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Efficacy of Clove flower bud powder as anaesthetic for three life stages of *S. melanotheron, O. niloticus and T. Guineensis*

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ABSTRACT: Clove bud powder was used on three life stages of *Sarotherodon melanotheron, Oreochromis niloticus and Tilapia guineensis* to test its efficacy as an anaesthetic. Different concentrations were used in triplicate on 10 samples of adults, juveniles and fingerlings in 30L plastic aquaria and induction and recovery times were recorded. In *S. melanotheron*- The range of the induction time for the fingerlings, juveniles and adults from 5mg/l to 30mg/l were: 52.00 ± 4.71 - 107.40 ± 12.43s, 43.00 ± 4.83 - 98.70 ± 3.92s, and 45.60 ± 4.48 - 89.80 ± 3.93s, respectively. The recovery time at 5mg/l compared with that at 30 mg/l were: fingerlings- 62.0 ± 4.45 and 125.40s, 84.80 ± 6.76 and 165.20 ± 10.05s, and 94.80 ± 6.49 and 194.40 ± 10.84s. Times of induction and recovery differed ($p < 0.05$) among the life stages. *In O. niloticus,* induction was effected in the fingerlings, juveniles and adults in 118.10 ± 4.46s, 114.00 ± 5.25s and 52.60 ± 4.55s at 5 mg/l; 60.60 ± 9.75s, 52.60 ± 4.56s and 55.60 ± 4.48s for 30mg/l, respectively. The induction time in juveniles across the concentrations was generally lower than that for the other life stages. In *T. guineensis,* the range of the induction time for the fingerlings, juveniles and adults from 5mg/l to 30mg/l were: 52.00±4.71-107.40±12.43s, 43.00±4.83-98.70±3.92s, and 45.60±4.48-89.80±3.93s, respectively. The recovery time at 5mg/l compared with that at 30mg/ was: fingerlings- 62.0±4.45 and 125.40s, 84.80±6.76 and 165.20±10.05s, and 94.80±6.49 and 194.40±10.84s. ©JASEM
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KeyWords: Anaesthetic, Clove powder (*Syzygium aromaticum),* Tilapia, Efficacy.

Fizsimmons (2016) observed that the tilapine species are the most cultured world over next to salmon and world tilapia production was expected to exceed 5,576,800mt and that from aquaculture was estimated 600,000mt produced in more than a hundred and forty countries. The genera *Oreochromis*, *Sarotherodon* and *Tilapia* are cultured both in brackish and freshwater areas under any culture systems in the Niger Delta because they can be easily managed with minimal skill and a good part of those engaged in the venture do so with minimal ease. Despite the high productivity and yields from the wild and culture facilities, tilapine species are very fragile under the various transport, evasive manipulations and other onfarm-related activities in intensive aquaculture. Anaesthtics have been effectively used to handle stress of transportation and other onfarm-related procedures in various fish species including tilapia (Matin, et *al.*, 2009) that are detrimental to the growth and productivity of farm raised fish (Petric, *et al*., 2006), and in extreme cases cause mortality (Akinrotimi *et al*., 2015). Both synthetic and non-synthetic anaesthetics are effectively used to manage and reduce stress to the barest minimum possible when carrying out various onfarm procedures in aquaculture

The use of some of these anaesthetics has health implications resulting in their ban in some countries. Hence, most of them have mandatory withdrawal period before fish so treated can be consumed. In most developing and third world countries, the synthetic anaesthetics are not readily available and easily accessible to farmers. Where they are available, the cost is generally beyond the reach of the average fish farmer. In recent times there is increased interest in the employment of eco-friendly anaesthetics particularly those from plant source (Nwakpa, *et al.,* 2014).

The search therefore is for anaethetics from natural sources termed "green anaesthetics" that are readily available, cheap, easy to apply, environmentally friendly with minimal or no impact to the consumers' health. Such anaesthetics are sourced from commonly available plants and should have the ability to effect induction in exposed fish within a short time (3min.) with a recovery time of about 5mins (Ross and Ross, 2008). A number of plants such as Indian almond (*Terminalia catappa*) leaves and clove flower bud powder (Akinrotimi, *et al.*, 2015) have been assessed and found to possess anaesthetic properties. Clove flower bud obtained from clove tree, *Syzygium aromaticum* (synonym: *Eugenia cariophylata*), family Myrtaceae is a median tree (8-12 m) native from the Maluku islands, East Indonesia (Cortés-Rojas, *et al*., 2014).

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The objective of the study was to assess the anaesthetic potential of the flower bud of clove tree on the induction and recovery times for the three life stages of three commercially important tilapine species: *S. melanotheron, O. niloticus* and *T. guineensis*.

MATERIALS AND METHODS

Dried clove flower of clove plant (*Syzigium aromaticum)* were purchased from Choba market, in Obio – Akpor Local Government Area of Rivers State. Plant authentication was done by Dr. B.E. Ekeke of the Department of Forestry and Environment, Rivers State University of Science and Technology, Port Harcourt, Nigeria. In the laboratory the buds were ground into powder using a kitchen blender (Model H12, Ken Wood, Japan). The milled clove calyx was sieved using 0.1micron nylon mesh to obtain the fine powder and stored in airtight container until used.

S. melanotheron fingerlings (mean length: 8.17±0.85cm, mean weight- 17.54±1.96g), juvenile: (mean length, 11.03±0.29cm, mean weight, 61.15g), adult (mean length, 20.41±1.21cm, mean weight, 224.64±44.24g), *O. niloticus* fingerlings (mean length, 7.06 ±0.47cm, mean weight, 9.17 ±1.75g), Juvenile (mean length 16.34 ± 0.91cm, mean weight 84 39 ±5.93g) and adult, (mean length 25 08 ±0.87cm, mean weight 176.75 ±14.33) and *T. guineensis*¸ fingerlings (mean length, 6.47 ±0.21cm, mean weight 7.05 ±1.57g), juvenile (mean length 17.35±1.68cm, mean weight 81.7 ±8.58g) and adult (mean length, 24.25±0. 84cm, mean weight 162.33±14.17g) where obtained from the African Regional Aquaculture Centre, Port Harcourt, Nigeria.

Water for the study was from borehole (Temperature, 28.1±1.20^{o}C; pH, 6.9±1.23, 0.03mg/l; dissolved oxygen, 5.6±1.9mg/l; electrical conductivity, 121.78±2.15µS/cm; nitrate, 0.07±0.10mg/l and ammonia, 0.04±0.003mg/l). The powder was weighed (5.0, 10.0, 15.0, 20.0 and 25.0 and 30mg/l) and applied directly in three replicates in to the water (10L) in 30L plastic aquaria. The mixture was stirred vigorously to ensure homogenous mixing. The fish were introduced into prepared experimental tanks at the rate of 10 fish per tank in triplicates. The management of the experimental setup, reading and recording of the induction and recovery times were done following the procedures in Akinrotimi, *et al*. (2016).

RESULTS AND DISCUSSION

S. melanotheron: The induction time of the anaesthesia in the fingerlings, juvenile and adult declined progressively with the level of powder, but the reverse ($p<0.05$) was the case with recovery time (Table 1). The induction time for the fingerlings, juvenile and adult at 30mgl/l was 2.4- 2.6 times that at 5mg/l. Recovery time for the respective life stages: fingerlings, juvenile and adults at 5mg/l and 30mg/l increased with the size of fish and concentration of powder. Time of recovery for the juvenile was generally lower than that for the other life stages (Table 1). Induction time for the fingerlings was different ($p<0.05$) from that of the juveniles and adults, whereas that for recovery differed ($p<0.05$) among the life stages.

O. niloticus: The trend of anaesthetic response of each of the life stages of *O. niloticus* to clove powder during induction and recovery was similar to that of *S. melanotheron* (Table 2). The concentration as well as the size affected ($p<0.05$) the induction and recovery period. Induction was effected in the fingerlings, juveniles and adults in 118.10±4.46s, 114.00±5.25s and 52.60±4.55s at 5mg/l; 60.60±9.75s, 52.60±4.56s and 55.60±4.48s for 30mg/l, respectively. The induction time in the juveniles across the concentrations was generally lower than that for the other life stages. However, the recovery time was higher with increase in the size of fish in all the concentrations (Table 2).

Table 1: Time to induction and recovery of fingerlings, juveniles and adults of *S. melanotheron* anaesthesized with flower of clove bud powder (mean±SD)

	Concentration of clove bud powder (mg/l)					
	5	10	15	20	25	30
Fish size	Induction					
Fingerling	92.55^{a}±8.49	80.3^{b}± 7.67	70.2^{c}± 9.57	54.85^{d}± 6.31	46.9^{e} ±4.71	38.35^{f} ± 2.35
Juvenile	103.8^{a}± 6.56	75.8^{b}±12.25	72.0^{b}± 6.72	58.4^{c}±4.7	52.8^{c}±2.53	40.5^{d}±3.31
Adult	87.55^{a}± 9.68	73.5^{b} ±2.46	63.1^{c}± 7.13	49.1^{d} ± 2.28	44.6^{de}± 2.84	39.2^{e} ± 1.32
Recovery duration						
Fingerling	79.2^{d}± 9.45	96.0^{cd}± 13.29	108.6^{c}± 15.13	120.7^{bc}± 0.73	137.9^{b}± 17.63	215.05^{a}± 2.28
Juvenile	59.4^{d}± 6.52	66.6^{d}± 5.10	76.2^{c}± 6.32	87.6^{b}± 8.0	89.4^{b}± 5.58	111.3^{a}±10.22
Adult	82.82^{d}± 12.02	107.6^{cd}± 6.77	118.1^{bc}± 5.15	126.6^{bc}± 6.17	147.5^{b}± 20.98	272.0^{a}± 59.86

Means in the same row with similar alphabets are not significantly different (Tukey Honest significant difference, HSD, $p<0.05$).

Table 2: Time to induction and recovery from anaesthesia of fingerlings, juveniles and adults of *O. niloticus* with flower of clove bud powder (mean±SD).

	Conc. of clover flower bud (mg/l)					
Fish size	5	10	15	20	25	30
	Induction duration (s)					
Fingerling	118[a] ± 4.45	105.3[b] ± 4.42	94.5[c] ± 4.77	83.4[d] ± 2.67	74.8[e] ± 3.26	60.6[f] ± 9.75
Juvenile	114.0[a] ± 5.25	96.6[b] ± 4.79	82.0[c] ± 3.27	65.9[d] ± 4.04	59.9[d] ± 7.43	52.6[e] ±4.55
Adult	100.9[a] ± 6.38	96.9[a] ± 8.4	84.7[b] ± 4.22	71.5[c] ±1.27	70.6[c] ± 2.27	55.6[d] ± 4.48
Recovery duration (s)						
Fingerling	72[e] ± 4.34	82.1[d] ± 5.15	94[c] ± 4.19	109.7[b] ±8.37	113.5[b] ± 3.35	136.1[a]± 10.37
Juvenile	95.8[f] ± 6.84	110.4[e]± 5.3	124.2[d]± 13.81	139.0[c] ± 6.8	152.5[b]± 5.15	182.9[a] ± 30.17
Adult	105.1[e] ± 8.57	120.7[de] ± 1.83	128.6[cd]± 3.2	145.4[bc]±8.95	157.1[b]±11.69	232.3[a]±34.45

Means in the same row with similar alphabets are not significantly different (Tukey Honest significant difference, HSD, p<0.05).

T. guineensis: The trends of the time of induction and recovery in all the life stages were similar to that recorded in the previous species (Table 3). Level of the clove powder and size of fish influenced (p<0.05) the induction and recovery period. The range of the induction time for the fingerlings, juveniles and adults from 5mg/l to 30mg/l were: 52.00±4.71-107.40±12.43s, 43.00±4.83-98.70±3.92s, and 45.60±4.48-89.80±3.93s, respectively. The recovery time at 5mg/l compared with that at 30mg/l at the various life stages were: fingerlings- 62.0±4.45 and 125.40s, 84.80±6.76 and 165.20±10.05s, and 94.80±6.49 and 194.40±10.84s. Times of induction and recovery differed (p<0.05) among the life stages.

The trend in the induction and recovery times in the different sizes of the species studied indicated progressive decline and increase, respectively with increase in the concentration of the powder, as was reported in several other studies (Kamble, *et al*., 2014; Balamurugan, *et al*., 2016). Results from several studies indicate that the induction times under anaesthetics may increase, decrease or remain unchanged with the concentrations of the anaesthetics, species and size of exposed fish (Walsh and Pease, 2003). In a study using juvenile angle fish *Pterophyllum scalare,* Mitjana *et al*. (2014) observed that at the highest doses 80mg/l for clove oil and 140mg/l for MS 222, the induction time remained unchanged. There also may be no dependent relationship between induction times and concentration of anaesthetics, as observed in *Dania rerio* exposed to clove oil (Grush, *et al*., 2004). The variability in species-specific response to the powder is aptly demonstrated in this study. The induction times in *S. melantheron* and *O. niloticus* at 5-10mg/l was shortest in the adult, followed by fingerlings and juveniles; however, in *T. guineensis* it was adults < juveniles <fingerlings

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Table 3: Time to induction and recovery from anaesthesia of fingerlings, juveniles and adults of *O. niloticus* treated with flower of clove bud powder (mean±SD).

	Conc. of clove flower powder (mg/L)					
	5	10	15	20	25	30
Life stage	Induction duration (s)					
Fingerling	107.6[a] ± 4.12	94.1[b]±2.73	85.9[c]±3.60	71.7[d]±2.06	63.6[e]±3.37	52.0[f] ± 4.71
Juvenile	98.7[a] ±3.92	83.9[b] ±4.82	69.9[c] ±2.13	57.9[d] ±3.77	56.7[d]±4.10	43.0[e] ±4.83
Adult	89.9[a]± 3.93	87.6[a] ± 7.42	73.5[b] ± 4.77	62[c] ±1.70	60.6 ±2.27[c]	45.6[d] ±4.48
Recovery duration (s)						
Fingerling	62.0[f]±4.45	72.0[e]± 4.89	83.3[d]±4.03	93.5[c]±4.06	100.6[b]± 3.63	125.4[a]± 8.95
Juvenile	84.8[f] ± 6.76	99.5[c] ± 4.65	113.5[d] ± 14.98	128.2[c] ± 7.11	140.2[b]± 3.55	165.2[a]±10.05
Adult	94.8[e] ±6.49	110.3[d]± 3.02	116.4[d]±3.84	131.5[c]±6.64	143.9[b]± 7.31	194.4[a]±10.84

Means in the same row with similar alphabets are not significantly different (Tukey Honest significant difference, HSD, p<0.05).

Beyond these concentrations, the trends for the three species was juveniles < adults < fingerlings. At the lower concentrations especially, 5 and 10mg/l it appeared that the active ingredients in the powder were not high enough to affect anaesthesia long enough leading to short recovery period from its effect on the organisms. However, generally from 15 to 30mg/l, both induction times followed the trends recorded (positive relationship between it and concentration) recorded in other studies. In the assessment of chemicals or plants to be used as anaesthetics in fish, primarily the time it takes to induce anaesthesia and recovery from the same is critical and forms the first basis for acceptance or rejection before any other secondary criteria (Husen and Sharma, 2014). Several authors have suggested what was considered to be ideal characteristics of anaesthetic agents. Neiffer and Stamper (2009) suggested an induction time of 5-10mins and recovery time within 5mins; whereas Ross and Ross (2008) stressed an induction and recovery times should be within 3mins and 5mins, respectively. The lowest induction times at which loss of equilibrium occurred for the species ranged from 1.5mins in adult to 0.65min. in fingerling for *S. melanotheron* at 5mg/l, whereas at 30mg/l, the highest was 1.97mins and 1.01mins in fingerling of *O. niloticus.* For recovery times it was between 0.99min in juvenile and 4.53mins in adult *S. melanotheron*. The values obtained in this study strongly suggest that unprocessed clove flower bud powder has one of the ideal characteristics of anaesthetic- short induction (3mins.) and recovery (5mins.) times for the three life stages of these tilapine species studied. These values compare favourably if not better than that recorded for other anaesthetics used in the tilapine species. In *O. niloticus,* the induction time using MS-222 was 3mins (Barretto, *et al*.,2007) and 2.6mins (Charoendat, *et al*., 2009). For clove oil, the induction times were between 3-10mins. with a recovery time of 3.67 - 6.28min (SimÕes, *et al.* 2012); clove oil and eugenol, 2.86 - 3.4min (Charoendat, *et al*., 2009). Considering the various induction (1.23-1.78mins) and recovery (1.1-2mins) times, at 10-30mg/l clove powder obtained in this study, for the three life stages of *S. melantheron, O. niloticus* and *T. guineensis,* the powder can be said to meet the ideal criteria for an anaesthetics for these species. Besides, the powder stores well, it is easy to apply, cheap, readily available and environmentally friendly. However, there is the need to further assess the physiological impact of the application of clove flower buds on the species studied.

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