EFFECTS OF AQUEOUS LEAF EXTRACT OF *TEPHROPSIA VOGELI* AS A TRAQUILIZER ON THE AFRICAN CAT FISH *HETEROBRANCHUS LONGIFILIS* VAL. (PISCES 1840).

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ABSTRACT

This study was carried out to evaluate the potential of the aqueous leaf Extract of *T. vogelii* as a tranquilizer on the African Catfish, *Heterobranchus longifilis* Juveniles (Mean weight 115.00±25.00g) were abtained from wild stock caught from the Benue River, Nigeria. The fish were acclimatized under laboratory conditions for two weeks in plastic tanks of 70 litre capacity filled with 40 litres of water prior to the commencement of the experiment. The fish were fed once a day at 09.00 hours at 4% body weight during the period of acclimatization. Each tank containing acclimatization water was aerated to enhance dissolved oxygen, and water was changed daily to prevent metabolic waste build up in order to maintain good water quality. Experimental fish were starved for 24 hours prior to sedation to prevent regurgitation from the gastro-intestinal tract (GIT). Four healthy *H. longifilis* were selected randomly from both control and treatment groups. Each fish was weighed and injected 0.05ml of the extract at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l using No.23 needle and a 2ml heparinized 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 distilled water. Injected fish were observed for behavioural responses. The result showed that *H. longifilis* injected with the aqueous leaf extract of *T. vogelii* passed sequentially through the first three stages of anaesthesia but could not attain total loss of equilibrium (stage 4 of anaesthsia). The result also showed that there was no significant difference in opercular beat after sedation in all concentration used (P>0.05). The result generally showed at higher concentration (0.06g/l) time of anaesthesia decrease to 44.67 seconds while at 0.02g/l concentration time of anaesthesia was 83.70 seconds Behavoural responses included mucus secretion, slow and erratic swimming, excrement discharge, increase in opercular beat rate, strong retension of reflex action, partial loss of equilibrium

and colour change. The effective concentration was 0.06g/l with an induction time of 44.67 seconds and a recovery time of 547.00 minutes.

Key words: Anaesthetics, tranquilizer, aquaculture, aqueous, induction, recovery, time.

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INTRODUCTION

The use of chemical anaesthetics in fisheries and aquaculture to immobilize fish started 60 years ago. Brown,(2011). Anaesthetics have been used in fisheries to minimize stress in fish caused by strainous sampling procedures such as weighing, tagging, collection of scales, transportation, treatment of disease etc. Anaesthetics also reduce excitement and hyperactivity – related trauma that occur during routine handling, and thus directly reduce mortality and morbidity. Cooked *et al*, (2004); Harms, (1999); Ross, (2001).

The restriction of several chemical anaesthetics for use with food fish is because they are not biodegradable, have negative environmental impact and health risk on both the fish and the handler. This inadequacy of chemical anaesthetics has resulted in a renewed interest to develop green (plant-derived) chemical anaesthetics with low environmental impact and health risk. Ramanayaka and Atapatu, (2006).

The leaves of plants are indiscriminately used for catching fish in water bodies in several parts of the Nigeria, yet there are no documented effects of the plant materials on important fish species. Gabiel and Okey, (2009). *Tephrosia vogelii*, commonly referred to as "fish poison bean" is one of such plant materials. Locally known as "Kuhwa Indyar" (in Tiv), *Tephrosia vogelii* has long been used by the Tiv people of Benue State in central Nigeria to kill fish in water bodies. *Tephrosia vogelii* is a perennial plant, grows 2 – 3 metres high, and ramified. The bar is grey–brown with yellowish or more or less fissured and lenticellet. Stems are grey–brown with yellowish or rust coloured dense pubescence, and flowers white, pink or purplish, assymetrical, 3 – 4 cm long with dense pubescent widely–toothed calyx.

The leaves of *T. vogelii* contain a number of different compounds commonly known as rotenoids which are effective against fish and various insects. These compounds include rotenone, dequelin, dehydrodeguelin, elliptone, 12a–hydronyrotenone, rotenone and tephrosin. Ingham (1983); Marston *et al* (1984); Mc David and Lesseps, (1994). Rotenone is reported to be the chemical that has pesticidal and piscicidal property in *T. Vogelii,* and with an acute toxicity of 132–1,500mg/kg is capable of killing fish and vegetable pests. Geradzirayi *et al*, (2009).

The African Catfish, *Heterobranchus longifilis* is one the most important cultured fish species in Nigeria. It has such important qualities as the ability to withstand handling stress, fast growth rate, high yield potential, high fecundity, palatability and consumers preference. Offem *et al*, (2008). This study was undertaken, primarily to evaluate the potential of *Tephrosia vogelii* aqueous leaf extract as a tranquilizer on *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 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 between july and september, 2011 and air-dried under shade for 21days. The samples were then oven–dried at 60^0c for 3 – 4 hours to constant weight (Omoniyi *et al*, 2002). The dried samples were pulverized to powder using an electric kitchen blender and store 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 phyto- chemical analysis to determine the chemical constituents (alkaloibs, Saponins, tannins, anthroquiniones, flavonoids etc) using standards methods described by Harbone, (1973) and Trease and Evans,(1989).

Prior to the administration of the aqueous leaf extract on the experimental fish a pre-experimental trial was carried out using standard procedures following the methods of APHA (1998) 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. Water quality parameters were measured and found to be within normal range as shown in table 2.

The administration of various concentrations of *T. vogelii* leaf 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 weighed and injected 0.5ml of the extract concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/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. Nelffer and Stamper, (2009). Fish in the control group were injected with the same done of distilled water. Gabriel et al.(2009). 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 abandoned after 60 minutes when the fish failed to reach anaesthetic stage 4, since periods greater than this were considered impractical for routine fish handling procedures. Agokei and Adebisi (2010).

The statistical analysis of the results obtained from the behavioural responses of the fish to aqueous leaf 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 results of the photochemical analysis of *T. Vogelii* leaf extract revealed the presence of tannins, saponins, flavoniods, phlobatannins, alkaloids, glycosides and phenols. The results of the pre-experimental test showed that all the fish injected with the various concentrations (0.01, 0.2, 0.3, 0.4, 0.5 and 0.6g/l) of *T. Vogelii* of leaf extract died in less than one hour. However, all those injected with the concentrations: 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 g/l survived without any mortality. Therefore, these later concentrations of *T. vogelii* leaf extract were considered ideal for use on the African Catfish *H. longifilis.* Some water quality parameters where monitored to ensure that there was no departure from the ideal as shown in Table 1.

Owing to the variations in the weight of the experimental fish the data was subjected to analysis of co-variance using weight as a covariate, as shown in table 2. The result of the behavioural responses in *Heterobranchus longifilis* exposed to the aqueous leaf extract of *T. Vogelii* revealed that the experimental fish attained the first three stages (tranquility period, excitation period and light anaesthesia) of anaesthesia. Behavoural responses included mucus secretion, slow and erratic swimming, excrement discharge, increase in opercular beat rate, strong retension of reflex action, partial loss of equilibrium and colour change. The time taken to traquilize (induction time) increased with increase in concentration of the anaesthetic solution.

Recovery time from anaesthesia followed the reverse order whereby faster recovery was observed with low concentration of the anaesthetic extract. The opercula beat rate (OBR) after sedation in the treatment group showed increase over that in the control and it increased with increase in concentration. The percentage change in opercular rate decreased marginally with increasing concentration of the anaesthetic extract.

Table 1: Mean values of water quality parameters during exposure of H. longifilis to various concentrations of T. vogelii aqueous leaf extract

Conc. (g/ℓ)	Water quality parameters			
	Temperature ˚C	Dissolved oxygen (mg/l)	PH	Alkalinity (mg/l)
0.00	27.30±0.00^{d}	7.24±0.01 ^{a}	7.12±0.02^{a}	-
0.01	27.31±0.00^{cd}	7.24±0.01 ^{a}	6.30±0.03^{e}	-
0.02	27.31±0.01^{cd}	7.24±0.01 ^{a}	6.32±0.01^{de}	-
0.03	27.32±0.01^{bc}	7.23±0.01 ^{a}	6.35±0.01^{d}	-
0.04	27.32±0.00^{bc}	7.23±0.01 ^{a}	6.36±0.01^{cd}	-
0.05	27.33±0.01^{ab}	7.24±0.00 ^{a}	6.43±0.01^{b}	-
0.06	27.34±0.01^{a}	7.24±0.01 ^{a}	6.41±0.01^{b}	-

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

Figure 1 shows the comparison of stages of anaesthesia in *Heterobranchus longifilis* injected with various concentrations of *T. Vogelii* aqueous extract. This result revealed that the highest value of induction time was 26.67±1.76 seconds at concentration 0.01g/l while the lowest value of induction time was 15.33±1.45 seconds at concentration 0.06g/l at anaesthesia stage 1. The highest value of induction time was 44.00±4.00 seconds at concentration 0.01g/l while the lowest value was 24.00±0.58 seconds at concentration 0.06g/l at anaesthesia stage 2. The highest value of induction time was 88.00±17.00 second at concentration 0.01g/l while the lowest induction time was 44.471.76 seconds at concentration 0.06g/l at anaesthesia stage 3. The highest value of opercular beat rate before sedation (OBR BFS) was 69.00±0.67 Beats/Minute at concentration 0.06g/l while the lowest value was 64.67±1.33 Beats/Minutes at concentration 0.01g/g. The highest value of opercula beat rate after sedation (0BR AFS) was 71.67±0.67 Beats/Minute at concentration 0.06g/l while the lowest value was 67.33 ± 1.20 beats/minute at concentration 0.01g/l. The highest value of percentage change in OBR was 8.59±1.92% at concentration 0.02g/l while the lowest value was 3.87 ± 0.49% at concentration 0.06g/l. The highest value of recovery time was 547.00±6.24 minutes at concentration 0.06g/l while the lowest recovery time was 503.33±3.33 minutes at concentration 0.01g/l. Mortality was not recorded among treatment groups in a post-anaesthetic period of 48 hours.

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

Conc. (g/ℓ)	Weight Of fish (g)	Volume injected (Ml)	Behavioural Responses							
			Induction Time(Seconds) in Stages of Anaesthesia			OBR(M^{-1})		Percentage increase in OBR (%)	Recovery time (Minutes)	Mortality After 24Hrs
			I	II	III	BFS	AFS			
0.01	73.33±1.67^{e}	0.5	26.67±1.76^{a}	44.00±4.00^{a}	88.00±17.00^{a}	64.67±1.3^{a}	67.33±1.20^{a}	4.14±0.56^{a}	503.33±3.33^{a}	-
0.02	73.33±1.67^{e}	0.5	22.67±1.20^{a}	35.33±2.40^{a}	83.70±13.40^{a}	66.33±0.8^{a}	72.00±0.58^{a}	8.59±1.92^{a}	511.67±4.40^{a}	-
0.03	87.33±3.33^{d}	0.5	20.33±1.45^{a}	33.00±7.09^{a}	65.30±18.30^{a}	66.67±1.2^{a}	70.33±1.45^{a}	5.49±0.44^{a}	516.67±4.8^{a}	-
0.04	103.3±3.33^{c}	0.5	18.33±1.45^{a}	32.00±1.53^{a}	55.33±6.89^{a}	67.00±1.5^{a}	70.00±0.5^{a}	4.55±1.63^{a}	524.67±4.9^{a}	-
0.05	124.00±1.00^{b}	0.5	16.33±1.45^{a}	25.33±0.88^{a}	47.00±1.53^{a}	68.00±0.5^{a}	71.00±1.15^{a}	4.40±0.81^{a}	542.00±8.08^{a}	-
0.06	145.00±2.89^{a}	0.5	15.33±1.45^{a}	24.00±0.58^{a}	44.67±1.76^{a}	69.00±0.6^{a}	71.67±0.67^{a}	3.87±0.49^{a}	547.00±6.24^{a}	-

- ❖ 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 presence of tannins, saponins, flavonoids, phlobatannins, alkaloids, glycosides and phenols in the leaves of *T. Vogelii* following phytochemical analysis shows similarity with reported work of Olatayo, (2005) and Makoshi and Arowolo, (2011) who reported the presence of tannins, saponins, alkaloids and glycosides in the leaves of *T. vogelii*. Ingham, (1983) and Marston *et al*, (1984) reported the presence of rotenoids including deguelin, dehydrodeguelin, elliptone, 12a-hydroxyrotenone, rotenone and tephrosin in various parts of *T. vogelii* and attributed the insecticidal and pesticidal activities of the plant to these active ingredients. Previous studies have shown that the active ingredients found in *T. vogelii* can impact negatively on fish. For instance, saponins are ichthyotoxins which destroy erythrocytes (Epel,2000) while alkaloids inhibit oxidative phosphorylation and block the mitochondria enzyme, NADH ubiquinone reductase, thus impairing their oxygen consumption (Block, 1984; Tiwari and Singh, (2003).

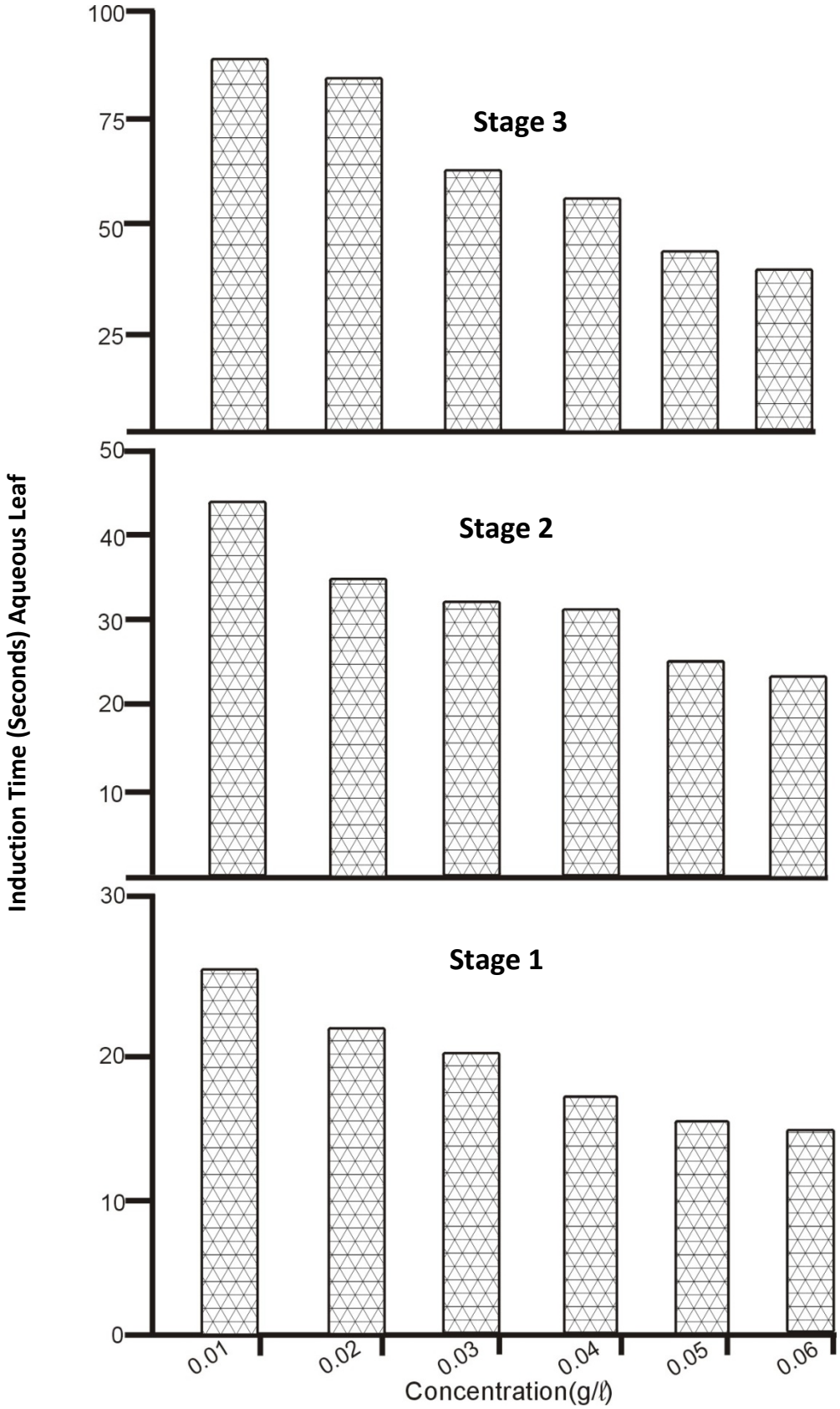

Figure 1: Comparison of Mean values of Induction Times in the Three Anaesthetic stages in *H. longifilis* injected with various concentrations of *T. vogelii* Aqueous leaf extract.

The route of administration of anaesthetics commonly used in research is immersion anaesthesia. However, in the present study the parenteral route of anaesthesia was adopted as recommended for air–breathing fish. Brucher and Graham, (1993). This is because such fish species in responding to confinement or hypoxic anaesthetic baths pull air from the surface water and reduce or temporarily stop opercular movement, and the decreased breanchial contact in water results in a slower rate of anaesthetic uptake. Hseu *et al*, (1997).

The result shows that *H. longifilis* juveniles treated with aqueous leaf extract of *T. vogelii* passed sequentially through the first three stages of anaesthesia (tranquillity period, excitement period and light anaesthesia) and the experimental fish were successfully tranquilized at all levels of concentration similar to the reported work of Altun *et al*, (2009) in common carp (*Cyprinus carpio)* juveniles exposed to sodium bicarbonate.

The time taken for the fish to enter the desired stage of anaesthesia (induction time) decreased with increasing concentration of the anaesthetic extract as reported in other studies Hseu, (1998); Yanar and Kumlu, (2000); Filicitto *et al*, (2012). This observation also agrees 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 study the increase in time taken to tranquilize the experimental fish with increased concentration of the *T. vogelii* leaf extract may be attributed to the accumulation of the active ingredients, in this case rotenoids, in the body system of the fish which impairs the activity of the CNS at a much faster rate. Solomon and Amali (2004). The failure of anaesthetized fish to enter deep anaesthesia (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 the anaesthetic than smaller individuals. Colye *et al*, (2004). This could also be due to the stage of the life cycle, age, lipid content and body condition, all of which are biological factors that influence metabolic rate and therefore the pharmacokinetics of the anaesthetic compound. Iversen *et al*, (2003). The result further indicated that the effective concentration of the aqueous leaf extract of *T. vogelii* was 0.01g/l at which the least induction and recovery times of 26.67 seconds and 503.33 minutes were obtained at anaesthetic stage 3. This induction time is similar to the induction time of 30.10 and 30.70 seconds reported for *Valamugil cunnesius* and *Monodactylus argenteus* respectively, following clove oil anaesthesia (Durville and Collet, (2001), and the 1.5minutes for *Acepenser perscicus* exposed to clove oil. Begheri 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, 2001) is considered the aqueous leaf of *T. vogelii* extract closely meet the requirement of an ideal anaesthetic.

Chemical agents have been used in the handling and transportation of fish to reduce mortality which occurs as a result of excitement and hyperactivity. Schoetgel *et al*, (1967). It has been suggested that the long recovery time of 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). It is has also been suggested that 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 to death. Dupree and Huner, (1984). Since transportation often involve long distances, the long induction time of *T. vogelii* leaf extract could be considered for use as a tranquilizer in the delivery of fish over long distances and other handling procedures such as morphological evaluation, biopsy and striping.

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