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Potentiality of *Moringa oleifera* aqueous extract as a growth modulator and antistress in acute hypoxic Nile tilapia *Oreochromis niloticus*

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Abstract

This study aims to get a comprehensive evaluation of the growth promoting effects and the hypoxic stress relief potentiality of *Moringa oleifera* aqueous extracts. *Oreochromis niloticus* fingerlings were arbitrarily allocated into five duplicated fish groups (30 fish tank^{-1}). The fish groups were labeled according to the *M. oleifera* aqueous extract dietary inclusion level (G_1:G_5). MOAE had fundamentally promoted tilapia growth. Serum total protein levels were considerably higher in the *M. oleifera* fed fish, whereas the levels of liver enzymes diminished significantly in G_5 fish. Additionally, the dietary *M. oleifera* resulted in a noticeable hypoglycemic effect together with a pronounced decline in the antioxidant activities. The use of *M. oleifera* supplemented diet decreased the hypoxia-related stress as conveyed by the gradual descent in the serum cortisol levels of the hypoxic-stressed tilapia. This study proposes the potentiality of *M. oleifera* aqueous extract as a growth promoter, antistress and antioxidants. It also validates its safe application in commercial tilapia culture. Future study is required to comprehend the influence of this plant extract in relieving chronic stress and its possible toxic effect as well. Feasibility study for its commercial usage is required too.

Keywords: Plant extracts, *Moringa oleifera*, *Oreochromis niloticus*, Growth, Antioxidant activity, Hypoxic stress.

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Introduction

Tilapia is a popular aquatic species being cultured worldwide. In 2012, the world tilapia production had boomed to reach 4.5 MT (FAO, 2014) and is anticipated to increase exponentially over the coming years. Indeed, Tilapia intensification is an essential requisite to cover the existent necessity for fish protein. The generated stress and limited disease control associated with intensive aquaculture have made sound management and biosecurity critical challenges to aquaculture (Kautsky *et al*., 2000; Schreck, 2000; Moss *et al*., 2012). As an important water quality criterion; Dissolved oxygen (DO) may represent a significant constraint for successful aquaculture. For instance oxygen depletion may occur under different culture conditions especially in intensive culture systems prompting fish hypoxia. Hypoxia has many adversative consequences on cultured fish. It can antagonistically affect varied kind of physiological and biochemical activities including growth (Wang *et al*., 2009) and hematological indices along with stress response stimulation (Simi *et al*., 2016).

Antimicrobials and other chemicals are regularly administered as additives in fish diets or water baths as growth promoters, prophylactics or therapeutants (Bulfon *et al*., 2015). Their continuous application resulted in various adversarial effects for the environment and health safety (Menz *et al*., 2015). Accordingly, numerous countries have rigid regulations that limit their application in aquaculture. In this concern, a new worldwide trend had evolved regarding the consumer perceptions as influenced by safety and quality of the animal crop. In this manner, imperative needs to search different choices to antimicrobials were of primary concerns. Such choices would guarantee better growth performance and pathogens control and afterward, would polish the sustainable fish production under intensive culture systems. Plants are the warehouses of safe and inexpensive chemicals. Natural plants may represent better substitutes for antimicrobials in aquaculture as they impound various actions like growth enhancement, antimicrobial and antioxidant activities together with their antistress potentialities (Reverter *et al*., 2014). Indeed, this is related to the several active constituents they have, such as pure volatile oils, alkaloids, phenolics, terpenoids, saponins, flavonoids and numerous other compounds (Anwar *et al*., 2007; Chakraborty and Hancz, 2011; Huyghebaert *et al*., 2011).

The drumstick tree, *Moringa oleifera*, is angiosperm plant demonstrating varied and valuable impacts, in accord to the plant part and inception. Different portions of this plant have been used all through history for its nourishing and healing importance (Mbikay, 2012; Leone *et al*., 2015). The seeds, for instance, possess potential antimicrobial action against certain pathogens. They also can be utilized for water cleansing since they contain particular proteins with coagulation properties (Nikkon *et al*., 2003; Suarez *et al*., 2003). Nevertheless, the information available

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about its influences on the immune response, antioxidant activity and growth of various fish species including Nile tilapia *Oreochromis niloticus.* To the best of our information, there is no data in the literature on the influence of dietary *M. oleifera*, in evoking a better fish response to stressful culture conditions, such as hypoxia. Therefore; this study aims to get a comprehensive evaluation of growth promoting effect of *M. oleifera* aqueous extracts (MOAE) on *O. niloticus* fingerlings. In addition, the MOAE influences on the enzymes antioxidant activities and some serum biochemical indices of acutely hypoxic Nile tilapia fingerlings.

Materials and methods

Fish and rearing

Apparently, healthy monosex tilapia fingerlings obtained from a private fish hatchery at Kafr El Sheikh Governorate, Egypt. The fish were transferred into polyethylene bags filled with oxygen to the Lab of Fish Breeding and production, Vet. Med, Alex. University, Egypt. Fingerlings were firstly acclimated in rectangular white plastic tanks (500 L capacity) supplied with 300 L of underground freshwater and equipped with individual biofilters along with constant air supply. During the adaptation period, fish were fed twice daily (09:00; 14:00) with a diet previously formulated to obtain a 30% crude protein.

Preparation of aqueous extract of M. oleifera leaves

Mature leaves of *M. oleifera* were selected and gathered from one area (Abu-hammad, Sharkia, Egypt). The air dried leaves were crushed using mortar and pestle. The Bioactive substances of *M. oleifera* leaves were extracted using infusion technique. The leaves were immersed in distilled water for 24 h using 1:2 ratios (weight /volume) (Fernandez, 1990). After that, a suction apparatus and doubled Whatman no. 1 filter paper used to spate the debris and filtrate. Then a concentrated filtrate was obtained through a vacuum rotary evaporator (Buchi R-110 Rotavapor, Switzerland) at (40°C) the dry extract kept frozen at 0°C.

Diet preparation

Five diets were prepared (Table 1), include the control one which was non-*M. oleifera* supplemented diet and the other four ones were supplemented with graded levels (50, 100, 200 and 400 mg Kg^{-1} diet) of *M. oleifera* watery extract. Then, tap water was added to each until a firm paste was acquired. Each doughy diet was then separately passed through a mincer with a 16 mm die, the resulted strands were gently broken into pellets, air dried at ambient temperature for two days and finally kept at 4°C in plastic bags till need.

Experimental setup and fish management

After adaptation periods, fish with an average body weight of 4.48±0.22 g and average body length of 6.27±0.19 cm were arbitrarily allocated into five replicated fish groups (two replicates $group^{-1}$). The fish groups were differentiated according to the MOAE

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dietary inclusion level. They were labeled as G_1; fed a control diet (0% MOAE kg^{-1} feed), G_2; fed 50 mg MOAE kg^{-1} feed, G_3; fed 100 mg MOAE kg^{-1} feed, G_4 was fed 200 mg MOAE kg^{-1} feed and G_5; fed 400 mg MOAE kg^{-1} feed. The stocking density was maintained at 30 fish $tank^{-1}$. One day after stocking, each fish group was hand-fed with its corresponding diet. The trial was continued for 60 days, with a feeding frequency two times per day (09:00; 14:00), six days a week until apparent satiation. Likewise, the adaptation period, the water quality of each tank was similarly preserved and managed throughout the experimental period. Moreover, 1/3 of the water in all tanks was replaced each other day. Accordingly, the tanks water quality was within the permissible limits (temperature; 26±0.42 °C, pH; 7.87±0.36, and DO; 6.94±0.56 mg L^{-1}).

Table 1: Composition and proximate analysis of the basal diet (g 100 g^{-1} dry matter).

Feed ingredients	Proportion
Fish meal (60%)	16
Soybean meal (47%)	27.5
Yellow corn	27
Rice bran	13
Wheat bran	15
Dicalcium phoshate	1
Methionin	0.05
Cholin chloride	0.05
Vitamin and mineral premix*	0.2
Binder	0.2
Total	100
Composition	**Proximate analysis (%)**
Dry matter	88.09
Crude protein	29.33
Crude Fiber	5.58
Ash	8.15

*Vitamin mineral premic (Technomix) (quantity per 1 kg).
Vitamin premix: Vitamin-A: 12,000,000 IU; Vitamin-D3: 2,000,000 IU; Vitamin-E: 10,000 mg; Vitamin-K_3: 2,000 mg; Vitamin-B_1: 1,000 mg; Vitamin-B_2: 5,000 mg; Vitamin-B_6: 15,000 mg; Vitamin-B_{12}: 1.0 mg; Biotin: 50 mg; Pantothenate: 10,000 mg; Nicotinic acid: 30,000 mg; Folic acid: 1,000 mg.
Mineral premix (mg per 2 kg): FeSO4: 30,000; ZnSO4: 50,000; MnSO4: 60,000; CuSO4: 10,000; calcium iodine: 1,000; cobalt: 100; choline: 250,000.

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Fish growth and survival

The body weight and the total length of all experimental fish groups were noted 24 h after the end of the feeding trial. The performance data and the survival rate (%) were appraised;

- Weight gain (g)=W_2-W_1
- Average daily gain (g day^{-1})= (W_2-W_1)/ T
- Specific growth rate (% day^{-1})= [loge (W_2)-loge (W_1)/ T]×100
- Condition factor (K)=(W/L^3)× 100

Where; W_2 is final weight (g), W_1 is initial weight (g), T is the trial period (days), L is total length (cm).

Hepatosomatic index (%)=[liver weight/W]×100

Survival rate (%)=[Number of survived fish / initial number of fish]×100

Biochemical parameters

To evaluate some of the biochemical parameters, blood samples (three anesthetized fish $tank^{-1}$) were collected from either the heart or the caudal vessels 24 h after the last diet. Hypodermic syringes were used to get the blood samples which were then transmitted to Wassermann tubes. The serum samples were obtained through allowing blood clotting at room temperature for 45 min then centrifuged at 3000 rpm (Hettich Centrifuge, Tuttlingen, Germany) for 15 minutes (Burnett *et al*., 2011). The sera were pipetted into Eppendorf tubes, labeled and hold on in deep freeze at -20 °C for further biochemical analysis.

Biochemical indices such as total protein and albumin were measured in step with Lowry *et al*. (1951) and Drupt *et al*.(1974). Immunoglobulin M (IgM) was assessed via spectrophotometric examination of fish serum. Also, the hepatic transaminase activities (ALT and AST) were tested in serum based on the method displayed by Reitman and Frankel (1957). Whereas serum urea levels were analyzed according to Fawcett and Scott (1960) and creatinine levels were estimated after Husdan and Raporpot (1968). Serum glucose levels were evaluated by the methodology of Cooper and Mc Daniel (1970).

Antioxidant enzymes assay

From each replicates three randomly selected fish were seined and transferred directly into an anesthetic containing water. Each fish was then dissected and the liver was extracted, cleaned by 0.9% NaCl solution. After that, each liver was homogenized in a cooled phosphate buffer saline (pH 7.2 at a ratio 1: 10) using electro-homogenizer (Heidolph, Germany). The homogenate was then centrifuged (13,000 ×g at 4°C for 10 min) and the supernatant was pipetted and stored at -80°C until analysis. The antioxidant enzymes assay comprised; superoxide dismutase (SOD), Glutathione peroxidase (GPX), (Glucose 6-phosphate dehydrogenase (G6PDH) and Nitric oxide (NO) which were analysed according to Paglia and Valentine (1967), Nishikimi *et al*. (1972), Bautista *et al*. (1988) and Van Bezooijen *et al*. (1998); respectively.

Acute hypoxic stress

Toward the completion of this trial,

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tilapia from various treated tanks were exposed to acute hypoxic stress. The tilapia fish were retained out of their tank and subjected to atmospheric air for three minutes. Investigation of cortisol profile was carried out through taking blood samples (5 fish treatment^{-1}). Thirty minutes and again two hours later from the same fish were sourced out of water for blood sampling to detect the post-hypoxia cortisol profile (Knobil, 1980).

Statistical analysis

One-way ANOVA was used to statistically analyze the data recorded for the differently treated fish groups under SAS (2008). Duncan's multiple range tests applied as well to detect any anticipated significant differences between treated fish groups at a significant level of 95%.

Results

Feeding MOAE supplemented diet had significantly enhanced tilapia growth and survivability in comparison to the MOAE non-supplemented one (Table 2). The FBW, WG, ADG, SGR and FL had significantly increased (p<0.05) in an ascending trend as the dietary inclusion level of MOAE increased from 50 mg kg^{-1} diet to 400 mg kg^{-1} diet. The condition factor was not significantly (p>0.05) differed by the MOAE dietary supplementation. However, higher estimates were recorded for the MOAE received fish. The MOAE supplemented diet expressively (p<0.05) increased the HSI, where the highest indices were assessed at 400 mg MOAE kg^{-1} diet inclusion level. Fish fed MOAE supplemented ration expressed significantly (p<0.05) better FCR values. Additionally, the lowest FCR values were achieved by those fish fed diets in 100 and 400 mg MOAE kg^{-1}, respectively. The Survival percentage noted in this study was not (p>0.05) affected by the MOAE diet supplementation.

Dietary MOAE significantly (p<0.05) influenced the serum TP level of *O. niloticus* fish (Table 3). The addition of 400 mg MOAE kg^{-1} diet resulted in an expressively higher serum TP level than the levels identified in the other treated fish groups. However, still comparable results were observed between the control MOAE non-supplemented group and the other treated fish groups (G2, G3, and G4). Meanwhile, albumin levels were not considerably (p>0.05) influenced by MOAE dietary supplementation. Additionally, IgM values noticeably increased (p<0.05) in the fish supplemented with 200 mg MOAE kg^{-1} diet. Liver and kidneys enzymes are demonstrated in Table 3; the G_5 exposed a noteworthy (p<0.05) drop in liver enzymes. The recorded urea levels diminished significantly in the fish fed 100 mg MOAE kg^{-1} diet. However, the serum creatinine levels did not elucidate any substantial differences (p>0.05) as an influence to dietary MOAE supplementation. MOAE considerably reduced the glucose levels. (p<0.05) in the sera of the MOAE supplemented fish (Table 3). Both G4 (200 mg kg^{-1}) and G1 (control one) displayed the highest

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serum glucose concentrations.

Table 2: Growth performance of *Oreochromis niloticus* fed dietary *Moringa oleifera* aqueous extract supplementation for 60 days.

	Treatments				
	Dietary *M. oleifera* extract mg kg^{-1}diet				
Parameters	**0 (Control)**	**50**	**100**	**200**	**400**
IBW(g)	4.45±0.24	4.53±0.23	4.53± 0.24	4.48±0.23	4.40±0.12
FBW (g)	24.88±1.97^{c}	28.58±0.76^{b}	29.58±0.72^{b}	26.88±1.94^{c}	31.76±1.09^{a}
WG (g)	20.43±2.18^{d}	24.05±0.76^{b}	25.05±0.72^{b}	22.40±1.88^{c}	27.36±1.02^{a}
ADG(g day^{-1})	0.34±0.03^{d}	0.40±0.01^{bc}	0.41±0.01^{b}	0.37±0.03^{c}	0.45±0.01^{a}
SGR (% day^{-1})	1.24± 0.09^{c}	1.33± 0.03^{b}	1.35±0.04^{b}	1.29±0.05^{bc}	1.43±0.02^{a}
FCR (%)	1.79±0.16^{a}	1.51±0.04^{b}	1.37±0.04c	1.55±0.18^{b}	1.28±0.06^{c}
FL(cm)	11.60±0.43^{b}	11.98±0.31^{b}	12.23±0.61^{ab}	12.01±0.51^{b}	12.66±0.59^{a}
CF	1.76±0.20	1.87±0.13	1.84±0.15	1.82±0.28	1.79±0.15
SR (%)	96.65±4.73	96.65±4.73	96.65±4.73	91.65±2.33	91.65±2.33
HSI (%)	1.74±0.27^{ab}	1.67±0.17^{b}	1.97±0.19^{a}	2.02±0.22^{a}	2.22±0.25^{a}

Data are expressed as means±standard deviations. Values with the same superscripts of the same row are not significantly different (p>0.05). Where, IBW= initial body weight, FBW=final body weight, WG=weight gain, ADG=average daily gain, SGR=specific growth rate, FCR=food conversion ratio, FL=fish length, CF=condition factor, HSI=Hepatosomatic Index.

Table 3: Some biochemical parameters, liver and kidney functions of *Oreochromis niloticus* 60 days after dietary *Moringa oleifera* aqueous extract supplementation.

	Treatments				
parameters	Dietary *M. oleifera* extract mg kg^{-1}diet				
	0 (Control)	**50**	**100**	**200**	**400**
Total protein (g)	4.0±0.80^{b}	5.16±1.26^{ab}	5.15±1.05^{ab}	5.23±1.43^{ab}	6.20±1.89^{a}
Serum IgM (µg ml^{-1})	117.34±14.24^{b}	127.19±34.94^{ab}	125.56±16.16^{ab}	150.15±44.86^{a}	112.77±11.62^{b}
Albumin (g dl^{-1})	1.88±0.14	1.96± 0.52	1.89± 0.39	2.10±0.34	2.03±0.52
AST (U L^{-1})	58.08±12.64^{a}	56.08±14.45^{a}	56.50±11.00^{a}	55.72±9.98^{a}	44.00±6.28^{b}
ALT (U L^{-1})	39.33±19.93^{a}	40.16± 14.62^{a}	38.26±16.13^{a}	40.98±18.64^{a}	31.33±10.35^{b}
Urea (mg dl^{-1})	13.14±1.78^{a}	10.89± 2.60^{ab}	9.10±1.57^{b}	11.16±3.37^{ab}	11.17±2.64^{ab}
Creatinine (mg dl^{-1})	0.73±0.09	0.73±0.10	0.71±0.08	0.65±0.20	0.80±0.11
Blood glucose (mg dl^{-1})	115.33±7.17^{a}	89.16±16.57^{b}	79.00±13.79^{b}	133.00±31.90^{a}	85.50±19.36^{b}

Data are expressed as means ± standard deviations. Values with the same superscripts in the same row are not significantly different (p>0.05).

Additionally, the dietary MOAE received fish groups showed a pronounced decrease in the SOD, GPX, G6PDH and NO activities (Table 4).

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The dietary MOAE supplementation had also triggered a substantial (p<0.05) deviation in the cortisol levels of the *M. oleifera* fed fish during the pre and post-hypoxic stress period (Fig. 1). Regarding the pre-hypoxic condition period; the MOAE supplemented fish (G1; 50 mg kg^{-1} diet) exhibited a significantly lower cortisol level as matched to both the control fish group and G_4 (200 mg MOAE kg^{-1} diet).

On the contrary to pre-hypoxic condition, elevated serum cortisol levels were equally assessed as a hypoxia-related response in all treated fish groups 30 minute's post-hypoxic stress. Nevertheless, the MOAE supplemented diets decreased the hypoxia-related stress in hypoxic tilapia as conveyed by the gradual drop in the cortisol level two hours after induction of hypoxia. The lowest serum cortisol values were detected in the G_4 and G_5.

Table 4: Antioxidant enzyme activities in *Oreochromis niloticus* 60 days after dietary *Moringa oleifera* aqueous extract supplementation.

	Treatments				
	Dietary *M. oleifera* extract mg kg^{-1}				
Parameters	**0 (Control)**	**50**	**100**	**200**	**400**
SOD (U mg^{-1})	16.69±0.82^{a}	12.16±2.63^{b}	7.24±2.02^{c}	7.14± 2.79^{c}	5.56±0.60^{c}
G6PD (mU mg^{-1})	6.21±3.27^{a}	6.10±1.05^{a}	2.05±1.08^{b}	1.56±0.52^{b}	2.17±1.32^{b}
GPX (mU mg^{-1})	4.56±0.56^{a}	4.11±0.26^{a}	2.29±0.28^{b}	2.11±0.28^{b}	1.13±0.11^{c}
(NO) activity (µ mol L^{-1})	26.43±3.55^{a}	18.97± 8.82^{abc}	20.07±5.25^{ab}	15.35±4.53^{bc}	9.01±0.96^{c}

Data are expressed as means ± standard deviations. Values with the same superscripts in the same row are not significantly different (p>0.05).

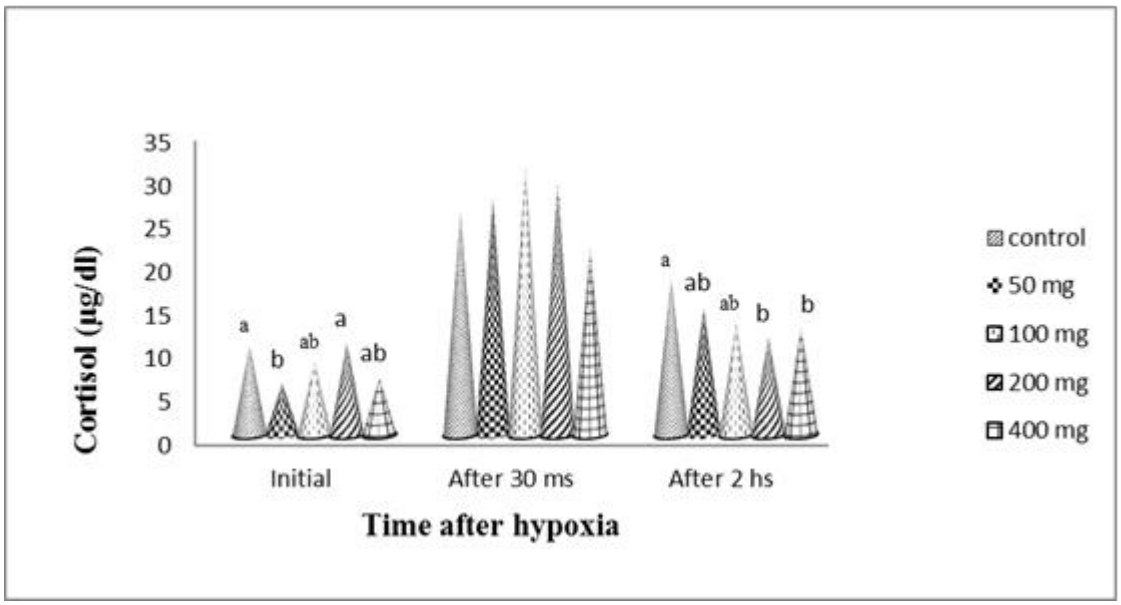

Figure 1: Serum cortisol levels of *Oreochromis niloticus* challenged with acute hypoxia after 60 days of dietary *Moringa oleifera aqueous extract* supplementation. Values with the same superscript letters at initial, 30 ms and 2 hours post-hypoxic stage are not significantly different (p>0.05) between different treated groups.

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Discussion

MOAE was recently verified to possess a valuable range of active substances. They possess growth promoting, antioxidant, tissue protective (liver, kidneys, heart and testes), anti-stressor, and immunomodulatory activities in fish (Alishahi *et al*., 2010; Stadtlander *et al*., 2013). This study clarified that feeding tilapia with MOAE containing diet enhanced the growth performance of *O. niloticus* fingerlings distinctly. The FCR was improved as the dietary inclusion level of MOAE increased. Fish received 400 mg MOAE kg^{-1} had a markedly high WG, SGR, and ADG. The ethanolic extract of *M. oleifera* flower (250 and 500 mg kg^{-1} diet) as well had improved tilapia growth (Tekle and Sahu, 2015). Also, a growth-promoting potential of *M. oleifera* seed protein extracts was reported by Stadtlander *et al*. (2013) when added to tilapia diet at 400 mg kg^{-1} in an eight weeks experimental period. Not only this but also the dried powder of *M. oleifera* leaves (10 mg kg $diet^{-1}$) was able to promote the growth of juveniles of *Penaeus indicus* as reported by Rayes (2013).

M. oleifera leaves is said to contain vast amount of immuno- nutritional components such as proteins, lipids, vitamins, enzymes, minerals, sugar, saponin and salicylates and other active constituents (Leone *et al*., 2015). Moreover, Heidarieh *et al*. (2013) indicated that *M. oleifera* had enhanced the gastrointestinal morphology of *O. mykiss* (increased villi length) which improved the food digestibility and absorption capacity. Herein, the increment in growth and diet utilization could be related to its immuno-nutritional components and the high feed digestibility, absorption and assimilation ability, through the augmented digestive enzymes and healthy intestinal microflora boosted by the *M. oleifera* prebiotic activity.

In the meantime, the usage of either raw *M. oleifera* or its extracts as a dietary supplement or protein replacement has shown variable results. Feeding Moringa leaf meal to Nile tilapia at a dietary inclusion level up to 8-10% of the diets hindered tilapia growth (Richter *et al*., 2003; Yuangsoi and Charoenwattanasak, 2011; Abo-State *et al*., 2014). Similarly, Afuang *et al*. (2003) incorporated methanol-extracted leaf meal containing fewer saponins and phenolics in the diets (at a 30% inclusion level) of tilapia fingerlings. The fish performance was not hindered, but the body protein content reduced. Whereas, the aqueous extracted leaf meal (15% of the diet) had reduced the feed intake, feed utilization and fish performance (Madalla, 2008). Also, Dongmeza *et al*. (2006) reported a considerable decrease in the Nile tilapia feed intake and consequently its growth after receiving different dietary moringa leaf extracts (tannin-reduced, saponin-reduced and saponin-enriched).

Measurement of the plasma biochemical parameters is a fundamental step to judge the health integrity of any aquatic species (Ferreira *et al*., 2007). Plasma proteins carry out many functions, such as adjusting the water balance in fish

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(Rudneva and Kovyrshina, 2011) and the protective effects implemented by the body for the elimination of any microorganisms (Gerwick *et al*., 2002). This study displayed an increased level of plasma protein in the dietary MOAE supplemented fish. Similar conclusions were revealed by Alishahi *et al*. (2010) and Haghighi *et al*. (2014) who informed that 0.5% and 1% *M. oleifera* dietary supplementation had significantly improved the serum total protein and globulin in *Cyprinus carpio* and *O. mykiss* respectively. Conversely, the serum total protein levels obtained in this work could not agree with either Dotta *et al*. (2014) for *O. niloticus* (0.5% *M. oleifera*) nor Kavitha *et al*. (2012) for *Cyprinus carpio* (exposed to 124.0 mg L^{-1} *M. oleifera* extract). They reported that *M. oleifera* dietary supplementation was unable to considerably elevate the total serum protein in those fish as an outcome of the metabolic degradation and utilization of protein.

Transaminase enzymes are of dynamic importance in protein and carbohydrate metabolism. Their levels (i.e. transaminases; AST and ALT) are used for judging the physiological and healthy state of aquatic species (Vutukuru *et al*., 2007). Therefore, they are indicative of organ dysfunction, especially for hepatic dysfunction with a subsequent leakage of enzymes into the blood (Gabriel and George, 2005). The aminotransferase activities were comparable among all the treated groups except for the group fish supplemented with 400 mg MOAE kg $diet^{-1}$ which displayed a reasonable diminishing in the activity aminotransferase enzymes. This result supports the previous studies that convey the potentiality of *M. oleifera* leaves extract to protect the membrane integrity of tilapia hepatocytes against stressors (Tekle and Sahu, 2015). Also, the increased growth rate conveyed in the current study give further support for the liver integrity as a vital organ implemented in the normal metabolic function of any aquatic organism.

Creatinine and urea are among the dominant biochemical indices particularly determined to assess renal condition (Gross *et al*., 2005; Adeyemi and Akanji, 2012). The fish received 100 mg MOAE kg^{-1} exhibited a significant decrease in the urea level. Such reduction may offer a further support of the nephron-protection activity of *M. oleifera* as reported by Anwar *et al*. (2007) and Sharma and Paliwal (2012). Also, the comparable creatinine levels distinguished in differently treated fish groups provide further support to the nephron-protective potentiality of MOAE. Nevertheless, our results are dissimilar to Mazumder *et al*. (1999) who clarified that a weekly dose (>46 mg kg^{-1} b.wt extract) of *M. oleifera* methanol root extracts had impaired function of mice kidney. Oyagbemi *et al*. (2013) as well reported elevated serum creatinine levels in rats administered *M. oleifera* (200 and 400 mg kg^{-1} b.w.).

Regarding the blood glucose levels verified in the current study; the MOAE supplemented diet had hypoglycemic effect in all of the treated fish groups. Similarly, fish received herbal additives

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showed hypoglycemia (Metwally, 2009; Banaee *et al*., 2011; Ojha *et al*., 2014). MOAE had successfully governed the glucose level in rabbits (Makonnen *et al*., 1997) and rats (Amin *et al*., 2016). The moringa leaf juices contain α-glucosidase and amylase inhibitors. These enzymes prevent the assimilation of glucose into absorbable metabolites, hence preventing the increase in blood glucose (Abdulkarim *et al*., 2005).

On the contrary; diabetes inducing property was verified to green tea extract indicated by the observed hyperglycemia in green tea extract exposed Nile tilapia (Abdel-Tawwab *et al*., 2010).

SOD and GPX are sensitive oxidative stress biomarkers. They are considered the core line of the body antioxidant defensive mechanism (Jiang *et al*., 2009). SOD is a dynamic enzyme responsible for both scavenging ROS and protection of cells from being damaged by free radicals (Chien *et al*., 2003). In our study, the MOAE supplementation considerably diminished the SOD and GPX activity, indicating that *M. oleifera* might have the capability to reduce peroxide radicals and converting it into oxygen and water due to an antioxidant potential (Atli and Canli, 2007). *M. oleifera* could also preserve the cell redox state by restricting the damaging effects of ROS (Halliwell and Gutteridge, 2007). Furthermore, the MOAE dietary supplementation resulted in reduced activity of other antioxidant enzymes. *M. oleifera* contains active biological compounds (polyphenols, glycosides, anthocyanin, tannins and thiocarbamates). These active compounds expel free radicals, active antioxidant enzymes and inhibit oxidases (Luqman *et al*., 2011) which give a further clarification for the *M. oleifera* associated antioxidant activity.

Furthermore, this study revealed that acutely hypoxic tilapia had exhibited an elevation in the cortisol level 30 minute's post-hypoxic stress. The elevated cortisol level was reduced distinctly two hours post-hypoxic stress in those fish received 200 and 400 mg MOAE kg^{-1} feed. The retrieval towards normal cortisol level following MOAE pretreatment proposed hypocortisolemic effects of MOAE. Additionally, the potent antioxidants of Moringa supplemented diets was said to be useful in compromising the adversative properties of stress related hypoxia. Production of oxidative radicals (especially ROS) is thought to be boosted under hypoxic stress (Chandel and Budinger, 2007). Accordingly, these inclusion levels were possibly capable of blocking the hypercortisolemia elicited by the acute stress caused by hypoxia or efficiently restored the homeostatic equilibrium of the life helping physiological systems (Van Rijn and Reina, 2010). Hammed *et al*. (2015) also reported that extracts of Moringa leaves infiltrate to the cell membrane lipid bilayer, resulting in improved permeability, and ROS elimination. Hence these fish groups could successfully tolerate and recover the hypoxic stress than the others. This study verified the potentiality of *M. oleifera* aqueous extract as a growth

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promoter, antistress and antioxidant for *O. niloticus* fingerlings. *M. oleifera* aqueous extracts are proposed to replace synthetic antimicrobials and growth promoters. This study is valuable to validate the safe administration of *M. oleifera* in commercial tilapia culture. In this concern, future studies are required to comprehend the influence of this plant extract in relieving chronic stress and its possible toxic effect as well. Feasibility study for its commercial usage is required too.

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