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Comparative Effects of Aqueous and Freeze Dried Leaf Extracts of *Tephrosia vogelii* on *Heterobranchus longifilis* Juveniles Val. (Pisces: 1840)

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Abstract: The present study was carried out to evaluate the potential of the aqueous leaf and freeze-dried leaf extracts of *Tephrosia vogelii* as tranquilizers on an African catfish, *Hetetobranchus longifilis* post juveniles (mean weight 115.00±25.00g) obtained from wild stock. They were acclimatized under laboratory conditions for two weeks prior to the commencement of the experiment. The fish were fed once a day at 9.00 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. Experimental fish were starved for 24 hours prior to sedation to prevent regurgitation from the gastro-intestinal tract (GIT). Four healthy *Hetetobranchus longifilis* were selected randomly from both the control and treatment groups. Each fish was weighted and injected 0.05ml of aqueous and freeze-dried leaf extracts at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/ℓ using №. 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 freeze-dried 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). Behavioural 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 induction time decreased with increasing concentration of the anaesthetic extract. The recovery time followed the reverse order. The opercular beat rates, before and after sedation in the treatment groups showed increase over that in the control group and it increased with increasing concentration. The result of the experiment with aqueous leaf extract showed that there was no significant difference in opercular beat rate after sedation in all centrations used (P>0.05). The result also showed that at higher concentration (0.06g/ℓ) induction was time 44.67 seconds while at concentration 0.02g/ℓ induction time was 83.70 seconds. In the case of the freeze-dried leaf extract of *T. vogelii* the induction at concentration 0.06g/ℓ was 49.33 seconds while at concentration 0.02g/ℓ induction time 76.67 seconds.

Keywords: *Heterobranchus longifilis*, Aqueous Leaf Extracts, Freeze Dried Leaf Extracts, *Tephrosia Vogelii*

1. Introduction

Chemical anaesthetics are now widely used for fish anaesthtics. However, inspite of the growing number of chemical anaesthetics now used in aquaculture, only MS-222 has been approved for use with food fish in the USA by the Food and Drug Administration (FDA) and in the European Union (EU) [40, 8]. This approval of MS-222 for use on edible fish notwithstanding, fish treated with MS-222 must be held for 21 days as required by law in those countries before human consumption. The restriction of several chemical anaesthetics for use with food fish is because they are not biodegradable, have negative environmental impact and health risks on both the fish and the handler. This

inadequacy of chemical anaesthetic has resulted in a renewed interest to develope green (plant–derived) anaesthetics with low environmental impact and health risks [33].

Tephrosia vogelii, locally known as "Kuhwa Indyar" (in Tiv) has long been used by the Tiv people of Benue State of Nigeria to kill fish in natural waters. Some 30-40 years ago, when community fishing was still a common practice this plant was very popular among the Tivs. However, with the advent of modernization many communities are no longer involved in fishing so the plant is becoming extinct.

The use of *T. vogelii* vary from one part of the world to another. The leaves are used for the treatment of dyspepsia and are highly toxic to cold blooded animals like mollusks, frogs and toads, worms and insects. They highly effective fish poisons for killing fish [4]. The roots are used for the treatment of tooth decay and rheumatism while the barn is used to treat boils [25].

The choice of the African catfish *Hetrobranchus longifilis* as the experimental fish in the present study is based on the economic importance of this fish species among cultured fish species in Nigeria. This fish species has such important qualilties as the ability to withstand handling stress, fast growth rate, high yield potential, high fecundity, palatability and consumer's preference. [29]. The objective of the present study is to investigate the possible use of *T. vogelii* as a tranquilizer for fish and to compare the effect of the aqueous leaf and freeze dried leaf extracts on *H. longifilis.*

The objective of the present study is to determine if there are remarkable differences in the induction and recovery times of the aqueous and the freeze-dried leaf extracts of *Tephrosia vogelii* as an anaesthetic drug.

2. Materials and Methods

Fresh samples of *T. vogelii* leaf were collected and air-dried under shade for 21 days. The samples were then oven-dried at 60˚C for 3-4 hours to constant weight [30]. The dried samples were pulverized to powder using an electric kitchen blender and stored in air-tight bottles for subsequent use.

The experimental fish *Heterobranchus longifilis* were purchased from wild stock caught from the River Benue. The fish were acclimatize in 70 litre-capacity plastic tanks filled with 40 litres of deionized water under laboratory conditions at the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi in Nigeria for a period of two weeks before exposure to sedation. During the period of acclimatization the fish were fed once daily at 09.00 hours at 4% of their body weight with commercial diet. Each tank containing acclimatization water was aerated. Water was changed daily to prevent metabolic waste build up to maintain good water quality. Some water quality parameters were measured using Hanna multiparater water Tester Model HI98129 and found to be desirable levels.

Preliminary experimental tests were carried out to determine suitable concentrations of aqueous leaf and freeze-dried freeze dried leaf extracts of *T. vogelii* that would be used for the sedation of the fish which would not result in their death. These anaesthestic solutions were administered on the fish through the parenteral route of anaesthesia. The fish were injected with 0.05ml of anaesthetic solution and immediately placed in anaesthetic free water and observed for behavioural responses such as stage of anaesthesia, opercular beat, mortality etc.

The aqueous extracts were prepared by desolving graded series of stored samples of *T. vogelii* in de-ionzed water contained in 2.5 litre air-tight bottles under laboratory conditions (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) at room temperature (27.0±0.4˚C) and allowed to stand for 24 hours after vigorous shaking. The settled portion was decanted and filtered through No. 1 Whatman filter paper. The filtrate was kept in air tight bottles and used as appropriate.

The preparation of the freeze-dried extract of *T. vogelii* leaf was done in the extraction laboratory of the National Institute for Pharmacitical Research (NIPR), Idu Industrial Estate, Abuja. 200g of the stored leaf samples of *T. vogelii was* weghed into a flat bottom flask of 2.5 litre capacity. 1 litre of water was added to cover the plant sample. The flask was shaken, covered and allowed to stand for 24 hours. The mixture was filtered using Mushlin Cloth and sunction filtration. For freeze-dried extact filtrate was concentrated using Rotary Evaporator and then dried using Iyovat gt3 freeze-drying machine. The dried extracts were weighed into sample bottles and stored. The anaethestic solutions of the freeze-dried extracts were prepared by dissolving graded series of the stored samples of *T. vogelii* in de-ionized water under laboratory conditions (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) for 24hours at room temperature (27±0.4˚C) and the mixture filtered.

The administration of the various *T. vogelii* aqueous and freeze-dried leaf extracts anaesthetic solutions was carried out in exactly the same maner, using the parenteral (injection) method of anaesthesia. Before sedation the fish were starved for 24 hours to prevent regurgitation from gastro-intestinal tract (GIT), and observation and recovery baths provided with aeration. Three healthy *Heterobranchus longifilis* post juveniles were selected randomly from both the control and treatment groups. Each fish 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 [28]. Fish in the control group were injected the same dose of distilled water [15]. Injected fish were observed for behavioural responses and transfered into 70 litre plastic tanks containing 40 litres of water for recovery, and time taken to recover noted. Continuous observation of the behavioural responses was abandoned after 60 minutes when the fish failed to reach the fourth stage of anaesthesia, since periods of time greater than this were considered impractical for routine fish handling procedures [1].

The statiscal analysis of the results obtained from the behavioural responses of test organisms to aqueous and freeze-dried leaf and bark extracts of *T. vogelii* was carried out using Genstat Discovery Edition 4 for one - way Analysis of Variance (ANOVA) to determine differences in

behavioural responses. Differences in the water quality parameters across the concentration used were also determined by ANOVA using Genstat Discovery Edition 4. Graphpad Prim 5 and SSC Stat V2.18 were used to test if differences existed between the behavioural variables measured for both the aqueous and freeze-dried leaf extracts of *T. vogelii*. Summmary statistics were obtained for the variables using Minitab 14 for windows.

3. Result

Table 1 shows that *H. longifilis* exposed to aqueous leaf extract of *T. vogelii* attained the first three stages of anaesthesia. The time taken to enter anaesthesia (induction time) decreased with increase in concentration of the anaesthetic solution. Recovery time from anaesthesia ranged from 503.3±3.33 minutes (8.30 hours) to 547.00±6.24 minutes (9.4 hours). The recovery time decreased marginally with increasing concentration of the anaesthetic extract. With the exception of weight significant differences (P>0.5) were not recorded in all variables measured.

Table 2 shows the result of *H. longifilis* injected with various concentrations freeze-dried leaf extract of *T. vogelii*. The result shows that the time taken for anaesthetized fish to enter anaesthesia decreased with increasing concentration of the anaesthetic extract. The recovery time followed the reverse order where faster recovery was observed with low concentrations of the anaesthetic extract. Recovery time ranged from 506.67 minutes (8.45 hours) to 553.67 minutes (9.23 hours) increasing with increase in concentration of the anaesthetic solution. Recovery time differed significantly among treatments groups (P<0.05). Anaesthetic effects were not observed in the control fish which behaved normally without reaching any stage of anaesthesia during the 60 minutes period of observation.

Comparative analysis of anaesthetic stages: The comparative analysis of the mean values of induction time in the three stages of anaesthesia in *H. longifilis* injected with aqueous leaf and freeze-dried leaf extracts of *T. vogelii is* shown in Figure 1. At anaesthetic stage 1 the highest mean values of induction time were 26.67±1.76 seconds for the aqueous leaf and 24.67±1.76 seconds for the freeze-dried leaf extracts obtained at concentration 0.01g/ℓ while the lowest mean value of induction time were 15.33±1.45 seconds recorded for both the aqueous leaf and freeze-dried leaf extracts at concentration 0.06g/ℓ. At anaesthetic stage 2 highest values of induction time were 44.00±4.00 seconds for the aqueous leaf and 47.67±9.56 seconds for the freeze-dried leaf extracts at concentration 0.01g/ℓ while the lowest induction times were 24.00±0.58 seconds for the aqueous leaf and 26.33±1.19 seconds for the freeze-dried leaf extract recorded at concentration 0.06g/ℓ. At anaesthetic stage 3 the highest values of induction time were 88.00±17.00 seconds for aqueous leaf and 79.33±23.07 seconds for the freeze-dried leaf extracts obtained at concentration 0.01g/ℓ while the shortest values of induction time were 44.67±1.76 seconds for the aqueous leaf and 49.33±4.79 seconds for the freeze-dried leaf extract obtained at concentration 0.06g/ℓ

Table 1. *Behavioural responses H. longifilis injected various concentrations of Tephrosia vogelii Aqueous leaf extract.*

Concentration (g/l)	Fish Weight (g)	Volume Injected (ml)	Behavioural Response							
			Time of Anaesthesia Induction (S)			OBR (M^{-1})		Percentage increase in OBR (%)	Recovery Time (Minutes)	Mortality After 48hrs
			I	II	III	BFS	AFS			
0.01	82.3±3.71^{b}	0.5	24.67±1.76^{a}	47.67±9.56^{a}	79.3±23.10^{a}	57.00±1.53^{c}	61.33±1.67^{b}	7.60±0.54^{a}	506.67±3.33^{c}	-
0.02	89.00±5.57^{b}	0.5	23.33±0.88^{ab}	42.33±5.04^{a}	76.67±5.49^{a}	60.67±2.40^{bc}	67.00±1.53^{ab}	10.61±2.35^{a}	518.33±4.41^{bc}	-
0.03	87.33±7.22^{b}	0.5	21.00±0.58^{abc}	37.67±4.84^{a}	65.67±5.49^{a}	61.33±1.76^{bc}	66.67±3.18^{ab}	8.58±2.13^{a}	526.33±2.91^{b}	-
0.04	100.67±8.09^{ab}	0.5	20.00±1.15^{bc}	30.67±4.67^{a}	57.00±8.54^{a}	64.00±1.53^{ab}	70.33±2.96^{a}	9.83±2.72^{a}	531.33±2.91^{b}	-
0.05	92.00±4.62^{b}	0.5	17.67±0.88^{cd}	29.33±2.03^{a}	57.33±3.93^{a}	64.67±0.88^{ab}	71.00±1.00^{a}	9.84±2.38^{a}	547.33±5.21^{a}	-
0.06	115.33±7.42^{a}	0.5	15.33±0.88^{d}	26.33±2.19^{a}	49.33±4.98^{a}	67.00±0.58^{a}	71.00±1.53^{a}	5.96±1.68^{a}	553.67±4.91^{a}	-

1) OBR BFS = Opercular Beat Rate Before Sedation
2) OBR AFS = Opercular Beat Rate After Sedation
3) Data were subjected to analysis of co-variance using weight as covariate
4) Means in the same colum followed by different subscripts differ significantly (P<0.05)

Table 2. *Behavioural Responses H. longifilis injected various concentrations of Tephrosia vogelii Freeze-dried leaf extract.*

Concentration (g/l)	FishWeight (g)	Volume injected (ml)	Behavioural Response							
			Time of Anaesthesia Induction (S)			OBR (M^{-1})		Percentage increase in OBR (%)	Recovery time (Minutes)	Mortality After 48hrs
			I	II	III	BFS	AFS			
0.01	82.3±3.71^{b}	0.5	24.67±1.76^{a}	47.67±9.56^{a}	79.3±23.10^{a}	57.00±1.53^{c}	61.33±1.67^{b}	7.60±0.54^{a}	506.67±3.33^{c}	-
0.02	89.00±5.57^{b}	0.5	23.33±0.88^{ab}	42.33±5.04^{a}	76.67±5.49^{a}	60.67±2.40^{bc}	67.00±1.53^{ab}	10.61±2.35^{a}	518.33±4.41^{bc}	-
0.03	87.33±7.22^{b}	0.5	21.00±0.58^{abc}	37.67±4.84^{a}	65.67±5.49^{a}	61.33±1.76^{bc}	66.67±3.18^{ab}	8.58±2.13^{a}	526.33±2.91^{b}	-
0.04	100.67±8.09^{ab}	0.5	20.00±1.15^{bc}	30.67±4.67^{a}	57.00±8.54^{a}	64.00±1.53^{ab}	70.33±2.96^{a}	9.83±2.72^{a}	531.33±2.91^{b}	-
0.05	92.00±4.62^{b}	0.5	17.67±0.88^{cd}	29.33±2.03^{a}	57.33±3.93^{a}	64.67±0.88^{ab}	71.00±1.00^{a}	9.84±2.38^{a}	547.33±5.21^{a}	-

1) OBR BFS = Opercular Beat Rate Before Sedation
2) OBR AFS = Opercular Beat Rate After Sedation
3) Data were subjected to analysis of co-variance using weight as covariate
4) means in the same colum followed by different subscripts differ significantly (P<0.05)

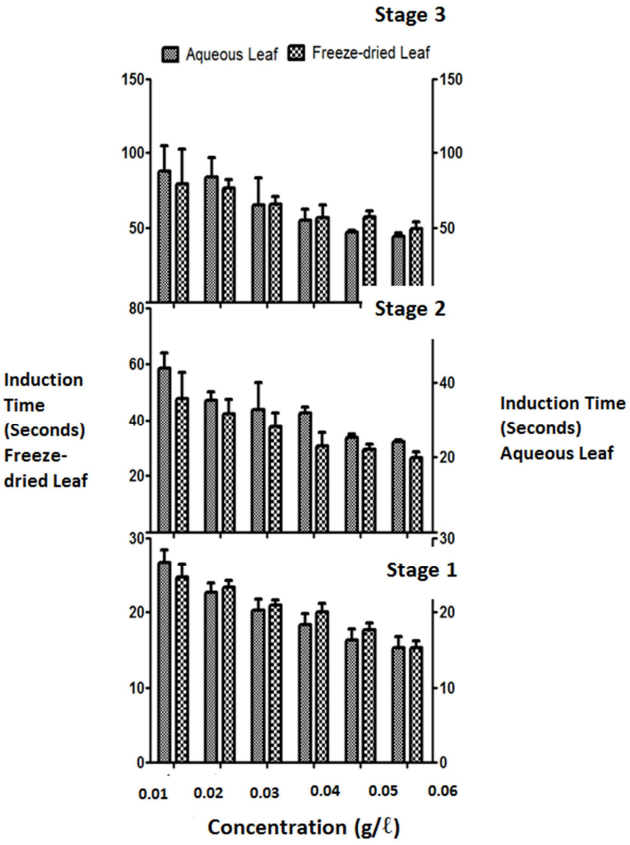

Figure 1. *Comparison of Mean values of Induction Times in the Three Anaesthetic stages in H. longifilis injected with various concentrations of T. vogelii Aqueous leaf and freeze-dried leaf extracts.*

4. Discussion

The route of administration of anaesthetics commonly used in research is immersion anaesthetic. However, in the present study the parenteral (injectable) route of anaesthesia was chosen. Brucher and Graham [9] 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 [20].

Evaluation of Anaesthetic Qualities of *T. vogelii* Extracts: The results obtained from the present study shows that in all the four experiments involving *H. longifilis* post-juveniles injected with aqueous and freeze-dried leaf extracts 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 [2]. 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 [21, 17, 36, 27]. This observation is also in agreement with Trevor and Miller, [39] 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 active ingtredients, rotenoids, in the body system of the fish which impairs the activity of the CNS at a much faster rate [36]. 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 [11]. 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 the anaesthetic compound [22]. In addition young fish were reported to be more sensitive to 2-phenoxyethanol anaesthetic than old fish (Barton and Helfrich, [7]. This could as well apply to this present study.

When the time taken for *H. longifilis* to enter anaesthesia or to be tranquilized (induction time) and recovery time are considered in the present investigation, there were no significant differences ($P<0.5$) in the mean values of induction in the experiments with aqueous leaf extract at all levels of concentrations implying that the induction of the fish anaesthetized with these extracts was not affected by concentration [22]. The experiments with the freeze-dried leaf extract showed significant differences ($P>0.5$) in induction time at anaesthetic stage 1 which depicts the effect of concentration on induction time [4].

The induction times of 88.00 seconds obtained with aqueous leaf and 79.30 seconds with the freez-dried leaf extracts of *T. vogelii* are 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 [13], 1-2 minutes for light sedation of common carp (*Cyprinus carpio)* juveniles exposed to sodium bicarbonate [2] and the 1.5 minutes reported for *Acipenser persicus* exposed to clove oil [6]. When the rapid induction time (3-5 minutes) required of an ideal anaesthetic [23, 22, 11, 27, 8] is considered the experiments with these two extracts of *T. vogelii* closely meet the requirement of an ideal anesthetic.)

The recovery times in agreement with other researchers [32, 17, 36, 14] tended to increase with increasing concentration of all the two anaestheic extracts of *T. vogelii*. Hseu *et al, [21]* reported that higher drug concentration or dose increase recovery time. In the case of immersion anaesthetics. Griffiths, [17] and Tort *et al*, [38] 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 anaesthetic in the central nervous system CNS of the experimental fish [39], 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. When the recovery time is considered in relation to fish heamatology, the heamatological indices were not significantly different from their controls indicating that the fish recovered fully from anaesthetic extracts [4]. The recovery time of 503.33 minutes (8.38 Hrs) obtained with the aqueous leaf and 506.67 minutes (8.43 Hrs) with the freeze-dried leaf extract are compartible with the 12 Hr recovery time reported for *Oreochromis niloticus* anaesthetize with quinaldine [34]. Chemical anaesthetic agents have been used in handling and transportation of fish to reduce mortality which occurs as a result of excitement and hyperactivity [35]. 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 [3, 26, 31]. It has also been suggested that light sedation is desirable during transportation of fish [37]. 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 [12]. Since transportation of fish often involve long distances and time, the long recovery time of *T. vogelii* extracts 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.

From the result of these experiments it can be concluded that there are no remarkable differences in the induction and recovery times of the aqueous and freeze-dried leaf extracts of *Tephrosia vogelii.*

References

[1] Agokei, O. E and Adebisi, A. A (2010). Tabacco as an anaesthetic fish handling procedures. *Journal of medical plant research 4: 1396-1399*.

[2] Altum, T and Danabas, D (2006). Effects of short and long exposure to the anaesthetic 2- phenoxyethanol mixed with ethyl alcohol on common carp (Cyprinus carpio, L, 1758) fingerlings. *The Israeli Journal of aquaculture-Bmidgeh* 58 (3) 178-182.

[3] 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. *N. Am. J. Fisher. Manag. 17: 301-307.*

[4] Ayuba, V. O; Ofojekwu, P. C (2004). Effects of extracts of dried seeds of toloache, *Datura innoxia* as anaesthetic on the African catfish *Clarias gariepinus* fingerlings. *In: 18th annual conference of the fisheries society of Nigeria (FISSON), 8-12h December, 2003. Pp 25-29.*

[5] Ayuba, V. O. and Ofojekwu, P. C (2010). Growih and Heamatological changes in *Clarias gariepinus* exposed to sub - lethal concentrations of *Datura innoxia* extrat for 12 weeks. *Hamdard Medicus. 53 (1): 91-98.*

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

[7] Barton, B. A and Helfrich, H (1981). Tune-dose responses of juvenile common carp to 2- phenoryethanol. *Progressive fish culturist 43: 223-231.*

[8] 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.

[9] Brucker, p, and Graham, M. (1993). The effect of anaesthetic Katamine hydrochloride on oxygen consumption rates and behaviour in the fish Heros (*Cichlasoma citrinellium*, gunther; 1964). *Com. Biochem, Physiol. 104C: 5.*

[10] 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 transporting large mouth bass *Micropterus salmoides. Aquaculture, 239: 509-529.*

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

[12] Dupree, H. K. and Huner, J. V. (1984). Third report of the fish farmers. The status of warm water fish farming and progress in farming research. U. S. fish and wildlife service, W ashington D. C.

[13] Durville, P and Collet, A. (2001). Clove oil used as an anaesthetic with juvenile tropical marine fish. *SPC Live Reef fish Information Bulletin #9*.

[14] 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 (*Dicentrachus labrax* L). *Journal of Animal and Veterinary Advances. 11 (4): 494-502.*

[15] Gabriel, U. U, Obomanu, F. G and Edori, O. S (2009). Heamatology, plasma enzymes and organ indices of *clarias gariepinus* after intramuscular injection with aqueous leaves extracts of *Lepidagathis alopecuroides. African journal of biochemistry research Vol. 3 (9): 312-316.*

[16] Gomulka P, Wlasow T, Velisek J, Svobodova Z, Chemielinska, E. (2008). Effects of Eugenol and MS–222 anaesthesia on Siberian Sturgeon *Acipenser baerii* Brandt. *ACTA VET. BRNO 77: 447-453.*

[17] Grifiths, S. P (2000). The use of clove oil as an anaesthetic for sampling intertidal rockpool fishes. *J. Fish Biol. 57: 1453-1464.*

[18] Gabriel, U. U, Obomanu, F. G, Orlu, E. E and Oreh, O. D (2010). Fulton's condition organ indices and heamotological response of catfish hybrid (*Heterobranchus longifilis, clarias gariepinus)* aqueous extract of leaves of *Lepidagathis alopecuroides. Ethiopian journal of environmental studies and management Vol. 3 (1): 30-36.*

[19] Gadzirayi, C. T, Mutandwa, E., Mivale, M and Chindundu, M (2009). Utilization of *Tephrosia vogelii* in controlling ticks in dairy cows by small scale commercial farmers in Zimbave. *African journal of Biotechnology. Vol 9 (17): 4134-4136.*

[20] Hseu, J. R, Yeh. S. L, Ting, U. Y (1997). Different Anaesthetic effects of 2-phenoxy ethanol on four species of teleost. *J. Fish Soc. Taiwan. 24. 185-191*.

[21] Hseu, J. R, Yeh, S. L, Chu, Y. T, Ting, Y. Y (1998). Comparison of efficacy of five anaesthetics in godlined seabream (*Sparus sarba)*. *Acta zool Thaiwan 9: 11-18*.

[22] 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 (*Salmo salar)*. Smoolts and their potential stress-reducing capacity. *Aquaculture 221: 549-566.*

[23] Marking, L. L. and Meyer, F. P. (1985). Are better anaesthetics needed in fisheries? *Fisheris 10 (6): 2-5.*

[24] McDavid, A and Lesseps, A (1994). Effects of *Tephrosia vogelii* leaf extracts on several crop pests. Natural plants products as pesticides. Proceeding from the first national symposium in Zambia held in Lusaka, 2nd - 5th August, 1994.

[25] Mshana, N. R, Abbiw, D. K, Addae-Mensah, F., Adjanouhoun, E., Ahyi, M. R. A., Ekpera. J. A., Enow-Drock, E. G., Noamesi, M. A., Odunlami, H., Oteng-Yeboah A. A, Sarpong, K., Sofowora, A., Tackle, A. N (2000). Traditional medicine and pharmacpocia: contribution to the revission of Ethnobotanical and flonistic studies in Ghana. O.A.U / scientific, technical and research commission. Pp 383.

[26] Munday, p. L. and Wilson s. k (1997). Comparative Efficacy of Clove oil and other Chemicals in Anaesthetization of *Pornacentras ambionensis*, a Coral Reef Fish. *Journal of Fish Biology. 51 (5): 931-938.*

[27] Mylonas, C. C, Cardinalethi, G, Sigelaki, I, Polzonethimagni, A. (2005). Comparative efficacy of clove oil and 2-phenoryethanol as anaesthetics in the aquaculture of European sea bream (*Sparus aurata*) at different temperatures. *Aquaculture, 246: 467-481.*

[28] 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.*

[29] Offem, B. O, Ayotunde, E. O, and Ikpi, G. U (2008). Dynamics in the Reproductive Biology of *Heterobranchus longifilis* val (piscess: 1840) in the Inland Waters of Cross River. *Nigeria. Research journal of fisheries and Hydrobiology. 3 (1): 22-31.*

[30] Omoniyi, I., Agbon, A. O and Sodunke, S. A (2002). Effect of lethat and sublethal concentrations of tabocco (*Nicotiana tobaccum)* leaf dust extract on weight and heamatological changes on *clarias gariepinus* (Burch). *J. App. Sci 2 (4): 382-387.*

[31] Park, M. O, Im, S. Y, Seol, D. W and Park, I. S (2009). Efficacy and physiological responses of rock bream (*Oplegnathus faciatus)* to anaesthetization with clove oil. *Aquaculture. 287: 427-430.*

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

[33] Ramanayaka, J. C and Atapatu, N. S. B. M (2006). Fish anaesthetic properties of some local plant materials. Tropical Agricultural Research Extension 9. http://www.agri.ruhrac.UC/tare/pdf/v-9/A.G.9.14pdf: Accessed: 24.03.2011.

[34] Sado, E. K.(1985). Influence of the anaesthetic quinaldine on some tilapias. *Aquaculture 46: 35-62.*

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

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

[37] 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.*

[38] Torts, L., Puicerver, M., Crepso, S. and Padros, F. (2002). Cortisol and heamatological response of sea bream and trout subjected to the anaesthetic clove oil and 2- phenoxyethanol. *Aquaculture Research 33: 907-910.*

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

[40] Yoshimura, H., Nakamura, M and Koeda, T (1981). Mutagenic screen of anaesthetics for fishes. *Mut. Res. 90: 119-124.*