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ORIGINAL ARTICLE

Immunomodulatory Effect of *Moringa oleifera* Lam. Aqueous Extract on the Burrowing Crab, *Cardiosoma guanhumi* (Latreille, 1828)

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SUMMARY

Moringa oleifera has impressive range of nutritional and medicinal values which when consumed have influence on hematological profile which is applied as an index of physiological condition of various organisms and thus provide information about the health status of local populations. The study aimed to determine the effects of *M. oleifera* aqueous leaves extract on the hematology, serum biochemical profile and antioxidant enzyme activities of the burrowing crab, *Cardiosoma guanhumi.* Thirty juveniles of *C. guanhumi* (Average weight 43.20±0.05 g) were tested, where six crabs were randomly selected and distributed per each tank (1 x 1x 0.6m^3) of 1litre of water. Different diets containing *M. oleifera* aqueous extract at inclusion levels of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and control were used to feed the crabs. Total Haemocyte Count ranged between 2733.33±0.90 mL and 6350.00±0.60 mL; there were significant differences among the treatment groups ($p < 0.05$). Haemocyte sub-population variables showed that crabs fed the control and 2.0ml *M. oleifera* kg^{-1} diet had increased in the granulocyte and monocyte populations but a decrease in the agranulocytes. The results of the serum enzymes showed an increase as the level of *M. oleifera* aqueous extract increases in the diet. Highest superoxide dismutase (75.43 ±21.25 min/mg pro) and catalase activities (2.96±0.18 min/mg pro), malondialdehyde (12.05±2.09 nmol/L) and glutathione concentrations (0.19 ±0.02 µmol/L) were recorded in crabs fed diet T4 (2.0ml *M. oleifera* kg-^{1}), while the lowest were obtained in crabs fed control diet. The present study showed that the inclusion of *M. oleifera* aqueous extract up to 1.0ml kg^{-1} will have immunomodulatory performance on *Cardiosoma guanhumi* without any deleterious effect on the crab's health status.

Keywords: Hemato-Biochemicals, Land Crab, Lagos Lagoon, Plant Extract, Nigeria

INTRODUCTION

Crabs have flourished predominantly as invertebrate fauna because of their ubiquitous existence in almost all parts of the world oceans, fresh water, and even on land (Akin-Oriola *et al*., 2005). Many species actively forage on land and several species have become semi-terrestrial. *Cardiosoma* is a genus of Land crab. Juveniles are often very colourful with a purple-blue carapace and orange-red legs and exhibit colour change when old age is reached (Ng *et al*., 2008). This crab is omnivorous and has been found abundant in mangrove habitat of the Lagos Lagoon, which has been subjected to reclamation by anthropogenic activities.

Crab hematology is important as an effective biological tool in the bio monitoring of marine ecosystem health status (Lawal-Are *et al*., 2019a). There has been a greater understanding of the need to establish reference hematological and biochemical values in crab in order to assess the health status and the subsequent diagnosis of diseases (Moruf and Lawal-Are, 2018). Hematological characteristics of most invertebrates have been studied with the aim of establishing normal value range, and deviation where it may indicate a disturbance in the physiological process. Certain serum chemistry could be used to identify tissue damage (Patti and Kulkarni, 1993; Sanni *et al*., 2020). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) are normally found within the cells of the organs but their increase in the plasma indicate tissue injury or organ dysfunction (Shalaby, 2009).

Environmental and physiological factors are known to influence crab hematology. These include stress due to capturing, transportation and sampling. There are many sources of chemical compounds such as alkaloids, flavonoids, pigments, phenols, terpenoids, steroids and essential oils in plants that possess diverse range of bioactivity (Iwalewa *et al*., 2007). These compounds produce definite physiological actions in the body like anti-stress, growth promotion, appetite stimulation, antimicrobial activities and immune-stimulation (Citarasu, 2010). Recently, researchers have increasingly been paying attention to moringa (*Moringa oleifera* Lam.), which is a widespread, drought – tolerant tree. Moringa fresh foliage has been included into the diet of different fish species. Positive effects on feeding behaviour and health status of *Oreochromis niloticus* (Richter *et al*., 2003), *Clarias gariepinus* (Bello and Nzeh, 2013) and *Cyprinus carpio* (Adeshina *et al*., 2018) have been reported. However, the inclusion of plant protein sources in the ration of crab requires investigation as the presence of certain limiting factors in plant ingredients such as high crude fibre content and antinutritional factors have been demonstrated (Nwanna *et al*., 2008). Excessive consumption could cause slow growth rates and poor performance which may result in mortalities if condition persists (Francis *et al*., 2001). Hemato-biochemical characteristics help carcinologist to interpret physiological responses by crab and

deviation from normal response may indicate a disturbance in the physiological process. Hence, the aim of the research was to determine the effect of *M. oleifera* aqueous extract on the hematological, serum biochemical profile and antioxidant enzyme activities of the burrowing crab, *Cardiosoma guanhumi* (Latreille, 1828).

MATERIALS AND METHODS

Collection of plant materials and extraction

The plant materials, fresh *Moringa oleifera* leaves were obtained from Ago in Okota, Lagos, Nigeria. The leaves were dried, ground to powder and kept in air tight container. The extract of aqueous *M. oleifera* was carried out according to the method suggested by Fatope *et al*. (1993) with minor modification. 50 g of the powdered leave was weighed and poured into 500 ml conical flask in which 200 ml of distilled water was added. The mixture was kept for 12 hours with constant agitation at 30 minutes intervals. The extract was filtered using muslin bag. The filtrate was concentrated using water bath at 50 ºC according to the method of Ojekale *et al*. (2006). The semi-solid extract was preserved in the refrigerator at 4°C.

The choice of aqueous extraction came from previous experiment in which two different solvents were used (ethanol and aqueous extraction) and the aqueous extraction gave more phytochemicals (Nweze and Nwafor, 2014). The extract was added to the ingredients which have been mixed together before pellets were made, one gram (g) of the extract was added to 25 mls of distilled water to make a solution. The solution gotten from mixture of the extract and distilled water was then added to water. Mixture was added to the ingredients using a pipette to measure in milliliters that is (0.5ml/kg of feed) for T1 (Treatment 1), (1.0ml/kg of feed) for T2 (Treatment 2), (1.5ml/kg of feed) for T3 (Treatment 3), (2.0ml/kg) for T4 (Treatment 4), (0.0ml/kg of feed) for T5 (Treatment 5).

Collection and acclimatization of experimental crab

This study was carried out at the Shellfish Unit of the Department of Marine Sciences, University of Lagos, Akoka, Nigeria. The organisms for this project were collected from the coastal and mangrove area of University of Lagos Lagoon front, located opposite the Lagos Lagoon, with the geographical latitude of 6°26"N and 6°39"N and longitude 3°29"E and 3°50"E (Lawal-Are *et al*.,2019b). The shores of the Lagos Lagoon and adjacent creeks are lined in undeveloped areas by mangrove swamps. An elaborate description of the study area is provided in Moruf *et al.* (2018). The Crabs were caught using a torch and a thick glove to remove them from their holes between the hours of 19.00 - 22.00 hours. Thirty juveniles of *C. guanhumi* (Average weight 43.20±0.05 g) were placed in plastic tanks (52.5 × 33.5 × 21cm) and allowed to acclimatize for a week before the commencement of the experiment. During this period they were fed with 4mm size pelleted five experimental diets (Table 1) twice daily (for a period of 60 days). They were fed 3 % of their body weight at 5.00 - 6.00 hours and 19.00 - 22.00 hours daily because the species is a nocturnal feeder. Six crabs were randomly selected and distributed per tank (1 x 1x 0.6m^3) of 1L of

water. The tanks had perforated plastic covers from these holes were passage for the pellets into the tank. At the end of feeding trial, the crabs were not fed for 24 hour prior to the day of serum samples collection

Table I: Percentage composition of the different experimental diets

Ingredients	T1 (0.5ml *M. oleifera*)	T2 (1.0ml *M. oleifera*)	T3 (1.5ml *M. oleifera*)	T4 (2.0ml *M. oleifera*)	T5 (Control)
Fish meal 72%	30	30	30	30	30
Fish meal 65%	20.32	20.32	20.32	20.32	20.32
Soybean meal	20	20	20	20	20
Wheat bran	14.5	14.5	14.5	14.5	14.5
Indomie	15	15	15	15	15
Vitamin-Mineral mix	0.08	0.08	0.08	0.08	0.08
Soybean oil	0.1	0.1	0.1	0.1	0.1
M. oleifera	5ml	10ml	15ml	20ml	0.0ml

Collection of haemolymph

Crab haemolymph was drawn with a 23G Syringe from the juncture between the bases of the ischium of the fifth walking leg. The haemolymph was collected into a syringe flushed with 1mL of anticoagulant (0.3 M NaCl, 0.1 M glucose, 30 mM Sodium citrate and 26 mM Citric acid), transferred into a 5 mL lithium heparin bottle kept in an ice chest and haemolymph of crabs were analyzed immediately for haemocyte morphology, hematological and biochemical indices (Adeogun *et al.*, 2015).

Analytical procedures

Total haemocyte counts (THC) of haemocyte population were determined using an improved Neubaeur heamocytometer according to methods described by Blaxhall and Daisley (1973). One of the aliquots of the haemolymph of individual crabs was transferred into the haemocytometer and counted manually. Haemocyte morphotypes were identified and a total number of 100 cells from each slide were counted. The percentage of each counted cell type was calculated and multiplied by total haemocyte population count to obtain absolute count.

Haemolymph samples were centrifuged for 10 minutes at 5000 g with a Hawskley micro haematocrit centrifuge and the serum derived was stored at -20ºC for further analysis. The serum was assayed for transaminases such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the phosphatase alkaline phosphatase (ALP) activities according to methods described by Coles (1986). Total protein was determined by the Biuret method.

Statistical analysis

Values were recorded with mean standard error and subjected to single factor ANOVA and Duncan Multiple Range Test (DMRT) for separating significant differences in means of haematological and serum biochemical parameters between crab

treatments. Differences in means were considered significant when $p < 0.05$.

RESULTS

Hematological profile

The effect of *M. oleifera* aqueous extract on the hematological indices of *C. guanhumi* is shown in Table II. Total Haemocyte Count (THC) ranged between 2733.33 ±0.90 mL and 6350.00 ±0.60 mL with crabs fed 0.5ml *M. oleifera* having the highest value of 6350.00 ±0.60 mL. There was significant differences among the treatment groups ($p<0.05$). Haemocyte sub-population variables showed that crabs fed the control and 2.0 ml *M. oleifera* kg-^{1} diet had increased in the granulocyte and monocyte populations but a decrease in the agranulocytes. The values of granulocytes in control (35.67±2.67 %) and in 2.0ml *M. oleifera* kg-^{1} diet (33.83 ± 1.67 %) showed significant ($p<0.05$) difference from the values obtained in crabs fed diet containing 0.5ml, 1.0ml and 1.5ml *M. oleifera* kg-^{1} aqueous extracts.

Table II: Effect of *Moringa oleifera* aqueous extract on the hematological indices of *Cardiosoma guanhumi*

		T1 (0.5ml *M. oleifera* kg-^{1})	T2 (1.0ml *M. oleifera* kg-^{1})	T3 (1.5ml *M. oleifera* kg-^{1})	T4 (2.0ml *M.oleifera* kg-^{1})	T5 (Control)
Total Haemocyte Count (mL)		6350.00 ±0.60^{a}	3733.33 ±0.90^{ab}	4975.00 ±1.64^{a}	5716.67 ±0.11^{a}	3500.00 ±0.94^{b}
Haemocyte Subpopulation	Granulocyte (%)	30.50 ±0.50^{b}	31.33 ±2.33^{b}	29.00 ±1.00^{b}	35.67 ±2.67^{a}	33.83 ±1.67^{a}
	Agranulocyte (%)	69.5 ±0.50^{ab}	67.33 ±1.67^{bc}	71.00 ±1.00^{a}	62.67 ±2.33^{b}	65.33 ±1.33^{c}
	Monocyte (%)	0.00 ±0.00^{b}	0.33 ±0.67^{b}	0.00 ±0.00^{b}	1.667 ±0.33^{a}	1.33 ±0.33^{a}

Keys: Mean±Standard Error; ND= Not detected; Values with different superscripts across row are significantly different at ($P < 0.05$)

Serum Biochemical Profile

The effect of *M. oleifera* aqueous extract on the serum biochemical parameters of *C. guanhumi* is shown in Table III. Lowest value for serum protein (24.99 ± 0.52 gL^{-1}) was observed in the crabs fed 0.5ml *M. oleifera* based diet. The result of Aspartate aminotransferase (AST) showed that crab fed 2.0ml *M. oleifera* based diet recorded the highest value of 25.00±0.69 µI^{-1} and lowest value of 12.56 ±5.43 µI^{-1} was observed in crab fed control diet. Alanine aminotransferase (ALT) results revealed that crab fed 0.5ml *M. oleifera* diet had the highest value of 17.60 ±0.26 µI^{-1} which was not significantly different ($p > 0.05$) from values of 17.17 ±1.19 µI^{-1} and 15.04 µI^{-1} obtained in crabs fed with 1.5ml and 2.0ml *M. oleifera* diet. The results obtained for Alkaline phosphatase (ALP) revealed that crab fed with 0.5ml *M. oleifera* diet recorded the highest value of 113.16±2.76 µI^{-1} and was not significantly different ($p < 0.05$) from the values obtained in crabs fed 1.5 to 2.0ml *M. oleifera* diets. Crabs fed 1.5ml *M. oleifera* based diet had the highest

lactate dehydrogenase (LDH) of 153.66 ±2.87 μI^{-1} and Urea of 46.29±1.65 $mg.dL^{-1}$ while the lowest LDH of 97.67±5.51 in 1.5ml *M. oleifera* fed crabs and Urea of 30.89±1.07 $mg.dL^{-1}$ in crabs fed control diet

Table III: Effect of *Moringa oleifera* aqueous extract on the serum biochemical indices of *Cardiosoma guanhumi*

	T1 (0.5ml *M. oleifera* kg^{-1})	T2 (1.0ml *M. oleifera* kg^{-1})	T3 (1.5ml *M. oleifera* kg^{-1})	T4 (2.0ml *M. oleifera* kg^{-1})	T5 (Control)
PRO (gL^{-1})	24.99 ±0.52^{a}	25.29 ±2.88^{a}	29.27 ±4.80^{a}	30.44 ±4.47^{a}	31.09±0.69^{a}
AST (μI^{-1})	18.52 ±2.52^{b}	18.56 ±5.88^{ab}	16.67 ±3.28^{b}	25.00 ±0.69^{a}	12.56 ±5.43^{ab}
ALT (μI^{-1})	17.60 ±0.26^{a}	13.67 ±1.67^{bc}	17.17 ±1.19^{a}	15.04±2.40^{ab}	10.93±1.07^{c}
ALP(μI^{-1})	113.16±2.76^{a}	92.00 ±0.92^{b}	100.05±4.83^{ab}	112.57±15.37^{a}	95.68±0.92^{b}
LDH(μI^{-1})	117.62±2.06^{b}	97.67 ±5.51^{c}	153.66 ±2.87^{a}	127.94 ±0.00^{b}	123.81±0.91^{b}
UREA($mg.dL^{-1}$)	36.81 ±2.84^{ab}	37.45 ±3.13^{ab}	46.29 ±1.65^{a}	32.71 ±11.83^{b}	30.89 ±1.07^{b}

Keys: aminotransferase (ALT), alkaline phosphatase (ALP), Aspartate (AST), lactate dehydrogenase (LDH) and protein (PRO). Mean±Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

Antioxidant Enzyme Activity

Antioxidant variables of the crab fed diets fortified with *Moringa oleifera* aqueous extract were increased (Table IV). There was a greater increase in SOD and CAT activities, MDA and GSH concentrations of crabs fed experimental diets than in the crabs fed control diet. Highest SOD (75.43 ±21.25 min/mg pro) and CAT (2.96±0.18 min/mg pro) activities, and MDA (12.05±2.09 nmol/L) and GSH concentrations (0.19 ±0.02 µmol/L) were recorded in crabs fed diet T4 (2.0ml *M. oleifera* kg^{-1}), while the lowest were obtained in crabs fed control diets.

Table IV: Effect of *Moringa oleifera* aqueous extract on the antioxidant enzyme activity of *Cardiosoma guanhumi*

	T1(0.5ml *M. oleifera* kg^{-1})	T2(1.0ml *M. oleifera* kg^{-1})	T3(1.5ml *M. oleifera* kg^{-1})	T4 (2.0ml *M. oleifera* kg^{-1})	T5 (Control)
PRO (g/L)	24.11 ±0.60^{a}	25.99 ±3.10^{a}	25.42 ±2.18^{a}	28.19 ±0.98^{a}	26.62 ±0.83^{a}
SOD (min/mg/protein)	57.85 ±1.92^{a}	83.17 ±7.92^{b}	62.34 ±6.44^{b}	75.43 ±21.25^{b}	46.15 ±3.71^{a}
CAT (min/mg protein)	2.02 ±0.02^{a}	2.08 ±0.74^{a}	2.71 ±0.62^{a}	2.96±0.18^{a}	2.01±0.19^{a}
MDA (nmol/L)	6.83 ±0.74^{a}	11.62 ±2.20^{b}	12.89 ±5.15^{b}	12.05±2.09^{b}	6.32±0.34^{a}
GSH (µmol/L)	0.10 ±0.01^{a}	0.16 ±0.01^{a}	0.18 ±0.00^{a}	0.19 ±0.02^{a}	0.11±0.00^{a}

Keys: a, b, c, means on the same row with different superscripts are statistically different (P>0.05), catalase (CAT), malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and protein (PRO). Mean±Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

DISCUSSION

Hematological and biochemical changes in crabs are often modulated in response to environmental factors to bring about homeostatic control within the organism and as a result are used as diagnostic tools for assessing the health of wild populations (Moruf and Lawal-Are, 2018). Moringa fresh foliage has been included into the diet of different fish species, positive effects on feeding behaviour in *Clarias gariepinus* (Bello and Nzeh, 2013), growth rate and health condition in *Cyprinus carpio* (Adeshina *et al*., 2018) have been reported. In this study, crabs fed with *M. oleifera* aqueous extract based diet had increased Total Haemocyte Count (THC) than the control and there is no significant difference among the treatment groups. Increased THC in the crabs appeared to be associated with lower dissolved oxygen. Reports have indicated that the increase in number of circulating haemocytes under hypoxic condition is a compensatory response to maintain oxygen tissue perfusion in crabs (Sussarellu *et al*., 2012). Haemocyte sub-population variables in crabs fed the control diet had increased granulocyte and monocyte populations but a decrease in the agranulocytes. Granulocytes have been reported to play a significant role in the crustacean defense system because of their antibacterial activity and function in secreting extracellular matrix proteins that stops the action of invading organisms, when the host is attacked by either extremely large particles or numerous tiny particles (Chisholm and Smith, 1995). Similar to this result, Moruf and Lawal-Are (2018) also reported significant higher levels of granulocyte in the mangrove crabs, *S. huzardii* and *U. tangeri*. The significantly higher level of granulocytes in crab may be a stress response to unfavorable environmental conditions. Lowest value for serum protein was observed in the crabs fed 0.5ml *M. oleifera* aqueous extract based diet and this, according to Adeogun *et al*. (2015), may be attributed to increased breakdown of serum peptidic material and modulation of their involvement in various biological processes due to environmental stress. Most environmental factors have been reported to affect serum protein concentration in crustaceans by influencing appetite and feeding behaviour rather than having a direct effect on serum protein itself (Scott and Solman, 2004). The results of the serum biochemicals showed an increase as the level of *M. oleifera* aqueous extract increased in the diet. Crabs fed *M. oleifera* aqueous extract diet had the highest value of AST and ALT. Increased AST, ALP and LDH activities in *C. guanhumi* treated with the leaves extract may be a direct consequence of stress induced protein metabolism in the tissue of the crabs. The results of innate immune response parameters (SOD and CAT activities, MDA and GSH concentration) were greatly higher in crabs fed *Moringa oleifera* aquaous extract fortified diets. The result of the present study is in agreement with the findings of Bello and Nzeh (2013) and Adeshina *et al*. (2018).

CONCLUSION

The increase in haematological, biochemical and innate immune response parameters of *Cardiosoma guanhumi* fed *Moringa oleifera* aqueous extract fortified diets reveal immunomodulatory effect of *Moringa oleifera* aqueous extract in the diet. In this study, crabs fed diet containing 2.0ml *M. oleifera* kg^{-1} aqueous extract had the innate

immune response. The study has shown that hematological and biochemical parameters are good indicators of health status of a crab. Further studies should be performed with *Moringa oleifera* leaf as a meal for complete understanding of immune responses of *Cardiosoma guanhumi.*

REFERENCES

ADEOGUN, A.O., SALAMI, O.A., CHUKWUKA, A.V. and ALAKA, O.O. (2015). Haematological and serum biochemical profile of the Blue Crab, *Callinectes amnicola* from two *Tropical Lagoon Ecosystems. African Journal of Biomedical Research*, **18**: 233 - 247.

ADESHINA, I., SANI, R.A., ADEWALE, Y.A., TIAMIYU, L.O. and UMMA, S.B. (2018). Effects of dietary *Moringa oleifera* Leaf meal as a replacement for soybean meal on growth, body composition and health status in *Cyprinus carpio* juveniles. *Croatian Journal of Fisheries*, **76**: 174-182.

AKIN-ORIOLA, G., ANETEKHAI, M. and OLOWONIREJUARO, K. (2005). Morphometric and meristic studies in two crabs: *Cardiosoma armatum* and *Callinectes pallidus. Turkish Journal of Fisheries and Aquatic Sciences*, **5**: 85-89.

BELLO, N.O. and NZEH, G.C (2013). Effects of varying levels of *Moringa oleifera* leaf meal diet on growth performance, hematological indices and biochemical enzymes of African catfish *Clarias gariepinus* (Burchell 1822). *Elixir Aquaculture*, **57**A: 14459-14466

BLAXHALL, P.C. and DAISLEY, K.W. (1973): Routine haematological methods for use with fish blood. *Journal of Fish Biology,* **5**: 771-781

CHISOLM, L.A. and SMITH, C.L. (1995). The sea urchin complement homologue, SpC3, functions as an opsonin. *Journal of Experimental Biology*, **207**: 2147–2155.

CITARASU, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, **18**: 403–414.

COLES, E.H. (1986). Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, PA, USA, pp1-42.

FATOPE, M.O., IBRAHIM, H. and TAKEDA, Y. (1993). Screening of higher plants reputed as pesticides using the Brine Shrimp Lethality Assay. *International Journal of Pharmacognosy,* **31** (4): 250-254.

FRANCIS, G., MAKKAR, H.P.S. and BECKER, K. (2001). Antinutritional factors present in plant – derived alternative fish feed Ingredients and their effects in fish. *Aquaculture*, **199** (3-4): 197-228.

IWALEWA, E.O., MCGAW, L.J., NAIDOO, V. and ELOFF, J.N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology*, **6**: 2868-2885.

LAWAL-ARE, A.O., MORUF, R.O., OLUSEYE-ARE, S.O. and ISOLA, T.O. (2019a). Antioxidant defense system alternations in four crab species as a bio-Indicator of

environmental contamination. *Bulletin UASVM Veterinary Medicine*, **76**(1): 73-80.

LAWAL-ARE, A.O., MORUF, R.O., AKUBUEZE, K.L. and ADEWOLE, O. (2019b). Morphometric characteristics of two walking crabs, *Cardiosoma armatum* (Gecarcinidae) and *Goniopsis pelii* (Graspidae) from a tropical mangrove swamp in Lagos, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, **15**(1): 83-88.

MORUF, R.O. and LAWAL-ARE, A.O. (2018). Haemato-Biochemical variations in estuarine crabs from a Lagoon ecosystem. *Journal of Applied Sciences and Environmental Management*, **22**(12): 1899–19033.

MORUF, R.O., BOLAJI, O.D. and LAWAL-ARE, A.O. (2018). Biometrics, gut contents and sexual dimorphism of the West African Mud Creeper, *Tympanotonus fuscatus* var *radula* from the mangrove swamps of a coastal estuary in Nigeria. *Egyptian Journal of Aquatic Biology and Fisheries*, **22**(1): 87- 96.

NG, P.K.L., GUINOT, D. and DAVIE, P.J.F. (2008). Systema Brachyurorum. Part I. An annotated checklist of extant brachyuran crabs of the world. *Raffles Bulletin of Zoology*, **17**: 1-286.

NWANNA, L.C., FALAYE, A.E. and SOTOLU, A.O. (2008). Water hyacinth *Eichhhomia crassipes* (Mart solms): a sustainable protein source for fish feed in Nigeria. Food health and environmental issues in developing countries: the Nigeria situation. O.C. Adeboye, K.A. Taiwo and A.A. Fatufe (Eds.) Alexander Von Humboldt Foudation, Bonn – Germany. pp. 187 – 194.

NWEZE, N.O. and NWAFOR, F.I. (2014). Phytochemical, Proximate and Mineral Composition of Leaf Extracts of *Moringa oleifera* Lam. from Nsukka, South-Eastern Nigeria. *Journal of Pharmacy and Biological Sciences* **9**: 99-103.

OJEKALE, A.B., LAWAL, O.A., LASISI, A.K. and ADELEKE, T.L. (2006). Phytochemistry and spermatogenic potentials of aqueous extracts of *Cissus populnea* (Guill and Perry) stem bark. *The Scientific World Journal*, **6**: 2140-2146.

PATTI, M. and KULKARNI, R.S. (1993), Ovarian and hepatic biochemical response to sumaach (a crude form of HCG) in fish *Notopterus notopterus* Pallas, under pesticide treatment. *Geobios*, **20**: 255-259.

RICHTER, N., SIDDHRURAJU, A. and BECKER, K. (2003). Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as alternative protein source for Tilapia (*Oreochromis niloticus* L.). *Aquaculture*, **217**: 599-611.

SANNI, Z.A., MORUF, R.O. and LAWAL-ARE, A.O. (2020). Hemato-Biochemical profiling of a burrowing crab exposed to polystyrene microplastic contaminant. *FUDMA Journal of Sciences*, **4**(2): 380 - 385

SCOTT, G.R. and SOLMAN, K.A., (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of

toxicity. *Aquatic Toxicology*, **68**: 369-392.

SHALABY, A.M.E. (2009). The opposing effects of ascorbic acid (Vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*) http://www.ag.arizona.edu/ista/ista6 web/pdf/209.pdf. Retrieved: 05-04-09. 2009.

SUSSARELLU, R., FABIOUX, C., CAMACHO, S.M., LE GOÏC, N., LAMBERT, C., SOUDANT, P. and MORAGA, D. (2012). Molecular and cellular response to short-term oxygen variations in the Pacific oyster *Crassostrea gigas*. *J. Exp. Mar. Biol. Ecol*. **412**: 87-95.