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Research Article

Chemical Profile and Surgical Anaesthesia Dosage of Dried Clove Bud in Brooders of African Catfish (*Clarias gariepinus*, Burchell 1822)

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ABSTRACT

The need to characterise optimum dosage of farmer preferred cheap and locally available dried clove bud (DCB), liable to induce anaesthesia to surgical stage in *Clarias gariepinus* brood-stocks necessitated this study. DCB was characterised for content of essential oil (CEO) and its chemical composition through hydro-distillation and gas chromatography (GC-MS) respectively. Ten random samples (5male and 5female) of C. gariepinus brooders(mean weight, 1.63±0.33kg) were immersed in 0.00 g/l (T1), 0.1g/l (T2), 0.2g/l (T3), and 0.3g/l (T4) concentrations of aqueous DCB, observed for time (minutes) to reach anaesthesia induction-IT, sedation-SeT, surgery-ST; and recovery-RT; then, survival (%) at 3weeks post-experimentation period. Data were analysed for differences and regression at $P<0.05$. DCB yielded 1.60% CEO; composed 52 chemicals containing 91.76% eugenol derivatives. Significantly, IT and SeT varied from 2.63±0.21min (T4) to 4.23±0.27mins (T2) and 5.73±0.22mins (T4) to 8.74±0.50mins (T2); ST occurred and was similar across T3 (13.95±1.69mins) and T4 (14.15±1.65mins); RT varied at 1.49±0.13mins post-SeT (T2) to 3.78±0.36 mins post-ST (T4), while survival was 100.00% in T1 - T4. The IT, SeT, ST, RT, and survival were similar across sexes in T2 - T4. Significantly, DCB regressed at concentration = 0.016 + 0.936 (RT) – 0.074 (SeT). DCB has low CEO but contain chemicals liable to induce surgical anaesthesia at 0.3g/l (T4) for about 4mins, without causing mortality in *C. gariepinus* brooders. Meanwhile, increased concentration would further delay recovery time.

Keywords: *Essential Oil, Clove Bud Extract, Fish Anaesthesia, Clarias gariepinus, Fish surgery/ablation*

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INTRODUCTION

Anaesthesia is any chemical tool employed in inducing sleep. Chemical compounds such as MS 222 have been widely utilised as anaesthesia in fish, but this is relatively expensive and not readily available to rural farmers, especially in Africa. Widayat *et al*., (2014) observed that there is the need for a more cost-effective anaesthetic for use in fisheries. The increasing concern about food safety of the recipient fish when synthetic chemicals are utilised for fish culture has led to the development of natural compounds for use (Nevas *et al*., 2004).

Eugenia caryophylata commonly known as "clove" is a member of the Myrtaceae Family, a perennial tree found in the tropics and well distributed in the north central region of Nigeria (Omotayo *et al*., 2013). Extract of the plant had been utilised to reduce stress in reared meagre in Portugal (Barata *et al*., 2016). Parts of clove plants are usually fried in broken pots to induce sleep in human patients in traditional African medicine, while its flower and buds are used as additives for local beverages that induce sleep. Clove plant materials has potentials as antibiotic, antiseptic, antimicotic and antibacterial agents, and clove oil has been observed to have anaesthesia properties useful for fish production (Adeshina *et al*., 2016). Diyaware *et al*., (2015) reported that clove seeds extract are suitable anaesthetic agent for handling, restraint and immobilisation in fish transportation. There is a growing interest to explore the anaesthesia potentials of clove plant materials in farmed fish production, especially in Africa.

The active compound in clove plant materials, liable to induce anaesthesia is contained mainly in its essential oil. Hence, the use of clove materials as anaesthesia has concentrated on its oil extract. Meanwhile, the technology of oil extraction seem cumbersome to some end users such as fish farmers. Interestingly, wet clove bud could be dried for preservation, powdered (for easy dissolution) and subsequently soaked in water for use. This approach would be more useful for farmers who may not have access to the technology of oil extraction, but have access to the clove plant. Moreso, dried clove bud powder are more readily available compared to the clove oil extract at local retail markets in Africa, especially in Nigeria. However, there is the need to characterise the essential oil yield and its content of active anaesthesia compounds in typical dried clove bud. Such action would bridge the existing knowledge gap needed in a more precise use of the dried clove bud as anaesthesia in fish culture, especially in rural Africa and other developing countries.

Fish farmers and breeders would prefer the use of aqeous concentrations of dried clove bud against the popular synthetic MS 222 chemicals based on the afore-mentioned reasons. However, information on dosage for achieving anaesthesia stage would be needed. Anaesthesia concentrations could produce induction, sedation and surgical stages in anaesthetised specimens (Iwama *et al*., 1989). Hence, anaesthesia could be induced at sedation up to surgical level and recovery may not be immediate. Meanwhile, extensive recovery time would be necessary to allow ample time in scenarios of surgery activities. Knowledge on dimensions of surgical anaesthesia is much more important in fish species such as *Clarias gariepinus* who need restriction of female brooders in egg spawning and minor surgery in milt collection through ablation.

The African catfish, *Clarias gariepinus* is the second most cultured fish species in Africa. It is the most widely accepted and highly valued fish, cultivated in Nigeria (Adewumi and Olaleye, 2011). Its production is increasing, its culture is spread globally and it has been the highest finfish produced in either fresh or brackish water aquaculture in Malaysia (Dauda *et al*., 2018). Natural spawning in this species has complications; hence artificial spawning is mostly practised by fish breeders and farmers for its seed production. The induced stress on male and female brooders of the species during spawning process constitutes a major challenge on their physiology, which sometimes results in post-spawning mortality. Complication in obtaining semen of male *C. gariepinus* often necessitates sacrificing of male specimens during each spawning exercise.

Attempt to stitch back the dissected portion to sustain the live of the milt-extracted male would require anaesthesia to reduce stress and to restrict the specimens during such surgical operation. Egg stripping, and milt collection among major stress in fish production (Adeshina *et al*., 2016). Use of natural anaesthesia such as the dried clove bud for anaesthesia of the species' brooders for spawning would be helpful for sustenance of its aquaculture. Meanwhile, there is the need to establish the dosage of the dried clove bud, needed to anaesthetise *C. gariepinus* brooders to surgical stage with allowance of ample time before recovery. This action would be of immense help in reducing broodstock mortality associated with seed production in *C. gariepinus*. However, there is an existing knowledge gap in this regard.

It is important to clarify the hypothesis on whether dry clove bud would not contain clove oil and the active eugenol anaesthesia compounds; whether none of the tested concentration of dry clove bud powder will be able to anaesthesise brooders of *C.* gariepinus to surgical anaesthesia stage; and whether anaesthesised brooders would recover and would not die during post anaesthesia period. This curiosity persuaded our aim to assess the potentials of the use of dried clove bud in anaesthesia of *C. gariepinus* brooders to surgical stage with specific objectives of 1) Evaluating the essential oil yield of the dried clove bud, and its chemical constituent, 2) Investigating the concentration of the dried clove bud, liable to induce surgical anaesthesia in brooders of *C. gariepinus* and the corresponding recovery time of the anaesthetised specimens and 3) Assessing the post-anaesthesia survival of the dried clove bud anaesthetised *C. gariepinus* brooders.

MATERIALS AND METHODS

Experimental Site

The experimental site for this study is the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria in West Africa. The experimental site is located at Ibadan City, Oyo State, Nigeria. Ibadan is located in Southwestern region of Nigeria, on coordinates 7.401962 and 3.917313. Aquaculture in Nigeria started from Southwestern Nigeria specifically in Oyo State, and the region is still one of the main hubs of aquaculture in Nigeria. Map of Nigeria showing the location of Ibadan inside Oyo State is presented (Plate 1).

Plate 1:
Map of Nigeria Showing Location of Ibadan in Oyo State
Source: Ojo and Awokola (2012)

Assessment of Dried Clove Bud for Content of Essential Oil and Chemical Composition

Procurement of Dried Clove Bud and Extraction of its Essential Oil: Dried clove bud was procured from commercial herb-seller in Ibadan City. Subsample of the dried clove buds were submitted to the Department of Chemistry, University of Ibadan, Nigeria for clove oil extraction and subsequent

analysis of its chemical compound. Clove oil extraction was carried out using hydrodistillation technique, following Safrudin *et al*., (2015). Clove buds were placed in an all glass Clavenger-type apparatus designed to British Pharmacopeia specifications (Zead *et al*., 2014) and hydro-distilled for 3 hours. Extracted oil were decanted, quantified and refrigerated until analyses.

Determination of Chemical Components of the Extracted Essential Oil of the Dried Clove Bud: The essential oil from the dried clove buds was analyzed for chemical components using the Gas Chromatographic method (GC-MS) through a Shimadzu GCMS-QP2010, Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software. The GC column was a ZB-5 fused silica capillary column with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 µm. The carrier gas was helium with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. Injector temperature was 250°C and the ion source temperature was 200°C. The GC oven temperature program was programmed for 50°C initial temperature, temperature increased at a rate of 2 °C/min to 260°C. A 5% *w*/*v* solution of the sample in CH_2Cl_2 was prepared and 0.1 µl was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with reference NIST library, following Adams (1995).

Assessment of Anaesthesia and Recovery Stages and Survival Rate in *C. gariepinus* Brooders Immersed in Solutions containing Dried Clove Bud

Experimental Fish: Forty brooders (20 males and 20 females) of *Clarias gariepinus* (1.63±0.33kg mean weight) were procured from private fish farms in Ibadan City and preconditioned for two weeks in concrete fish tank in the Department of Aquaculture and Fisheries Management University of Ibadan prior to their utilisation for the experiment. The fish were fed with commercial floating fish feed (Skretting) of 8.0 mm diameter, containing 43% of crude protein, at 1% bodyweight/day during the conditioning periods.

Preparation of Aqueous Solutions of Dried Clove Bud for the Experiment: In order to increase solubility, subsamples of the dried buds were prepared to powder form by simple milling process using a blender. The grinded dried clove bud were prepared into solution to form four treatments containing 0.0 g/litre, 0.1 g/litre, 0.2 g/litre, 0.3 g/litre of the clove bud in water (solutions I, II, III and IV). The clove solutions were produced by mixing the respective quantity of the grinded samples in the specified water volume in a circular plastic bowl. These were allowed to stay for 5minutes, at which total dissolution was observed, and colouration of each of the aqueous medium of the treatments noted. A 1.0 litre of each of the solution was prepared and utilised for each treatment.

Determination of the Time for Anaesthesia and Recovery Stages in Treatments: Randomly selected ten preconditioned experimental fishes (5 males and 5 females) were grouped to four groups and each group immersed into 1.0 litre each of the aqueous solutions I, II, III and IV inside the plastic bowl to represent treatments I, II, III and IV respectively. The immersed brood-fishes were assessed for time to reach each of the stages of anaesthesia and full recovery as described in Iwama *et al*., (1989), presented in Table 1. Specifically, time at which each of the induction, sedation and surgical anaesthesia stages, and full recovery was reached in each treatment was noted. The anaesthetised specimens were removed to clean water after full recovery.

Table 1:
Description of the Assessed anaesthesia and recovery stages

Anaesthesia Stages	Description
I (Induction)	Loss of equilibrium
II (Sedation)	Loss of gross body movements but with continued opercular movements
III (Surgical)	As in Stage II with cessation of opercular movements
Full Recovery	Equilibrium regained and pre-anaesthetic appearance

Source: (Iwama et al., 1989)

Determination of the Survival Rate in Treatments during Post-Anaesthesia Period: Samples in all treatments were assessed for mortality during a three weeks post experimentation period. Feeding conditions followed similar pattern as carried out during conditioning period. However, weekly water exchange was carried out during the 3 weeks period.

Water Quality Parameters of the Clove bud Treatments During 3-week Period: Water quality was monitored for temperature, pH, and dissolved oxygen, following Diyaware *et al*., (2015). Temperature was measured using mercury in glass thermometer, pH through electronic pen type pH meter (Hana Scientific) and dissolved oxygen through water test kit using Winkler's method (AOAC, 2000).

Statistical Analysis
Data were presented using descriptive tools: percentage, mean and standard deviation. Differences across treatments were assessed through one-way ANOVA of the IBM SPSS statistica 21.0 software. Significant differences were taken at $P<0.05$. Multivariate regression analysis was utilised for determination of relatedness of the concentration of the solution with anaesthesia and recovery times in treatments

RESULTS

Yield of Essential Oil and Its Chemical Composition in Dried Clove Bud
Results on oil yield from the dried clove bud are presented in Table 2. A total of 4.22±0.88 g of oil was extracted from 264.61±7.31 g dried clove bud, indicating the oil yield of about 1.60±0.21%. The chemical composition of the essential

oil as revealed by the GC-MS analyses (Table 3) indicated fifty-two identifiable chemical components representing 99.98% total oil content. The most abundant compound was meta-Eugenol, also known as Chavibetol (79.19%), followed by trans-caryophyllene (12.57%).

Table 2
Yield of Essential Oil in the Dried Clove Bud

Parameter	Values
Weight of the CBP used for oil extraction (g)	264.61±7.31
Quantity of extracted clove oil (g)	4.22±0.88
Clove oil yield (%)	1.60±0.21

**Percentage oil yield = weight of oil / weight of the sample x 100*

Table 3: Chemical Profile of the Essential Oil in the Analysed Dried Clove

S/N	Compounds	RT	RI	% Composition
1	Ethyl 2-methylbutyrate	10.779	846	0.03
2	3-methyl-3-Buten-1-ol acetate	12.746	881	0.03
3	3-Hepten-6-ol	13.035	888	0.01
4	2-Heptanone	13.109	889	0.7
5	2-Heptanol	13.821	905	0.17
6	Methyl hexanoate	15.281	933	0.03
7	Alpha-Pinene	15.817	933	0.01
8	Ethyl tiglate	16.247	939	0.04
9	Camphene	16.954	950	0.01
10	Benzaldehyde	18.009	965	0.02
11	Beta-Pinene	18.889	978	0.02
12	6-methyl-5-hepten-2-one	19.491	983	0.06
13	Myrcene	19.712	986	0.01
14	2-Octanone	19.846	989	0.03
15	Mesitylene	20.054	992	0.01
16	Ethyl hexanoate	20.457	998	0.11
17	Para-Cymene	22.296	1026	0.01
18	Limonene	22.598	1030	0.03
19	1,8-Cineole	22.85	1033	0.05
20	(Z)-Beta-Ocimene	23.105	1037	0.01
21	2-Heptyl acetate<2>	23.354	1040	0.59
22	(E)-Beta-Ocimene	23.872	1047	0.15
23	Gamma-Terpinene	24.739	1058	0.01
24	Acetophenone	25.455	1068	0.02
25	3Z-Hexenyl methyl carbonate	26.488	1082	0.02
26	2-Nonanone	27.228	1091	0.34
27	Methyl benzoate	27.562	1096	0.08
28	Linalool	27.876	1100	0.03
29	2-Nonanol	28.033	1102	0.05
30	2-Methyl-6-methylen-octa-1,7-dien-3-one	28.798	1111	0.1
31	Benzyl acetate	32.582	1155	0.1
32	Ethyl benzoate	33.135	1166	0.09
33	Terpinen-4-ol	33.849	1181	0.01
34	Methyl salicylate	34.	1193	0.48
35	Ethyl octanoate	34.95	1196	0.01
36	2-Nonyl acetate	37.547	1231	0.02
37	Chavicol	38.979	1251	0.03
38	2-Undecanone	41.742	1305	0.01
39	Alpha-Cubebene	45.286	1348	0.01
40	Chavibetol	46.909	1371	79.19
41	Alpha-Copaene	47.405	1377	0.1
42	Trans-Caryophyllene	50.388	1419	12.57
43	Alpha-Humulene	52.487	1457	1.44
44	Trans-Cadina-1(6),4-diene	53.431	1473	0.01
45	Gamma-Muurolene	53.616	1477	0.02
46	Germacrene D	54.021	1484	0.04
47	Beta-Selinene	54.537	1492	0.01
48	(E,E)-alpha-Farnesene	55.374	1505	0.08
49	Delta-Amorphene	55.976	1515	0.01
50	Eugenol acetate	56.271	1519	2.82
51	Caryophyllene oxide	60.164	1580	0.14
52	Caryophylla-4(12),8(13)-dien-5-b-ol	63.347	1630	0.01

Anaesthesia and Recovery Stages, and Survival Rate in *C. gariepinus* Brooders Immersed in Solutions containing Dried Clove Bud

Time of Anaesthesia and Recovery Stages, and Post Anaesthesia Survival Rate in Pooled Sexes: As shown (Fig. 1), the grinded clove bud in concentrations I to VI totally dissolved within 5 minutes. The aqueous solution's colour ranged from colourless (treatment I) to deep brown colour (treatment IV

Figure 1
Colour Chart Showing Colour Changes Associated with the Concentrations of Clove Bud Powder in Aqueous Solutions

Table 4 shows the results on the mean time of reaching anaesthesia stages and the survival rates at 3weeks post anaesthesia rearing period in specimens (pooled sexes). The targeted anaesthesia stages (induction, sedation, surgical and anaesthesia recovery stages) were not detected in T1 (control). Induction time varied from 3.03±0.21 mins (T4) to 4.23±0.27 mins (T2) post immersion while sedation time varied from 6.13±0.22 mins (T4) to 9.14±0.50 mins (T2) post immersion. The induction and sedation time were significantly highest in T2 compared to T3 and T4. Surgical stage was not detected in T2. However, fishes in T3 and T4 showed evidence of surgical stage. The surgical stage ranged from 14.35±1.69 mins post immersion in T3 to 14.15±1.65 mins post immersion in T4. These values were statistically similar. Recovery occurred at post-sedation stage in T2, and post-surgical stage in T3 and T4. Final recovery time ranged from 1.49±0.13 min post sedation time in T2 to 4.18±0.36 postsurgical time in T4. The final recovery time was significantly different across T2 to T4. Mortality did not occur in any of the treatment during anaesthesia stages and survival was 100.00±0.00% in T1 to T4 during anaesthesia and the 3 weeks post anaesthesia rearing period.

Time for Anaesthesia and Recovery Stages and Post treatment Survival in Separated Sexes: Table 5 shows that induction, sedation, surgical and recovery stages of anaesthesia were not detected in male and female subgroups of T1 (control). Induction time were statistically similar in sexes of T2 (4.20±0.46 mins in female and 4.26±0.33 mins in male), T3 (3.07±0.57 mins in female and 3.12±0.33 mins in male), and T4 (3.02±0.32 in female and 3.03±0.29 in male). However, induction time in T2 male and T2 female were different from values in corresponding sexes in T3 and T4. Sedation time were statistically similar in sexes of T2 (8.36±0.54 mins in female and 9.52±1.60 mins in male), T3 (5.48±0.98 mins in female and 6.16±0.6 mins in male) and T4 (5.32±0.19 mins in female and 6.14±0.30 in male). However, values in T2 male and T2 female were different from values in corresponding sexes in T3 and T4. Surgical stage was not reached in female and male in T2; but surgical time was 15.10±2.00 mins in female, 16.06±2.43 mins in male in T3 and 12.24±2.18 mins in female, 13.20±2.80 mins in male in T4. The surgical time in male and female sexes in T3 and T4 were not significantly different. However, surgical time in male and female in T3 were significantly different from their counterparts in T4.

Recovery occurred at post-sedation stage in T2, and post-surgical stage in T3 and T4. Recovery time were at 1.36±0.22 mins in male and 1.62±0.53 mins in female in T2, post sedation; 2.42±0.29 mins in male, 3.10±0.25 mins in female, post-surgical stage in T3; and 3.30±0.70 mins in male, 4.26± 0.6 mins in female, post-surgical stage in T4. Recovery time across male and female in T2, T3 and T4 were not significantly different; however, respective sexes in T2, T3 and T4 were significantly different. Survival rate was 100.00±0.00% in all sexes in T1 to T4 during anaesthesia and 3weeks post anaesthesia rearing period.

Relationship between Concentrations and time to Reach Anaesthesia Stages in *C. gariepinus* Brooders: TableS 6a, b and c show the respective prediction model summary, analysis of variance (ANOVA) and coefficients of model predictors for the relationship between concentrations of clove bud and time for reaching anaesthesia and recovery stages in the experimental brood fishes. At R=1.00; R^2=1.00, p=0.00, the model extracted recovery and sedation as significant predictors of concentration of clove bud powder. The coefficient of matrix of these parameters (Table 6c) showed that at Beta constant of 0.016, standardised Beta for sedation time was -0.074 while Beta for recovery time was 0.936. The standardised regression equation showed: Concentration = 0.016 + 0.936 (RT) – 0.074 (SeT), where RT= Recovery time; SeT= Sedation Time.

Table 4:
Mean Time of Reaching Anaesthesia and Recovery Stages and the 3weeks Post Anaesthesia Survival in Pooled sexes of *C. gariepinus* Brooders

Treatment	Induction Time (min)	Sedation time (min)	Surgical Time (min)	Recovery Time (min)	Survival rate (%)
T1	ND	ND	ND	ND	100.00±0.00
T2	4.23±0.27^a	8.74±0.50^a	NR	1.49±0.13^c	100.00±0.00
T3	2.89±0.32^b	5.82±0.56^b	13.95±1.69^a	2.56±0.14^b	100.00±0.00
T4	2.63±0.21^b	5.73±0.22^b	14.15±1.65^a	3.78±0.36^a	100.00±0.00

Mean values with same superscript along same column are not significantly different (p>0.05); ND indicate not detected; NR indicate not reached at 20 minutes post immersion.
T1, T2, T3, T4 represent Clove bud powder (CBP) concentrations at 0.00 g/l, 0.1g/l, 0.2g/l, and 0.3g/l respectively

Table 5:
Mean Time of Reaching Anaesthesia and Recovery Stages and the 3weeks Post Anaesthesia Survival in Separate Sexes of *C. gariepinus* Brooders

	T1		T2		T3		T4	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female
Induction time	ND	ND	4.26±0.33[a]	4.20±0.46[a]	3.12±0.33[b]	2.67±0.57[b]	2.63±0.29[b]	2.62±0.32[b]
Sedation time	ND	ND	9.52±1.60[a]	7.96±0.54[a]	6.16±0.64[b]	5.48±0.98[b]	6.14±0.30[b]	5.32±0.19[b]
Surgical time	ND	ND	NR	NR	16.06±2.43[a]	15.10±2.00[a]	11.84±2.18[b]	13.20±2.80[b]
Recovery time	ND	ND	1.36±0.22[c]	1.62±0.53[c]	2.42±0.29[b]	2.70±0.25[b]	3.30±0.70[a]	4.26±0.63[a]
Survival Rate (%)	100	100	100	100	100	100	100	100

Mean values with same superscript along same column are not significantly different (p>0.05); ND indicate not detected; NR indicate not reached at 20 minutes post immersion.
T1, T2, T3, T4 represent Clove bud powder (CBP) concentrations at 0.00 g/l, 0.1g/l, 0.2g/l, and 0.3g/l respectively

Table 6a.

Model	R	R Square	Adjusted R Square	S.E
1	1.00[b]	1.00	0.00	0.00

b Predictors: (Constant), recovery, sedation

Table 6b.
ANOVA for the Regression of concentration, and time for reaching anaesthesia and recovery Stages in *C. gariepinus* Brooders

Model	Sum of Squares	df	Mean Square	F	Sig.
1. Regression	0.02	2	0.01	0.00	0.00[c]

a Dependent Variable: Conc; c predictors: (constant), recovery, sedation

Table 6c.
Coefficients of Model Predictors for the Regression of concentration, and time for reaching anaesthesia and recovery Stages in *C. gariepinus* Brooders

Model	Unstandardized Coefficient B	S.E	Standardized Coefficient B	t	Sig.
1(Constant)	0.016	0.00		0.00	0.00
RT	0.082	0.00	0.936		
SeT	-0.004	0.00	-0.074		

a Dependent Variable: Concentration of Aqueous Clove Bud Powd
Regression equation: *CONC = 0.016 + 0.082 (RT) – 0.004(SeT), where RT= Recovery time; SeT= Sedation Time*.

Water Quality Parameters of the Clove bud Treatments During 3weeks Period: Result on water quality (Table 7) shows that respective temperature, pH and dissolved oxygen ranged between 27.56±0.28^{0}C (T1) to 28.03±0.16^{0}C (T2), 7.01±0.48 (T3) to 7.42±0.27 (T4), and 4.01±0.81 mg/l (T1) to 4.41±0.30 mg/l (T3) during the experimentation period. These values were not significantly different across treatments.

Table 7:
Mean Experimental Fish Medium's Water Quality during 3Weeks Period

Treatment	Temperature(^{0}C)	pH	DO (mg/l)
T1	27.56±0.28	7.07±0.67	4.01±0.81
T2	28.03±0.16	7.04±0.97	4.10±0.17
T3	27.91±0.34	7.01±0.48	4.41±0.30
T4	27.71±0.17	7.42±0.27	4.12±0.11

**Clove bud powder (CBP) concentrations, 0.00 g/l (T1), 0.1g/l (T2), 0.2g/l (T3), and 0.3g/l (T4); Mean with same superscript along the same column are not significantly different (p>0.05)*

DISCUSSION

Clove flower buds and its essential oils are desirable, being environmentally friendly. Meanwhile, cloves in either powder or essential oil form are very promising for use in agriculture (Ibrahim and Alahmadi, 2015). The essential oil from the clove has been observed to induce anaesthetics in coral reef fish, rabbit fish and rainbow trout aquaculture (Keene *et al*., 1998). Scientific interest in utilising the clove bud powder for local anaesthesia in fish has been demonstrated by Diyaware *et al*., (2015); but the quantity of essential oil yield, its chemical composition and surgical dosage was not established.

Knowledge on quantity of essential oil yield from specific parts of a plant can facilitate estimation of quantity of the active compound in the plant part, that have been utilised to achieve a level of anaesthesia in local or primitive condition. The utilised clove bud powder in the current study had 1.6% essential oil yield indicating that this quantity of oil could have supplied the active chemicals that induced the observed anaesthesia. The oil yield in the current study is within 1.5 - 3% oil yield reported by Chemik (2012) but lower to the reported values in Milind and Deepa, (2011). This divergence could be as a result of processing methods as inferred by Safrudin *et al* (2015). Clove of different regions could reflect differential oil yields (Isa and Nazarifah, 1986), hence, the variation could also be related to divergent place of origin from which the bud was obtained, as supported by Chemik (2012). However, chemical profile of the extracted oil indicated presence of the required active clove chemicals needed for inducing anaesthesia.

The extracted oil contained fifty-two identifiable chemical components, among which meta-Eugenol and trans-caryophyllene contributed 79.19% and 12.57% respectively. The bioactive or functional component of clove plant materials is Eugenol (Khalil *et al*., 2017). Buchbauer *et al*., (1996) observed 50 compounds in clove flower buds which is similar to the 51 compounds in the utilised clove bud powder in the current study. The 79.19% Chavibetol/meta-eugenol content of the oil agreed with obtained values reported by other scholars (Alma *et al*., 2007; Omotayo, *et al.*, 2013; Widayat *et al*., 2014). The number of chemical compounds in the experimental oil is higher compared to the one reported by Bhuiyan *et al*., (2010), who reported 34 chemical constituents in oil extract from clove bud. Variation in composition of clove oil could emanate from quality of soil and cultural technic (Pitarevic *et al*., 1985; Verzar-Petri *et al*., 1985; Arslan

et al., 2004). However, it is of note that the essential oil mainly contained the derivatives of eugenol, in which Chavibetol/meta-eugenol, Eugenol acetate and caryophyllene<trans> accounted for about 95% of the oil.

Anaesthesia induction and sedation stages were earliest in T4, but latest in T2. The 0.1 g/l clove powder concentration in T2 is equivalent to 100 mg powder/l. At the 1.6% oil yield, the concentration would contain 1.6 mg oil/litre of water. The clove oil content in the T2 was therefore higher than the 0.05mg of clove oil/l of water, which induced anaesthetics in tropical marine fish, *Valamagugil cunnesius* and *Monodactylus argenteus*, (Keene *et al*., 1998). The concentration of active clove oil content in the utilised concentrations of the powdered clove bud would therefore be potent to induce anaesthesia even in the highlighted fishes. The provision of the baseline data on the oil yield of the clove bud powder enabled an estimation of oil content in the treatment, thus providing standard for comparison of our values with other studies that utilised clove oil extract. It also highlighted the powder as a potent clove product in inducing anaesthesia, similar to the use of oil extract. The advantage of the estimation can be utilised by local farmers when dried powdered forms of clove bud is utilised on-farm.

The mean values of induction and sedation time were highest in T2 but lowest in T4. This showed that the time to reach induction and sedation stages would have linear relationship with concentration of clove bud powder utilised. Similar relationship was also reported by Diyaware *et al*., (2015). The T2 had the significantly longest sedation time (9.14 min), while T3 and T4 being statistically similar, had the earliest sedation time. This indicate that although sedation is obtainable using the concentrations in T2 to T4, the concentration in T2 was relatively weak in getting the brooders to sedation stage, hence, the lateness of the fish to reach the stage. The T3 and T4 could have been more effective in this regard, hence, there earlier time of reaching the stage. The similarity between sedation time in T3 and T4 indicate that both concentrations would be potent in sedating *C. gariepinus* brooders.

The surgical anaesthesia stage was reached only in T3 and T4 and surgical time were 14.35±1.69 mins in T3, and 14.15±1.65 mins in T4, and these values were statistically similar. This implies that concentration starting from 0.2 g of powder/litre of water (T3) would be able to induce anaesthesia to surgical stage. Since surgery would require some minutes of operation, it would be important to characterise the time taken for the anaesthetised specimens to recover from the anaesthesia. Specifically, recovery time is very important in planning surgical activities, as this would enable the breeder to know how long to hold the fish in the passive condition of anaesthesia. However, recovery time is better determined per stage of anaesthesia, as different activities requiring passiveness may target any of the anaesthesia stages.

The trend of recovery time in T2 to T4 implies increased recovery time with increased concentration of clove bud powder. The latest recovery time was achieved in T4, indicating that concentration in T4 would not only induce surgical anaesthesia, but it would also give the comparatively better time allowance for the fish to recover when compared to T3. This treatment would be preferable for activities that require relatively longer time in surgically anaesthetised brooders. Interestingly, all the assessed anaesthesia concentrations did not result in mortality during 21days post anaesthesia exposure (100% survival was observed in all the treatments). This could imply that the use of aqueous clove bud powder in the specified concentrations would not be lethal to *C. gariepinus* brooders within 21days post-exposure period.

Sexes may react differently to chemical treatments. Knowledge on responses of different fish sexes to dosages of anaesthesia would be very important in planning and time budgeting for anaesthesised fish of separate sexes. In the current study, induction, sedation, surgical and recovery times, as well as survival were not significantly different between male and female sexes of the aqueous clove bud exposed *C. gariepinus* brooders and the survival were 100. These results shows that anaesthesia induction, sedation, surgical and recovery stages alongside survival were not significantly affected by the sex of the fish. Meanwhile, T4 having had the highest recovery time, would be more desirable for operations requiring relatively longer period of surgical anaesthesia.

Ideal anaesthetic agent should have quick recovery at less than 5 mins (Brown *et al*., 2010). This agreed with the obtained approximately 4mins in both the unsexed and sexed (T4) cases for recovery time in the current study. However, the assertion of Brown *et al*., (2010) with respect to recovery time may not be applicable to the conditions of fish spawning and surgery. The dissection of male fish, removal of gonad for artificial spawning and stitching back the dissected fish may take up to 5- 10 mins, ditto to artificial stripping of *C. gariepinus* eggs for spawning. Therefore, these activities would require longer recovery time than the obtained value in the current study. It would be ideal to assess the possible predictors of recovery time in aqeous clove bud powder anaesthetised *C. gariepinus*. Such knowledge would be useful in influencing for extension of recovery time.

Predictive modelling could analyse data in a form of data mining to generate a model to help predict future outcomes (Kalechofsky, 2016). Multiple regression models are predictive, and are often utilised to establish relationship between multiple variables for prediction on the future action (Moustris *et al*., 2012; Castillo *et al*., 2017). The regression equation for the relationship between the concentrations of clove bud powder and time to reach anaesthesia and recovery stages in *C. gariepinus* indicated significant positive linear relationship between recovery time and concentration of the clove bud powder, while sedation time was significantly negative. This implies that increased concentration of the clove bud powder would increase recovery time while sedation time will decrease, vice versa. The trend shows that there is possibility of extending the recovery time by increasing the concentration beyond T4. Such action would also facilitate early time to reach the sedation stage. In the meantime, the prediction equation would be useful forecasting for extension of clove bud powder concentration with the aim of extending recovery time for the surgical anaesthesia stage in *C. gariepinus* brooders.

The colour changes in the anaesthetised treatment could be utilised as litmus test for local users, as the deepness of the brown colouration tends to follow the pattern of clove bud

powder concentration utilised. Across the treatment period, values of water quality (temperature, dissolved oxygen and pH) of the treatments medium were within the acceptable range for fish culture (Omitoyin, 2006; 2007). The treatments water quality were not significantly different (P>0.05), and are of standard for fish culture, thus implying that clove bud powder in the utilised concentrations would not introduce water quality challenges either to the anaesthesised fish, or to the immediate environment.

In conclusion, Clove bud powder contain low level of essential clove oil but it contained eugenol derivatives in desirable proportions. The clove bud powders did not inflict water quality challenge during anaesthesia. All the assessed clove bud powder concentrations 0.1 g/l to 0.3 g/l induced anaesthesia; however, only the T3 and T4 were able to get the brooders to surgical stage, while T4 had the latest recovery time. The T4 would be a better option for surgery and spawning operation in *C. gariepinus* brooders, having induced surgical anaesthesia stage at the relatively earliest time, for longer recovery time without brooders mortality during 21days post anaesthesia period, irrespective of sex. Extension of the recovery time to make way for extra time needed for surgery and spawning in *C. gariepinus* is achievable through increased concentration of the clove bud powder.

Powdered dried clove bud concentration of 0.3 g/l of water (T4) is recommended for inducing surgical anaesthesia stage in *C. gariepinus* brooders having had the latest recovery time. Extension of recovery time to give allowance for more time for spawning and surgery/ablation activities would be feasible through extension of the concentration of dried clove bud powder beyond 0.3 g/l.

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