 150.00, and 200mg/l. The results obtained indicated that the induction time in both sizes were found to decrease significantly ($P < 0.05$) as the concentrations of the R. Vomitoria root extracts increased, with the shortest induction time 145.00±3.00(s) observed in juveniles at the concentration of 200mg/L and the longest 266.66±12.12(s) in adult at the concentration of 50mg/L of the extracts. However, the recovery time increased significantly with increasing concentration of the R. Vomitoria root extracts, with the highest recovery time of 732.33±11.50 (s) observed in Adult size, at 200.00mg/L concentration and the lowest 434.33±28.29(s) in Juveniles at 50.00mg/L concentration of the extracts, with the optimum dosage of 50.00mg/L and 100.0mg/L for juvenile and Adult sizes respectively. Interestingly, the survival of the exposed fish during the trial was 100% in both sizes, as no mortality was recorded in all concentrations of the plant extracts. The findings of this trial, indicated that R. Vomitoria root extracts, was efficient with zero mortalities and therefore can be recommended as an effective Anaesthetic for use in aquaculture.

Keywords: Anaesthetic; Aquaculture; Plant extracts; Catfish; Stress

Introduction

Increased concern about animal welfare and potential pain caused culture fish species during routine manipulations has identified the search for ideal and appropriate anesthetics for use in aquaculture Southgate. The use of Anaesthetic enhance safety for both the fish and the handler during aquaculture operation and allows them to be performed out of the water with minimal stress for the fish Mylonas, et al. [1], Anesthesia minimizes pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness [2,3]. Anaesthetic in fish farms is

used to minimize mortality during handling and transport. This may reduce susceptibility to pathogens and infection [4]. Anaesthetic is also used in fish during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical procedures [5]. When choosing ananaesthetics, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment and the choice may also depend on the nature of the experiment and species of fish [6]. Knowledge about the ideal and optimum concentration of an Anaesthetic for various fish species is necessary because in-appropriate concentrations may lead to adverse effects on the fish.

One of the prominent plant that has Anaesthetic property is Rauwolfia Vomitoria, commonly called devil sizzle stick, belonging to the family Apocynaceae, is a rain forest shrub that grows in Nigeria having oval leaves with straight venation and cluster of tiny flowers [7]. It is widely distributed in West and East Africa. The members of this family usually have medicinal properties. The root bark extracts are reportedly being used in various ways in many African countries, for example, in Nigeria; it is used by traditional healers in treating psychiatric patient. While in the Democratic Republic of Congo it used to treat leprosy. In Ghana it is used to treat arthritis [8]. The barks has purgative, sedative and emetic properties and the major physiochemical constituents of this plant include alkaloids, glycosides, polyphenols and reducing sugar [9]. The alkaloid has been reported to include reserpine, a well-known antihypertensive substance found in this plant Amole, et al. [10]. Usages of R. Vomitoria in fish Anaesthetic are very limited. Hence, this study therefore assessed the efficacy of R. Vomitoria root extracts as anesthetics agents in two sizes of C. Gariepinus.

Materials and Methods

Project Location and Sources of Experimental Fish

This study was carried out at the Genetic family testing unit hatchery of African Regional Aquaculture Centre (ARAC) Alou, Port Harcourt. A total of four hundred and eighty (480) apparently healthy C. Gariepinus which consists of 240 each of juvenile (mean length 26.64cm ± 3.11SD and mean weight 356.21±12.91SD) and adult (mean length 52.13±12.91 and mean weight 1100.38g±41.61SD) were sampled from ARAC rearing concrete tanks adjacent to the experimental site. These tanks are being stocked for at least two production cycles annually. The juveniles were of the age (12 weeks) and the adult (24 weeks). The fish were harvested from the tanks after draining, using drag net. They were immediately transferred into holding tanks at the experimental site.

Fish Acclimation

In the hatchery, the fish were acclimated to laboratory conditions for a period of three days. 100litres size 4 rectangular tanks during this period the fish were fed daily at 0.5% body weight and the water in the holding tanks were renewed daily.

Source of Anaesthetic Agents

Rauwolfia Vomitoria

R. Vomitoria roots were obtained from a forest near senior staff quarters in Rivers State University of Science and Technology, Port Harcourt. Forest experts from the Department of Forestry of the same University authenticated a sample of plant and confirm it to be the roots of R. Vomitoria. The roots were cleaned with clean water, in bowl, and made free from sand. They were later cut into pieces and dried in the laboratory. The fresh air-dried roots were macerated into smaller pieces using a kitchen blender (Model, H112, and Glenwood, Japan). After this 5kg was weighed and soaked in 5 liters of water for 24 hours for the extract of the plant following the method of Peri.

Preparation of Test Solution

A stock solution of the Anaesthetic was prepared by adding 1ml of the Anaesthetic concentrate to 1 liter of water. Exposure concentration of anesthetics were 0.00ml/L (control); 50, 100, 150, and 200ml/L. Thirty 50L plastic containers were labeled each filled with water from borehole to the 30L mark, and another 30 tanks filled with fresh water without anesthetics were placed side by side. The different concentrations were prepared by serial dilution by measuring 50ml, 100ml, 150ml and 200ml of the stock solutions (x30) that was made into 30L with the borehole water that gave the desired concentrations.

Experimental Procedure

The mixture of anesthetics with water given the desired concentrations was then stirred with a glass rod of 50cm in length for homogeneous mixture. Within 10 minutes the tanks were randomly stocked with 10 juveniles per tank, while 4 were stocked for adult fish using a scrap net. The tanks were not aerated during the experimental period.

Determination of Induction and Recovery Time

The time for onset of anesthesia for the exposed fish was measured using a digital stopwatch. Fish behaviour was monitored individually through the induction and life

recovery stages in each life stage and concentrations for all the eight anesthetics. In the induction stage, 5 different behaviors were observed [11] slow swimming, slight increase in opercula beat frequency. Due to loss of equilibrium, loss of reflexes, movement and lastly deep anesthesia, where the fish was lying on one side and lay at the bottom of the tanks. After the anesthesia, 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 were observed. Recovery time was then recorded.

Survival of Exposed Fish

After the recovery stage in clean water, the exposed fish were monitored for any mortality. This was done by physical observation and any mortality was recorded. The percentage survival was calculated using the formula

$$\frac{No\ of\ survival\ fish}{Total\ \ No.of\ \ fish\exp osed} \times \frac{100}{1}$$

Source: FAO (2000).

Evaluation of Water Quality Parameters

Water quality parameters: dissolved oxygen, nitrite, and ammonia and supplied were evaluated using LaMotte fresh water test kit (Model AQ4, Chest own, Maryland, USA). PH and DO were determined with pH/DO meter (Model, H 9812, Hannah Products, and Portugal).

Statistical Analysis

The data obtained from the study were collected and analyzed using statistics software 8.0 for windows/Data were first tested for normality (Kolmogorov - Smirnov test) and homoscedasticity of variance (Barletta test). When these conditions were satisfied, a one way analysis of variance (ANOVA) was employed to reveal significant differences in measured variables among control and experimental groups. When a difference was detected ($P < 0.05$), Tukey's multiple comparison test was applied to identify which treatment were significantly different

Results

The water quality parameters in Rauwoijia vorrutoria root extracts experimental tanks were shown in Table 1. Significant variations ($P < 0.05$) comparable to control with 200mlL^{-1} were noticed in pH (6.92±0.13 to 9.20±0.18) and shipside (0.04±0.01 to 1.96±0.12) while other parameters evaluated were within the same range with no significant difference ($p > 0.05$).The induction time recorded in both sizes of C. Gariepinus exposed to Rauwolfia Vomitoria root extracts were within the same range, as low values were observed at different concentrations Table 1. Also, the recovery time, which tends to increase corresponding with the increase in concentrations of the root extracts, were observed to be in the same range for both juveniles and adult sizes, with same value of 350.00(5) recorded at 200m/L^{-1}) Table 2. The survival rates were 100.00% in both sizes.

Concentrations (mgL^{-1})					
Parameter	**0**	**50**	**100**	**150**	**200**
Temperature (°C)	28.96± 0.30a	28.96± 0.41 a	28.86 ± 0.21 a	29.00± 0.53 a	29.20± 0.72 a
PH	6.92± 0.13 a	7.50± 0.10 ab	7.90± 0.17 ab	8.56± 0.42 b	9.20±0.18 c
Dissolved Oxygen (mgL^{-1})	6. 04± 0.31 b	6.07±0.34 b	6.13± 6.41 ab	6.10± 0.12 ab	6.18± 0.41 a
Nitrite (mgL^{-1})	0.06± 0.13 a	0.05± 0.02 a	0.06± 0.02 a	0.06± 0.11 a	0.06±0.01a
Ammonia (mgL^{-1})	0.34± 0.05 a	0.34± 0.05 a	0.34± 0.04 a	0.32± 0.02a	0.33± 0.04 a
Sulphide (mgL^{-1})	0.05± 0.05a	0.05± 0.01 a	0.06± 0.01 a	0.09± 0.02 ab	0.09±0.02 ab

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

Table 1: Water Quality Parameters in Experimental Tanks of C. Gariepinus exposed to Rauwolfia Vomitoria root Extracts (Mean ± SD).

Concentrations (mgL^{-1})						
Life Stage	**Parameter**	**0**	**50**	**100**	**150**	**200**
Juvenile	Induction time (s)	0.00± 0.00	180.0±7.93	168.33±1.52	150.66±3.052	145.00± 3.60
	Recovery time (s)	0.00± 0.00	434.33± 28.29	584.32±8.50	596.14± 49.21	604.0±15.390
	Survival (%)	100.0± 0.00	100.00±0.01	100.00± 0.00	100.00± 0.01	100.00±0.01
Adult	Induction time (s)	0.00± 0.00	206.66± 12.12	156.33±36.50	126.66±1.52	119.00±1.00
	Recovery time(s)	0.00± 0.00	581.0±12.61	616.00±21.63	700.33± 15.4	732.33± 11.50
	Survival (%)	100.0± 0.00	100.00± 0.01	100.00±0.01	100.00± 0.01	100.00±0.01

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

Table 2: Induction, Recovery and Survival C. Gariepinus exposed to Rauwolfia Vomitoria Root Extracts (Mean ± SD).

Discussion

The water quality parameter of the various test media did not vary significantly (P<0.05) from those of the control. Since the parameters examined were within acceptable ranges for fish culture and toxicity test [12]. They may not have acted synergistically with the anesthesia to affect the behavior observed during the research. The result from this study compares favorably with those conducted to compare the efficacy of commonly used anesthetics on various fishes [13]. The present study revealed that R. Vomitoria root extracts acted as an Anaesthetic agent in sedating both sizes of C. Gariepinus at different concentrations. It acted by widespread depression of the central nervous system produced by an action on nerve axons, transmitter release or membrane excitability [14-16] reported that anesthesia is achieved by placing the fish into an Anaesthetic solution that is absorbed through the gills and enter the arterial blood to the central nervous system. Anaesthetic are needed for easy handling, sorting, measuring, transporting and surgical procedures in aquaculture and fisheries to facilitate application procedure. The induction time recorded in this work, reduced as the dosage of R. Vomitoria root extracts increased, while the recovery of anaesthetized fish increased notably with increasing concentrations of the extracts. This observation agrees with the findings of Akinrotimi, et al. [17] in two species of mullets exposed to clove seed extracts. This trend followed the pattern of typical fish anesthetics Mylonas, et al. [1]. In terms of induction and recovery time to Anaesthetic, the aqueous extracts of R. Vomitoria root met Markings, et al. [18] criterion for ideal anesthetics suitable for application in teleost fish. Generally, an ideal Anaesthetic ought to induce anesthesia quickly in less than 6min, permit a fast recovery in 10min or less, not poisonous to fish, nor hazardous to human, available and must be inexpensive Markings, et al. [18]. With the optimum dosage of 100.00mg/L and 150.00mg/L for juveniles and adults sizes respectively, R. Vomitoria root extracts to meet many of the criterions used in evaluation of an ideal Anaesthetic and compared favorably with conventional anesthetics like MS-222, etomidate and 2-phenoxethanol. Its main advantage lie in its availability all the year round, low cost, easy to use and high safely margins to humans [8]. Conversely, earlier reports indicate that size does not have a direct influence on the time required to induce anesthesia in fish [19,20]. However, a considerable variation exists within sizes of the same species in response to anesthetics application, in aquaculture [21]. Variations in Anaesthetic response in two sizes of yellow perch (Perce flavescent) exposed to MS- 222, eugenol and 2-phenoxylethanol were observed Feng, et al. [22]. 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) anaesthetized with metomidate Fichi, et al. [23]. 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 [24]. Furthermore, the same trend was reported in Nile tilapia (Oreochromis niloticus) exposed to Sodium bicarbonate [25]. In the present study, the sedation and anesthesia induction times were higher in adult size of C. Gariepinus exposed to R. Vomitoria root extracts as anesthetics than in juveniles. 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 anesthetics diffusion [26].

Conclusion

The results in this study concluded that applications of R. Vomitoria root extracts as anesthetics did not affect the water quality parameters. Though, R. Vomitoria root extracts acted as an Anaesthetic agent in sedating three sizes of C. Gariepinus. The induction time recorded in this work, reduced as the dosage of R. Vomitoria root 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 R. Vomitoria root extracts met the criterion which for an

ideal anesthetics. An effective concentration of 100.00mg/L and 150.00mg/l of R. Vomitoria root extracts could be used to sedate juveniles and adult sizes respectively

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