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Anaesthetic effects of aqueous crude leaf extract of desert date (*Balanites aegyptiaca*) on Nile tilapia (*Oreochromis niloticus*) fingerlings

Audu Idi Wakawa, Audu Bala Sambo and Suleiman Yusuf

Abstract
Desert date (Balanites aegyptiaca) is an evergreen plant which is readily available, inexpensive, and non toxic to human. The leaves contain, among other phytochemicals, flavonoids and alkanoids as active ingredients that could be responsible for sedating or anaesthetizing fish. Many expensive, unavailable chemical and plant anaesthetics toxic to human have been reported but there is paucity of information on anaesthetic potentials of B. aegyptiaca on fish. This study is aimed at determining the anaesthetic potency of leaf extract of B. aegyptiaca on Oreochromis niloticus fingerlings. A total of 120 O. niloticus fingerlings (mean weight 23.13±2.43g and mean total length 12.51±0.39cm) were exposed to 4.00, 3.50, 3.00, 2.50, and 2.00g/L of aqueous crude leaf extract of B. aegyptiaca in 12 rectangular glass tanks (40x25x23cm) filled with 10L each of dechlorinated municipal tap water. Tank with 0.00mg/L had no plant material and served as control. Exactly ten (10) O. niloticus fingerlings were introduced into each treatment and control Tanks which were arranged in randomized block design each with replicate. Anaesthetized fish showed decreased induction time with increase in concentration of the plant extract while recovery time increased with increase in concentration of the extract. The best anaesthesia induction dose was 2.50g/L with mean induction and recovery times of 12.15±0.12 and 11.32±1.18 min respectively. Opercula ventilation rate per minute (OVR/min) decreased with increase in concentration of the plant extract. Induction time and OVR/min were significantly different ($P<0.05$) in all the treatments. Due to the effectiveness & safety margin, availability, and affordability; B. aegyptiaca is close to an ideal anaesthetic. Farmers could therefore use 2.50g/L dose of the extract for transportation and handling of O. niloticus fingerlings.

Keywords: anaesthesia, induction, *B. aegyptiaca*, *O. niloticus*

1. Introduction
When fish are handled out of their water environment they become stressed to the extent that their physiology and anatomy are affected and can even be lethal depending on the severity of the stress (Ramanayaka & Atapattu, 2006; Adebayo & Olufayo, 2017) [1, 2]. In aquaculture anaesthetics are used during transportation to prevent stress, physical injury and reduce metabolism (Shawn, Robert & James, 2004) [3]. Anaesthetic is any substance that reduces sensitivity to pain and may cause unconsciousness which could be used to immobilize fish so they can be easily handled during aquaculture practices such as harvesting or capturing, sorting, tagging, sampling, artificial reproduction procedures and surgery (Matin, Hussain, & Hashim, 2009; Neiffer & Stamper, 2009; Javahery & Moradlu, 2012,) [4, 5, 6]. According to Ross & Ross (1984) [7], immobilization of fish is necessary before attempting to perform even the simplest aquaculture task. Many authors (Agokei & Adebisi, 2010; Hekimoglu & Ergun, 2012; Kamble, Saini & Ojha, 2014; Adebayo & Olufayo, 2017) [8, 9. 10, 2] reported anaesthetic effects of plant extracts on different fish species with a view to determining plants with ideal anaesthetic properties. An ideal anaesthetic, according to Brown (2011) [11]; Marking and Meyer's study (as cited in Agokei & Adebisi, 2010) [8], exhibits the following qualities: induction time of less than 15 min; recovery time of 5 min or less; nontoxic to fish and has a large safety factor; easy to handle and not harmful to humans; no persistent effect on fish physiology and behaviour; rapidly excreted or metabolized leaving no residues and requiring no withdrawal time; no cumulative effect or problem from repeated exposure and inexpensive.
Desert date (*Balanites aegyptiaca*) is a common plant in arid and semi-arid regions of Northern Nigeria especially in Adamawa, Bauchi, Borno, Gombe, Jigawa, Kano, Katsina

States, Sokoto, Yobe and Zamfara States. It is also found in some parts of Plateau and Nassarawa States, Nigeria. The plant is called 'Aduwa' in Hausa language while the Fulani tribe calls it 'Tanni' (Oyema, Chinedu & Ahmad, 2017) [12]. Arabs refer to it as 'Heglig' while Swahili calls it 'mchunju' (Tesfaye, 2015) [13]. Some parts of *B. aegyptiaca* have been reported as poisonous to fish but not to man (Heuze & Tran, 2012) [14]. Phytochemical screening of *B. aegyptiaca* leaf revealed the presence of alkanoids, flavonoids, tannins saponins, balsam and phenols (Abdulhamid & Sani, 2016) [15]. Tsuchiya (2017) [16] earlier reported that alkanoids and flavonoids are phytochemicals with anaesthetic properties which could explain the potentials of *B. aegyptiaca* to anaesthetize fish.

Nile tilapia, according to Trewavas' study (as cited in Anonymous, 2015) [17], is easily recognized by its caudal fin, which is distinctively striped, with 30-34 lateral scales and 20-26 gill rakers on the lower limb of the first gill arch. This fish is highly sensitive to poor water quality conditions and susceptible to stress during handling, transportation, reproductive procedures, weighing, tagging and surgery hence considered for the experiment.

Many workers (Marking & Mayer, 1985; Hajek, Klyzejko & Dziaman, 2006; Javahery & Moraldu, 2012) [18, 19, 6] have documented several chemical compounds such as Tricane Methanesulphate (MS-222), Benzocaine, phenoxyethanol, Chlorobutanol which are expensive; with deleterious effects on fish (Absalom, Nwadiaro, & Wophill, 2013) [20] and many plant extracts such as Clove oil (Agokei & Adebisi, 2010) [8], AQUI-S (Javahery & Moradlu, 2012) [6], *Cannabis sativa* (Audu, Adamu, & Ufodike, 2013) [21] and *Datura stramonium* (Adebayo and Olufayo, 2017) [2] as fish sedatives and/or anaesthetics; there is paucity of information on anaesthetic properties of leaf extract of *B. aegyptiaca* on fish. The objective of this study is to investigate the anaesthetic effect of the crude leaf extract of *B. aegyptiaca* on *O. niloticus* fingerlings

2. Materials and Methods

2.1 Location, Collection and Preparation of Leaves of *B. aegyptiaca*

The leaves of *B. aegyptiaca* were collected from Gashua, Bade Local Government Area of Yobe State; North-eastern Nigeria. The leaves were washed with clean water several times to remove soil, dust or dirt. A quantity of 3kg was shade dried and sample pulverized with pestle and mortar; sieved into fine powder (0.5mm) and stored in airtight plastic container.

2.2 Acclimation of the Experimental Fish

Exactly 120 *O. niloticus* fingerlings (mean weight 23±0.03g and mean total length 12501±0.39cm) were purchased from Alpaks Fish Farm, Rantia, Jos, Plateau State, Nigeria and transported in two oxygenated polythene bags to Aquaculture laboratory of Hydrobiology and Fisheries Unit of University of Jos, Jos, Nigeria. Fish (20 fingerlings/tank) were acclimated (7 days) in six 35L capacity round plastic tanks each filled with 20L of water. The water in the holding tanks was changed once daily (8.00 hours) and fish were fed to satiation with artificial diet (Coppens®) twice daily at 10.00 and 17.00 hrs.

2.3 Experimental Design

The experiment consists of 12 rectangular glass tanks (40x25x23cm) and 120 *O. niloticus* fingerlings (mean weight 23.13±2.43g and mean total length 12.51±0.39cm) in a randomized blocks design. Each of the six glass tanks were filled with 10L of dechlorinated municipal tap water, with five of the filled tanks inoculated with varying concentrations (4.00, 3.50, 3.00, 2.50 and 2.00g/L) of aqueous crude leaf extract of *B. aegyptiaca* and 10 *O. niloticus* fingerlings each were introduced into all the tanks while the sixth tank served as the control and was not inoculated with the test material. The setup was replicated and five (20L capacity) round plastic containers were used as recovery tanks.

2.4 Monitoring Water Quality Parameters

Water quality parameters such as temperature (daily), Dissolved Oxygen (DO_2), free carbon dioxide (CO_2), alkalinity, and hydrogen ion concentration (pH) of the experimental tanks were monitored biweekly using the standard methods of APHA (1985) [22].

2.5 Determination of Anaesthetic Concentrations

Prior to the administration of the extract on the experimental fish, a series of preliminary experimental trials were conducted to determine suitable anaesthetic concentrations to be used in the experiment resulting to five definitive sedative test concentrations (4.00, 3.50, 3.00, 2.50 and 2.00g/L) of the crude leaf extract of *B. aegyptiaca* on *O. niloticus* fingerlings

2.6 Anaesthesia of *O. niloticus* Fingerlings with Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca*

A total of 120 Fingerlings of *O. niloticus* (mean weight 20.13±2.43g) were exposed to 4.00, 3.50, 3.00, 2.50 and 2.00g/L of the extract in 12 rectangular glass tanks (40x25x23cm) arranged in randomized block design (Rezende, Pascoal, Vianna & Lanna, 2017) [23]. To each concentration, 10 *O. niloticus* fingerlings were exposed by immersion method (Neifer & Stamper, 2009) [5] in glass tanks labeled A1, B1, C1 D1 and E1 and were replicated as tanks A2, B2, C2, E2, and E2. The remaining batches of 10 fingerlings each were not exposed to the test material and served as control (0.0g/L) tanks (F1 & F2). The stages of sedation and anaesthesia were; normal stage, light sedation, deep sedation, partial loss of equilibrium, total loss of equilibrium, total loss of reflex and recovery.

Table 1: Stages of Anaesthesia

Stages	Notable behaviours
Normal (NM)	Normal swimming & regular opercula ventilation rate, reactive to external stimuli
Light Sedation (LS)	Slight loss of reactivity to external stimuli & slightly decreased opercula ventilation rate
Deep Sedation (DS)	Deep fall in opercula ventilation rate, slow swimming & partial loss of reactivity to external stimuli
Partial Loss of Equilibrium (PE)	Partial loss of muscular tone, erratic swimming, increased opercula rate & reaction only to strong tactile or vibration stimuli
Total Loss of Equilibrium (TE)	Total loss of muscular tone & equilibrium, slow & regular opercula movements
Total Loss of Reflex (TR)	Opercula movement is slow & irregular , total loss of reflex & reaction to external stimuli
Recovery (RC)	Restart of opercula movement, erratic movement of the body

***Source*:** Summerfelt & Smith (1990) and Javahery & Moraldu (2012)

2.7 Procedure for Anaesthesia Experiment

Anesthetic stages were determined as suggested by Summerfelt & Smith (1990) [24]; Javahery & Moraldu (2012)

[6] (Table 1). *O. niloticus* Fingerlings exposed to the treatment tanks were observed for behavioural responses compared with those in the control. The behavioural changes at each stage were noted and any fingerling on attainment of anaesthesia (Total loss of reflex) in each tank was removed and placed in corresponding recovery tank.

2.8 Determination of Opercula Ventilation Rate (OVR/min) of *O. niloticus* Fingerlings Exposed to Aqueous Crude Leaf Extract of *B. aegyptiaca*

Prior to the introduction of the experimental fish into each concentration of the test material the opercula ventilation rate (OVR) for each fish was determined using a digital stop watch. The total number of times the operculum opens and closes per minute was counted. The OVR/min of each fish in the recovery tank was also recorded by counting the number of times the operculum opens per minute.

2.9 Statistical Analyses

Statistical analyses were performed using IBM SPSS (version 20) software. Data were analyzed by one-way Analysis of Variance (ANOVA) and Pearson's correlation coefficient. ANOVA was used to determine the difference between treatment means while the level of interaction between concentrations, induction and recovery times were analyzed by Pearson's correlation coefficient. Level of significance was determined at P=0.05 level of probability and a P<0.05 value was considered statistically significant. Tukey's multiple comparisons test was applied to identify the significant treatments. Data were presented as means standard error (±SE).

3. Results

3.1 Water Quality Parameters

The water quality parameters for anaesthesia of *O. niloticus* with aqueous crude leaf extract of *B. aegyptiaca* are presented in Table 2. DO decreases as the concentration of the extract increases. The highest DO concentration (4.6±0.09g/L) was recorded in the control while the lowest (1.0±0.00g/L) was in 4.00g/L concentration. There was significant difference (P<0.05) in the DO value of all the treatments compared with the control tank.

The water temperature (°C), however, did not show any variation with increase in concentration of the test material. The various treatment tanks and the control maintained a constant mean temperature of 23±0.00 °C with no significant difference (P>0.05) between the treatment and the control tanks.

Hydrogen ion concentration (pH) showed variation with increase in concentration of the test material. The highest mean pH (6.7±0.13) was recorded in the control (0.00g/L) while in the treatment tanks the pH progressively decreased with increase in concentration of the plant material. Lowest pH (6.4±0.09) was recorded in the highest concentration (4.00g/L) of the plant extract (Table 2). There was no significant difference (P>0.05) in the pH of the treatment tanks compared with the control.

Table 2 revealed that Free CO_2 increased with increase in concentration of the aqueous crude leaf extract of *B. aegyptiaca*. The control recorded 5.00±0.70mg/L while the highest Free CO_2 (46.00mg/L) was recorded in the highest concentration (4.00g/L) of the test material. Free CO_2 values were significantly different (P<0.05) in all the treatment tanks compared with the control.

Total alkalinity (TA) increased with increase in concentration of aqueous crude leaf extract of *B. aegyptiaca* in the treatment tanks (Table 2). Mean TA was lowest (35.50±0.70mg/L) in the control tank and highest (50.00±0.70mg/L) in the highest concentration (4.00g/L) of the extract. There was no significant difference (P>0.05) between the TA of the treatment tanks compared with the control.

Table 2: Mean Water Quality Parameters of Experimental Tanks during Anaesthesia of *O. niloticus* Fingerlings with Concentrations of Aqueous Crude Leaf Extract of *B. Aegyptiaca*

Parameters	Concentrations (g/L)					
	0.00	2.00	2.50	3.00	3.50	4.00
DO (mg/L)	4.60±0.09	2.50±0.09*	2.20±0.09*	1.30±0.20*	1.00±0.00*	1.00±0.00*
Temp.(°C)	23.00±0.00	23.00±0.00	23.00±0.00	23.00±0.00	23.00±0.00	23.00±0.00
pH	6.70±0.13	6.60±0.07	6.50±0.06	6.40±0.08	6.40±0.04	6.40±0.09
Free CO_2 (mg/L)	5.00±0.09	8.00±0.09*	13.00±0.20*	13.00±0.00*	15.00±0.00*	46.00±0.09*
TA (mg/L)	35.50±0.70	39.50±2.17	45.00±1.22	45.50±0.64	48.20±0.65	50.0±0.70

Values with Asterisks (*) in the same row are significantly different compared to the control

3.2 Anaesthetic Effects of Leaf Extract of *B. aegyptiaca* on *O. niloticus* Fingerlings

The mean behavioural responses, induction and recovery times of *O. niloticus* fingerlings during anaesthesia with aqueous crude leaf extract of *B. aegyptiaca* are presented in Table 3. Behavioural response of the fingerlings was a function of the concentration of the extract and time of exposure. Induction time decreased with increase in concentrations of the extract while recovery time inversely increased with increase in concentrations of the extract. *O. niloticus* fingerlings exposed to the lowest concentration (2.00g/L) of the plant extract recorded the longest mean induction time of (18.32±0.08 min) and shortest mean recovery time of (5.23±0.12 min). Invariably, no mortality was recorded in the lowest concentration which coincided with the results obtained at 2.50g/L test material concentration which had mean time of 12.15±1.05 min for the fingerlings to attain anaesthesia while mean recovery time was 11.32±1.18 min. Different trends were observed at concentrations of 3.00 and 3.50g/L of the test material which recorded 10% mortality with decreased mean induction time of 9.00±0.04 and 6.27±1.03 min respectively while mean recovery time increased to 15.42±1.87 and 21.05±1.52 min respectively. The shortest (5.18±0.07 min) and longest (30.19±1.23 min) mean induction and recovery times respectively were recorded in the highest plant extract concentration (4.00g/L) and had the highest (30%) mortality. Statistical analyses revealed significant difference (P<0.05) between induction time of all the treatments tanks compared to the control.

Table 3: Mean Behavioural Responses and Survival Rate of *O. niloticus* Fingerlings during Anaesthesia with Aqueous Crude Leaf Extract of *B. Aegyptiaca*

Tank	Conc.(g/L)	No. of Fish	Behavioural Responses (min)							No. of mortality	No. of Survival	Mortality Rate (%)	Survival Rate (%)
			Stage 1 (NM)	Stage 2 (LS)	Stage 3 (DS)	Stage 4 (PE)	Stage 5 (TE)	Stage 6 (TR)	Stage 7 (RC)				
A	2.00	10	4.08 ±0.07	6.41 ±0.13*	8.03 ±0.38*	9.26 ±0.52*	15.15 ±0.40*	18.32 ±1.01*	5.23 ±0.12	0	10	0	100
B	2.50	10	3.28 ±0.10	5.60 ±0.58*	7.24 ±0.09*	8.21 ±0.24*	10.15 ±0.05*	12.15 ±1.05*	11.32 ±1.18*	0	10	0	100
C	3.00	10	2.05 ±0.35	2.83 ±0.33*	3.32 ±0.18*	4.29 ±0.04*	7.10 ±0.40*	9.00 ±0.40*	15.42 ±1.87*	1	9	10	90
D	3.50	10	1.15 ±0.14	2.26 ±0.01*	3.08 ±0.02*	4.25 ±0.27*	5.22 ±0.67*	6.27 ±1.03*	21.05 ±1.52*	1	9	10	90
E	4.00	10	1.13 ±0.04	2.24 ±0.08*	3.23 ±0.28*	3.35 ±0.15*	4.19 ±0.07*	5.18 ±0.07*	30.19 ±1.23*	3	7	30	70

Values with Asterisks (*) in the same row are significantly different from the Stage 1 (NM)
NM=Normal behaviour: Normal swimming and regular opercula ventilation rate, reactive to external stimuli
LS=Light Sedation: Normal swimming and regular opercula ventilation rate, reactive to external stimuli
DS=Deep Sedation: Deep fall in opercula ventilation rate, slow swimming and partial loss of reaction to external stimuli.
PE=Partial loss of equilibrium: Partial loss of muscular tone, erratic swimming, increased opercula ventilation rate and reaction only to strong tactile or vibration stimuli.
TE=Total loss of equilibrium: Total loss of muscular tone and equilibrium, slow and regular opercula movements
TR=Total loss of reflex: Opercula movement is slow and irregular, total loss of reflex and reaction to external stimuli
RC=Recovery: Restart of opercula movement, erratic movement of the body

3.3 Effect of aqueous crude leaf extract of *B. aegyptiaca* on opercula ventilation rate (OVR min) of *O. niloticus* fingerlings

The mean OVR/min of *O. niloticus* fingerlings after exposure to concentrations of aqueous crude leaf extract of *B. aegyptiaca* is presented in Figure 1. Mean OVR/min decreases with increase in concentrations of the plant extract. The highest mean OVR/min (101.40±3.01) was recorded in the control while the lowest mean OVR/min of 50.00±0.65 was recorded in the highest treatment concentration (4.00g/L). There was significant difference (P<0.05) in the OVR/min of the fingerlings exposed to the aqueous crude leaf extract in all the concentrations compared with the control.

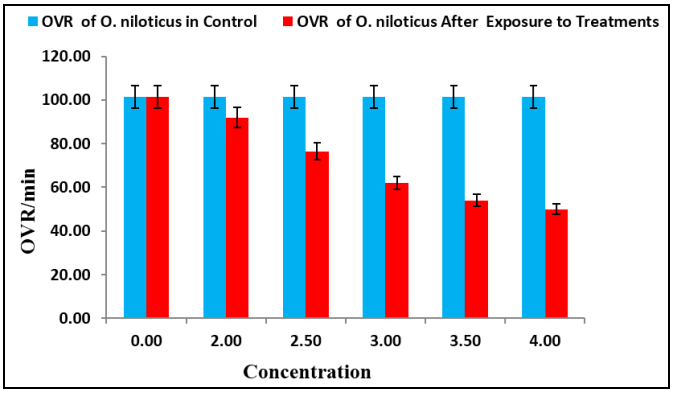

Fig 1: Mean OVR/min of *O. niloticus* Fingerlings Exposed to various Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* during Anaesthesia and the control group

4. Discussion

The results of the present study showed evidence of potency in anaesthetization of fingerlings of *O. niloticus* by the aqueous crude leaf extract of *B. aegyptiaca*. The decrease in DO with increase in concentration of the plant extract in this study corroborates the report of Audu *et al*. (2013) [21] that DO increase with increase in concentration of *C. sativa* but disagreed with Anju, Solomon, & Cheikyula (2015) [26] who recorded constant DO as concentration of *Tephropsia vogelii* aqueous crude leaf extract increases. The observed respiratory distress observed in *O. niloticus* fingerlings in this study may be due to decrease in the DO concentration (Oluwatoyin, 2011) [27] since the minimum DO required by *O. niloticus* is 3 mg/L (David, 2012) [28]. The mean temperature (23±00 ºC) recorded in this study was within the normal range (23.89-34.45 ºC) reported by David, 2012 [28], therefore could not have been responsible for the observed changes in behaviour or physiology of *O. niloticus* fingerlings. Similarly, pH could not have been responsible for the observed behavioural and physiological changes in *O. niloticus* fingerlings since the mean pH values (6.4±0.09-6.7±0.13) in this study are within the normal range (5-8) as reported by Nobre, Lima, & Magalhaes (2014) [29]. Free CO_2 increases acidity of water which could result into physiological distress as well as affect the pH of the blood and cause severe imbalance in fish (Jobling, 1994) [30]. The increase in free CO_2 (5.00±0.70-46.00 mg/L) in this study could therefore be a factor in the behavioural and physiological changes observed in the fingerlings of *O. niloticus*. The stages of anaesthesia in this experiment followed the pattern of fish anaesthesia reported by Summerfelt & Smith (1990) [24]; Javahery & Moradlu (2012) [6]. Results showed that all the fingerlings were anaesthetized in all the concentrations within 5 to 18 min while time of recovery was within 5 to 30 min. This coincides with the report of Adebayo and Olufayo (2017) [2] that the mean induction time of *H. bidorsalis* to leaf extract of *D. stramonium was* within 18 to 25 min with mean recovery time within 30 to 40 min. There was decrease in transition time to induction as the concentration of the plant extract increases while recovery time increases with increasing concentration of the extract. The longest induction time was recorded in the lowest concentration (2.00g/L) while the shortest time was in the highest concentration (4.00g/L concentration of the plant extract. This result is in line with the findings of Kamble, *et al*. (2014) [10] who reported reduction in induction time with increased concentration of clove oil on *Cyprinus carpio*. Similar result was recorded by Agokei & Adebisi (2010) [8] when Nile tilapia (*O. niloticus*) was exposed to different concentrations of aqueous and alcoholic extract of Tobacco. The shortest (5.18±0.07min) and the longest (18.32±1.01min) mean induction times as well as shortest (5.23±0.12min) and

longest (30.19±1.23min) mean recovery times in this study were lower than 42.15±2.75 and 54.28±4.66, shortest and longest mean anaesthetization times respectively reported by Audu, *et al*. (2013) [21] after exposing *O. niloticus* fingerlings to different concentrations of leaf extract of *Cannabis sativa*. The decrease in OVR/min with increase in concentration of *B. aegyptiaca* in this study agrees with the report of Akinbulumo (2005) that fish exposed to anaesthetics usually exhibit decrease in opercula movement. Considering the fast induction and short recovery times, and the apparent zero toxicity effect on fish at lower concentration, coupled with reported harmlessness to humans, and its availability as well as inexpensiveness; *B. aegyptiaca* could be inferred as satisfying the requirement of an ideal anaesthetic (Brown, 2011) [11]; Marking and Meyer's study (as cited in Agokei & Adebisi, 2010) [8].

5. Conclusion

Though the effective concentrations of the aqueous leaf extract of *B. aegyptiaca* is rather high compared to conventional anaesthetics, it could be safely and effectively used for anaesthetization of *O. niloticus* fingerlings, owing to its safety margin, availability, and affordability. It can be concluded that *B. aegyptiaca* is a potent anaesthetic with efficacy at low concentration of 2.50g/L, short induction and recovery times and zero percent mortality; therefore recommended when transporting and handling of *O. niloticus* fingerlings.

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