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Evaluation of Mustard Seed (*Brassica Nigra*) Powder as Anaesthetic Agents in Different Life Stages of Black Jaw Tilapia (*Sarotherodon Melanotheron*)

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Abstract

This study assessed the anesthetic effects of mustard seed (*Brassica nigra*) powder in different life stages of Black Jaw Tilapia (*Sarotherodon melanotheron*). A total of 540 fish comprising of 180 each of: fingerlings (mean length 5.54cm ± 1.08SD and mean weight 12.04g ± 2.09SD); juveniles (mean length 10.02cm ± 3.91SD and mean weight 30.91g ± 4.02SD) and adult (mean length 13.01cm ± 2.66SD and mean weight 70.82g ± 7.01SD) of S. *melanotheron* were used for the study. They were exposed to aqueous extracts of mustard seed (*Brassica nigra*) powder at different concentrations: 0.00-control; 10.00; 20.00; 30.00; 40.00 and 50.00 mg/L at the rate of 10 fish per tank in triplicates. The results obtained indicated a size related response of the fish to mustard seed extracts. The induction time decreased significantly ($P < 0.05$) as the concentrations of the mustard seed extracts increased. The recovery time in the three sizes of exposed fish, generally increased as the concentrations of the anaesthetics increased. The survival rate was 100.00% in adult sizes of the exposed fish in all concentrations. While in fingerlings and juveniles, 90-95% survival rates were recorded at higher concentrations of the anaesthetics. This study therefore revealed that mustard seed can effectively be used as anaesthetics agent in S. *melanotheron* at optimum dose of 30mg/L.

Keywords: Anaesthetics; Tilapia; Aquaculture; Stress; Life stages.

1. Introduction

Anaesthetics have been used extensively in aquaculture activities in different parts of the world to immobilize fish [1]. Application of anaesthetics with its concomitant sedative effect usually reduce stress on the fish during handling procedures such as weighing, measurement, tagging, transportation, capture, breeding, and surgical activities [2]. Conversely, anaesthetics also reduce excitement and hyperactivity-related trauma that occur during handling and consequently reduces mortality and morbidity in the culture medium [3, 4]. However, the use of chemical anaesthetics during aquaculture operations has some challenges during its applications, this include poor solubility in water and long induction and recovery time (quinaldine), acute toxicological effects at high concentrations and potential health risks on humans including development of rashes (2-phenoxyethanol), and high cost of these chemicals especially in developing countries of the world [5-7].

In recent times, natural products of plant origin have been proposed by many researchers as viable alternatives to synthetic anaesthetics in aquaculture operations. Since the beginning of human civilization, herbs have been used by mankind for its therapeutic value and medicinal purposes. A large proportion of modern drugs in circulation have been isolated and developed from natural sources [8]. Many of these isolations and developments were based on the uses of these plants in traditional medicine [9]. Ethno veterinary medicine is widely practiced in rural areas of Africa, including Nigeria. Most of the rural fish farmers in Nigeria do not have access to most of the chemical anaesthetics that are being used in developed countries of the world nor the financial resources to procure synthetic drugs used in aquaculture activities [10]. One of the plant products with promising qualities for anaesthetic properties that could be use during aquaculture operations is mustard seed. 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 [11].

The specie *Sarotherodon melanotheron* belongs to the family Cichlidae. It is commonly referred to as black jaw tilapia. This specie is primarily estuarine domiciled specie, but extends its distribution to fresh and salt waters, and has the ability to tolerate waters of high salinities [12]. They are primarily detritivores, feeding on alga, phytoplankton and aquatic vegetation and can grow and reproduced within a pH range of 3.5-5.2. *S. melanotheron* is endemic to Africa, with a wide coverage from Senegal to Zaire [13]. It can be differentiated from other tilapiine

species by black patch jaw or chins, slightly emerginate caudal fin and pale blue body colouration on its lower flank and becoming orange or metallic golden yellow at times with black patches [14]. Anaesthetic work on *S. melanotheron,* are not too common [15]. Hence, information on anaesthetic response of *S. melanotheron* exposed to various concentration of nutmeg is important so as to establish its efficacy as an effective replacement for chemical anesthetics. This therefore study assessed the efficacy of nutmeg as plant based anaesthetics in different life stages of *S.melanotheron.*

2. Materials and Methods

2.1. Experimental Location and Fish

The study was carried out in African Regional Aquaculture Center, an outstation of Nigerian Institute for Oceanography and Marine Research, Buguma, Rivers State, Nigeria. A total of 540 fish comprising of 180 each of: fingerlings (mean length 5.54cm ± 1.08SD and mean weight 12.04g ± 2.09SD); juveniles (mean length 10.02cm ± 3.91SD and mean weight 30.91g ± 4.02SD) and adult (mean length 13.01cm ± 2.66SD and mean weight 70.82g ± 7.01SD) of *S. melanotheron* were used for the study. They were sourced from ponds during the low tide. The fishes were transported in six open 50l open plastic containers to the laboratory and acclimated for a period of seven days.

2.2. Preparation of Mustard Seed

Dried Mustard Seed was purchased from fruit garden market at Kaduna Street, D-Line, and Port Harcourt in Port Harcourt City Local Government Area of Rivers State, Nigeria. 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.

2.3. 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 54 plastic basins of dimension (52 x 44 x 34 cm^3) each were used for the experiments. The basins were labeled based on life stages of the fish, treatment levels and replicates. Each basin was stocked with 10 fish per tank.

2.4. Experimental Procedure

The powder was weighed into different concentrations (0.00-control; 10.00; 20.00; 30.00; 40.00 and 50.00 mg/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.

2.5. Determination of Induction and Recovery Time

Induction time is the time for onset of anaesthesia in 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. 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 [1]. Recovery time was then recorded.

2.6. Evaluation of Water Quality Parameters

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. 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 were evaluated using LaMotte fresh water test kit (Model AQ4, Chestown, Maryland, USA).

2.7. Statistical Analysis

The data obtained from this study was collated and analyzed using statistics software SPSS version 22. 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.

3. Results

The water quality parameters in the experimental tanks of *S.melnotheron* exposed to mustard seed extracts are presented in Table 1. The results obtained indicated a significant reduction ($P<0.05$) in the values of dissolved oxygen which reduced with increasing concentration of the anaesthetics agent. While, other water quality parameters were within the same range with no significant different in relation to the concentration of the anaesthetics agent ($P>0.05$). The induction time in three life stages of *S. melanotheron* exposed to mustard seed extracts are presented in Figure 1. The use of mustard seed extracts as anaesthetics resulted in different induction times depending on the dosage and sizes of the fish. Furthermore, the induction times in adult size of *T.guineensis* were higher than that

juveniles and fingerlings which reduced with increasing concentration of anaesthetics agent. The results of the recovery time in *S. melanotheron* exposed to mustard seed extracts indicated a significant ($P < 0.05$) increase in the recovery time, as the concentrations of the extracts increased (Figure 2). The survival rates of the exposed fish are presented in Figure 3. The results revealed that 100.00% survival rate were recorded in the adult fish in all concentrations of the anaesthetics agent. While in fingerlings and juveniles 5-10% mortality were recorded at higher concentrations of the anaesthetics agent (Figure 3).

Table-1. Water Quality Parameters in Experimental Tanks of *S.melanotheron* exposed to Mustard Seed Extracts (Mean ± SD)

Concentrations (mgL^{-1})					
Parameter	**0.00**	**10.00**	**20.00**	**30.00**	**40.00**
Temperature (°C)	29.11± 0.75[a]	29.03± 0.99[a]	29.06 ± 1.44[a]	28.99± 2.04[a]	29.01± 2.77[a]
pH	6.11± 0.33[a]	6.61± 0.38[a]	6.38± 1.77[a]	6.52± 0.47[a]	6.57±0.71[a]
DO(mgL^{-1})	6. 75± 1.92[c]	6.41±0.88[c]	5.82± 0.81[b]	5.39± 0.22[b]	4.07± 0.66[a]
Nitrite (mgL^{-1})	0.0081± 0.01[a]	0.049± 0.02[a]	0.053± 0.02[a]	0.057± 0.02[a]	0.068±0.02[a]
Ammonia (mgL^{-1})	0.21± 0.01[a]	0.23± 0.01[a]	0.22± 0.02[a]	0.23± 0.03[b]	0.22± 0.01[b]

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

Figure-1. Induction time in Three Life Stages of *S.melanotheron* Exposed to Mustard Seed Extracts

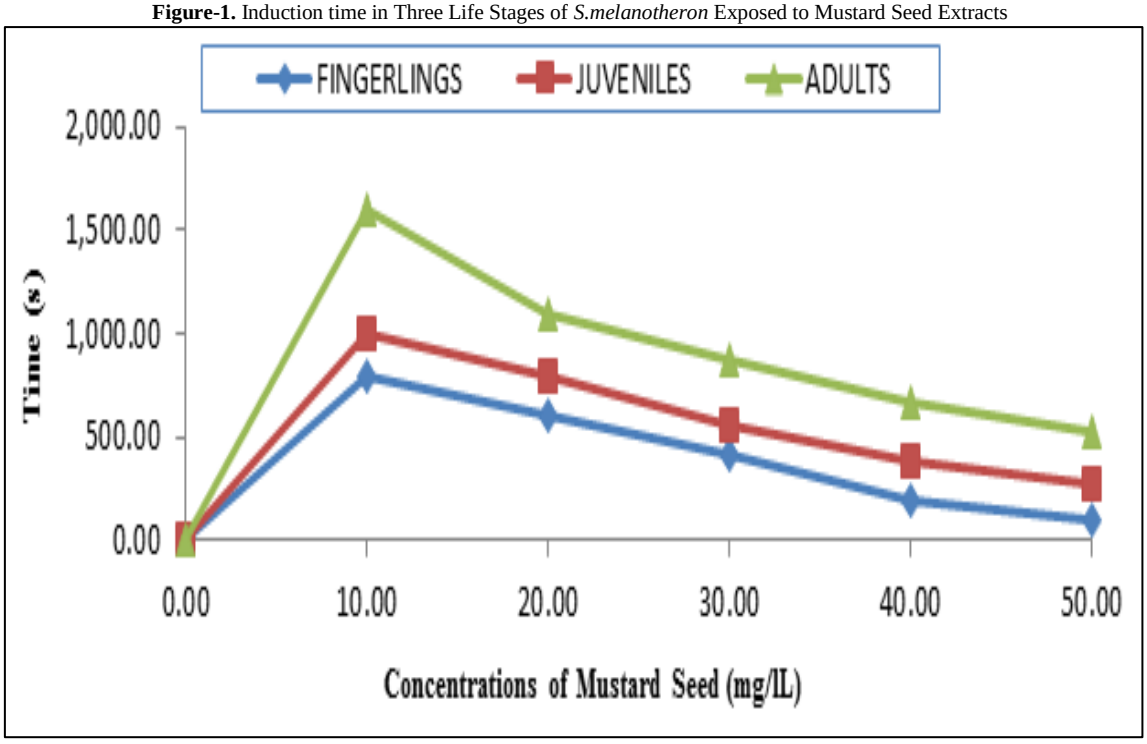


Figure-2. Recovery time in Three Life Stages of *S.melanotheron* Exposed to Mustard Seed Extracts

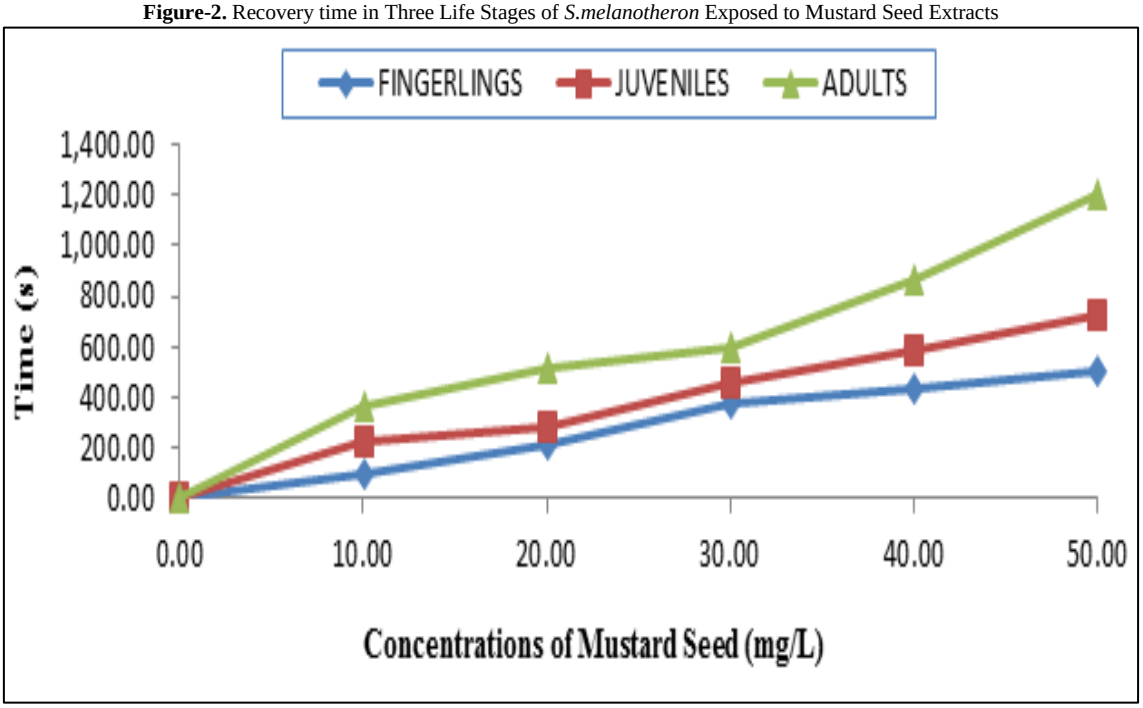
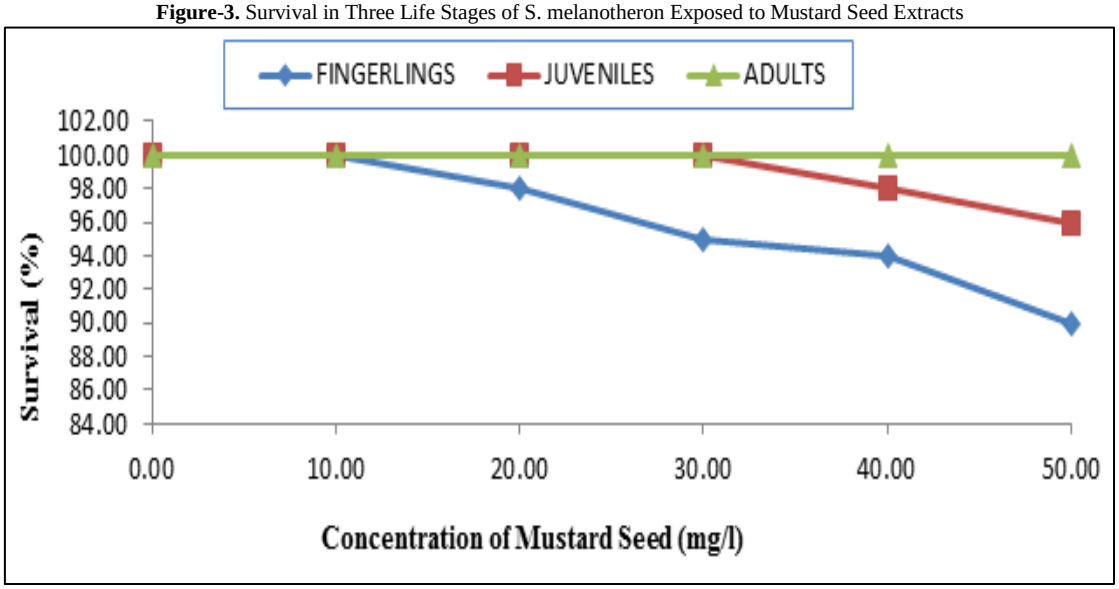


Figure-3. Survival in Three Life Stages of S. melanotheron Exposed to Mustard Seed Extracts



4. Discussion

The water quality parameters in experimental tanks in three life stages of *S. melanotheron* 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 considered during the study were within the same range, with no significant different ($P>0.05$), in relation to the concentration of the anaesthetics. This result agrees with the report of Akinrotimi, *et al.* [17], in the experimental waters of *C.gariepinus* exposed to clove seed extracts.

Anesthetic concentration and various biological or environmental factors significantly affect the anesthesia of fish [18, 19].The effect of the aqueous extract of mustard seed on three life stages of *S. melanotheron* appeared to be concentration dependent. Faster tranquilization and sedation was achieved at higher concentrations of the extracts as reported in other studies using plant based anaesthetics [15, 20-22]. This observation is also in agreement with the report of Feng, *et al.* [23] that the degree of anaesthesia is influenced by the concentration of the anaesthetic in the central nervous system (CNS) of the organism. Induction times in all the sizes of the treated fish decreased significantly with increasing concentrations of aqueous extracts of mustard seed powder. There was a strong negative relationship between mustard seed powder and induction time. Similarly, negative relationship between anesthetic agent concentrations and induction time of anesthesia were reported in European bass and *S. aurata* Mylonas, *et al.* [24], Senegalese sole [25], Russian sturgeon [20], Rivulated rabbitfish or Surf parrotfish [21]. Therefore, in the present investigation the shorter induction time taken to tranquilize the experimental fish, *S. melanotheron*, with increased concentration of the anaesthetic extract may be attributed to the accumulation of the active ingtredients, allyl isothiocyanate, in the body system of the fish which impairs the activity of CNS at a much faster rate [8].

In this study, a positive correlation exists between anesthetic concentration and recovery time in this study. Similar effects have been demonstrated in several studies such as reported in Grey mullets [15], *C. gariepinus* [26], *T.guineensis* [27] and Rainbow trout [28]. However, Mirghaed, *et al.* [29] and Bolasina, *et al.* [30] could not find positive relationships between anesthetic concentration and recovery time. Conversely, Weber, *et al.* [25] and Mitjana, *et al.* [31] explained that differences among the studies might be due to the specific properties of each species which determine the physiological responses of fish to anesthetic agents. In addition, it is stated that the pharmacokinetics of the anesthetic agent may cause differences among studies [32].

In the present study, exposure of *S.melanotheron* to aqueous extracts of mustard seed powder resulted in zero mortalities for the adult fish. However, low mortalities were recorded in fingerlings and juveniles sizes at higher concentrations of the anaesthetics agent. This results agrees with the findings of Akinrotimi, *et al.* [17] in *Tilapia guineensis* exposed to clove extracts. These authors recorded no mortality in the adult fish exposed to nutmeg extracts, but observed a low mortality in fingerlings of the specie. These authors attributed the death of experimental fish to the capacity of the clove to markedly induce anaesthesia which could cause death. The absence of mortalities in the experiments with mustard seed extracts in this study may therefore be due to the fact that the various concentrations of the mustard seed powder extracts used in this research lack the capacity to induce deep anaesthesia in the experimental subjects.

5. Conclusion

Aquaculture industry needs more effective anesthetics drugs which are inexpensive and environmentally friendly, also harmless for fish and labour-saving. The mustard seed extracts assessed in the present study have proved to be effective anesthetics without causing fish mortality. Based on the results from this work, an effective concentration of 30.0mg/L of mustard seed extracts could be used to sedate fingerlings, juveniles and adult sizes of S. melanotheron . This highlights the potential of this extracts as fish anesthetics which can be effectively utilized for the sustainability of aquaculture industry in Nigeria.

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