# 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 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 2007; etChakraborty 2011: and Hancz, Huyghebaert et al., 2011).

The drumstick Moringa tree, 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 nourishing and healing its 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 al..2003). Nevertheless, the information available about its influences on the immune antioxidant activity response, 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 response to stressful culture conditions, such as hypoxia. Therefore; this study aims to get a comprehensive evaluation of growth promoting effect M. oleifera aqueous (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 Kafr hatcherv at E1Sheikh 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 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<sup>-1</sup> 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<sup>-1</sup>). The fish groups were differentiated according to the MOAE

dietary inclusion level. They were labeled as  $G_1$ ; fed a control diet (0% MOAE kg<sup>-1</sup> feed),  $G_2$ ; fed 50 mg MOAE kg<sup>-1</sup> feed,  $G_3$ ; fed 100 mg MOAE kg<sup>-1</sup> feed,  $G_4$  was fed 200 mg MOAE kg<sup>-1</sup> feed and  $G_5$ ; fed 400 mg MOAE kg<sup>-1</sup> feed. The stocking density was maintained at 30 fish tank<sup>-1</sup>. 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<sup>-1</sup>).

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

diet (g 100 g 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		

<sup>\*</sup>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<sub>3</sub>: 2,000 mg; Vitamin-B<sub>1</sub>: 1,000 mg; Vitamin-B<sub>2</sub>: 5,000 mg; Vitamin-B<sub>6</sub>: 15,000 mg; Vitamin-B<sub>12</sub>: 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.

# 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<sup>-1</sup>)=  $(W_2-W_1)/T$
- Specific growth rate (% day<sup>-1</sup>)=  $[\log (W_2)-\log (W_1)/T]\times 100$
- Condition factor  $(K)=(W/L^3)\times 100$

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 anesthetized fish tank<sup>-1</sup>) 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 3000 (Hettich Centrifuge, rpm 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 electrohomogenizer (Heidolph, Germany). The homogenate was then centrifuged  $(13,000 \times g \text{ at } 4^{\circ}\text{C for } 10 \text{ min}) \text{ and the}$ supernatant was pipetted and stored at -80°C until analysis. The antioxidant enzymes assay comprised; superoxide dismutase (SOD), Glutathione peroxidase (GPX), (Glucose 6phosphate 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,

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<sup>-1</sup> diet to 400 mg kg<sup>-1</sup> 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. **MOAE** supplemented 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<sup>-1</sup>, 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<sup>-1</sup> diet resulted in an expressively higher serum TP level than the levels identified in the other treated fish However, still groups. comparable were observed between the results 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 glucose levels. (p<0.05) in the sera of the MOAE supplemented fish (Table 3). Both G4 (200 mg kg<sup>-1</sup>) and G1 (control one) displayed the highest

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 <sup>-1</sup> 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°	$28.58\pm0.76^{b}$	$29.58\pm0.72^{b}$	26.88±1.94°	31.76±1.09 <sup>a</sup>	
WG (g)	$20.43\pm2.18^{d}$	$24.05 \pm 0.76^{b}$	$25.05 \pm 0.72^{b}$	$22.40 \pm 1.88^{c}$	$27.36\pm1.02^{a}$	
ADG(g day <sup>-1</sup> )	$0.34\pm0.03^{d}$	$0.40\pm0.01^{bc}$	$0.41\pm0.01^{b}$	$0.37\pm0.03^{c}$	$0.45{\pm}0.01^a$	
SGR (% day <sup>-1</sup> )	$1.24 \pm 0.09^{c}$	$1.33 \pm 0.03^{b}$	$1.35\pm0.04^{b}$	$1.29\pm0.05^{bc}$	$1.43\pm0.02^{a}$	
FCR (%)	$1.79\pm0.16^{a}$	$1.51\pm0.04^{b}$	1.37±0.04c	$1.55 \pm 0.18^{b}$	$1.28\pm0.06^{c}$	
FL(cm)	11.60±0.43 <sup>b</sup>	11.98±0.31 <sup>b</sup>	$12.23 \pm 0.61^{ab}$	12.01±0.51 <sup>b</sup>	12.66±0.59 <sup>a</sup>	
CF	1.76±0.20	1.87±0.13	$1.84\pm0.15$	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 (%)	$1.74 \pm 0.27^{ab}$	$1.67 \pm 0.17^{b}$	1.97±0.19 <sup>a</sup>	2.02±0.22 <sup>a</sup>	2.22±0.25 <sup>a</sup>	

Data are expressed as means $\pm$ 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 <sup>-1</sup> diet				
	0 (Control)	50	100	200	400
Total protein (g)	4.0±0.80 <sup>b</sup>	5.16±1.26 <sup>ab</sup>	5.15±1.05 <sup>ab</sup>	5.23±1.43 <sup>ab</sup>	6.20±1.89 <sup>a</sup>
Serum IgM (µg ml <sup>-1</sup> )	117.34±14.24 <sup>b</sup>	127.19±34.94 <sup>ab</sup>	$125.56 \pm 16.16^{ab}$	150.15±44.86 <sup>a</sup>	112.77±11.62 <sup>b</sup>
Albumin (g dl <sup>-1</sup> )	1.88±0.14	$1.96 \pm 0.52$	$1.89 \pm 0.39$	2.10±0.34	2.03±0.52
AST (U L <sup>-1</sup> )	58.08±12.64 <sup>a</sup>	56.08±14.45 <sup>a</sup>	56.50±11.00 <sup>a</sup>	55.72±9.98 <sup>a</sup>	$44.00\pm6.28^{b}$
ALT ( $UL^{-1}$ )	39.33±19.93 <sup>a</sup>	40.16± 14.62 <sup>a</sup>	38.26±16.13 <sup>a</sup>	40.98±18.64 <sup>a</sup>	$31.33 \pm 10.35^{b}$
Urea (mg dl <sup>-1</sup> )	$13.14 \pm 1.78^{a}$	$10.89 \pm 2.60^{ab}$	$9.10\pm1.57^{b}$	11.16±3.37 <sup>ab</sup>	11.17±2.64 <sup>ab</sup>
Creatinine (mg dl <sup>-1</sup> )	0.73±0.09	0.73±0.10	$0.71 \pm 0.08$	$0.65\pm0.20$	$0.80\pm0.11$
Blood glucose (mg dl <sup>-1</sup> )	115.33±7.17 <sup>a</sup>	89.16±16.57 <sup>b</sup>	79.00±13.79 <sup>b</sup>	133.00±31.90 <sup>a</sup>	$85.50\pm19.36^{b}$

Data are expressed as means  $\pm$  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).

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<sup>-1</sup> diet) exhibited a significantly lower cortisol level as matched to both the control fish group and  $G_4$  (200 mg MOAE kg<sup>-1</sup> diet).

On the pre-hypoxic contrary to condition. elevated serum cortisol levels were equally assessed as a hypoxia-related response in all treated fish groups 30 minute's post-hypoxic Nevertheless, **MOAE** stress. the supplemented diets decreased 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<sub>4</sub> and G<sub>5</sub>.

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

otetjera aqueous extract supplementation.					
	Treatments				
	Dietary M. oleifera extract mg kg <sup>-1</sup>				
Parameters	0 (Control)	50	100	200	400
SOD (U mg <sup>-1</sup> )	16.69±0.82 <sup>a</sup>	12.16±2.63 <sup>b</sup>	7.24±2.02°	$7.14\pm 2.79^{c}$	5.56±0.60°
G6PD (mU mg <sup>-1</sup> )	$6.21\pm3.27^{a}$	$6.10\pm1.05^{a}$	$2.05\pm1.08^{b}$	$1.56\pm0.52^{b}$	$2.17\pm1.32^{b}$
GPX ( mU mg <sup>-1</sup> )	$4.56\pm0.56^{a}$	4.11±0.26 <sup>a</sup>	$2.29\pm0.28^{b}$	$2.11\pm0.28^{b}$	1.13±0.11 <sup>c</sup>
(NO) activity (μ mol L <sup>-1</sup> )	26.43±3.55 <sup>a</sup>	$18.97 \pm 8.82^{abc}$	$20.07 \pm 5.25^{ab}$	15.35±4.53 <sup>bc</sup>	9.01±0.96°

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

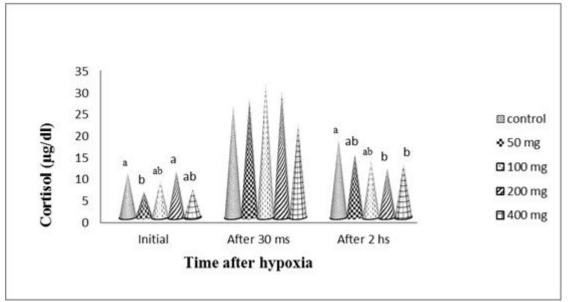


Figure 1: Serum cortisol levels of *Oreochromis niloticus* challenged with acute hypoxia after 60 days of dietary *Moringa oleifera aqueous extract* supplementation. Values with the same superscript letters at initial, 30 ms and 2 hours post-hypoxic stage are not significantly different (p>0.05) between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

#### **Discussion**

MOAE was recently verified to possess a valuable range of active substances. 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<sup>-1</sup> had a markedly high WG, SGR, and ADG. The ethanolic extract of M. oleifera flower (250 and 500 mg kg<sup>-1</sup> diet) as well had improved tilapia growth (Tekle and Sahu, 2015). Also, a growth-promoting potential oleifera seed protein extracts was reported by Stadtlander et al. (2013) when added to tilapia diet at 400 mg kg <sup>1</sup> in an eight weeks experimental period. Not only this but also the dried powder of *M. oleifera* leaves (10 mg kg diet<sup>-1</sup>) was able to promote the growth of iuveniles of Penaeus indicus 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 supplement dietary 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 (2003) incorporated methanolextracted leaf meal containing fewer saponins and phenolics in the diets (at a inclusion 30% 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

(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 supplementation dietary 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<sup>-1</sup> M. oleifera extract). They reported that M. oleifera dietary supplementation was unable considerably elevate the total serum protein in those fish as an outcome of the metabolic degradation and utilization of protein.

Transaminase of enzymes dynamic importance in protein 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<sup>-1</sup> 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 biochemical dominant indices particularly determined to assess renal condition (Gross et al., 2005; Adeyemi and Akanji, 2012). The fish received 100 mg MOAE kg<sup>-1</sup> 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 distinguished creatinine levels differently treated fish groups provide support the further to nephronprotective potentiality of MOAE. Nevertheless, our results are dissimilar to Mazumder et al. (1999) who clarified that a weekly dose (>46 mg kg<sup>-1</sup> 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<sup>-1</sup> 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

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 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 **GPX** and 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 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 post-hypoxic minute's stress. The elevated cortisol level was reduced distinctly two hours post-hypoxic stress in those fish received 200 and 400 mg MOAE kg<sup>-1</sup> 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 useful in compromising adversative properties of stress related hvpoxia. Production of oxidative radicals (especially ROS) is thought to boosted under hypoxic stress and Budinger, 2007). (Chandel 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

promoter, antistress and antioxidant for O. niloticus fingerlings. M. oleifera aqueous extracts are proposed to replace synthetic antimicrobials and growth promoters. This study validate the valuable to safe administration of М. 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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