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Journal of Fisheries Science
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ARTICLE

Effects of Different Combinations of Two Spices: Clove and Nutmeg Seed Extracts on Antioxidants Levels in African Catfish (Clarias gariepinus)

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ARTICLE INFO

Article history
Received: 23 September 2021
Accepted: 8 October 2021
Published Online: 15 October 2021

Keywords:
Spices
Nutmeg
Clove
Anaesthetics
Fish
Antioxidants

ABSTRACT

The effect of different combinations of botanical spices such as clove and nutmeg in different proportion on the antioxidants activities which include lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxide (GPX) in juveniles and adults sizes of *Clarias gariepinus* was investigated using different combinations of clove (C),and nutmeg (C.N 0:0- Control 0% of Clove and Nutmeg; C.N 1:3- 25% Clove and 75% Nutmeg; C.N 3:1- 75% Clove and 25% Nutmeg; C.N 2:2- 50% Clove and 50% Nutmeg; C.4 - 100% Clove; N4- 100%) in triplicates. The results from the study indicated that the anaesthetic caused a substantial ($p<0.05$) modifications in the five antioxidants under examination. The highest deviations in the studied antioxidants were observed in the fish exposed to C4 combination of the anaesthetics and the lowest in the control. The results from this work therefore suggest that the anaesthetics can alter antioxidants levels in the fish which was more noticeable in the fish exposed to C.N 3:1- 75% Clove and 25% Nutmeg; C.N 2:2- 50% Clove and 50% Nutmeg; C.4 - 100% Clove; N4- 100%. Hence fish farmers and scientists are advised to take caution when combining these plant extracts for use in aquaculture.

1. Introduction

Stress is often associated with disease outbreak in cultured fish [1]. Stress in aquatic organisms such as fish can trigger substantial losses of both animals and capital in both capture and culture systems [2-4]. In reducing these losses to the barest minimum necessitate information about the nature of the stressors, stress intensity and fitness effect [5]. In the culture medium, the welfare of cultured fish is often compromised by stress and has become an increasing concern in most of the aquaculture facilities [6-8]. Furthermore, the influence of a variety of different segment of aquaculture operations such as transportation, handling and netting, confinement and short-term crowding, inappropriate stocking densities, water quality deterioration on fish welfare and the

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DOI: https://doi.org/10.30564/jfsr.v3i2.3741

consequent effects on to fish welfare must be minimized, so as to enhance productivity [9].

One of the predominant and common types of stress in a living organism is oxidative stress. Oxidative stress can be described as a disruption of the pro-oxidant-antioxidant equilibrium in favour of the former, resulting in possible impairment of cells [10]. It occurs as a result of an elevation in Reactive Oxygen Species (ROS) and destruction of antioxidant defence systems or incapacity to repair oxidative damage. The main damage induced by ROS results in alterations of cellular macromolecules such as membrane lipids (lipid peroxidation), DNA [11]. The resulting damage may alter cell task, eventually leading to cell death. Enzymatic (CAT) and non-enzymatic antioxidants such as reduced glutathione (GSH) and its precursor normally counteract damaging effects of ROS by either repairing the oxidative damage or precisely foraging oxygen radicals. Catalase is a common enzyme found in almost all the organisms which are exposed to oxygen, where it functions to catalyze the breakdown of hydrogen peroxide to oxygen and hydrogen oxide. Glutathione (GSH) is an antioxidant which helps to protect the cells from the ROS such as free radical**s** and peroxides. The glutathione defense enzyme systems in living cell detoxifies and eradicates the xenobiotics leading to the arrangement of products that can easily dissolve in water and their quick removal from the cell of the organism [12,13] .

Anaesthesia is a biological condition with the partial or total loss of feeling or loss of voluntary neuromotor control induced by chemical or nonchemical means [14]. Anaesthesia minimizes pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness [15,16]. Anaesthetics are chemical substances used by fish farmers to minimize the rate of mortality during handling and transport. This may also reduce the susceptibility of cultured fish to pathogens and infection [17]. Anaesthetics are also used in fish farming during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical process [18]. When choosing ananaesthetics, a number of factors are put into consideration which include efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment [19], and the choice may also depend on the nature of the experiment and species of fish under consideration [20,21].

In application of anaesthetics during aquaculture operations, information about the appropriate and most favourable concentration of an anaesthetic for a range of fish species is essential because improper concentrations might lead to harmful effects such as stress [22-24]. Hence, access to nontoxic and efficient fish sedatives is a crucial necessity of fisheries researchers, manager, and culturists [25]. Recently, nutmeg (*Myristica fragrans)* and clove bud (*Syzigium aromaticum*)h ave received favorable reviews as an alternative fish anesthetic for a number of fish species as well as for crustaceans [26,27]. Nutmeg has been utilized in different parts of the world for medicinal purposes. Nutmeg (*Myristica fragrans*) is a member of the Myristicaceae family. It is a perennial tree found ecologically in the tropics and well distributed in the north-central region of Nigeria. Extracts from its nuts contains 70-90% myristidate4-21% beta-caryophyllene, 1-21% eugenyl acetate and 10 19% tannin [28].

Despite the several studies that have been done on some physiological changes in *C. gariepinus* exposed to clove and nutmeg [29,30], however there is no available literature on the combined effects of clove buds and nutmeg on the these variables. This study was conducted out to assess the effect of these plant materials as anaesthetics on the selected antioxidants in the plasma of juvenile and adult sizes of C. *gariepinus*. This study will shed more light on stress management procedures in fish farming, using combination of two plants extract as anaesthetics. The aims of the present study therefore, are to evaluate the effects of anaesthesia with mixture of clove and nutmeg buds on the antioxidants in the plasma of juvenile and adult of sizes of *C.gariepinus.*

2. Materials and Methods

Sources and acclimation of Experimental Fish

A total of 180 specimens of *Clarias gariepinus* comprising of 90 each of juveniles (mean length17.78 cm±2.88 SD mean weight 106.99g± 4.78SD), and adults (mean length 29.33 cm±3.01 SD and mean weight 654.43g±11.89SD) were obtained from African Regional Aquaculture Centre, (ARAC), Aluu, Rivers State of Nigeria. They were transported in four 50 L jerry cans to the Rivers State University Fish farm and acclimated for a period of seven days. During this time the fish were fed with a commercial feed (45.0% CP) at 3% body weight. The water in acclimation tanks was changed every two days [26].

Preparation of Clove bud and nutmeg

Dry nutmeg seeds (Plate 1) and dried buds of clove plant **(**Plate 2) were purchased from Choba Market in ObioAkpor Local Government Area of Rivers State, Nigeria. Plant verification was done by means of the keys described by Agbaje [31]. The seeds were taken to the Fisheries laboratory and milled into power using a kitchen

 DOI: https://doi.org/10.30564/jfsr.v3i2.3741

blender (Model H2, Ken wood, Japan). The pulverized seeds were afterwards sieved using 0.1 micro nylon meshes to get the fine particles of the spices.

Plate 1. Nutmeg seeds

Plate 2. Clove seeds

Experimental Design and procedures

The design of the experiment is a Randomized Complete Block Design (RCBD) having six treatments levels each with three replicates for each of the life stages. A total of 36 plastic aquaria of dimension (52 x 44 x 34 cm^3) each were used for the experiments. The 36 aquaria were labelled based on the experimental units and replicates. Each aquarium was stocked with five fish. The powder was combined based on ratio of Clove : nutmeg into different proportion of C.N 0:0- Control 0% of Clove and Nutmeg; C.N 1:3- 25% Clove and 75% Nutmeg; C.N 3:1- 75% Clove and 25% Nutmeg; C.N 2:2- 50% Clove and 50% Nutmeg; C.4 - 100% Clove; N4- 100% Nutmeg. This was achieved by using weighing balance. It was applied directly in three replicates into the water (10 L) in 30 L experimental plastic aquaria. The mixtures were stirred extensively to ensure homogenous mixture. They were then introduced into prepared experimental aquaria, containing 5 concentrations of powdered nutmeg and clove at the rate of ten fish per tank in triplicates. The fish was then introduced into the tank. When the fish has attained stage five anaesthesia (the point when the fish has lost sensitivity to gentle prodding with rod), blood samples were collected from the caudal vein with syringe and blood samples were preserved in heparinized bottle for analyses.

Evaluation of Water Quality Parameters

Water quality variables such as: dissolved oxygen, nitrite, ammonia and sulphide were evaluated using LaMotte fresh water test kit (Model AQ4, Chestown, Maryland, USA). pH was determined with pH meter (Model, H 9812, Hannah Products, Portugal). The dissolved oxygen level was evaluated by the Winkler method [32].

Sample Preparation

At the end of each experimental period, 2 ml of fresh blood sample was collected by making a caudal puncture with the help of fine needle and transferred into heparinized sample bottles. Blood samples were centrifuged immediately for 15 minutes at 5000 rpm. Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20°C until assayed. The results were read using a universal microplate reader on a Jenwayvisible spectrophotometer (Model 6405). The activity of antioxidants in centrifuged plasma was determined spectrophotometrically using the method of Eales [33].

Statistical Analysis

All the data were expressed as mean and standard deviation of mean. The statistical package, SPSS Version 22 was used for the data analysis. The means were separated using two-ways ANOVA and the two means were considered significant at 5%($P<0.05$).

3. Results

The physico-chemical indices of water in the experimental tanks of *C.gariepinus* exposed to combined nutmeg and clove seed powder is shown in Table 1. The results indicated significant differences ($p<0.05$) only among the dissolved oxygen and ammonia in the control and treated group range. The values of the other variables were not significantly different ($P>0.05$). Difference in the values of antioxidants in the juveniles of C.*gariepinus* juveniles exposed to different combination of nutmeg and clove seed powder were presented in Table 2. There was significant reductions ($P<0.05$) comparable to the control values in GSH, SOD and GPX, whereas the values of CAT and LPO increased. Moreover, the same trends were also recorded in adult size of C.*gariepinus* exposed to these plant powder (Table 3). Comparative values of GPX in juveniles and adults of C.*gariepinus* exposed to different combinations of nutmeg and clove seed extracts are presented in Figure 1. The highest value of 50.00 was recorded in the adult fish exposed to the control, while the lowest was in C.N 3:1. However, GPX values in the adult fish were consistently higher than that of the juveniles (Figure 1).

DOI: https://doi.org/10.30564/jfsr.v3i2.3741

Table 1. Physio-Chemical Parameters of Water in the Experimental Tanks of C.*gariepinus* Exposed to Combined Nutmeg and Clove bud Powder.

Parameters	Combinations of Clove and Nutmeg in Different Proportion					
	C.N 0:0	C.N 1:3	C.N 3:1	C.N 2:2	C 4	N 4
DO (mg/l)	6.33±0.30[d]	5.33±0.57[c]	4.00±0.00[b]	4.33±0.57[b]	3.66±0.57[a]	5.00±0.00[c]
NH3 (mg/l)	0.02±0.01[a]	0.05±0.00[b]	0.04±0.01[b]	0.03±0.00[a]	0.05±0.00[a]	0.07±0.00[a]
Tem 0C	29.66±1.15[a]	30.00±1.00[a]	29.33±0.57[a]	29.33±0.57[a]	29.33±0.57[a]	29.33±0.57[a]
pH	5.33±0.57[a]	5.33±0.57[a]	6.00±0.00[a]	5.33±0.57[a]	5.50±0.50[a]	5.33±0.57[a]
Condt (S/m)	115.0±1.0[a]	123.00±1.0[a]	128.66±1.52[a]	126.66±1.52[a]	131.33±1.15[b]	119.00±1.00[a]
Nitrite (mg/l)	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
Nitrate (mg/l)	0.13±0.01[a]	0.15±0.02[a]	0.13±0.02[a]	0.13±0.02[a]	0.12±0.01[a]	0.14±0.01[a]

Means within the same row with different super scripts are significantly different (P<0.05)

Key: C.N 0:0- (Control) 0% of Clove and Nutmeg; C.N 1:3- 25% Clove and 75% Nutmeg; C.N 3:1- 75% Clove and 25% Nutmeg; C.N 2:2- 50% Clove and 50% Nutmeg; C.4 - 100% Clove; N4- 100% Nutmeg.

Table 2. Antioxidants Levels in the blood of C.*gariepinus* Juveniles Exposed to Different Combination of Nutmeg and Clove bud Powder.

Concentration	GPX	CAT	GSH	SOD	LPO
C:N 0:0	39.66±1.52[b]	62.00±2.00[a]	5.33±0.57[c]	10.33±0.57[b]	7.33±0.57[a]
C:N 1:3	38.08±3.46[b]	65.66±1.15[a]	4.66±0.57[c]	9.33±0.57[a]	8.66±0.57[a]
C:N 2:2	30.66±1.15[b]	74.33±1.15[b]	2.33±0.57[b]	6.00±1.00[a]	12.00±1.00[b]
C:N 3:1	26.66±2,08[a]	76.00±1.00[b]	3,33±0.57[b]	6.00±0.00[a]	13.00±1.00[b]
C 4	28.33±1.52[a]	79.00±1.00[b]	1.66±0.57[a]	5.66±0.57[a]	14.66±0.57[b]
N 4	35.33±5.89[b]	70.00±1.00[b]	3.33±0.57[b]	6.66±0.57[a]	9.66±0.57[a]

Means within the same column with different super scripts are significantly different (P<0.05)

Key: C.N 0:0- (Control) 0% of Clove and Nutmeg; C.N 1:3- 25% Clove and 75% Nutmeg; C.N 3:1- 75% Clove and 25%:2- 50% Clove Nutmeg; C.N 2and 50% Nutmeg; C.4 - 100% Clove; N4- 100% Nutmeg.

Table 3. Antioxidants Levels in the blood of C.*gariepinus* Adults Exposed to Different Combination of Nutmeg and Clove budPowder.

Concentration	GPX	CAT	GSH	SOD	LPO
C:N 0:0	50.00±2.00[a]	82.00±2.00[a]	7.66±0.57[a]	15.33±0.57[a]	12.66±1.15[a]
C:N 1:3	48.33±3.78[a]	86.00±1.00[a]	7.33±1.15[a]	14.33±0.44[a]	13.66±0.57[a]
C:N 2:2	41.00±1.00[a]	95.00±1.00[a]	5.00±1.00[a]	11.33±0.57[a]	17.00±1.00[a]
C:N 3:1	37.66±2.08[a]	92.66±4.93[a]	5.33±9.57[a]	11.33±0.51[a]	18.66±1.52[a]
C 4	38.66±2.08[a]	99.00±1.00[a]	5.00±0.00[a]	10.33±0.52[a]	19.66±0.57[a]
N 4	46.00±1.00[a]	90.66±1.15[a]	5.66±0.57[a]	11.33±0.09[a]	14.33±1.54[a]

Means within the same row with different super scripts are significantly different (P<0.05)

Key: C.N 0:0- (Control) 0% of Clove and Nutmeg; C.N 1:3- 25% Clove and 75% Nutmeg; C.N 3:1- 75% Clove and 25% Nutmeg; C.N 2:2- 50% Clove and 50% Nutmeg; C.4 - 100% Clove; N4- 100% Nutmeg.

 DOI: https://doi.org/10.30564/jfsr.v3i2.3741

The activity of Glutathione perioxide in both juveniles and adult declined below their control values under the exposure to the anaesthetics (Figure 2). The highest value of CAT (99.0 IU/L) was recorded in C4 (100 % Clove), while the lowest (62.0 IU/L) was recorded in juvenile fish in the control (Figure 2). Despite this, the values of CAT were higher in the adult fish when put side by side to the juveniles in all the exposure combinations. Furthermore, the similar inclinations were also recorded in the values of GSH (Figure 3), with the values in adult fish higher than that of the juveniles in all combinations of clove and nutmeg extracts. The values of SOD and LPO in both sizes of *C.gariepinus* exposed to these plant extracts are presented in Figures 4 and 5 respectively. However, these values were higher in adult fish when compared to the juveniles.

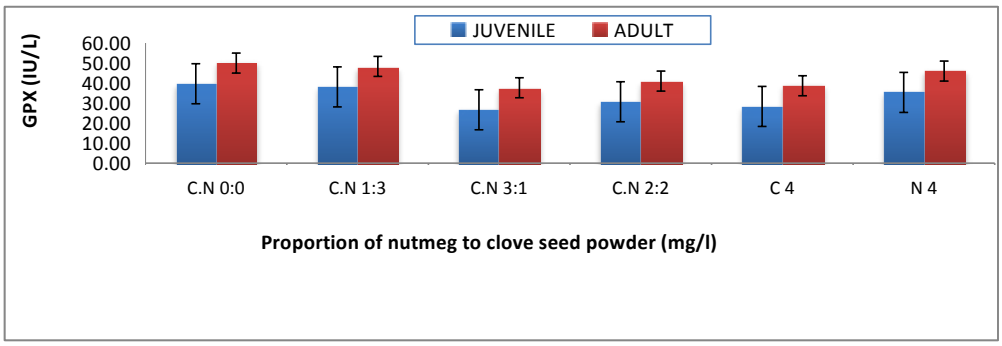

Figure 1. Effect of different combinations of Nutmeg seed and Clovebuds powder on Glutathione peroxide (GPX) activities in the sera of juvenile and adult *C. gariepinus*

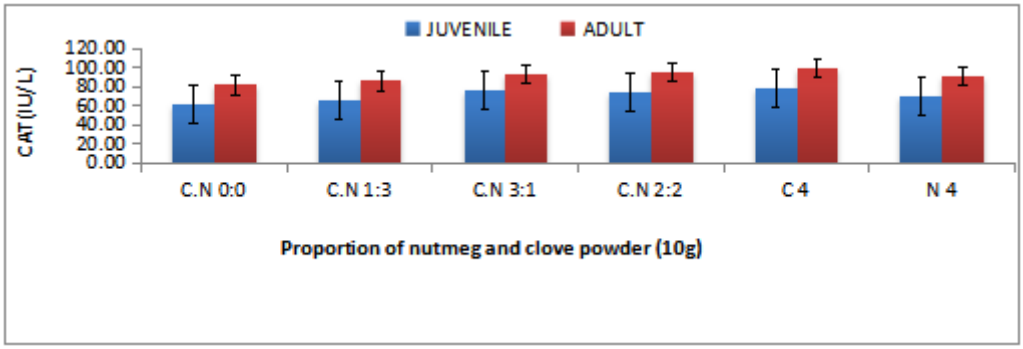

Figure 2. Effect of different combination of nutmeg and clove bud powder of Catalase (CAT) activities in the blood of juvenile and adult *C. gariepinus*

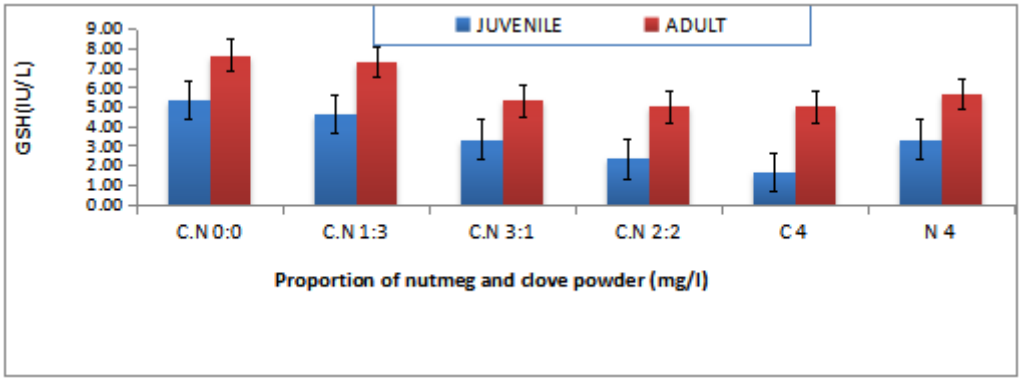

Figure 3. Effect of different combination of Nutmeg and Clove bud powder of Glutathione GSH activities in the plasma of juvenile and adult of *C. gariepinus*

DOI: https://doi.org/10.30564/jfsr.v3i2.3741

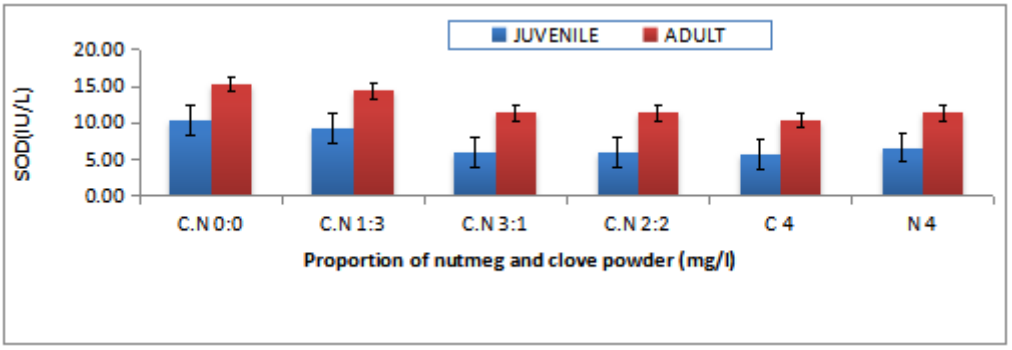

Figure 4. Effects of different combination of nutmeg and clove bud powder on SOD activities in the plasma of juvenile and adult of *C. gariepinus*

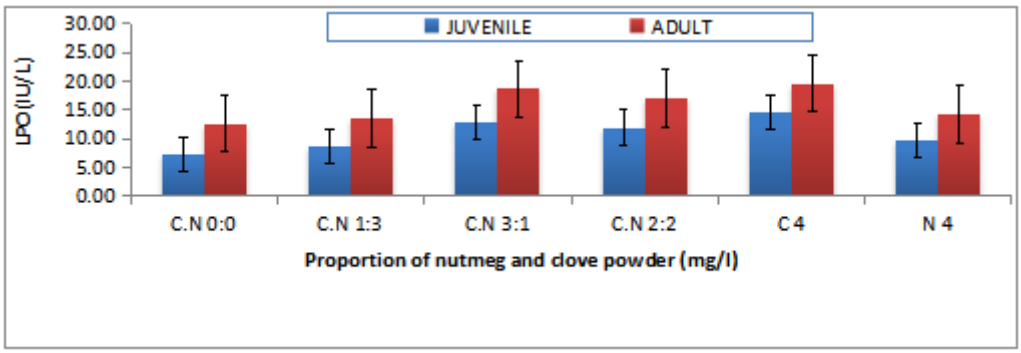

Figure 5. Effects of different combination of Nutmeg and Clove bud powder on Lipid peroxidation LPO activities in the plasma of juvenile and adult of *C. gariepinus*

4. Discussion

Oxidative stress is a condition when stable position ROS concentration is transiently or chronically enhanced, distressing cellular metabolism and its regulation and damaging cellular constituents [34]. It can also be described as a condition when antioxidant defences are overwhelmed by pro-oxidant forces [35]. The commencement of oxidative manifestations leads to the response of antioxidants activation in articulation of genes encoding antioxidant enzymes. Nevertheless, there are substantial disparities in the knowledge on response to oxidative stress, specifically in aquatic animals. Antioxidant enzymes are included in stress evaluation because of their indelibility under conditions of mild oxidative stress and their potential role in adaptation to aquaculture -induced stress. It is expected that they may be more responsive at revealing preliminary stress induced insult on the cells [36]. Laboratory studies established that the dimension of variations in the manifestation of a large number of specific genes or performances of certain enzymes of antioxidant defence can be investigated in an early warning system of toxicant exposure [34]. Apparently, the early caution can be used at what time temporal effect of stress is expected. Typically, the range of oxidative stress indices in fish includes the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), lipid peroxidase (LPO) and glutathione s transferase (GST). The evaluation of these has most frequently been utilized in eco-toxicology programmes for fish [37].

In this work, increased values of CAT and LPO activities in combination with the decrease in the values of SOD, GSH and GPX were recorded in the plasma of *C.gariepinus* sampled in both juveniles and adult sizes of C.*gariepinus* exposed to different combinations of nutmeg and clove seed extracts. A similar result was observed in *C. carpio* exposed to higher concentrations of clove oil [38]. Moreover, the concerted elevation of LPO and CAT activities was equally indicated in the plasma of starlet fish (*Acipenser ruthenus*) exposed to 2-phenoxy- ethanol as anaesthetics [39]. In the study of three populations of brown trout (*S. trutta*) treated with elevated levels of anaesthetic MS222, all the exposed fish recorded higher activities of CAT in their plasma compared to unexposed trout [40] (Hansen *et al*., 2006). Conversely, imbalanced antioxidant activities were observed in the various oxidative stress biomarkers in the Indian freshwater fish,

 DOI: https://doi.org/10.30564/jfsr.v3i2.3741

Wallagoattu exposed to anaesthetics metomidate [41].

The increase recorded in the values of catalase activity or its steadiness alongside with reduction in SOD and GST activities have been reported by some authors [39,40]. In spite of Lowry *et al*. [42] observed that in plasma, hepatic and adrenal tissues of white sucker (*C. commersoni*) exposed to some anaesthetics from some farms in Agricultural region in Québec (Canada) had their catalase activities higher than those fish from the control. On the other hand, the same trend was observed in this study. Nevertheless, Falfushynska and Stolyar [43], ascribed the soaring catalase activity in fish exposed to a quantity of extracts, to little production of oxygen, which has been reported to boost the making of catalase as cell stability in the case of surplus infusion of pollutants [44]. On the contrary Catalase decline and activation can be considered as a last resort of antioxidant defence in teleost fish. The catalase role in the antioxidant defence of fish was reported by Porte [45], based on the information on its activation by hydrogen peroxide at high concentrations. They suggested that catalase on the whole plays a comparatively inconsequential role in hydrogen peroxide catabolism at low rates of peroxide generation, but it becomes crucial when the rate of hydrogen peroxide production is enhanced, for example, at oxidative stress.

GSH is a foremost cytosolic low molecular weight sulfhydyl compound that functions as cellular reducing and protective reagent against a wide range of contaminant through SH-group. It directly acts a scavenger of oxyradical and also as an antioxidant enzyme base. Apparently, GSH is essential in protecting against deleterious effects of the cell exposed to ROS by reacting with them to form glutathione disulphide (GSSG). This antioxidant defence effect occurs spontaneously through GSH or by GST. It acts as cofactor for glutathione transferase, which make it easy for the elimination of some chemicals and erstwhile reactive molecules from the cells [46]. Thus a change in GSH levels may be a vital indicator of detoxification capacity of an organism. During present investigation, significant decrease in GSH level observed in the plasma of both sizes of *C.gariepinus* at different exposures could be due to its utilization to confront the current oxidative stress under the influence of ROS produced from anaesthetics exposure. Reduced GSH and its metabolizing enzyme make available the principal resistance in opposition to ROS induced cellular destruction [47]. This reduction may be because of increased utilization of GSH, which can be transformed to oxidized glutathione and potentially fragile GSH regeneration. Furthermore, these authors affirmed that GSH depletion point to its exhaustive phase II biotransformation; by this means boost the risk of oxidative stress due to reduced cell protection activity [48]. Similar decrease in the plasma of *Cyprinus carpio* have also been reported on exposure to clove at higher concentrations [49] . Present observations are in agreement with the findings of Avilez *et al.* [15] who studied effect of propiconazole on *Oncorhynchus mykiss,* these researchers reported a decrease in GSH level in the plasma of the fish and pointed it to be a primary protective response of the cell against oxidative stress induced by pollutants.

GPX is a group of multifunctional isoenzymes, which play an important role in detoxification of toxic electrophiles by catalyzing the conjugation of a wide variety of electrophilic substrates to GSH and thus protects the cell from oxidative stress. It is considered as first line of defence against oxidative stress injury, decomposing superoxide radicals and hydrogen peroxide before interacting to form the reactive hydroxyl, which has a number of adverse biological effects when present in high amounts [50, 51]. The reduced GPX activity is concomitant to the decrease in GSH level in the plasma liver and gills. From the above discussion, it could be inferred that GPX utilizes GSH for the xenobiotic detoxification.

5. Conclusions and Recommendations

The parameters measured provided useful information for evaluating the toxicological effects of different combinations of nutmeg and clove buds in *C.gariepinus*. From the results of this study, it was revealed that the combination of C.N at ratio 1:3 which consists of 25% Clove and 75% Nutmeg is suitable for use in aquaculture, because of its minimal alterations in the antioxidant activities in *C.gariepinus* when compared to other concentrations. From this study it is obvious that this combination can be effectively applied to anaesthesized fish during aquaculture operations.

References

[1] Small, B.C., Sladky, K.K & Davis, K.B. (2004). Validation of a time-resolved fluuroimmunanoassay for measuring Plasma cortisol in channel, catfish (*Ictalurus punctatus)*. *Journal of World Aquaculture Society,* 33, 184-187.

[2] Adeyemo, M.I., Umoh. I .B., & Eyong, E. U. (2009). Influence of *Nauclea latifolia* leaf extracts on some hepatic enzymes of rat fed on coconut oil and non-coconut oil meals. *Pharmaceutical Biology,* 43 (2), 153-157.

[3] Davis, E. W., (2010). Physiological responses of rainbow trout (*Oncorhychus mykiss)* to crowding and anaesthesia wish Aquis-S. *Journal of World Aquaculture Society,* 31(1), 105-114.

DOI: https://doi.org/10.30564/jfsr.v3i2.3741

[4] Akinrotimi, O.A, Wilfred-Ekpripo, P. C & Ukwe, O.I.K (2018). Oxidative Stress in *Tilapia guineensis* Exposed to Paraquat Dichloride in the Laboratory. *Journal of Advanced Research in Medical & Pharmaceutical Sciences* (IJARMPS-ISSN-2455-6998), 3(5), 14-18.

[5] Portz, D.E., Woodley, C.M., & Cech, J.J. (2006). Stress-associated impacts of short-term holding on fishes. *Reviews in Fish Biology and Fisheries,* 16, 125-170.

[6] Chandroo, K.P., Duncan, I.J.H., &Moccia, R.D. (2004). Can fish suffer? Perspectives on sentience, pain, fear and stress.*Applied Animal Behaviour Science,* 86*,* 225-250.

[7] Turnbull, L., Carrick, T.R., & Pottinger, T.G. (2007). Divergence of endocrine and metabolic responses to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress. *General and Comparative Endocrinology,* 133, 332-340.

[8] Brownmall, R.E., Gontijo, A.M., & Volputo, G.L. (2009). MS-222 does not induce primary DNA damage in fish. *Aquacutlure International,* 15, 163-168.

[9] Gabriel, U.U., & Akinrotimi, O.A. (2011). Management of Stress in fish for sustainable aquaculture development. *Research,* 3(4), 28-38.

[10] Agviar, L.A., Kalinin, A.L., &Rantin F.T. (2002). The effects of temperature on the neotropical fish *Piaractousmesopotamicus. Journal of Thermal Biology,* 27, 299-308.

[11] Ahmed, S.M., Swami V., Dhanapai, P.G.R., & Chandrasekhar, V.M. (2005). Anti-Diabetic activity of *Terminalia catalppas*leaf extracts in arlexian-induced diabetic rats. *Iranian Journal of Pharmacology and Therapeutics,* 4 (1), 36-39.

[12] Adamek M, Jung-Schroers V, Hellmann J, Teitge F and others (2016) Concentration of carp edema virus (CEV) DNA in koi tissues affected by koi sleepy disease (KSD). Dis Aquat Org 119: 245-251.

[13] Akinrotimi, O.A., Aranyo, A.A., & Ibemere, I.F. (2011a). Physiological response in *Tilapia guineensis* subjected to handling stress. *Advances in Agriculture science* and Engineering Research, 1(2), 34-39.

[14] Summerfelt, R.C., & Smith, L.S. (199O). Anaesthesia, surgery and related techniques. *Methods for Fish Biology* (Schreck, C.B.,& Moyel,P.B). Bethesda, MD, USA: American Fisheries Society.

[15] Avilez, I. M., Altran, A E., Agira, L. H., & Morales, G. (2004). Haematological responses of the neotropically teleost matrixa (*Bryon cephalous*) to environmental nitrite.*Comparative Biochemistry* and *physiology,* 139,135-139.

[16] Akinrotimi, O.A., Uedeme-Naa, B. and Agokei, E.O. (2010). Effects of acclimation on haematological parameters of *Tilapia guineensis. Science World Journal.* 5(4):1-4.

[17] Wood, L.C., Thiesen, D.D., & Hej, S. (2008). Efficacy of Aqui-S as an anaesthetic for market-sizes striped bass. *North American Journal of Aquaculture,* 70, 219-222.

[18] Adamek, Z., Tasic, K., Paul, K., &Lamasic, M. (1993). The effect of 2-phnenoxyethanol narcosis on blood of young carp, *Veterinary Archives,* 63, 245-250.

[19] Sovio, A., Nylom, K., Huhti, M (2007), Effects of anaesthesia with MS -222, and benzocaine on the blood constituents of rainbow trout (*Salmo gairdineri). Journal of Fish Biology,* 10, 91-101.

[20] Monday, P.L., & Wilson, S.S. (1997). Comparative efficacy of clove oil and other chemicals in anesthetization of *Pomacentrus amboinensis* a coral reel fish. *Journal of Fish Biology,* 51, 931-938.

[21] Akinrotimi, O.A., Aranyo, A.A., & Ibemere, I.F. (2011b). Effects of capture, handling and confinement on the glucose levels of black jaw tilapia *Sarotherodon melanotheron. Advances in Students Research*, 1(2), 27-30.

[22] Martins, S.D., Redfish, M.F., O'Dea,N. G., & Shrimpton, J.M. (2009). Are we missing a mineral corticoid in teleost fish? Effects of cortisol deoxycorticosterone and aldosterone on osmo regulation in Atlantic salmon. *General Comparative Endocrinology,* 157, 35-40.

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

[24] Popovic, T.N., Strunjak-Perovic, I., Coz-Pakovac, R., Barisic, J., Jadan, M., & Saverbum, K. (2012). Tricaine methane-sulfonate (MS-222) application in fish anaesthesia a review. *Journal of Applied* I*chthyology,* 28, 553-564.

[25] Akinrotimi, O.A., Gabriel, U.U., & Edun O.M. (2015). The efficacy of clove seed extracts as an anaesthetic agent and its effect on haematological parameters of African catfish (*Clarias gariepinus*). *International Journal of Aquaculture and Fishery Sciences* 1(2), 042- 047.

[26] Gabriel, U.U., Ezeri G. N. O., &Opabumi O. O. (2004). Influence of sex, source, health status and acclimation on the haematology of *Clariasgariepinus. African Journal of Biotechnology,* 3 (9), 463-437.

[27] Gholipourkanani, H., Versteeg, D.J., &Ahadizadeh, W. (2013).Use of propofol as an anaesthetic and its efficacy on some haematological values of ornamental fish (*Carassiusauratus). Spring Plus,* 2, 76-81.

 DOI: https://doi.org/10.30564/jfsr.v3i2.3741

[28] Acrete, L., Espinosa, E., Josa, A., &Tont, L. (2016). Physiological response of hybrid striped bass subjected to anaesthesthetics . *Aquaculture,* 321, 44-54.

[29] Akinrotimi O.A., Ansa, E.J & Edun, O.M (2016). Effectiveness of clove seed extracts as anaesthetics in transportation of *Tilapia guineensis* juveniles. *Journal of Aquaculture Engineering and Fisheries Research* 2(2), 67-75.

[30] Akinrotimi, O.A and Aiyeloja, J.O (2018). Effectiveness of Nutmeg ectracts as anaesthetics in transportation of Tilapia guineensis .Pp 67-70. In Ndimele, P.E. (eds). 33rd Annual Conference of the Fisheries Society of Nigeria, October 29th -November 2nd , 2018, Ikorodu, Lagos State Nigeria.

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

[32] APHA, (2001). *Standard methods for the examination of water and waste water* (17th edition). Washington D. C, USA . American Public Health Association Press.

[33] Eales, J.G. (1985). The peripheral metabolism status in poiklotherms in response to chemical application. *Canadian Journal of Zoology,* 64, 1817-1828.

[34] Lushack, C. (2011). Toxicological effect of anaesthetic exposure on haematological, biochemical and liver transaminases activity in an Indian major carp *Catla catla*. *Food Chemistry and Toxicology,* 48, 2848-2854.

[35] Livingstone, D.R. (2001). Contaminant-stimulated Reactive Oxygen Species Production and Oxidative Damage in Aquatic Organisms. *Marine Pollution Bulletin,* 42,656-666.

[36] Adams, S.M. and Greeley, M.S. (2000). Ecotoxicological Indicators of Water Quality: Using Multi-response Indicators to Assess the Health of Aquatic Ecosystems. *Water, Air and Soil Pollution*, 123: 103-115.

[37] Viarengo, M.M., Pereira, C., Grav, E.G., & Iwama G.K. (2007). Metabolic responses associated with continental stress in tilapia, the role of cortisol. *Comparative Biochemisry and Physiology,* 166, 89-95.

[38] Huang C., Chao, L.C., & Lin, H.A. (2007). Na^+, k^+ AT pase and vacuolar-type H^+-AT pase in the gill of the aquatic air breathing fish (*Trichogaster microlepis)* in response to salinity variation. *Comparative Biochemistry and Physiology,* 155, 309-318.

[39] Stanic, B., Andric, N., Zoric, S., Grubor-Lajsic, G. and Kovacevic, R. (2006). Assessing Pollutionin the Danube River Near Novi Sad (Serbia) Using Several Biomarkers in Sterlet (*Acipenser ruthenus* L.). *Ecotoxicology and Environmental Safety,* 65: 395-402.

[40] Hansen C.S., Clyde V.L., Wallace R.S., Paulmurphy J., Patterson T.A., & Sladky K.K. (2006) Effects of anaesthesia and surgery on serial blood gas values and lactate commendations in yellow perch (*Perca flavescens*). *Journal of the American Veterinary Medical Association,* 236, 1104-1108.

[41] Portz, D.E., & Martinez, B.M. (2009). Farmed fish welfare suffering assessment and impact on product quality. *Italian Journal of Animal Science,* 8(1), 139-160.

[42] Lowry, O.M., Rosenbrough, N.J., Farr, A.C. and Randall, R.F. (2010). Protein estimation with Folin Phenol reagent. *The Journal of Biological Chemistry,*193: 265-275.

[43] Falfushynska, H.I. and Stolyar, O.B. (2009). Function of Metallothioneins in Carp *Cyprinus carpio* from Two Field Sites in Western Ukraine. *Ecotoxicology and Environmental Safety*, 72, 1425-1432.

[44] Akinrotimi, O.A, Wilfred-Ekpripo, P. C & Ukwe, O.I.K (2018). Effects of 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) on leukocyte profiles in juveniles and adult sizes of *Tilapia guineensis*. MOJ Immunology, 6(5), 173-176.

[45] Porte, B.M. (2002). Farmed fish welfare suffering assessment and impact on product quality. *Italian Journal of Animal Science,* 8(1), 139-160.

[46] Ortuno, J., Argeles-Esteban, M and Mesegver, J (2002). Lack of effect of combining different stress on innate immune response of sea bream (Sparus aurata). *Veterinary Immunology and Immunopathology,* 84:17-27.

[47] Acker, P.A. (2000). Stress hormones and the cellular stress responses in salmonids. *Journal of Fish Physiology and Biochemistry,* 23(2), 236-240.

[48] Kono, Y. and Fridovich, I. (2012). Superoxide Radical Inhibits Catalase. *The Journal of BiologicalChemistry*, 257, 57-51.

[49] Carter, E., Marco, P.D., Madich, A ., & Cataudella, S. (2011). Serum parameters of Adriatic sturgeon (*Acipenser naccarii*) effects of temperature and stress. *Comparative Biochemistry and Physiology,* 121, 351-354.

[50] Arjona, F.J., Ruiz-Jarabo, I., Vargas-Clacoff, L., Flik, G., Mancera, J.M., &Klaren, P.H.M. (2010). Acclimation of *Soleasenegalensis*to different ambient temperatures implications for thyroidal status and osmoregulation.*Marine Biology,* 157, 1325-1335.

[51] Akinrotimi, O., A., & Achilike N.M. (2019). Efficacy of nutmeg (*Myristica fragrans*) extracts as anaesthetics in transportation of *Sarotherodon melanotheron.* Pp 35-39. In Lelei, K.E. (Eds). 34rd Annual Conference of the Fisheries Society of Nigeria, October 28th -November 1st, 2019, Abakaliki, Ebonyi State, Nigeria.

DOI: https://doi.org/10.30564/jfsr.v3i2.3741