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Eugenia cayrophyllata Oil as Anesthetic in Cultured African Catfish, (*Clarias gariepinus*, Burchell 1822) Juveniles

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ABSTRACT
The study investigated the use of clove oil as anaesthetic in *Clarias gariepinus*. One hundred and eight juvenile fish (40.6±1.52 to 50.0±3.20 g body weight and 10.0±1.35 to 18.67±2.81 cm total length) were treated with **(**0, 65, 75, 85, 95, and 105 mg/L) of clove bud extract per litre of water in plastic tanks in triplicates. Blood samples were taken for haematology, plasma biochemistry, and serology parameters. The results show that stage 3 induction and recovery were achieved in fish treated with 95mg/l and 105mg/l of *E. caryophyllata* per litre of water. There was a strong relationship between induction/recovery periods and concentration relationship; y=57. 91-27.45 induction time (x), R^2 = 0.907/concentration of oil (y) =50. 08-29.41 recovery time (x), R^2 = 0.921). Fish treated higher concentrations (85 to 105 mg/l) had higher haematology and blood enzyme parameters, but similar to lower plasma biochemistry than the control. Fish treated with 95 and 105 had PVC (27.00±1.00 and 32.33±1.15%), Hb (8.73±0.31 and 11.07±0.75dl), RBC (3.25±0.50 and 3.45±0.04 $\times 10^6$/µl), WBC (18.72±0.44 and 18.77±0.18$\times 10^6$/µl), AST (194.17±10.11 and 197.33±7.37 µl), ALP (232.17±63.64 and 270.00±66.30 µl), urea (8.30±0.72 and 7.13±0.23µl), creatinine (0.72±0.19 and 0.83±0.15mg/dl), glucose (277.50±90.36 and 300.67±30.62mg/dl) and cholesterol (243.83±92.57 and 279.33±41.02mg/dl) higher than the control PVC (24.78±4.31%), Hb (7.96±1.36 cl), RBC (1.72±0.87 $\times 10^6$/µl), WBC (17.10±1.15 $\times 10^6$/µl), AST (181.06±41.75µl), ALP (187.83±51.22µl), urea (6.86±0.53µl), creatinine (0.68±0.15mg/dl), glucose (232.44±104.23mg/dl) and cholesterol (184.33±92.57mg/dl) respectively. There were statistical differences ($p<0.05$) in the haematology, plasma biochemistry and blood enzymes of fish. *E. caryophyllata* oil is a suitable, cheaper, and a naturally-derived anaesthetic material for *the C. gariepinus* juveniles.

Key words: *E. caryophyllata*, anaesthetic, haematology, plasma biochemistry, serology cultured *C. gariepinus*

INTRODUCTION

Aquaculture is a profit-oriented venture. Apart from inputting and quality, quantity of fish determines profitability. Stress is a factor that impedes growth performance, inhibit optimal physiological function and sound health of the fish. However, fish is subjected to unavoidable stress in today aquaculture practices such as handling, transportation, weighing, blood, egg collection, stripping, milt collection and other management practices. These affect biological, haematological and serological profiles of the fish (Ellis *et al*., 2012). When fish are stressed, the nervous tension, hassle and anxiety may cause loss of mobility, loss of balancing, changes in hematological values, off-feed, loss weight, loss of reflexes, increase/decrease in respiratory activities, increased disease susceptibility and mortality (Kreiberg, 2000). Chemical such as tricaine methanesulphonate, TMS (MS-222), Benzocaine, Lidocaine, etc has been used in aquaculture practices. Some of these chemicals had been used in Nigeria to reduce stress through an aesthesis in aquaculture during transportation. The health status of animal, including fish is important because poor health condition may directly or indirectly affect the consumer.

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The growing concern about safety of foods has led to the development of natural compounds with no residual effect in animal tissues (Nevas *et al*., 2004). Oil from aromatic plant contains phenolic compounds has been reported to have anti-stress ability (Barata, 2016) and because it is generally recognized as safe (GRAS) thereby promoting its usage in the food industry (Ponce *et al*., 2003; Bayoub *et al*., 2010).

Clove (*Eugenia caryophyllata*) is a member of the Myrtaceae family. It is a perennial tree found ecologically in the tropics and well distributed in the north-central region of Nigeria (Omotayo, *et al.*, 2013). Oil from its buds contains 70 – 90% eugenol, 4-21% beta-caryophyllene, 1-21% eugenyl acetate and 10 – 19% tannin (Chaieb *et al*., 2007; Adeshina, 2015). Iwama *et al*. (1989) reported three anaesthesia and recovery stages. Iwama indicated that the time in which fish is exposed to anaesthetic until the fish shows total loss of equilibrium as stage 1 (SA1). Stage 2 is when the fish shows loss of body movement (SA2) and when opercula movement stops or ceases or hardly noticeable is stage 3 (SA3). Recovery time is also in three stages as described by Iwama *et al*. (1989), when anaesthetized fish regain opercula movement as stage 1 (SR1), gross body movement as stage 2 (SR2) and regain of equilibrium as stage 3 (SR3) after anaesthetized fish is transferred into recovery tanks (Barata *et al*., 2016). Ross and Ross, (2008) defined effective concentration of anaesthesia as the concentration that causes fish to lose opercula movement within three minutes.

Despite the huge activities on *Clarias gariepinus* the information on the use of naturally derived anaesthetics to manage both intentional and unintentional stress across the *C. gariepinus* production chain is scare, hence the need for this study. This study sheds more light on stress management in fish farming using plant extract as an aesthetics in Nigeria. The objectives of this study are to investigate the efficacy and dosage of clove oil as an aesthetic in *C. gariepinus.*

MATERIALS AND METHODS

Study area

The study was conducted in the research laboratory of the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria located on Latitude 10°23'0"N and Longitude 12°5'0"E. Oyo state has relatively two seasons: dry season starts around November to March while rainy season starts March to October (OYSG, 2015) with annual rainfall ranges 1500 to 1600 mm in 2015 (Lade and Oloke, 2015).

Plant collection and identification

The buds of *E. caryophyllata* were purchased from Agbowo Market, Ibadan and identified at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan where a voucher specimen was deposited under FHI 125057.

Extract and dosage preparation

Five grams (5g) of *E. caryophyllata* was weighed on a digital Scout Pro scale (Model: M1207) and transferred into Soxhlet apparatus. The Hema*et al.* (2010) and Bayoub *et al.* (2012) extraction procedures were adopted. One hundred and seventy (170) ml of 95% ethanol was poured into a spiral tube of the equipment. The extraction last for six hours and repeated until the required volume was achieved. The filtrate was concentrated on a rotary evaporator at 45°C for chemicals elimination, stripped into sterile bottles to prevent contamination and stored in a refrigerator until use. The mean yield of the extract was 0.94 g per 5 g of *E. caryophyllata* buds.

Experimental fish

Clarias gareipinus juveniles of body weight (40.6±1.52 to 50.0±3.20 g) and total length ranges between 10.0±1.35 to 18.67±2.81 cm were purchased from the Department of Aquaculture and Fisheries

Management Fish Farm, University of Ibadan. The fish were transported to the Departmental Research Laboratory immediately in a plastic container half-filled with water from the rearing tanks and conditioned for seven (7) days in a rectangular plastic tank (50x34x27cm) and fed twice a day (5% of body weight) with 40% crude protein commercial floating pelleted (Durantee) feed at 08:00 am and 06:00 pm.

Experimental design

Six (6) different concentrations (0, 65, 75, 85, 95, and 105 mg/L) were prepared using 1:9 of clove oil and ethanol as described by Barata (2016). Each clove oil concentration was placed in a transparent rectangular tank (induction tank) in replicates. One hundred and eighty (180) *C. gariepinus* juveniles were allocated into the transparent rectangular plastics (50 x 34 x 27cm) containing 35 litres of water in a completely randomized designed. Each tank was allotted ten juvenile fish and observed for twenty minutes. The fish were later transferred into recovery tanks. The observations were recorded. Water in each tank was replaced every three (3) day, sourced from a borehole.

Monitoring of water quality parameters

Water parameters were monitored twice daily at 08:00 am and 6:00 pm (just before feeding). Dissolved oxygen were measured using digital D.O. meter [LABTECH (R)] Model AVI-660 (Power: 220V, AC: 50 Hz: Sr./No. 376), pH was measured with the aid of a digital pH meter [LABTECH (R)] Model Photoic 20 (Power: 230V AC: 50 Hz: Sr./No. 1223) and temperature was measured with the aid of mercury-in-glass thermometer. The D.O., pH and temperature recorded ranges between 4.57±0.73 to 6.558±0.38 mg/l, 7.21±0.31 to 7.82±0.12 and 25.33±1.07 to 26.82±1.19 ^{0}C respectively throughout the experimental period.

Blood collection

Fish were then placed on a clean board with the belly facing upward. The puncture was carried out at about 3 – 4cm away to the genital opening using a 5 ml sterile syringe and was dry using tissue paper. The needle was inserted gently perpendicularly in the vertebral column of the fish and a blood was drawn immediately it touches caudal blood vessel. Blood was taken under gentle aspiration and withdrawn. The blood was gently transferred into tube containing lithium heparin at about 25^{0}C and transferred to the Clinical Pathology Laboratory of the Department of Veterinary Pathology, University of Ibadan for haematological analysis (the blood stain method were adopted for differential blood cell count), plasma biochemistry and serological analysis. The slides were placed in a staining jar which has May-Grunwald's stain in it. It was diluted with buffered water of an equal volume for about 5 minutes. The slides were transferred into a second staining jar containing fresh Giemsa's stain (diluted with nine volumes of buffered water) for 15 minutes. The slides were transferred into another stain jar containing buffered water and rapidly washed in three changes of water and finally stand in buffered water for 5 minutes for differentiation, air dried and mount on light microscope. The following Haematological parameters for each treatment were recorded: Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood cells (RBC), White Blood Cells (WBC), Platelets, Lymphocytes, Neutrocytes, Monocytes, Eosinophil, Basophil. Others were estimated using the following formulae: Mean Cell Haemoglobin (MCH in pg) = Hb ÷ RBC x 100, Mean Cell Haemoglobin Concentration (MCHC) = HB ÷ PCV x 100, Mean Cell Volume (MCV in FL) = PCV ÷ RBC x 100. Plasma biochemistry (Total protein (TP), Albumin (ALB), Globulin), Blood enzyme analysis (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Urea, Creatinine, Glucose, and Cholesterol) using randox kits.

Statistical analysis

The data obtained from the experiment were subjected to one-way analysis of variance (ANOVA). Variation between the means were determined using Duncan's multiple range test (DMRT) ($p = 0.05$) with the aid of SPSS statistical package version 20.

RESULTS

Table 1 shows the anaesthetic effect of *E. caryophyllata* buds extract of *C. gariepinus* juveniles. Changes in responses of fish to increase in concentration of *E. caryophyllata* buds extract were observed. Numbers of fish anaesthetized were higher (8.67±1.53 and 9.67±0.58) in fish treated with higher dosages (95 and 105 mg/l respectively) of *E. caryophyllata* bud extract while no induction (0.00±0.00) was observed in fish treated with 65 mg/l. Significant variation (P<0.05) was observed in fish treated with 95 and 105 mg/l of *E. caryophyllata* buds extract. More so, time of induction were faster (4.99±0.42, 3.35±0.31 and 2.89±0.27 mins) in fish treated with higher dosages (85, 95 and 105 mg/l respectively) of *E. caryophyllata* bud extract than (10.03±0.39 and 7.58±0.23 mins) as observed in fish treated with lower dosages (65 and 75 mg/l) of *E. caryophyllata* buds extract. However, there is significant variation (p<0.05) in fish treated with 65 mg/l compared with other dosages. During the induction period fish anaesthetized with 65mg/l, 75mg/l, and 85mg/l concentration of *C. caryophyllata* did not show stage 3 of anaesthetization within three minutes. There were loss of equilibrium and body movements but the opercula were actively moving at these concentrations. However, opercula movement ceased at 95mg/l within 3 to 4 minutes. The induction time was reduced with an increase in the concentration of the clove oil concentration.

More so, the number of fish recovered were higher (10.00±0.00, 10.00±0.00, 10.00±0.00, and 9.67±0.58) in fish treated with a lower concentration (65, 75, 85 and 95 mg/l, respectively) than fish (5.67±1.53) treated with higher dosage (105 mg/l). Significant variation (p<0.05) was observed in fish treated with 105 mg/l (Table 1). Responses at recovery time show that there were earlier (1.30±0.26 and 3.02±0.53 mins.) recovery period in fish treated with 65 and 75 mg/l, respectively but recovery period were delayed (7.73±0.43 mins.) in fish treated with 105 mg/l. There was a significant difference ($p < 0.05$) between the recovery period of fish treated with 105 mg/l compared to other treatments of the experiment.

Table 1: Mean (±SD) induction and recovery at stage 3 of *C. gariepinus* juveniles treated with *E. caryophyllata* oil

Activities	***E. caryophyllata* oil inclusion levels (mg/l)**						**F-cal** ($\alpha_{0.05}$)	**F-tab** ($\alpha_{0.05}$)
	0 (control)	**65**	**75**	**85**	**95**	**105**		
NFI	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.33 ± 1.53^{a}	4.00 ± 1.00^{b}	8.67 ± 1.53^{c}	9.67 ± 0.58^{c}	46.44	3.36
NFR	0.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	9.67 ± 0.58^{a}	5.67 ± 1.53^{b}	20.44	3.36
IT (mins)	0.00 ± 0.00^{a}	10.03 ± 0.39^{e}	7.58 ± 0.23^{d}	4.99 ± 0.42^{c}	3.35 ± 0.31^{b}	2.89 ± 0.27^{b}	420.62	3.36
RT (mins)	0.00 ± 0.00^{a}	1.30 ± 0.26^{b}	3.02 ± 0.53^{c}	3.61 ± 1.00^{c}	5.50 ± 1.18^{d}	7.73 ± 0.43^{e}	61.89	3.36

Mean within the same row having different superscripts letters are significantly different ($P < 0.05$).
NFI = Number of fish anaesthetized; NFR = Number of fish recovered; IT =Induction time; RT = Recovery time.

Figure 1 shows the induction relationship and the concentration of oil (y =57. 91- 27.45x, $R^2 = 0.907$). Where, y = concentration of oil and x = induction time. For any unit increased concentration of oil, there will be (-27.45) decrease in time of induction.

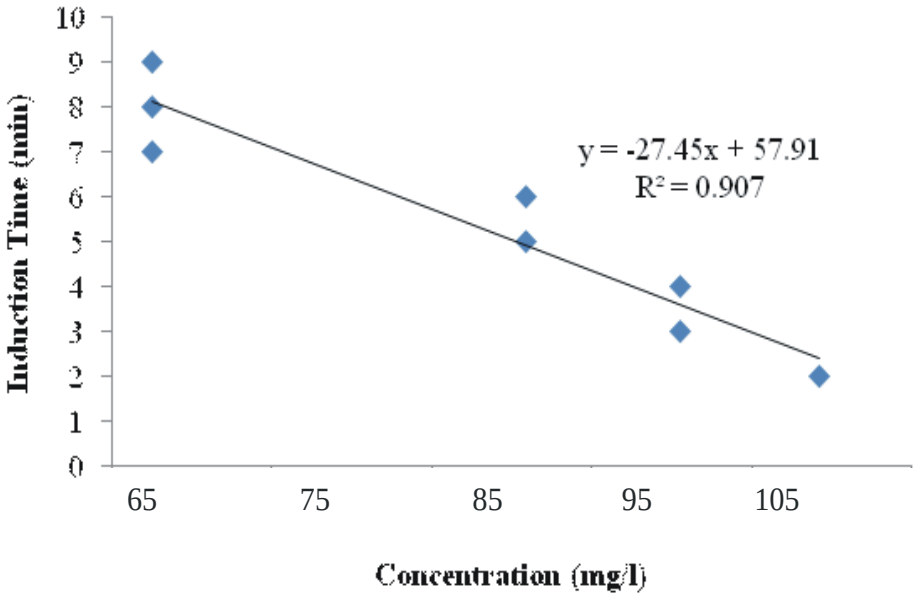

Fig. 1: Relationship between induction time and concentration of *E. caryophyllata* oil

Figure 2 shows the recovery time relationship and the concentration of *E. caryophyllata* oil (y = 50.08-29.41x, R^2 = 0.921). Where, y = concentration of oil and x = recovery time. For any unit increased concentration of oil, there will be (-29.41) decrease in recovery time of induction.

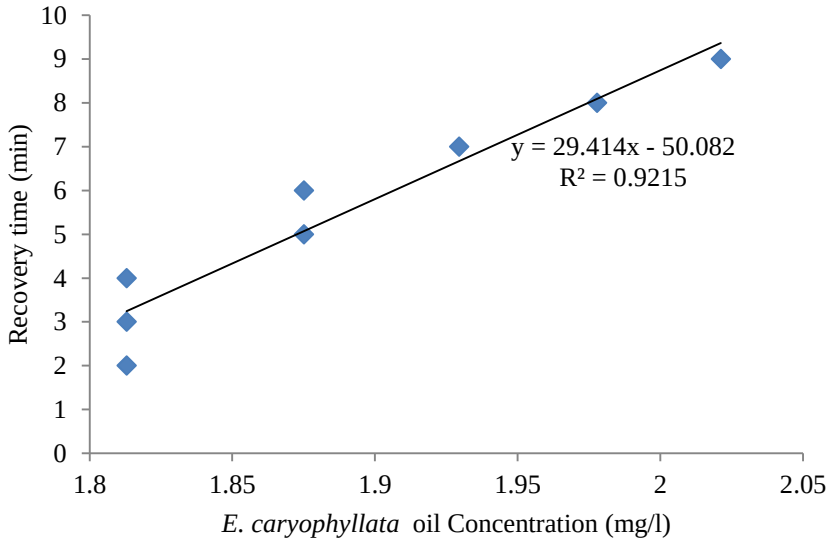

Fig. 2: Relationship between recovery time and concentration of *E. caryophyllata* oil

Table 2 shows the haematological parameters of *C. gariepinus* treated with *E. caryophyllata* buds extract at different levels (0, 65, 75, 85, 95, and 105 mg/l). There was a significant difference in the haematological parameters of the fish (P<0.05). Fish treated 75, 85, 95 and 105 mg/l of *E. caryophyllata* buds extract had significantly higher PCV (26.00±1.00, 27.00±1.00, 27.00±1.00 and 32.33±1.15 % respectively), Hb (8.93±0.40, 9.07±0.67, 8.73±0.31 and 11.07±0.75 dl respectively) RBC (.93±0.40, 2.98±0.69, 3.25±0.50

and 3.45±0.04 $\times 10^6/\mu l$, respectively) and Platelet (173.67±1.53, 187.67±10.26, 224.00±3.61 and 188.00±2.00 $10^3/\mu l$ respectively) than the control (24.78±4.31%, 7.96±1.36 dl, 1.72±0.87x $10^6/\mu l$ and 160.56±37.28 x $10^3/\mu l$ respectively) and 65 mg/l treatment (23.00±1.00%, 7.40±0.35 dl, 1.21±0.10x $10^6/\mu l$ and 126.33±2.52 x $10^3/\mu l$ respectively), while WBC was statistically significantly higher in fish treated with 85, 95 and 105mg/l (18.50±0.87, 18.72±0.44, and 18.77±0.18 x $10^6/\mu l$) than the fish in the control treatment (17.10±1.15 x $10^6/\mu l$). More so, basophils, eosinophils ad monocytes were not significantly ($p>0.05$) in all treatment compared with the control.

Table 2: Mean (±SD) haematological parameters of *C. gariepinus* juveniles treated with *E. caryophyllata* buds extract

Parameters	***E. caryophyllata* oil extract inclusion levels (mg/l)**					
	0 (Control)	**65**	**75**	**85**	**95**	**105**
PCV (%)	24.78±4.31^a	23.00±1.00^a	26.00±1.00^b	27.00±1.00^b	27.00±1.00^b	32.33±1.15^c
Hb (dl)	7.96±1.36^b	7.40±0.35^a	8.93±0.40^{ab}	9.07±0.67^{ab}	8.73±0.31^b	11.07±0.75^d
RBC($\times 10^6/\mu l$)	1.72±0.87^b	1.21±0.10^a	3.65±0.03^c	2.98±0.69^{bc}	3.25±0.50^b	3.45±0.04^c
WBC($\times 10^6/\mu l$)	17.10±1.15^c	16.10±0.15^b	13.92±0.20^a	18.50±0.87^d	18.72±0.44^d	18.77±0.18^d
Platelet ($10^3/\mu l$)	160.56±37.28^b	126.33±2.52^a	173.67±1.53^c	187.67±10.26^c	224.00±3.61^d	188.00±2.00^a
Lymph (%)	63.39±5.71^a	68.00±2.00^c	65.33±2.52^b	66.33±1.15^c	71.33±1.53^a	60.00±1.00^a
Neutro(%)	30.11±5.76^b	25.67±3.21^a	29.33±2.89^b	26.67±1.16^b	22.33±1.53^a	43.00±2.00^c
Mono (%)	3.00±0.97^b	2.67±0.58^a	2.00±1.00^a	2.67±0.58^a	3.33±0.58^b	3.33±0.58^b
Eos (%)	3.06±1.26^a	3.33±2.08^a	3.00±0.00^a	4.33±0.58^b	3.00±0.00^a	2.67±1.53^a
Bg (%)	0.33±0.49^a	0.33±0.58^a	0.33±0.58^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
MCV(FI)	163.40±44.23^b	191.13±11.19^b	71.19±3.33^a	93.90±21.50^a	84.20±10.44^a	93.75±4.26^a
MCH (Pg)	52.77±14.95^d	61.45±2.32^d	24.46±1.31^a	31.31±5.72^a	27.24±3.62^b	32.10±2.48^c
MCHC (%)	32.15±1.13^a	32.18±0.77^a	34.36±0.78^b	33.55±1.48^b	32.36±0.95^a	34.20±1.13^b
Neutro:Lymph	0.49±0.14^b	0.38±0.06^a	0.45±0.06^b	0.40±0.02^a	0.31±0.03^a	0.57±0.05^b

Mean within the same row having different superscripts letters are significantly different ($P < 0.05$).

Key: PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; Lymph = Lymphocytes; Neutro = Neutrophil; Mono = Monocytes; Euos = Eosinophil; Bg = Basophil; MCV = Mean Celll Volume; MCH = Mean Cell Haemoglobin; MCHC = Mean Cell Haemoglobin Concentration; Neutro:Lymph = Neutrophil/Lymphocytes ratio

Table 3 shows the plasma biochemistry of the fish treated with *E. caryophyllata* buds extract. Total protein in fish treated with 65mg/l (4.25±0.72 g/dl) was significantly different ($p<0.05$) from the other treatments however, other concentrations including control had higher values. There was a reduction in the levels of albumin and globulin of fish treated with *E. caryophyllata* bud extract. The values were lower than those obtained in fish in the control group except albumin of fish treated with 95mg/l (1.53±0.06 g/dl) and globulin of fish treated with 105mg/l (4.67±0.06g/dl). However, A-G ratios were higher in fish treated with 65mg/l (0.50±0.01) while the leas were observed in fish treated with 105mg/dl (0.17±0.06).

Table 3: Mean (±SD) plasma Biochemistry of *C. gariepinus* juveniles treated with *E. Caryophyllata* buds extract

Parameters	***E. caryophyllata* extract inclusion levels (mg/l)**					
	0 (Control)	**65**	**75**	**85**	**95**	**105**
Total protein (g/dl)	5.79±0.81^{b}	4.25±0.72^{a}	5.37±0.15^{b}	5.4±0.20^{b}	5.47±0.12^{b}	5.60±0.27^{b}
Albumin (g/dl)	1.32±0.68^{b}	0.95±0.14^{a}	1.27±0.50^{b}	0.90±0.10^{a}	1.53±0.06^{b}	1.03±0.15^{a}
Globulin (g/dl)	4.46±0.40^{b}	3.30±0.40^{a}	4.10±0.46^{b}	4.43±0.21^{ab}	3.93±0.15^{a}	4.67±0.06^{c}
A-G. ratio	0.27±0.16^{a}	0.50±0.01^{b}	0.27±0.15^{a}	0.17±0.06^{a}	0.33±0.06^{a}	0.17±0.06^{a}

Mean within the same row having different superscripts letters are significantly different (P < 0.05).

Table 4 shows the blood enzymes of *C. gariepinus* juveniles treated with *E. caryophyllata* buds extract. Values AST were higher in all concentration than the control (181.06±41.75 µl) with 105mg/l concentration taking the lead (197.33±7.37 µl) showing significant difference (p<0.05). Highest urea (8.30±0.72 µl), creatinine (0.83±0.15 mg/dl), glucose (306.00±39.23 mg/dl), and cholesterol (279.33±41.02 mg/dl) was observed in fish treated with 95mg/dl, 105 mg/l, 85 mg/dl and 105mg/dl respectively.

Table 4: Mean (±SD) blood enzymes parameters *C. gariepinus* juvenile treated with *E. caryophyllata* buds extract

Parameters	***E. caryophyllata* extract inclusion levels (mg/l)**					
	0 (Control)	**65**	**75**	**85**	**95**	**105**
AST (µl)	181.06±41.75^{a}	193.33±3.51^{b}	176.00±2.65^{a}	193.00±5.57^{b}	194.17±10.11^{b}	197.33±7.37^{c}
ALT (µl)	37.11±14.72^{ab}	20.00±1.00^{a}	19.33±0.58^{a}	29.67±24.83^{b}	30.33±16.75^{b}	24.00±7.81^{a}
ALP (µl)	187.83±51.22^{a}	198.00±2.65^{b}	192.00±9.54^{a}	310.33±61.23^{d}	232.17±63.64^{c}	270.00±66.30^{c}
Urea (µl)	6.86±0.53^{b}	6.20±0.20^{a}	6.20±0.36^{a}	7.10±0.61^{b}	8.30±0.72^{c}	7.13±0.23^{b}
Creat. (mg/dl)	0.68±0.15^{b}	0.47±0.06^{a}	0.57±0.57^{a}	0.70±0.10^{a}	0.72±0.19^{a}	0.83±0.15^{a}
Glucose (mg/dl)	232.44±104.23^{b}	142.00±1.00^{a}	263.00±45.04^{ab}	306.00±39.23^{c}	277.50±90.36^{ab}	300.67±30.62^{c}
Cholesterol (mg/dl)	184.33±92.57^{a}	207.00±6.08^{b}	192.00±7.00^{c}	290.67±60.47^{a}	243.83±92.57^{b}	279.33±41.02^{c}

Mean within the same row having different superscripts letters are significantly different (P < 0.05).
Key: AST = Aspertate aminotransferase, ALT = Alamine aminotransferase, ALP = Alkaline Phosphatase, Creat. = Creatinine

DISCUSSION

Decrease in induction time was observed in this study as the concentration of *E. caryophyllata* bud extract increases. This pattern reflects a direct proportional functionality between the clove extract and the induction time. Meanwhile, there was increase in recovery time to increase in extract inclusion concentrations which reflects an inverse proportional trend between recovery time and clove inclusion levels. The findings of this study in terms of trend of concentration and induction time are in agreement with the works of Akinrotimi *et al.* (2013), Diyaware *et al.* (2015) and Barata *et al.* (2016). The induction times of 3.35 to 4.99 minutes observed at 95 mg/l and 85 mg/l which is in agreement with Barata *et al.* (2016) who reported 3minutes cessation of opercula movement in *Arygyosomus regius* and fall within acceptable time of induction as opined by Marking and Meyer (1985) and Iwama *et al*. (1989) as the third stage of anaesthetization is the cessation of the operculum.

Furthermore, 85 and 95 mg/l inclusion concentration induced the fish within the recommended period observed in this study were higher than 40 mg/l reported by Weber *et al.* (2011) in *Solea senegalensis*, 10 mg/l reported by Akinrotimi *et al.* (2016). However, the finding shared similar view with Weber *et al.*

(2011) who reported 85 mg/l in *Arygyosomus regius*. The differences in the effective concentration could be attributed to variation in fish species.

Recovery period shows that fish with lower concentration of the oil recover quickly than the higher concentration. This further suggests that, holding other factors constant (water temperature, salinity, size of fish) the recovery time is longer in fish with higher concentrations. The recovery time of fish induced with 95mg/l and 105mg/l was highest but close to each other thus called for attention. This trend was similar to the result reported by Akinrotimi *et al.* (2013), Diyaware *et al.* (2015) and Barata *et al.* (2016) and values are in agreement with the work of Weber *et al*. (2009). The high values of R^2 of induction and recovery (0.907 and 0.921) study show that the oil works effectively and can be relied upon.

The result of haematology indices, plasma biochemistry and blood enzymes shows that the oil used for inducement did not negatively affect the blood profile of the fish and thus could be used in aquaculture practices including research. The higher albumin and AG ratio suggested that the fish blood was in good condition. The results of haematology, plasma biochemistry and blood enzyme of anaesthetized fish are in agreement with the work of Osuigwe *et al.* (2005), Omitoyin (2006), Bello *et al*. (2012), Onyia *et al.* (2015), Okorie-Kanu and Unakalamba (2015) and were within the recommended values for *C. gariepinus*. Test for significance on induction and recovery time shows that F-calculated are 46.44 and 20.44 for induction and recovery higher than F-tabulated of 3.48 thus the null hypothesis is rejected. This result further revealed that *E. caryophyllata* oil has a significant effect on induction and recovery time. Aquaculture and research activities such as surgical operation, milt collection, morphometric and meristic studies, tagging, blood sampling, transport of juvenile *C. gariepinus* could be conducted using *E. caryophyllata* oil as an anaesthesia to avoid stress.

E. caryophyllata oil is an effective anaesthetic material. The clove oil concentrations of 85-95 mg/l could be used to anaesthetize *C. gariepinus* juveniles without altering blood indices of the fish. Therefore, uses of naturally produce anaesthetics will reduce the residual effect of continued use of chemicals in aquaculture.

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