2(2): 67-75 (2016) doi: 10.3153/JAEFR16009

Journal of

Aquaculture Engineering and Fisheries Research

E-ISSN 2149-0236

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

EFFECTIVENESS OF CLOVE SEED EXTRACTS AS ANAESTHETICS IN TRANSPORTATION OF *Tilapia guineensis* JUVENILES

Ojo AKINROTIMI, Ebinimi ANSA, Olajumoke EDUN

African Regional Aquaculture Center/Nigerian Institute for Oceanography and Marine Research P.M.B, Port Harcourt, Rivers State, Nigeria

Received: 08.03.2015

Accepted: 27.10.2015

Published online: 16.12.2015

Corresponding author:

Ojo AKINROTIMI, African Regional Aquaculture Center/Nigerian Institute for Oceanography and Marine Research P.M.B 5122, Port Harcourt, Rivers State, Nigeria

E-mail: ojoakinrotimi@yahoo.com

Abstract:
The effectiveness of clove seed extracts as a viable anaesthetic agent in transportation of *Tilapia guineensis* were evaluated. Juveniles of *T. guineensis* (mean length 13.12cm ±1.20; mean weight 45.64g ±1.84) were exposed in three replicates to different concentrations (0.00mg/L- control; 5.00; 10.00; 15.00; 20.00 and 25.00mg/L) of clove seed aqueous extracts, extracted in water . The exposed fish were later transported in open plastic tanks from Buguma to Aluu, in Port Harcourt over a distance of 50km. Before and after transportation, glucose and cortisol levels in the plasma of the fish were assessed. The result of the study indicated a significant reduction ($P<0.05$) in the levels of cortisol and glucose in the plasma of the fish with increasing concentrations of the anaesthetics. The lowest survival rate (30.0%) was recorded in the fish transported with no anesthetics, while 100% survivals were recorded in fish exposed to 15.0 and 20.0 mg/L of the extracts. In conclusion, this study suggests that application of clove seed extracts within the range of 10.00 and 20.00 mg/L reduced the stress response in *T. guineensis* during transportation, thereby enhances their survival.

Keywords: Aquaculture, Clove, Anaesthetics, Tilapia, Transportation

Introduction

Fish transportation is an integral aspect of aqua cultural practices, it involves movement of small or large quantity fish over some distances to the waters where they are to be stocked (Orji, 2005; Akinrotimi *et al.,* 2007), usually fish are transported for a number of reasons which include: collection and transportation of brood stock, fingerings, juveniles and adult fish to stocking sites, to market and also for the purpose of introduction of fish species into a new culture environment (Berka, 1986; Cooke *et al.,* 2004; Akinrotimi *et al.,* 2013*a).*Several authors have reported that fish transportation from one location to another can elicit stress, which affects fish performance negatively and consequently reduced its survival in the culture conditions (Robertson *et al.,* 1987; Kiessling *et al.,* 2009; Akinrotimi *et al.,* 2011a). Conversely, huge loses and severe mortality in newly stocked fish farms has been associated with acute stimulus such as handling and transportation (Iwama *et al.,* 1998; Akar, 2011). As part of the handling procedures in intensive fish farming, transportation times may vary considerably, depending on the distance covered. As a rule, fingerlings and juveniles are usually transported from the hatchery to culture site. In this process, the fish should arrive the farm in good physiological conditions to meet the criteria demanded by the farmer (Carneiro *et al.,* 2002; Akinrotimi *et al.,* 2011b).

The rapid expansion of fish farming activities in recent times raised an issue about the sustainability of the aquaculture sector. Akinrotimi *et al.* (2013b) noted that animal welfare is an important aspect of aquaculture sustainability. Although animal welfare has been defined in different ways by different authors (Broom 2011; Hagen *et al*., 2011; Ohl and van der Staay 2012), the most important aspect of their assertions is that poor welfare of fish in aquaculture is associated with overstocking, and stretches the adaptive capacity of the animals in different ways (McEwen and Wingfield 2003 Akinrotimi *et al.,* 2011c;), which may result in chronic and acute stress-related physiological and behavioural changes, which in extreme cases may lead to mortality. Successive or cumulative exposure to stressors may compromise the adaptive capacity of an animal and lead to allostatic overload and poor welfare (Korte *et al*., 2007; Akinrotimi *et al.,* 2011d). It is therefore important to identify the effects of stressors such as transportation on fish welfare using glucose and cortisol level as indicators.

Under stressful conditions, fish body responds to the stress immediately to the stress known as primary and secondary response. The primary responses involve the perception of an altered state by central nervous system and the release of stress hormones, which include cortisol into the blood circulation system of the fish (Martinez-Porchas *et al*., 2009). Cortisol is the principal glucocorticoid secreted by the interrenal tissue located in the head-kidney of teleost fish (Iwama *et al.,* 1999; Harper and Wolf, 2009; Akinrotimi *et al.,* 2011e). This hormone is released by the activation of the hypothalamus-pituitary-interrenal axis (Mommsen *et al.,* 1999). While primary stress responses trigger the sequential secondary response such as elevation of glucose levels (Mommsen *et al*., 1999; Barton 2002). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Gorissen *et al*., 2012). In stressful situations the chromaffin cells release catecholamine hormones (Nikoo and Falahatkar, 2012). Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways (Gust *et al*., 1991; Matsunaga and Watanabe, 2010) to cope with the energy demand by the stressor in adverse conditions

Anaesthetics are being used to minimize the stress associated with aquaculture procedures. Hence, anaesthetizing fish prior to transport can reduce metabolic rate, oxygen demand, reduce general activity, increase case of handling and mitigate the incidence of stress response (Carmichael *et al.,* 1984; Akinrotimi *et al.,* 2013b). Plant extracts is a potential source of new and effective anaesthetics in fish handling and transportation in intensive aquaculture (Akinrotimi *et al.,* 2014a). With the recent awareness on safe aquaculture practices, to develop "green" anaesthetics with low environmental and health risks, coupled with the prohibitive cost and scarcity of conventional anaesthetics (Akinrotimi *et al.,* 2014b), there is the need therefore to develop a viable alternative anaesthetics of plant origin which could be used in fish transportation. In this study the main substance used is an aqueous extracts of seed of the clove plant (*Sygium aromaticum)*. Its major an active ingredient is eugenol (70-90%). Clove extracts is considered an appropriate anaesthetic for fish because of its low cost, availability and safety to fish and humans (Small 2004; Sinka and Nealb, 2009).

Tilapia guineensis is an important culturable species in the brackish water zone of Nigeria, that are usually transported by fish farmer to the site where they are to be cultured. In most cases, they arrived at the stocking site in deplorable physiological conditions which negatively impacts on their performance in the culture condition. Hence the need for their proper transportation using natural anaesthetics. This study therefore assesses the effectiveness of clove seed as anaesthetic agent in transportation of *T. guineensis.*

Materials and Methods

Experimental fish

A total of 150 *T. guineensis* juveniles (mean length 13.12cm ±1.20; mean weight 45.64g ±1.84) were harvested at low tide from the recruitment ponds at African Regional Aquaculture Center, (ARAC), Brackish Water Research Station. Buguma, Rivers State, Nigeria. They were transferred immediately to the hatchery where they were acclimated to laboratory conditions for seven days (Gabriel *et al.,* 2004). During this period they were fed with ARAC feed (35.0% C.P) at 3% body weight per day, with the water in acclimation tanks being renewed every two days (Akinrotimi, 2012).

Preparation of Clove Plant

Dried buds of clove plant (*Syzigium aromaticum)* were purchased from Choba market, in Obio–Akpor Local Government Area of Rivers State. Plant identification was done using the keys of Agbaje (2008). The buds were taken to the laboratory where they were ground into powder using a kitchen blender (Model H12, Ken Wood, Japan). The milled clove buds were sieved into fine powdery form using 0.1u nylon mesh.

Experimental Procedure

Graded series of ground and filtered seeds were weighed and applied directly at (5.0, 10.0, 15.0, 20.0 and 25.0 mg/L) in three replicates in to the water in 20L experimental tanks filled to 10L mark, the mixture was stirred vigorously to allow homogenous mixing. The fish were later introduced into prepared experimental tanks (15 in number) at the rate of 10 fish per tank in each triplicate. Then they were transported to Port Harcourt, over a distance of 50km.

Laboratory Analysis

Before and after transportation, blood samples (l mL) for glucose and cortisol analysis were taken from the *vena caudalis* of the fish using 5ml syringe fitted with 21G needle. The glucose level was evaluated with glucose meter (Accuchek model RS 910, China) while cortisol was done with aid of cortisol analyzer (RTTG, Model, China). The number of survival of the experimental fish were noted and recorded in each of the concentration.

Water Quality Analysis

Water quality parameters such as nitrite, ammonia and sulphide were evaluated using LaMotte salt water test kit (Model AQ–4, Chestown, Maryland, USA), pH was determined with pH meter, (Model, HI 9812, Hannah Products, Portugal). The dissolved oxygen levels were evaluated by the Winkler method (APHA, 1985).

Statistical Analysis

Data from this experiment were analyzed with one way analysis of variance (ANOVA) test at 0.05 probability and difference among mean where existed were determined by Tuckeys multiple comparison test (Zar, 1996).

Results and Discussion

The water quality parameters in experimental tanks were of the same range with no significant difference (Table 1). The water quality variables monitored in this study were within the same range with the control (without anaesthetics). Similar result was observed in the transportation waters of largemouth bass (*Micropterus salmoides)* using clove (Cooke *et al.,* 2004). This is because use of anaesthetics in fish transport minimizes its activity, and the excretion of ammonia through the gills. Hence clove seed extracts maintain a relatively good water quality during fish transportation (Akinrotimi, 2014).

The concentration of clove extracts used in this study induced all anaesthesia stages in fish; however, the fish anaesthetized with 20 $mg.L^{-1}$ reached total loss of equilibrium and had lower opercula movement rates at a significantly shorter time, as compared to other lower concentrations tested (Table 2). In brief procedures, adequate anaesthetic concentration should promote total loss of equilibrium without necessarily inducing all anaesthesia stages (Akinrotimi *et al*., 2013a). In the present study, the concentrations of 10, 15, and 20 mg.L^{-1} of clove extracts promoted total loss of equilibrium at similar intervals, and equally promote the fast recovery of fish anaesthetized with the extracts (Table 3) Therefore, 10-20 mg.L-1 is the optimal concentration range to induce anaesthesia in handling of *T. guineensis* in aquaculture procedures. This is because an ideal anaesthetic agent for use in aquaculture must induce fish within 3 mins and recover within 5 mins, these two criteria was found within the range of 10-20 mg.L^{-1} in application of the clove extracts.

The effect of clove seed extracts on the glucose and cortisol levels of *T. guineensis* during transportation is shown in Figure 1 and 2 respectively. The result indicated that the levels of glucose and cortisol in the plasma of the fish reduced as the concentrations of the clove seed extracts increased, with the highest values recorded in the control, and the lowest in the fish transported with 20.00mg/L concentration of the anaesthetics.. The lowest survival rate (30%) was observed in the fish with no anaesthetics, while 100% survival were recorded in fish exposed 10.00, 15.0 and 20.0mg/l of the extracts (Figure 3). In aquaculture practices the understanding of fish stress response is essential to avoid stress-related problems, and to improve fish quality in rearing conditions so as to optimize production. As in other vertebrates, fish experiencing stress show a number of physiological changes that are expressed through a number of particular indicators (Broom, 2011). Stress has been described as an energy drain of energy that might be utilized in growth diverted to catabolic utilization (Gorrissen *et al*., 2012). This study evaluated the use of different concentration of clove seed extracts as a tool for sedating fish for handling and transportation in aquaculture.

Table 1. Water Quality Parameters in Experimental Tanks of *T.guinneensis* Exposed to Clove Seed Extracts (Mean ± SD).

	Concentrations (mL.L^{-1})				
Parameters	**Control**	**5.00**	**10.00**	**15.00**	**20.00**
Salinity (‰)	12.61 ±0.31^a	12.84 ±0.24^a	12.52 ±0.67^a	12.61 ±0.47^a	12.89 ±0.31^a
Temperature (°C)	28.96 ±0.3^a	28.87 ±0.37^a	29.03 ±1.10^a	28.70 ±0.60^a	29.03 ±0.41^a
pH	6.91 ±0.16^a	6.83 ±0.21^a	6.83 ±0.22^a	6.68 ±0.17^a	6.79 ±0.21^a
DO (mgL^{-1})	6.89 ±0.27^a	6.89 ±0.26^a	6.74 ±0.21^a	6.34 ±0.55^a	6.93 ±0.57^a
Nitrite (mgL^{-1})	0.047 ±0.01^b	0.047 ±0.01^b	0.050 ±0.03^a	0.005 ±0.01^a	0.0047 ±0.03^a
Ammonia (mgL^{-1})	1.32 ±0.01^b	0.32 ±0.01^a	0.21 ±0.03^a	0.31 ±0.04^a	0.31 ±0.02^a
Sulphide (mgL^{-1})	0.24 ±0.01^b	0.04 ±0.01^a	0.04 ±0.01^a	0.04 ±0.10^a	0.35 ±0.03^a

Means within the row with different superscripts are significant (P<0.05)

Table 2. Induction time in Different *Anaesthesia* Stages in *T. guineensis* Exposed to of Clove Seed Extracts.

	Behavioural Description (seconds)			
Clove seed extracts (mg/L)	**Slow swimming**	**Loss of equilibrium**	**Loss of opercula momovement**	**Loss of movement**
Control	0.00 ±0.00^a	0.00 ±0.00^a	0.00 ±0.00^a	0.00 ±0.00^a
5.00	51.61 ±1.11^d	88.61 ±1.11^c	120.61 ±2.62^d	190.71 ±1.21^c
10.00	59.81 ±1.21^d	80.11 ±2.61^c	110.71 ±1.02^c	180.64 ±1.01^c
15.00	42.74 ±0.11^c	76.71 ±1.17^{ab}	105.71 ±1.17^{ab}	152.72 ±2.61^{ab}
20.00	34.61 ±1.71^{ab}	70.81 ±1.02^{ab}	87.11 ±1.21^{ab}	134.61 ±1.09^{ab}

Means within the column with different superscripts are significantly different (P<0.05)

Table 3. Recovery time in Different *Anaesthesia* Stages in *T. guineensis* Exposed to Clove Seed Extracts.

Behavioural Description (seconds)				
Clove seed extracts (mg/L)	**Reappearance of opercula movement**	**Irregular balance**	**Total recovery equilibrium**	**Normal swimming**
0.00	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
5.00	79.61 ±1.12^{b}	91.24 ±1.78^{b}	119.81 ±1.71^{b}	146.88 ±2.1^{b}
10.00	96.84 ±1.69^{ab}	118.71 ±1.64^{ab}	127.62 ±2.11^{ab}	166.71 ±2.61^{ab}
15.00	108.88 ±2.11^{c}	141.11 ±1.71^{c}	141.77 ±2.02^{c}	188.11 ±1.78^{c}
20.00	131.11 ±0.18^{d}	156.78 ±2.01^{d}	159.71 ±1.96^{d}	231.14 ±2.61^{d}

Means within the column with different superscripts are significantly different ($P<0.05$)

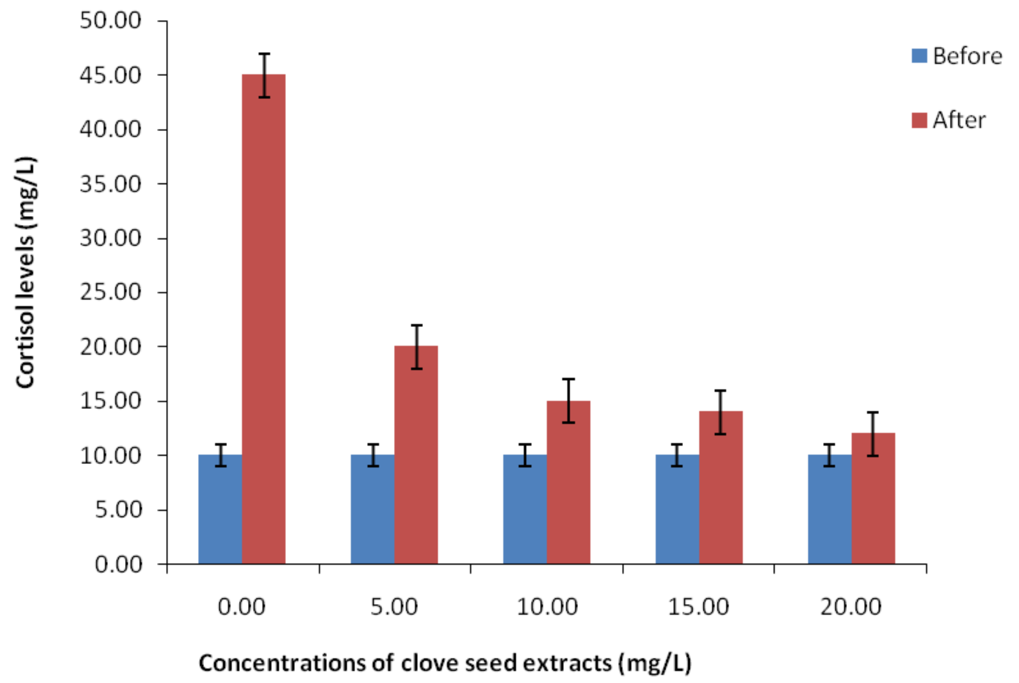

Figure 1: Effect of clove seed extracts on the cortisol of *T. guineensis* during transportation

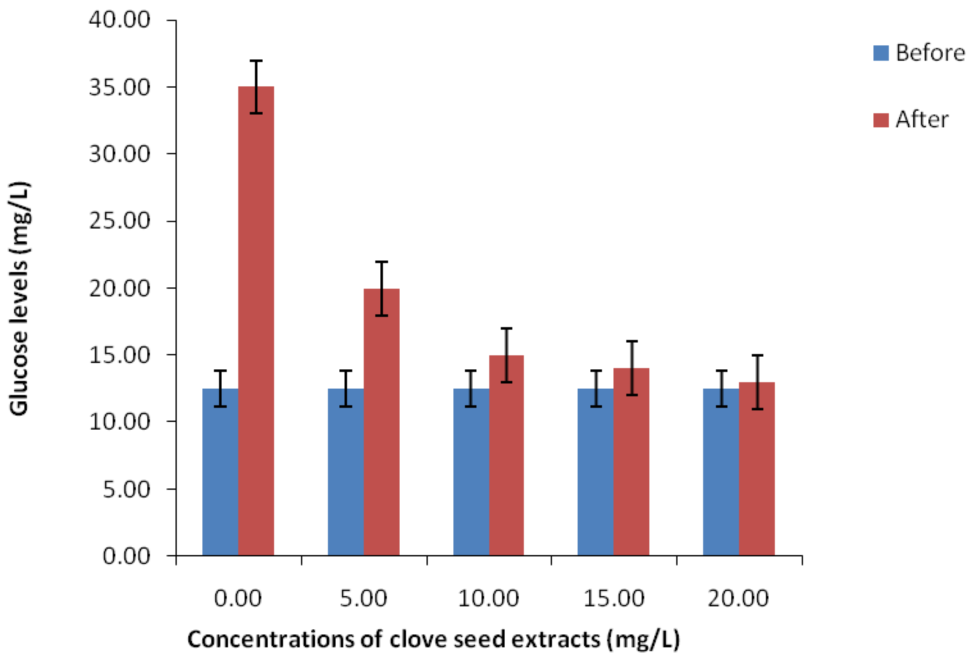

Figure 2: Effects of clove seed extracts on the glucose levels of *T. guineensis* during transportation

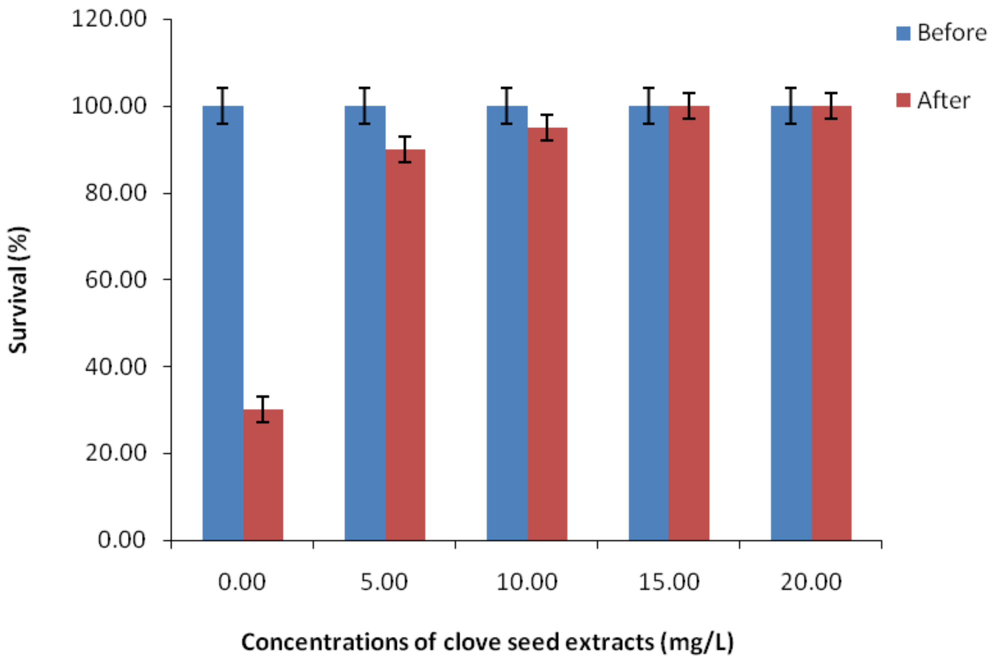

Figure 3: Effect of clove seed extracts on the survival of *T. guineensis* during transportation

Changes in the concentration of plasma cortisol depend upon the nature of stress stimuli and the duration of the stress as well as also the magnitude and severity of stress (Barton, 2002) and species under investigation (Metz *et al*., 2003; Nikoo and Falahatkar, 2012). In the present study, since there is a significant increase in the cortisol concentration immediately after traportation.

The control group (transport without anaesthetics) showed an increasing in plasma cortisol levels when compared with anaesthetized ones. Although exposure of the fish to clove extract reduced the cortisol response considerably, however, it did not abolish it completely. This result can compared favourably with that of Akar (2011) in blue tilapia (*Oreochromis aureus*) and small (2004) in chanell catfish (*Ictalurus punctatus)* transported with clove oil. These authors reported that cortisol level decreased with increasing concentration of the clove anaesthetics. This trend may be due to the blockage of sensory information to the hypothalamus in the brain consequent of high concentrations of anaesthetics applied (Iversen *et al.,* 2003).

Mobilization of readily available energy in the form of glucose is suggested to enhance the survival of fish It is perhaps, not surprising, therefore, that elevation of plasma glucose has been recognized as a part of generalized stress response in fish. The reduction in glucose levels of *T. guineensis* with increased concentrations of clove extracts in this work is similar to the findings of Larissa *et al.* (2011) in Nile tilapia (*Oreochromis niloticus)* transported with clove oil. However, Lambooij *et al.* (2009) observed an increase in glucose levels of common carp *(Cyprinus carpio)* transported with anaesthetic metomidate, this contradictory trend according to Di Marco *et al.* (2011) lies in the ability of clove to block cortisol secretion which stimulate glucose production. As cortisol is considered to be a major mediators of the increase in plasma glucose levels seen in stressful fish (Barton, 2002). In this work, high survival was recorded in the fish exposed to different concentrations of clove seed extracts. This observation corroborated that of Akbari *et al.* (2010) in transportation of Indian shrimp (*Fenneropenaeus indicus),* and that of Anyanwu *et al.* (2011) in transportation of *Penaeus monodon* with clove extracts.This according to Akinrotimi *et al.* (2014b) is due to light anaesthesia induced by the application of clove for the mitigation of stress associated with transportation, which allows the fish to breathe effectively, and maintain equilibrium during transportation, thereby enhances its survival.

Conclusion

In conclusion, this study suggested that the use of clove seed extracts reduced the stress response in juveniles of *T. guineensis* during transportation. Assessments of clove oil for aquaculture purposes have to be encouraged because this natural anaesthetic is becoming more evident as a safe and low cost alternative for the sustainability of aquaculture industry.

References

Agbaje, E.O., (2008). Gastro intestinal effects of *Syzigium aromaticum* in animal model. *Nigeria Quarterly Journal of Hospital Medicine,* 18(3), 137-141.

Akar, A.M., (2011). Effects of clove oil on the response of blue tilapia (*Oreochromis aureus)* by transportation stress. *Journal of the Arabian Aquaculture Society,* 6(1), 77-86.

Akbari, S., Khoshrod, M.J., Rayahian, H., & Asfharwasub, M., (2010). The use of eugenol as an anaesthetic in transportation of Indian shrimp post larvae. *Turkish Journal of Fisheries and Aquatic Science,* 10, 423-429.

Akinrotimi, A., Ansa, E.J., Owhonda, K.N., Ononkwo, D.N., Edun O.M., Anyanwu, P.E., Opara, J.U., & Cliffe, T., (2007). Effects of transportation stress on haematologcal parameters of black chin tilapia, *Sarotherodon melanotheron. Journal of Animal and Veterinary Advances,* 6(7), 841-845.

Akinrotimi, O.A., Abu, O.M.G., & Aranyo, A.A., (2011a). Environmental friendly aquaculture key to sustainable fish farming development in Nigerial. *Continental journal of Fisheries and Aquatic Science,* 5(2), 17-31.

Akinrotimi, O.A., Abu, O.M.G., & Aranyo, A.A. (2011b). Transforming aquaculture from subsistence to commercial level for sustainable development in Niger delta region of Nigeria. *Journal of Agriculture and Social Research,* 11(2), 34-42.

Akinrotimi, O.A., Ciffe, P.T., & Ibemere, I.F., (2011c). Integration of rural aquaculture into small scale farming system in Niger delta region of Nigeria. *Global Approaches to Extension Practice,* 7(1), 43-48.

Akinrotimi, O.A., Aranyo, A.A., & Ibemere I.F., (2011d). Effects of capture, handling and confinement on the glucose levels of black jaw tilapia (*Sarotherodon melanotheron*). *Advances in students Research,* 6(2), 27-30.

Akinrotimi, O. A., Opara, J. Y., & Ibemere, I.F., (2011e). Effects of handling stress on immune functions of black jaw tilapia *Sarotherodon melanotheron*. *Nigerian Journal of Fisheries,* **8(**1), 154-158.

Akinrotimi O.A., (2012). *The anaesthetic effects of clove seed in selected species of grey mullets Liza falcipinnis and Liza grandisquanmis*. Pp 287-291. In Adeyemo A., Otobotekere A.J.T., Keremah, R.I and Erhouse, E (Eds). Proceeding of the 27th annual conference and biennial general meeting of the fisheries society of Nigeria 2530th November, 12. Yenagoa, Bayelsa State.

Akinrotimi, O.A., Edun, O.M., & Mebe, E.D., (2013a). Effects of clove seed as anaesthetic agents in two species of grey mullets *Liza falcipinnis and Liza grandisqumis. Journal of Aquatic Science,* 1(1), 7-10.

Akinrotimi, O.A., Gabriel, U.U., & Orokotan, O.O., (2013b). Changes in enzymes activities of *Clarias gariepinus* brood fish exposed to anaesthetics metomidate. *Applied Ecology and Environmental Science,* 1(3), 37-40.

Akinrotimi, O.A., (2014). Assessment of the efficacy of synthetic and natural anaesthetics on the African catfish *Clarias gariepinus* (Burchell, 1822). PhD Thesis. Deparment of Fisheries and Aquatic Environment, Rivers State University of Science Technology Port Harcourt. 260pp.

Akinrotimi, O.A., Gabriel, U.U., & Deckae, S.N., (2014a). Anaesthetic efficacy of sodium bicarbonate and its effect on the blood parameters of African catfish, *Clarias gariepinus. Journal of Aquatic Sciences,* 29(1B), 233-246.

Akinrotimi, O.A., Gabriel, U.U., & Deekae, S.N., (2014b). Investigations on the potential of Indian almond free (*Terminalia catapaa)* leaf extracts as anesthetic agents in African catfish (*Clarias gariepinus). Journal of Aquatic Sciences* 29(1B), 223-231.

Anyanwu, P.E., Ayinla, O.A., Pepple, P.C., Ebonwo, B.I., Hamzat, M.B., Ihimekpen, A.F., Ayaobu-Cookey, I.K., Mantanmi, M.A., Afulabi, E.S., Akinrotimi, O.A. Ajijo, M.R., Olaluwoye, B.L., Achlike, N., & Abdulan, M., (2011). The use of clove seed as an anaesthetic agent for the transportation of *Macrobrachium vollenhovenii* brood stock NIOMR, 2011 Annual Report.

APHA, (1985). Standard method for the examination of water and waste water (16th Edition), Washington. Public Health Association.

Barton, B.A., (2002). Stress in fishes. A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative Comparative Biology*, 42, 517-525.

Berka, R., (1986). The transportation of live fish, a review, fisheries research institute, scientific information centre. *EIFAC Technical Paper*, 48, 1-48.

Broom D.M., (2011). A history of animal welfare science. *Acta Biotheory*, 59, 121-139.

Carmichael, G.J., (1998). Long distant truck transport of intensively reared large mouth bass. *Progressive Fish Culturist*, 46, 111-115.

Carneiro, P.C., Urbrinati, E.C. & Martins, M.L., (2002). Transport with different concentration of benzocairne concentrations and its consequences on haematological parameters and gill parasite populations of matrixa. *Acta Science,* 24, 555-556.

Cooke, S.J., Sunki, C.D., Ostrand, K.G., & Wahl, D.H. (2004). Behavioral and physiological assessment of low concentrations o clove oil anaesthetic for handling and transporting large mouth bass. *Aquaculture,* 239: 509-529.

Dimarco, P., Petch, T., Longobandi, A., Prioori, A., Finoia, M. G., & Marino, G., (2011). Efficacy of tricaine methanesulphonate and their side effects on the physiology of sturgeon hybrid. *Journal of Applied Ichthyology,* 27, 611-617.

Gorissen, M., Bernier, N.J., Manuel, R., de Gelder, S., Metz, J.R., Huising, M.O., & Flik, G., (2012). Recombinant human leptin attenuates stress axis activity in common carp (*Cyprinus carpio* L.). *Genaral Comparative Endocrinology,* 178: 75–81.

Gust, D.A., Gordon, T.P., Wilson, M.E., Ahmedansari, A., Brodie, A.R., & Mcclure, H.M., (1991). Formation of a new social group of unfamiliar female rhesus-monkeys affects the immune and pituitary adrenocortical systems. *Brain Behavioural Immunology,* 5, 296-307.

Hagen, K., Van den Bos, R., & de Cock Buning, T. (2011) Editorial: concepts of animal welfare. *Acta Biotheor,* 59, 93-103.

Harper, C., & Wolf J.C., (2009) Morphologic effects of the stress response in fish. *International Journal of Agriculture,* 50, 387-396

Iwama, G.K., Thomas, R.B., & Vijayan, M.M., (1999). Heat stock protein expression in fish. *Review in Fishery Biology*, **8**: 36 – 56.

Iverseen, M., Finstad, B., McKinley, B., & Elliassen, R., (2003). The efficacy of metomidate clove oil as anaesthetics in Atlantic salmon (*Salmo salar)* and their potential stress reducing capacity. *Aquaculture,* 221, 549-516.

Kiessling, A., Johansson, D., Zahl, I.H., & Samuelsen, O.B., (2009). Pharmokinetices, plasma cortisol and effectiveness of benzocaine, MS-222 and isoeogenol measured in individual dorsal aorta of Atlantic salmon (*Salmo salar)* following bath administration. *Aquaculture,* 286, 301-308.

Korte, S.M., & Olivier BKoolhaas, J.M., (2007). A new animal welfare concept based on allostasis. *Physiology Behaviour,* 92, 422-428.

Lambooij, B., Pilarcyzk, M., Bialowas H., & Hens, V.D., (2009). Anaesthetic properties of propiscin and 2- phenoxyethanol in the common carp (*Cyprinus carpio)* neural and behavioural measures. *Aquaculture Research,* 40, 1328-1333.

Larissa, N. S., Danielle, C.L., & Andrea, T.M., Levy C.G. (2011). Efficacy of clove oil as anaesthetic in handling and transportation of Nile tilapia juveniles. *Zoological,* 28(3), 285-290.

Martinez-Porchas, M., Martinez-Cordova, L. R., Ramos-Enriquez, R. (2009). Cortisol and glucose: reliable stress indicators of fish stress? *Pan-American Journal of Aquatic Sciences,* 4, 158–178.

Matsunaga, W., & Watanabe, E., (2010). Habituation of medaka (*Oryzias latipes*) demonstrated by open-field testing. *Behavioural Process,* 85, 142-150.

McEwen, B.S., & Wingfield, J.C., (2003). The concept of allostasis in biology and biomedicine. *Horm Behaviour,* **43**, 2-15

Metz, J.R., van den Berg, E.H., Bonga S.E.W., & Flik, G., (2003). Regulation of branchial Na/K-ATPase in common carp (*Cyprinus carpio* L.) acclimated to different temperatures. *Journal of Experimental Biology,* 206, 2273-2280.

Mommsen, T.P., Vijayan M.M., & Moon T.W., (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology,* 9, 211-268.

Nikoo, M., & Falahatkar, B., (2012). Physiological responses in wild brood stocks of the Caspian Kutum (*Rutilus frisii kutum*) subjected to transportation stress. *Journal of Applied Animal Welfare and Science,* 15, 372-382.

Ohl, F., & van der Staay F.J., (2012). Animal welfare: at the interface between science and society. *Vetrinary Journal,* 192, 13-19.

Orji, R.C.A, (2005). The effect of transportation stress on haematocrit level of *Orechromis niloticus*. *Animal Research International*, 2, 224-226.

Robertson, L.P., Thomas, C.R., & Arnold., Y.M., (1987). Plasma cortisol and secondary stress. Responses of red drum to handling, transport, rearing density and disease outbreak. *Progressive Fish Culturist,* 49, 1-2.

Sinka, T.D., & Nealb, W., (2009). Stress response and post transport survival of hybrid with or without clove oil. *North American Journal of Aquaculture,* 71, 267-275.

Small, B.C., (2004). Effects of isoeugenol sedation on plasma cortisol, glucose and lactate dynamics in channel catfish, *Ictalurus punctatus* exposed to three stressors. *Aquculture,* 238, 469-481.

Zar, J.H., (1996). *Biostatistical Analysis* (4th Edition. practice Hall, Publishers, New Jersey, U.S.A.