Journal of Research in Forestry, Wildlife & Environment Vol. 12(2) June, 2020
E-mail: jrfwe2019@gmail.com; jfewr@yahoo.com
http://www.ajol.info/index.php/jrfwe

ISBN: 2141 – 1778
Anju et al., 2020

EVALUATION OF ANAESTHETIC EFFECTS OF AQUEOUS BARK EXTRACT OF *Tephrosia vogelii* ON AN AFRICAN CATFISH *Heterobranchus longifilis* POST JUNVENILES VAL. (PISCES: 1840) AS A TRANQUILIZER

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ABSTRACT

This work was designed to investigate the potency of aqueous bark extract of Tephrosia vogelii as a tranquilizer on Heterobranchus longifilis, the concentration at which the fish is completely tranquilized and the recovery time of the extract. Fresh samples of T. vogelii bark were collected, air-dried under a shade for 21 days and oven-dried at 60^{o}C for 3–4 hours to constant weight. The dried samples were pulverized using an electric kitchen blender and stored in air tight bottles for subsequent use. Parts of the dried samples of T. vogelii bark were used to carry out phytochemical analysis to determine their chemical constituents. The bark was extracted in aqueous medium for the purpose of this experiment. The result of the sedation of Heterobranchus longifilis with the aqueous bark extract of Tephrosia vogelii showed that all H. Longifilis juveniles injected with the aqueous bark extracts were tranquilized up to anaesthetic stage 3 (light sedation). None of the experimental fish was able to enter anaesthetic stage 4 (deep) anaesthesia. The aqueous bark extract was found to have a significantly shorter (P<0.05) induction time of 52.33 seconds with a short recovery time of 125.33 minutes at concentration 0.01g/l at anaesthetic stage 3 depicting that the aqueous bark extract of T. vogelii has the high potency of a tranquilizer.

Keywords: *Heterobranchus longifilis*, Aqueous Leaf Extracts, Anaesthetic, *Tephrosia Vogelii*, Tranquilizer

INTRODUCTION

Anaesthesia is a biological reversible state induced by an agent which results in the partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non-chemical means (Summerfelt and Smith, 1990). Anaesthesia is required in aquaculture to help prevent injuries and to minimize stress in fish during routine handling and surgical procedures. Anaesthetic agents are often assumed to provide analgesia if they result in complete immobilization. However, the distinction between anaesthetic agents (with analgetic properties) and immobilization drugs is not always clear and depends on the properties of the particular drug, so that it is never appropriate to assume analgesia with all anaesthetics (Neiffer and Stamper, 2009). Anaesthetics have been also used in euthanasia: when necessary, overdose of an immobilization drug is an acceptable means of euthanasia, the most frequent choice being the use of immersion drugs (particularly MS-222) at concentration five to ten times for a particular species of fish, although injectable anaesthetic drugs are also effective (Ross and Ross, 1984).

The characteristics required of an ideal anaesthetic include: rapid induction time, 5 minutes recovery time, wide safety margin, easy to handle, effective at low dosages, anaesthetized fish must survive, non- toxic to fish, handler and the environment and must be rapidly extracted (Marking and Meyer, 1985; Iversen*et al*, 2003; Coyle *et al*, 2004; Mylonas*et al*, 2005; Brown, 2011).

Although chemical anaesthetics have long served as a valuable means of minimizing stress and preventing injuries and mortalities during routine handling, experimental and surgical procedures in fisheries and aquaculture, several of them impact

negatively on the fish, handler or the environment and are not biodegradable. These shortcomings of chemical anaesthetics coupled with the fact that ichthyotoxins of plant origin are less expensive, biodegradable, readily available, easy to handle and safe on the fish, human and environment (Singh *et al*, 1996) research effort is being intensified to investigate into plant extracts with potentials for anaesthetics as an alternative source of fish anaesthetic. The objective of the present study is to investigate into the possible use of the aqueous bark extract of *Tephrosia vogelii* as a tranquilizer for the African catfish *Heterobranchus longifilis*.

MATERIALS AND METHODS

Heterobranchus longifilis juveniles (mean weight 115.20±25g SD) were obtained from wild stock caught from the River Benue, Benue State, Nigeria. The fish were transported in Jerry cans to the general purpose laboratory of the Department of fisheries, University of Agriculture, Makurdi, Nigeria for acclimatization. Plastic tanks of 70 – litre capacity filled with 40 litres of water were used to acclimatize the fish under laboratory conditions for a period of two weeks before exposure to the anaesthetic extract. During the period of acclimatization the fish were fed once a day at 09.00 hours at 4% body weight with commercial fish diet. Each tank containing acclimatization water was aerated to enhanced dissolved oxygen. Water was changed daily to prevent metabolic waste build-up to maintain good water quality. Some water quality parameters were measured and found to be at desirable levels as shown in Table 1. The experimental fish were starved for 24 hours before commencement of the experiment to prevent regurgitation from the gastro-intestinal tract (GIT), and observation and recovery baths were provided with aeration.

Fresh samples of *T. Vogelii* were collected, air-dried under shade for 21days and then oven-dried at 60 ^{o}C for 3-4 hours to constant weight (Omoniyi*et al*, 2002). The dried samples were pulverized to powder using an electric kitchen blender and stored in air–tight bottles for subsequent use. A quantity of 200g of the stored sample was weighed into a 2.5 litre flat bottomed flask and 1 litre of de-ionized water was added to cover the sample. The mixture was shaken to ensure proper mixing and allowed to stand for 24 hours. The mixture was filtered with muslin cloth and then with sunction filtration. Then various quantities of the mixture were drawn to perform phytochemical analysis to determine the chemical constituents (alkaloids, Saponins, tannins, anthroquiniones, flavonoids etc) using standards methods described by Harbone, (1973) and Trease and Evans,(1989).

Prior to the administration of the effect of aqueous bark extract on the experimental fish a pre-experimental trial was carried out using standard procedures following the methods of APHA (1985) to determine suitable concentrations to be used in the experiment. Based on the pre-experimental test six concentrations of the aqueous leaf extract of *Tephrosia vogelii* were prepared by dissolving known weights of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l in 1litre of de-ionized water contained in 2.5 litres air–tight laboratory bottles at room temperature (27.00 ± 0.40^{0}C). The mixture was shaken to ensure proper mixing of the plant samples and water. The solution was allowed to stand for 24 hours, and the settled portion decanted and filtered with No. 1 Whatman filter paper. The filtrate was kept in air–tight bottles and used as appropriate.

The administration of various concentrations of *T. vogelii* aqueous bark extract was carried out in exactly the same manner, using the parenteral (injection) method of anaesthesia. Three healthy *H. Longifilis* were selected randomly from both the control and treatment groups. Each was weighed and injected 0.5ml of the extract concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06)g/l using No. 23 needle and a 2ml heparinised syringe. Injection was done intramuscularly (IM) at the dorsal saddle, just above the lateral line behind the operculum. Fish in the control group were injected with the same dose of distilled water. Injected fish were observed for behavioural responses and transferred to 70–litre capacity plastic tanks filled with 40 litres of water for recovery and time taken to recover noted. Continuous observation of the behavioural response was discarded after 60 minutes when the fish failed to reach anaesthetic stage 4, since periods greater than this were considered unreasonable for routine fish handling procedures.

The statistical analysis of the results obtained from the behavioural responses of the fish to aqueous bark extract of *T. Vogelii* was carried out using Genstat Discovery Edition 4 for one–way analysis of variance (ANOVA) to determine the differences in behavioural responses. The same software was used to determine differences in the water quality parameters across the concentrations used. Graph Pad Prim 5 and SSC Stat V2. 18 were used to test if differences existed between the variables measured. Summary statistics were obtained for the variables using Minitab 14 for windows**.**

RESULTS

The behavioural responses of *H. longifilis* exposed to*T.vogelii* aqueous bark extract is shown in Table 2.The decrease in time taken for fish to enter anaesthesia was more pronounced and significant differences ($P<0.05$) were observed among treatment groupsat all three stages of anaesthesia. The opercular beat rate before injection failed to show a definite pattern. However, the opercular beat rate after injection showed a declining trend with increasing concentration of the extract in the first four concentrations, but increased at the fifth and sixth concentrations. Recovery time ranged from 125.33 minutes (2.05hours) to 188.00 minutes (3.08hours) increasing with increase in concentration in the treatments groups. Recovery time differed significantly among treatments groups ($P< 0.05$).

Table 1: Mean water quality parameters during sedation of *H. longifilis* with *T. vogelii* aqueous bark extract.

Concentration (g/l)	Temperature (˚C)	Dissolved oxygen (mg/L)	PH	Alkalinity (mg/L)
0.00	25.41±0.02^{a}	6.81±0.02^{c}	7.05±0.5^{d}	30.18±0.01^{d}
0.01	25.38±0.02^{ab}	6.82±0.02^{c}	7.17±0.02^{c}	30.21±0.03^{d}
0.02	25.36±0.02^{bc}	6.88±0.01^{b}	7.24±0.01^{b}	30.34±0.01^{c}
0.03	25.35±0.01^{bc}	6.90±0.91^{b}	7.30±0.01^{ab}	30.38±0.02^{bc}
0.04	25.33±0.01^{c}	6.91±0.01^{ab}	7.29±0.01^{ab}	30.42±0.02^{ab}
0.05	25.34±0.00^{c}	6.95±0.02^{a}	7.32±0.01^{a}	30.39±0.02^{abc}
0.06	25.32±0.01^{c}	6.93±0.02^{a}	7.30±0.03^{ab}	30.45±0.03^{a}

Means in the same column followed by different superscript differ significantly (P<0.05)

Table 2: Behavioural responses of *H. longifilis*, injected with various concentrations of *Tephrosia vogelii* Aquesous bark extract.

Concentration (g/ℓ)	Fish Weight (g)	Volume injected (ml)	Behavioural Responses					Percentage increase in OBR (%)	Recovery time (Minutes)	Mortality After 48hrs
			Time of Anaesthesia Induction (S)			OBR (M^{-1})				
			I	II	III	BFS	AFS			
0.01	72.00±5.00[a]	0.5	20.67±1.76 [a]	26.00±1.15[a]	2.33±2.85[a]	49.67±2.33[a]	59.67±4.06[a]	20.40±8.34[a]	125.33±2.96 [e]	-
0.02	75.67±5.67[a]	0.5	19.00±1.53[ab]	23.67±0.33[a]	51.33±3.18[a]	51.00±1.00[a]	57.67±1.20[a]	13.81±3.64[a]	132.00±1.53[de]	-
0.03	79.33±4.33[a]	0.5	16.67±0.67[bc]	22.00±3.06[ab]	44.00±7.21[ab]	49.00±0.58[a]	55.67±2.60[a]	13.57±4.74[a]	135.67±2.60[d]	-
0.04	70.67±5.81[a]	0.5	15.33±0.88[bc]	20.00±1.15[abc]	44.00±1.73[ab]	49.67±0.58[a]	54.33±6.01[a]	9.34±0.44 [a]	150.67±8.97 [c]	-
0.05	73.33±1.67[a]	0.5	13.67±0.88 [cd]	16.00±0.58[bc]	38.33±1.20 [b]	57.33±1.33[a]	62.00±2.00[a]	8.09±0.95 [a]	163.33±8.11[b]	-
0.06	73.00±4.73[a]	0.5	11.00±1.73 [d]	14.00±1.73[c]	36.00±1.15[b]	55.67±7.97[a]	61.67±6.74[a]	12.17±5.30[a]	188.00±5.03[a]	-

Key: OBR BFS = Opercular Beat Rate Before Sedation; OBR AFS = Opercular Beat Rate After Sedation

- ❖ Data were subjected to analysis of co-variance using weight as covariate
- ❖ Means in the same colum followed by different subscripts differ significantly (P<0.05)

DISCUSSION

The results obtained from the present study show that in this experiment with *H. longifilis* juveniles injected with aqueous bark extract of *T. vogelii* there was sequential progression through the first three stages of anaesthesia and the experimental fish were successfully tranquilized at all levels of concentration. This is similar to the findings from the study on the effects of sodium bicarbonate on common carp (*Cyprinus Carpio*) juveniles which only reached the third stage of anaesthesia (Altun *et al*,2009). The effect of the anaesthetizing extract appeared to be concentration dependent since faster tranquilization was achieved at higher concentrations of the extract as reported in other studies (Hseu*et al*,1998; Griffiths,2000; Solomon and Amali, 2004; Mylonas *et al*,2005). This observation is also in agreement with Trevor and Miller,(1987) that the degree of anaesthesia is influenced by the concentration of the anaesthetic in the central nervous system (CNS) of the organism. Therefore, in the present investigation the shorter induction time taken to tranquilize the experimental fish, *H. longifilis*, with increased concentration of the anaesthetic extract may be attributed to the accumulation of the active ingtredients, rotenoids, in the body system of the fish which impairs the activity of CNS at a much faster rate(Solomon and Amali,2004). The failure of the anaesthetized fish to enter deep sedation (anaesthetic stage 4) could be due to the size and weight of the fish in relation to the low concentration used since larger individuals generally require a greater concentration of anaesthetic than smaller individuals (Colye*et al*, 2004). This may also be attributed to stage of the life cycle, age, lipid content and body condition of the fish, all of which are biological factors that influence the metabolic rate and therefore the pharmacokinetics of anaesthetic compounds (Iversen*et al*, 2003).

When the time taken for *H. longifilis*to enter anaesthesiaor to be tranquilized (induction time) and recovery time are considered in the present investigation,there were significant differences (P<0.05) ininduction time in the experiment with aqueous bark extract at all levels of concentration at anaesthetic stage 1-3 of anaesthesia depicting the effect of concentration on induction. The induction time of 52.33 seconds obtained with the aqueous bark extract is compartible with the average induction time of 30.10 seconds and 30.70 seconds reported for *Valamugil cunnesius* and *Monodactylus argenteus* respectively exposed to clove oil (Durvilleand Collect,2001), 1-2 minutes for light sedation of common carp (*Cyprinus carpio)* juveniles exposed to sodium bicarbonate (Altun*et al*,2009) and the 1.5 minutes reported for *Acipenserpersicus* exposed to clove oil (Bagheri and Imanpoor, 2001). When the rapid induction time (3-5 minutes) required of an ideal anaesthetic (Marking and Meyer,1985; Iversen,2003; Coyle *et al*,2004; Mylonas*et al*,2005; Brown,2011) is considered, the experiment with aqueous bark

extract of *T. vogelii* closely meet the requirement of an ideal anesthetic. Comparison of the potency of the aqueous bark extract of *T. vogelii* taking the corresponding recovery time into consideration revealed that the aqueous bark extract tranquilized *H. longifilis* with an induction time of 52.33 seconds and a recovery time of 125.33 minutes at concentration 0.01g/ℓ. Similar comparison between Eugenol and other anaesthetic agents reported by other researchers revealed the notable power of Eugenol as an anaesthetic agent (Keene *et al*,1998; Griffiths,2000;Cooke *et al*,2004; Filiciotto*et al.*, 2012).

The recovery time in agreement with other researchers (Peake, 1998; Griffiths, 2000; Solomon and Amali, 2004; Filiciotto *et al*,2012) tended to increase with increasing concentration of the aqueous bark extract of *T. vogelii*. Hseu *et al*,(1998) reported that higher drug concentration or dose increase recovery time. In the case of immersion anaesthetics Griffiths,(2000) and Tort *et al*,(2002) suggested that this may be due to the fact that higher dose induced anaesthesia more rapidly thus allowing the the experimental fish to be removed from the anaesthetic bath and placed in clean water earlier than fish exposed to lower doses. Since the degree of anaesthesia is influenced by the concentration of the anaestheticin the CNS of the experimental fish (Trevor and Miller,1987), in the present study where the parenteral route of anaesthesia was used this may be explained by the fact that more of the active ingredients of the anaesthetic extracts accumulated in CNS of the fish at higher concentrations thus suppressing the activity of the CNS to a greater degree than at lower concentrations and consequently prolonging the recovery time.

Chemical anaesthetic agents have been used in handling and transportation of fish to reduce mortality which occurs as a result of excitement and hyperactivity (Shoettgel *et al*, 1967). It has been suggested that the long recovery time observed with clove essence could be an added advantage in activities such as morphological evaluation, biopsy and stripping which require long handling periods outside water (Anderson *et al*, 1997; Munday and Wilson, 1997; Park *et al*, 2009). In addition, light sedation is desirable during transportation of fish (Summerfelt and Smith, 1990). This is because fish anaesthetized at deep sedation (anaesthetic stage 4) levels lose equilibrium and may sink to the bottom, pile up and finally suffocate (Dupree and Huner, 1984). Since transportation of fish often involve long distances and time, the long recovery time of *T. vogelii* aqueous bark extract could be considered as an advantage for use as a tranquilizer in the delivery of fish over long distances and other handling procedures such as morphological evaluation, biopsy and stripping.

In this experiment with aqueous bark extract of *T.vogelii* no mortalities were recorded in a 48 hour post-anaesthetic period similar to reports by Mohammed, (1999) and Altun and Danabas, (2006). However, three sea bass (*Dicentrarchuslabrax*) were reported dead at the two highest concentrations when anaesthetized with eugenol (Filiciotto*et al*, 2012), and the death of experimental fish was attributed to the capacity of eugenol to markedly induce anaesthesia which could cause death.

CONCLUSION

The absence of mortalities in the experiment with the aqueous bark extract of *T. vogelii* in the present study is because the various concentrations of the anaesthetic extract used in the research lack the capacity to induce deep anaesthesia in the experimental subjects. The colour change observed with experimental fish after anaesthetization with *T. Vogelii* aqueous bark extract from light brown to light orange colour could be due to the effect of the anaesthetic extract on the cells and tissues of the epidermis. We recommend however that this observation be subjected to further investigation.

REFERENCES

Altum T., Bilgin, R and Danabas, D (2009).Effects of sodium bicarbonate on anaesthesia of common carp (*Cyprinuscarpio* L, 1758). Juveniles. *Turkish journal of fisheries and aquatic sciences* 9:29-31.

Anderson, W.G, Mckinley, R.S and Colavecchia, M (1997). The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. *NorthAmerican Journalof Fisheries Management* 17:301-307.

APHA/AWWA/WPCF: American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1985). *Standard methods for the examination of water and wastewater*, 16thEdn., APHA, Washington D. C., USA,1265.

Bagheri, T and Imanpoor, M.R (2011).The efficacy, physiological responses andheamatology of Persian sturgeon *Acipenserpersicus* to clove oil as an anaesthetic agent.*Turkish journal of fisheries and aquatic sciences. 11:477-483*

Brown, L. A. (2011). Anaesthetic for fish. Seminar on application of anaesthetic agents in aquaculture held in Can tho on 1st December, 2010. http://www.vietfish.org./49c70/aqua.htm. Accessed: 17.06. 2011.

Colye, S.D, Durborow, R. M and Tidweu, J.H (2004).Anaesthetics in aquaculture. *SRAC publications* No. 3900.

Cooke, S.J, Suski, C.D, Ostrand, K.G, Tafts, B.L and Wahl, D.H (2004).Behavioural and physiological assessment of low concentrations of clove oil anaesthetic for handling and transportating largemouth bass *Micropterussalmoides. Aquaculture*, 239:509-529.

Filiciotto, F, Buscaino, G, Buffa, G, Bellante, A, Maccorrone V, and Mazzola, S. (2012).Anaesthetic qualities of Eugenol and 2 – phenoxyethanol and their effects on some heamtological parameters in farmed European Sea Bass (*Dicentrachuslabrax* L). *Journal of Animal and Veterinary Advances.* 11 (4): 494 – 502.

Hseu, J.R, Yeh, S.L, Chu, Y.T, Ting, Y.Y (1998). Comparison of efficacy of five anaesthetics in godlinedseabream (*sparussarba). Actazool Thaiwan*, 9:11-18.

Iversen, M, Finstad, B, Mckinley S, and Elisassen, R.A (2003). The efficacy of metomidate, clove oil, Aqui-Sk and Benjoak R as anaesthetics in Atlanta Salmon (*Salmosalar)* Smoolts and their potential stress-reducing capacity. *Aquaculture 221:549-566.*

Marking, L. L, and Meyer, F.P, (1985). Are t anaesthetics needed in fisheries.*Fish 10(6): 2–5*

Mohammed, S. J. (1999). Comparative efficacy of four anaesthetic on common Carp,*Cyprinus carpio* L. *Acta Ichthyological et piscatoria* 39: 91-97.

Munday P.L. and Wilson S.k (1997).Comparative Efficacy of Clove oil and other Chemicals in Anaesthetization of *Pornacentrasambionensis*, a Coral Reef Fish. *Journal of Fish Biology*. 51(5):931-938

Mylonas, C.C, Cardinalethi, G, Sigelaki, I, Polzonethimagni, A. (2005).Comparative efficacyof clove oil and 2–phenoxyethanol as anaesthetics in the aquaculture of European Sea bream (*Sparusaurata*) at different temperatures.*Aquaculture*, 246:467 – 481.

Neiffer, D.L and Stamper, M.A (2009). Fish sedation, Anaesthetic, Analgesia and euthanasia: considerations, methods and types of Drugs. *ILAR Journal*, 50:343-360.

Omoniyi, I., Agbon, A.O and Sodunke, S.A (2002). Effect of lethal and sublethal concentrationsoftabocco (*Nicotiana tobaccum)* leaf dust extract on weight and heamatological changes on *Clarias gariepinus* (Burch). *Journal of Applied Science*, 2(4):382-387.

Park, M.O, Im, S.Y, Seol, D.W and Park, I.S (2009). Efficacy and physiologicalresponses of Rock Bream (*Oplegnathus faciatus)* to anaesthetization with clove oil. *Aquaculture. 287:427-430.*

Peak, S. (1998). Sodium bicarbonate and Clove oil as potential anaesthetics for non-salmonid fishes.*North American Journal of Management*, 18:919-924.

Ross, L.G and Ross, B (1984).Anaesthetic and sedative techniques for fish. Glasgow: Nautical Press. Pp 122-123.

Schottgel, R.A, Walker, C.R., Marking, L.L and Julin, A.M (1967). Ms-22 as an anaestheticfor channel catfish; Its toxicity, efficacy and muscle residues. U.S Fish and Wildlife service investigation in fish control. Pp 17.

Solomon, S.G and Amali, E.I (2004).Preliminary investigation into the use of a local plant, *Daturainnoxia* as tranquilizer for Mudfish, *Clariasgariepinus*Burchell (1822). In: *18th Annual Conference of the Fisheries Society of Nigeria (FISON) 8-12th December,* 2003. Pp 43-47.

Summerfelt, R. C. and Smith, J. S/ (1990). Anaesthetic surgery and related techniques. In: C.B. Shreck and P.M. Moyle (eds). Methods of fish biology. American Fisheries society, Bethesda MD, Pp 213-172.

Torts, L. Puigcerver, M., Crespo, S. and Padros F. (2002). Cortisol and haematological response in Sea bream and Trout subjected to the anaesthetics clove oil and 2-phenolyttanol. *Aquaculture Research* 33:907-910.

Trease, G.E and Evans,W.C (1989). Textbook of pharmcognosy, 12thedn.Balliere, Tinadl. London. Pp45-50.

Trevor, A.J and Miller, R.D (1987).General anaesthetics.Basic and chemical pharmacology 93rd (ed) Pp 246-253.