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Anaesthetic effects and haematological responses of *heterobranchus bidorsalis* juveniles exposed to clove oil

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This study aims to investigate the use of clove oil as an anaesthetic agent for *Heterobranchus bidorsalis* juveniles and assess the effects on the test fish, using some hematological parameters. Different concentrations of clove oil (0.8 to 1.2 ppm) were used in static water to determine the induction time and recovery time of the test organisms. Behavioral changes of the test fish were observed, the time the fish became immobilized and recovery from anaesthesia was recorded. The results showed erratic swimming, loss of equilibrium, loss of body movement with continued operculum movements, and loss of reflex of the test organisms during the period of exposure at higher concentrations (1.0 to 1.2 ppm) of clove oil. The physiological stress responses of *H. bidorsalis* juveniles increase when compared with fish in the control experiment. The induction time (119 s) significantly decreased with increasing concentrations of clove oil (1.2 ppm), while the recovery times ranged between 180 to 906 s from lower concentration (0.8 ppm) of clove oil to the highest concentrations (1.2 ppm).There was a positive linear effect (R^2=0.95, P<0.05) between the concentration of clove oil and the induction time. The values obtained from hematological responses of the test organisms exposed to different concentrations of clove oil showed that the clove oil caused a concentration dependent changes in the blood of *H. bidorsalis* juveniles with significant reduction (P<0.05) in the values of packed cell volume (PCV) (26.00 to 16.00), haemoglobin (Hb) (8.70 to 5.90), mean corpuscular volume (MCV) (130.00 to 70.70) and the values of mean corpuscular haemoglobin (MCH) (38.61 to 32.41), and white blood cell (WBC) (29.50 to 73.50) increased significantly (P<0.05) with increasing concentrations of clove oil, while the values of red blood cell (RBC) and mean cell haemoglobin concentration (MCHC) varies with no definite pattern. Clove oil could be used as anaesthetic agent in fisheries because the induction and recovery times were within the recommendation range used in fishery management and does not pose any environmental hazard.

Key words: *Heterobranchus bidorsalis*, clove oil, anaesthetic effect, haematology examination, induction time, recovery time.

INTRODUCTION

Anaesthetics are often used in aquaculture, fisheries and biological researches as a way to minimizes fish hyper-motility which is a considerable source of injuries during handling procedures (Cho and Heath, 2000; Rose and Rose, 2008; Olufayo and Ola, 2010). The consequent damages from such accidents succumbed fish to increase the susceptibility to pathogens and infection diseases (Rose and Rose, 2008). Therefore, reducing

fish motility by anaethetics may decrease the undesirable handling consequences (Rose and Rose, 2008). There are many researches on the physiological effects of different anaesthetics on fishes and clove oil has received favourable reviews as an alternative anesthetic for different fish species (Okey et al., 2013; Hitoki et al., 2011) due to the antifungal and antibacterial properties (Keene et al., 1998; Cho and Heath, 2000). Plant naturally possess substances which are used by man in production of medicine especially antibiotics (Okwu and Josiah, 2006), thus, their importance in aquaculture and fishery management is very important.

Clove oil is an organic natural product, a dark brown liquid resulting from distillation of flowers, flower stalks and leaves of Eugenia aromatica (Nagababu and Lakshmaiah, 1992), its contains 70 to 90% eugenol, more than 17% eugenol acetate and 12% kariofilen. It is derived from the stem, leaves and buds of Eugenia caryophylatta tree and its active ingredients are eugenol (a-methoxy-4-2 (2-propenyl)-Phenol and isoeugenol (4-propenyl-2-methoxy phenol).

The use of clove oil is more potent than synthetic anaesthetics used in fish and its efficacy as an anaesthetic for various fish has been demonstrated in various fish species (Olufayo and Ola, 2010, Dang- Won Seol et al., 2007) and the U.S Food and Drug administration has considered it as safe compound (Summerfeit and Smith, 1990) for use in biological research.

Some research works have been carried out using clove oil as anaesthetics on different fish species: Alok et al. (2014) and Hajek (2011) worked on common carp (Cyprinus carpio); Cho and Heath (2000) worked on Chinook salmon (Oncorhynchus tshawytscha); Veliek et al. (2006) and Olufayo and Ola (2010) worked on African catfish; Keene et al. (1998) and Prince and Powell (2000) worked on rainbow trout (Oncorhynchus mykiss); Anderson et al. (1997) and Prince and Powell (2000) investigated the effects of clove oil rainbow trout; while Woody et al. (2002) worked on adult sockeye salmon. The main objective of this study was to investigate the effects of clove oil on H. bidorsalis juveniles using haematological parameters to assess the changes in the test fish induced by the anaesthetic.

MATERIALS AND METHODS

Experimental fish

One hundred and fifty healthy juveniles of H. bidorsalis (weight 25.8 g) were obtained from Ayoola Commercial Fish Farm, Nigeria and were transported in pond water at 23°C temperature to the Department of Fisheries and Aqua culture Technology Laboratory at Federal University of Technology Akure in a plastic container containing 50 L of fresh water to reduce stress. The fish were acclimated in the laboratory in a well aerated large fibre glass holding tank (1500 L volume) for one week. The fish were fed during the acclimation period with Coppen feed according to their body weight, but were unfed for 24 hours prior to the test in order to minimize the production of waste, thereby reducing ammonia production from the wastes.

Preparation of anaesthetic solution

Clove oil manufactured by B.D.H Chemical- Limited (Poole England), Boisar 401501 with Batch No: 0392-459-232422 and Product No: 46063 were obtained from Pascal Scientific Pharmaceutical Stores, Akure, Nigeria. The stock solution (100 ml) of clove oil was dissolved in 95% ethanol at a ratio of 1:10 (Cho and Health, 2000) before adding to fresh water, because it is insoluble at water temperature below 15°C. Different concentrations clove oil of 0.8, 0.9, 1.0, 1.1 and 1.2 ppm was applied to the tanks in duplicates.

Experimental procedure

Ten fish were distributed each into prepared anesthetic baths of different concentrations of clove oil in duplicates with a control with 10 L of water in twelve plastic tanks each (n=10 fish/tank) and the hyper-activities of the test fish, changes in the body color, loss of sensitivity to stimuli, motionless (induction time) and other behavioural activities were observed. Individual test fish were transfer to a recovery tank (50L) as they reach anesthesia and recovery time was established at the point when the animal recovered normal activities and regular breathing. Blood was collected into a syringes containing 0.5 mg disodium salt of Ethylene Diamine Tetra-acetic acid (EDTA) during anaesthesia state. This anticoagulant prevented the blood from clotting. The following haematological parameters were measured from the blood samples collected before, during and after recovery from anaesthesia: haematocrit (RBC),haemoglobin (Hb), erythrocytes (RBC), leucocytes counts (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration. All these parameters were determined using standard methods.

Water quality parameters

Dissolved oxygen (DO), pH, temperature and conductivity in each aquarium were measured and observed before the experiment, during the 96 h exposure and after the experiment using the methods described by APHA (1989).

Statistical analysis

Linear regression and graphs were used to determine the relationship between concentration, induction and recovery time while experimental data were analyzed using one way analysis of variance (ANOVA) to test for significant differences. Standard deviation (SD) and Pearson correlation coefficient were calculated. Significance was set at $P < 0.05$. All analyses were performed

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Table 1. Induction and recovery time and mortality in *H. bidorsalis* exposed to various concentration of clove oil.

Clove oil concentration(ppm)	Induction time (s)	Recovery time (s)	Mortality (%)
0	0	0	0
0.8	477.50±3.54^{e}	180.00±0.00^{a}	0
0.9	443.00±11.31^{d}	305.00±7.07^{b}	0
1.0	360.00±0.00^{c}	514.00±48.08^{c}	10
1.1	186.00±8.49^{b}	675.00±21.21^{d}	20
1.2	119.00±1.41^{a}	906.50±9.19^{e}	20

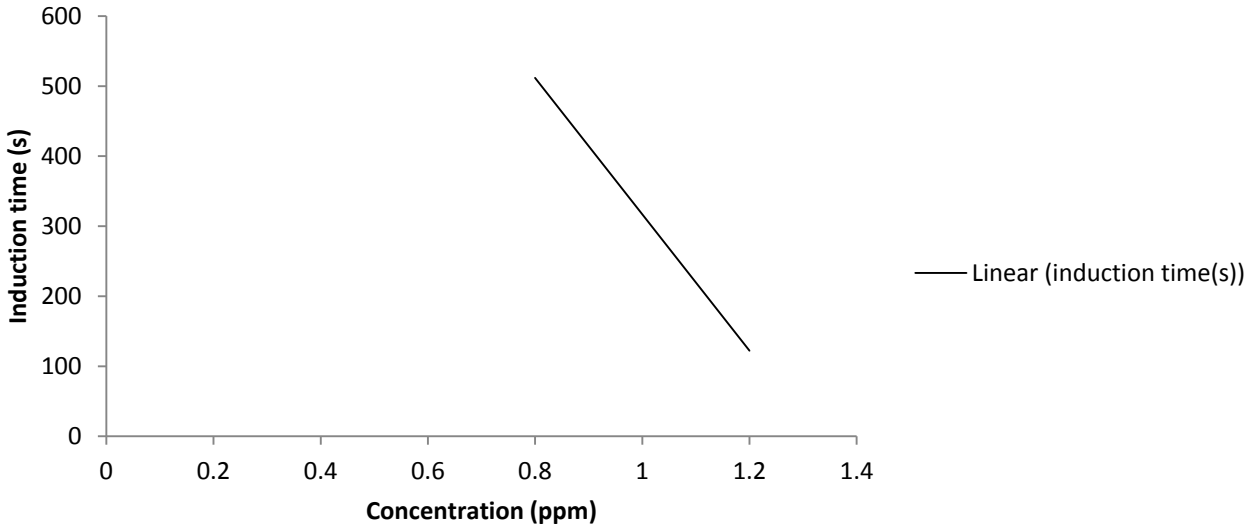

Figure 1. Relationship between concentration and induction time of *H. bidorsalis* exposed to different concentrations of clove oil: (P<0.05).

using statistical package for social sciences (SPSS) software (version 17.0, 2015).

RESULTS AND DISCUSSION

All the test fish reached anaesthesia within 2 min in the highest concentration (1.2 ppm) and 8 min in the lowest concentration (0.8 ppm). Results from the experiment revealed that there was relationship between concentration of clove oil, induction time, recovery time and mortality of *H. bidorsalis* juveniles exposed to various concentration of clove oil (Table 1).

Induction time

The induction time decreases with increasing concentration of clove oil (y= -974x + 1291, Figure 1). There was a positive linear effect (P<0.05) R^2=0.950 between the concentration of clove oil and the induction time. In this experiment, the induction time in highest concentration (1.2 ppm) is 119 s and took longer time to recover (906 s) (Table 1). The rate of mortality recorded at the highest concentrations (1.1 ppm and 1.2 ppm) of clove oil agreed with the study of Sladky et al. (2002); who reported that at higher concentration, there was high mortality rate

Recovery time

The recovery time increases with increase in concentration of clove oil (y=1822x – 1306, Figure 2). There was a positive linear effect (P<0.05) R^2=0.991 between the concentration of clove oil and the recovery time. The result clearly showed that, the higher the concentration of anaesthetic, the shorter the induction time and the longer the recovery time. The longer recovery time observed in fish anaesthetized with clove oil in higher concentrations could be as a result of stress incurred from the anaesthetic influence, metabolic rate, oxygen consumption, branchial respiration and the blood pressure of the test fish. Effects of dissolved oxygen were

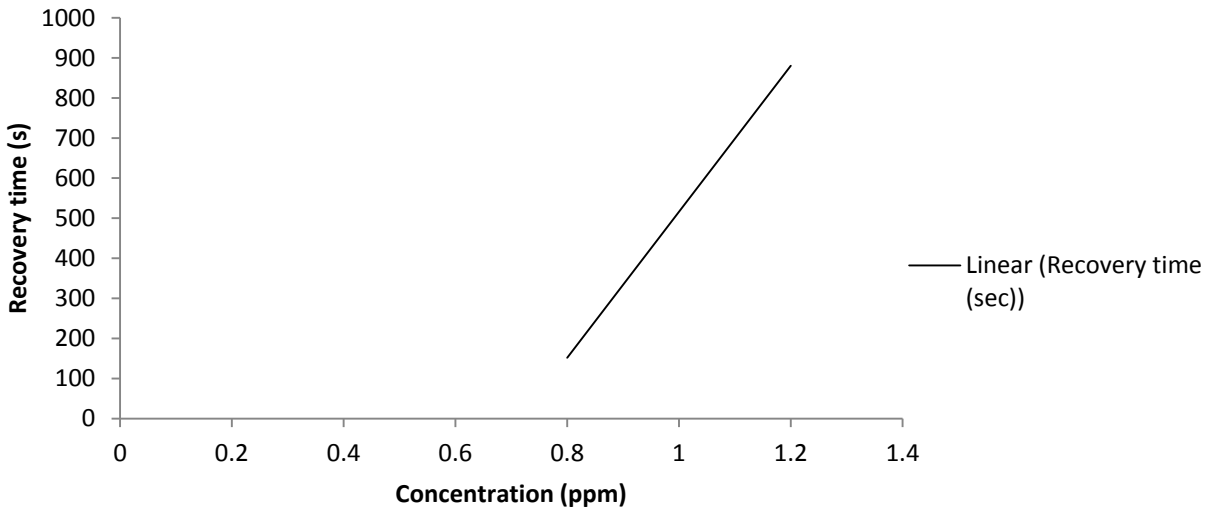

Figure 2. Relationship between concentration and recovery time of *H. bidorsalis* exposed to different concentration of clove oil (P<0.05).

Table 2. Physico-chemical analysis of water after subjecting to various concentration of clove.

Parameter	Concentrations (ppm)					
	0	0.8	0.9	1.0	1.1	1.2
Conductivity	2201.00±1.41^{a}	206.57±0.76^{b}	206.53±0.11^{b}	208.71±0.83^{b}	210.93±0.38^{c}	213.25±1.20^{d}
D.O (mgl^{-1})	6.57±0.21^{c}	2.95±0.35^{b}	2.54±0.03^{b}	2.47±.08^{b}	1.40±0.28^{a}	1.50±0.71^{a}
Temperature (°C)	27.28±0.75^{a}	26.65±0.92^{a}	25.70±0.08^{a}	26.15±0.80^{a}	26.36±0.52^{a}	26.37±1.03^{a}
$_{P}$H	7.30±0.08^{a}	7.36±1.11^{a}	7.48±0.12^{a}	8.15±0.21^{b}	8.28±0.18^{b}	8.40±0.28^{b}

Values on the same row with different superscript letters are significantly different (P<0.05).

obvious in all of the concentrations shows significant differences in both induction and recovery time.

Physiochemical parameters

Conductivity was observed to increase with increasing concentration of clove oil. The dissolved oxygen in the control was significantly higher than other varying concentrations of clove oil (0.8 to 1.2 ppm) and decreases drastically with the increase of concentration of clove oil. Temperature is observed to be fluctuating while pH increases with increasing concentration (Table 2).

Haematological parameters

Table 3 shows that the haematological parameters measured were significantly affected by clove oil. Treatment with clove oil caused a progressive decrease (P < 0.05) in the PCV values (26.00±2.83 to 16.00±1.41), HB (8.70±0.14 to 5.90±0.42), MCV (130.00±14.14 to 70.70±14.14) and increase in MCH values (32.41±5.43 to 38.61±6.12) and WBC (29.50±6.36 to 73.50±49.50) when compare with the treatment in the control tanks. The RBC and MCHC values vary within different concentrations of clove oil (1.80±0.14 to 2.85±0.07 and 26.30±0.14 to 41.75±2.47) respectively. Exposure of *H. bidorsalis* juveniles to clove oil caused significant decrease in PCV, HB, RBC and MCV. This significant reduction may be an indication of severe anaemia caused by clove oil on the exposed fish. This finding agreed with Olufayo and Adeyanju (2012) who worked on haematological effect of neem leaves (*Azadirachta indica*) on *H. bidorsalis* and reported that the toxicant caused a significant decrease in PCV, Hb, RBC, MCHC and MCV.of *H. bidorsalis* Otherwise, increase in the values of MCH and WBC of the fish, may be due to the physiological reaction of the fish to the effects of stress induced by the anaesthetics used. This was attributed to a generalized stress response resulting from increased pituitary inter-renal activity which agreed with Thompson and Eling (1989).

Behavioral changes

The behaviors exhibited by the test fish ranged from

Table 3. Hematology of *H. bidorsalis* juveniles exposed to different concentrations of clove oil.

Parameter	Concentrations (ppm)					
	0	0.8	0.9	1.0	1.1	1.2
PCV (%)	26.00±2.83^{c}	24.00±0.41^{bc}	22.00±1.41^{abc}	19.00±2.83^{ab}	16.00±1.41^{a}	17.00±4.24^{a}
RBC(mm)	2.00±0.00^{ab}	2.20±0.14^{b}	2.85±0.07^{c}	2.70±0.14^{c}	2.11±1.01^{b}	1.80±0.14^{a}
WBC(mm)	29.50±6.36^{a}	41.50±3.53^{ab}	56.50±9.19^{ab}	59.50±12.02^{ab}	64.50±12.02^{c}	73.50±49.50^{c}
HB(g 100 ml^{-1})	8.70±0.14^{c}	8.15±0.35^{b}	7.55±0. .21^{b}	7.20±0.28^{b}	5.90±0.42^{a}	6.10±0.14^{a}
MCH(pg cell^{-1})	32.41±5.43^{c}	32.83±3.94^{bc}	34.14±1.55^{a}	38.61±6.12^{a}	35.55±5.35^{a}	37.39±9.74^{b}
MCHC(g 100 ml)	41.75±2.47^{a}	35.80±4.53^{a}	26.30±0.14^{a}	26.80±1.13^{a}	26.85±1.63^{a}	34.25±3.04^{a}
MCV(µm-1)	130.00±14.14^{c}	109.05±0.64^{bc}	77.15±3.04^{a}	70.70±14.14^{a}	76.15±6.72^{a}	73.75±16.19^{a}

Values on the same row with different superscript letters are significantly different (P<0.05).

slight loss of reactivity to external stimuli at lower concentrations toincreased opercula rate; erratic swimming; partial and total loss of equilibrium and loss of reflexes in fish exposed to higher concentrations of clove oil. Results from this experiment agreed with previous works, which reported that *H. bidorsalis* juveniles exposed to toxicants or anaesthesia usually exhibits changes in opercula rate, erratic movement and different behavioral activities (Olufayo and David, 2009; Akinbulumo, 2004). The observation of fish responses in this experiment also agreed with Pascuel el al. (1994), which says fish settled at the bottom of experimental tanks indicates stress or weakness.

Conclusion

Clove oil is effective, relatively safe and economically sustainable anaesthetic agent. It is environmental friendly at low concentration and less persistence in aquatic environment. The results of this study provide baseline information on the responses of *H. bidorsalis* to various concentrations of clove oil and show that active anaesthetic components of clove oil could be used as potential anaesthesia at concentrations 0.8 to 1.0 pmm when associated with a reasonable recovery time and mortality. It does not cause irreversible damage in *H. bidorsalis*, therefore, the use of clove oil in fisheries should be encouraged in preference to synthetic anaesthetic such as MS22 when searching for medicinal plants with anasthetic active compound.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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