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ORIGINAL ARTICLE

Behavioural Responses of *Heterobranchus longifilis* Juveniles. Val (Pisces: 1840) Exposed to Freeze–dried Bark Extract of *Tephrosia vogelii* as an Anaesthetic

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This study evaluates the anaesthetic properties of freeze-dried leaf extract of *Tephrosia vogelii* on the African catfish *Heterobranchus longifilis* juveniles. Experimental fish of Mean weight 115.00 were obtained from River Benue at Makurdi, Nigeria and acclimatized at the hatchery of University of Agriculture Makurdi for two weeks. Four *H. longifilis* were selected randomly for both control and treatment groups. Each treatment fish was weighed and injected intramuscularly 0.05ml of the extract at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l using a 2ml heparinized syringe. The result showed that H. longifilis in treatment group passed sequentially through the first three stages of anaesthesia but could not attain total loss of equilibrium (stage 4 of anaesthesia). The result showed that treatment group of fishes passed sequentially through the first three stages of anaesthesia but could not attain total loss of equilibrium (stage 4 of anaesthsia). Behavioural responses included mucus secretion, slow and erratic swimming, excrement discharge, increase in opercular beat rate, strong retention of reflex action, partial loss of equilibrium and colour change. The induction time showed a declining pattern with increasing concentration of the extract in the treatment levels with significant differences ($P<0.05$) observed at all three stages of anaesthesia. Recovery time showed the reverse order with significant differences ($P<0.05$) at all levels of concentrations. The opercular beat rate before and after injection, did not follow a definite pattern ($P>0.05$). The most effective concentration was 0.06g/l with an induction time of 32.00±1.76 seconds and a recovery time of 182.00±3.46 minutes. The result of this study revealed that the freeze-dried bark extract of *T. vogelii* can be used as a tranquilizer for transporting fish over average distances, biopsy and morphological evaluation.

Key words: Anaesthetics, tranquilizer, freeze-dried, bark, induction, recovery, time

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The use of various piscicidal plants such as *Blighia sapida, Kiglia Africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa* and *Tephrosia vogelii* by fisher folks to catch fishes in Nigeria's has been reported (Obomanu, 2007). Several of these piscicidal plants including those that are not listed here have been used in various parts of Nigeria over a long time to catch fish but very few studies have so far been carried out to document the effects of these plants on the fish,

man, the environment and other aquatic fauna that co-inhabit the aquatic ecosystem with the target fish.

Tephrosia vogelii (fish poison bean) is a perennial shrub that grows 3–4 metres high, branching low and ramified, stems are grey – brown with yellowish or rust–coloured dense pubescence. Leaves alternate, imparipinnate, 10–25cm long. Leaflets elliptic, 2.5–8.5cm long and 0.6–2.5cm wide with a macronate–round apex and cuneate tapering base. Flowers white, pink or purplish, 3–4cm long with dense pubescent widely – toothed calyx (Michael, 2002).

Tephrosia vogelii contains rotenone, deguelin, dehydrodeguelin, elliptone, 12a–hydronyrotenone, and tephrosin in various parts of the plant and the insecticidal and pesticidal properters of the plant are attributed to these active ingredients (Ingham, 1983; Marston *et al*., 1984). Stevenson *et al*., (2012) reported that *T. Vogelii* C_1, found in the leaves contains rotenoids as the main flavonoid aglycone. This report further shows that *T. Vogelii* C_1 is an effective pest control alternative to synthetic pesticides owing to the occurrence of insectidical rotenones in the foliage whereas the flavonoid aglycone of T. *Vogelii* C_2 has no such pesticidal activity.

The various parts of the *T. Vogelii* are used for the treatment of dyspepsia, tooth decay, rheumatism and the effective control of ecto-parasites in dairy cattle (Zimbabwe) (Mshana *et al*., 2000; Gadzirayi, 2009).

Heterobranchus longifilis is one of the most important economic 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 consumer's preference (Offem *et al*., 2008).

MATERIALS AND METHODS

Fresh samples of *T. Vogelii* bark were collected during the rainy season between July and September 2011. The samples were air-dried for 21 days under shade and then, Oven- dried for 3-4 hours to constant weight (Ominiyi *et al*., 2003). The dried samples were pulverized to powder using an electric kitchen blender and stored in air-tight laboratory bottles. 200g of the stored bark samples of *T.vogelii* was weighed into a flat–bottom flask of 2.5 litre capacity and 1 litre of distilled water was added to cover the samples. The flask was covered, shaken and allowed to stand for 24 hours. The mixture was then filtered using Muslin Cloth and sunction fitration. The fitrate was concentrated using Rotary Evaporator and then dried using Iyovat gt3 freeze-drying machine. The dried extracts were weighed into samples bottle and stored. The anaesthetic solution of the freeze-dried bark extract of *T. vogelii* was prepared by dissolving graded series of stored freeze–dried samples of *T. vogelii* (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) in distilled water under laboratory condition for 24 hours at room temperature (27.00 ± 0.04^0C) and the mixture filtered using No. 1 Whatman filter paper.

The administration of the anaesthetic solution of the freeze–dried bark extract of *T. Vogelii* was carried out using the parentenal (injection) method of anaesthesia. Before sedation the fish were starved for 24 hours to prevent regurgitation from the gastro-intestinal tract (GIT), and observation and recovery baths provided with aeration. Four healthy *Heterobranchus longifilis* juveniles were selected randomly from both the control and the treatment groups. Each was weighed and injected 0.5ml of the extract concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) using No. 23 needle and a 2ml syringe. Injection was done

intramuscularly (IM) at the dorsal saddle, just above the lateral line, behind the operculum (Neiffer and Stamper, 2009). Injected fish were observed for behavioural responses and transferred into 70-litre plastic tanks containing 40 litres of water for recovery and time taken to recover recorded.

Statistical Analysis

Statistical analysis of the results obtained was carried out using Genstat Discovery Edition 4 for one–way Analysis of variance (ANOVA) to determine differences in behavioural responses. Summary statistics were obtained for the variables using Minitab 14 for windows. Significant differences were accepted if the P-value was less than 0.05

RESULTS

PRE-EXPERIMENTAL TESTS

The result of preliminary experimental tests to determine suitable concentrations of freeze-dried bark extracts of *T. vogelii* to be used in the research showed that all the fish injected with the various concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6g/ℓ) of *T. vogelii* died within a period of one hour. However all those injected with the concentrations: 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/ℓ survived without mortality. These concentrations of the various extracts of *T. vogelii* were therefore considered ideal for use in the administration of *T. vogelii extracts* on the African catfish *H. longifilis.*

ADMINISTRATION OF *TEPHROSIA VOGELII* FREEZE-DRIED BARK EXTRACTS AS A TRANQUILIZER TO *H. LONGIFILIS*.

Table 1 is the result of sedation of *H. longifilis* with freeze- dried bark extract of *T. Vogelii*. The entrance of experimental fish to anaesthesia (induction time) clearly shows a declining pattern with increasing concentration of the extract in the treatment levels. Significant differences ($P<0.05$) were observed in time taken for fish to enter anaesthesia at all three stages of anaesthesia. Opercular beat rate before and after injection, increased with increasing concentration without significant differences ($P>0.05$). Recovery time ranged from 140.67 minutes (2.20 hours) to 163.00 minutes (3.43 hours) and increased steadily with increasing concentration, with significant differences ($P< 0.05$).

The discolouration of anaesthetized fish from light brown to light orange was observed. In this experiment mortalities were not recorded after a post asnaesthetic period of 48 hours. Anaesthetic effects were not observed in the control fish which swam actively in water and behaved normally without reaching any stage of anaesthesia during the 60 minutes period of observation.

Figure 1 shows the result of recovery time in *H. longifilis* treated with various concentrations of freeze-dried bark extract of *Tephrosia vogelii*. The result showed that recovery time was longer with concentration of 0.06g/l.

Figure 2 shows the result of the opercular beat rate before sedation (OBR BFS) and opercular beat rate after sedation (OBR AFS) in *H. longifilis* obtained with the freeze-dried bark extract of *T. vogelii.* The result showed that OBR AFS was higher than OBR BFS in all the six concentrations used for this study.

Figure 3 shows the result of percentage change in opercular beat rate in H. longifilis treated with various concentration of freeze-dried bark extract of Tephrosia vogelii. The result showed that percentage change in opercular beat was highest at concentration of 0.01g/l.

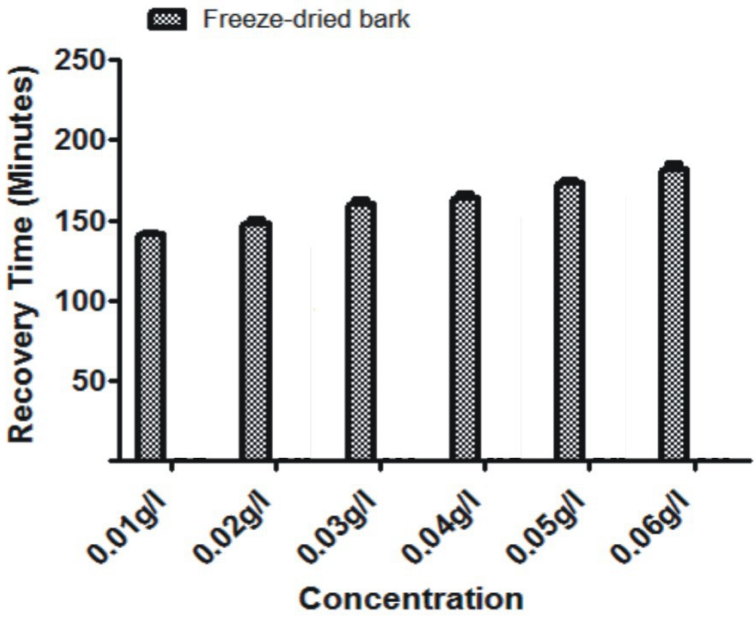

Figure 1 : Mean values of recovery time in *H. Longifilis* injected various concentration of *T. vogelii* freeze-dried bark extract.

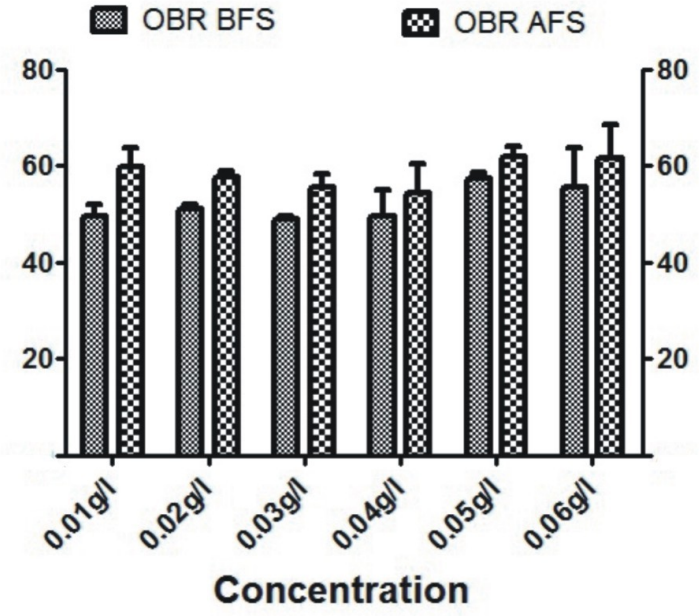

Figure 2 : Comparison of Mean values of opercular beat rate before and after sedation in H. longifilis injected various concentrations of T. vogelii Freeze-dried Bark extract.

OBR BFS = Opercular Beat Rate Before Sedation
OBR AFS = Opercular Beat Rate After Sedation

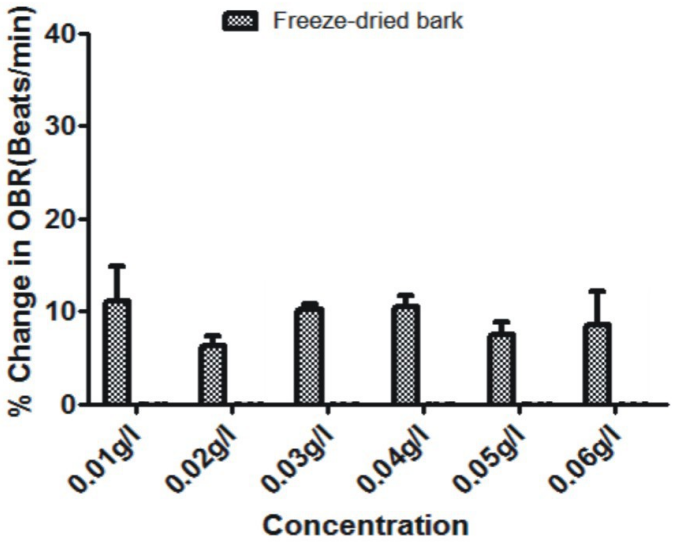

Figure 3 : Mean values of percentage change in opercular beat rate and recovery time in *H. longifilis* injected various concentration of *T. vogelii* freeze-dried Bark extract.

Table 1 : Behavioural responses of H. longifilis injected with various concentrations of *Tephrosia vogelii* Freeze-dried bark extract.

Conc. (g/ℓ)	Weight Of fish (g)	Volume injected (ml)	Behavioural Responses: Induction Time (Seconds) Stages of Anaesthesia I	II	III	OBR (M^{-1}) AI	BI	Percentage increase in OBR (%)	Recovery time (Minutes)	Mortality After 48hrs
0.01	77.33±3.38 [a]	0.5	24.67±1.76 [a]	35.67±4.48 [a]	57.33±6.69 [a]	56.33±3.18 [a]	62.33±1.76 [a]	11.06±3.78 [a]	140.62±1.76 [d]	-
0.02	80.67±3.48 [a]	0.5	22.00±1.15 [a]	29.33±0.33 [b]	43.00±3.2 [b]	58.00±1.00 [a]	61.67±1.67 [a]	6.28±1.02 [a]	147.33±3.53 [cd]	-
0.03	86.33±3.48 [a]	0.5	19.67±0.88 [b]	25.67±1.67 [bc]	39.33±2.03 [bc]	59.00±1.73 [a]	65.00±2.31 [a]	10.13±0.68 [a]	160.00±3.06 [bc]	-
0.04	74.33±3.48 [a]	0.5	18.33±1.20 [b]	23.00 [d]±2.08 [a]	35.00±1.53 [bc]	60.33±1.20 [a]	66.67±1.20 [a]	10.52±1.18 [a]	163.67±2.60 [bc]	-
0.05	75.00±2.89 [a]	0.5	14.00±1.15 [c]	21.00±0.58 [d]	34.33±2.85 [bc]	62.00±1.73 [a]	66.67±2.40 [a]	7.48±1.33 [a]	172.67±2.40 [ab]	-
0.06	78.33±1.67 [a]	0.5	11.67±0.88 [c]	19.33±0.88 [d]	32.00±1.73 [c]	62.67±2.03 [a]	68.00±3.21 [a]	8.50±3.39 [a]	182.00±3.46 [a]	-

OBR BFS = Opercular Beat Rate Before Seda

OBR AFS = Opercular Beat Rate After Sedation

Data were subjected to analysis of co-variance using weight as covariate

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

DISCUSSION

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

The results obtained from the present study shows that *H. longifilis* juveniles injected with various concentrations of freeze-dried bark extract of *T. Vogeliii* sequentially progressed 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 extracts appeared to be concentration dependent since faster tranquilization was achieved at higher concentration of the extracts as reported in other studies. (Hseu, 1998; Griffiths, 2000; Solomon and Amali, 2004; Mylonas *et al*., 2005). This observation is 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 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 individual generally require a greater concentration of anaesthetic than smaller individuals (Colye *et al*., 2004) or biological factors such as stage of the life cycle, age, lipid content and body condition of the fish, biological factors that influence the metabolic rate and therefore the pharmacokinetics of the anaesthetic compound (Iversen, 2003).

When the time taken for *H. longifilis* to enter anaesthesia (induction time) and recovery time are considered in the present investigation, significant differences ($P<0.05$) were recorded in the mean values of induction time obtained with the freeze-dried bark extract at all levels of concentration at anaesthetic stage 1-3 of anaesthesia depicting the effect of concentration on induction time. The induction time of 57.33 seconds obtained with freeze-dried bark extract of *T. vogelii* is in agreement with the average induction time of 1-2 minutes for the light sedation of common carp (*Cyprinus carpio)* juveniles exposed to sodium bicarbonate (Altun *et al*., 2009) and the 1.5 minutes reported for *Acipenser persicus* 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 the freeze-dried bark extract of *T. vogelii* closely meet the requirement of an ideal anesthetic.

The recovery time (Figure 1) is in agreement with other reported works (Peake, 1998; Griffiths, 2000; Solomon and Amali, 2004; Filiciotto, 2012). The recovery time increased with increasing concentrations. 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 induce anaesthesia more rapidly thus allowing the experimental fish to be removed from the anaesthetic bath and placed into clean water earlier than fish exposed to lower doses. However, since the degree of anaesthesia is influenced by the concentration of the anaesthetic in the CNS of the experimental fish (Trevor and Miller,1987), in the present study where the parenteral route of anaesthesia was used this may 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. The recovery time of 140.67 minutes obtained with the freeze-dried bark extract at concentration 0.01g/ℓ at anaesthetic stage 3 agrees with the recovery time of 135.3 minutes reported for the experiment with *Baringtonia raecemosa* extract on common carp (*Cyprinus carpio)* (Ramanayaka and Atapatu, 2006).

Chemical anaesthetic agents have been used in fish handling and transportation of live 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 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). It 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 botton, pile up and finally suffocate. Dupree and Huner, (1984). Since transportation of fish often involve long distances and time, the long recovery time of the freeze-dried bark extract of *T. vogelii* could be considered as an advantage for use as a tranquilizer in the delivery of fish over average distances and other handling procedures such as morphological evaluation, biopsy and stripping.

The opercular beat is an indicator of stress. It increases and decreases according to the type and concentration of anaesthetic (Altun and Danabas, 2006). Accordingly, it has been reported that enhancing the concentrations of quinaldine sulfate and diazapem from 2.5-20mg/ℓ increased the opercular rate in sea bream (*Sparus aurata*) while increasing the concentration of clove oil decreased opercular rate in sockeye salmon (*Oncorhyncus nerka*) (Kumlu and Yanar, 1999; Woody *et al*., 2002). In the present study involving *H. longifilis* with freeze-dried bark extract of *T. vogelii* the opercular beat rate (0BR AFS) after sedation of the extracts tended to be higher than values opercular beat rate before sedation (OBR BFS) of the extract (Figure 2) at all levels of concentration at light sedation (partial loss of equilibrium or stage 3 of anaesthesia). This observation is consistent with Solomon and Amali, (2004) that the OBR of *C. gariepinus* exposed to *Datura innoxia* increased as the fish attained partial loss of equilibrium. At deep sedation level i.e stage 4 of anaesthesia, the opercular rate of sea bass (*Dicentrarchus labrax*) exposed to quinaldine sulfate decreased until it almost ceased (Yanar and Kumijj, 2001). Filiciotto *et al*., (2012) similarly reported that the opercular rate of *Dicentrarchus labrax* treated with clove oil assumed values that were generally lower after administration of the anaesthetic and attributed this to the notable power of eugenol and its capacity to induce deep anaesthesia. In the present experiment the increase in the values of opercular rate after sedation of *H. longifilis* with the freeze-dried bark extract may be attributed to the failure of the anaesthetizing extract to induce deep anaesthesia in the experimental subjects since it is at deep anaesthesia level that the opercular rate after administration of an anaesthetic tends to decrease below the rate or value before administration of anaesthetic.

The mean values of percentage change in OBR in *H. longifilis* treated with the freeze-dried bark extract of *T. vogelii* showed that values obtained after the administration of the extract did not show marked deviations from the values obtained before sedation (Figure 3). It has been observed that

marked deviations in values of OBR from reference (control) suggests an adjustment in physical fitness as a result of stress condition (Edwards and Fushur 1991; Leight and Van Dolah, 1999). Since the values of percentage change in OBR after administration of the extracts in the present study did not show marked deviations from the values before sedation it appears reasonable to suggest that the experimental fish, *H.longifilis* were not negatively impacted by stress to warrant an adjustment in the physical condition.

In the present study with freeze-dried bark extract of *T. vogelii* extract no mortalities were recorded in a 48 hour post-anaesthetic period similar to reports of Mohammed, (1999) and Altun and Danabas, (2006). However, three sea bass (*Dicentrarchus labrax*) were reported dead at the two highest concentrations when anaesthetized with eugenol (Filiciotto *et al*., 2012), and the researchers attributed the death of experimental fish to the capacity of eugenol to markedly induce anaesthesia which could cause death. The absence of mortalities in the experiment with the freeze-dried bark extract of *T. vogelii* in the present study may therefore be explained by the fact that the various concentrations of the extract used in the research lack the capacity to induce deep anaesthesia in the experimental fish. The colour change observed with experimental fish after anaesthetization with the freeze-dried 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. This, however, is a matter for further investigation.

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