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Survival, Growth, Haematological and Biochemical Responses of *Clarias Gariepinus* Grown in *Moringa Oleifera* Seeds Treated Wastewater

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Abstract

The current study aimed to assess the feasibility of fish farming in treated wastewater using *Moringaoleifera* seeds extract (MSE). A native Catfish (*Clariasgariepinus*) specimens were grown in effluent water (Eff water), MSE treated effluent (MSE+Eff) and control conditions (dechlorinated water)for one month. The growth performance (*e.g.* mortality percent, behavioral variations and weight changes), hepatosomatic indices (HSI), hepatic DNA fragmentation, hematological, biochemical variables (e.g. cortisol activity, tumor necrosis factor-α (TNF-α) and acetylcholine esterase (AChE) level), oxidative stress and antioxidants were evaluated. In contrast to EFF water, the MSE treated water significantly increased survival percent, antioxidants enzymes glotathione (GSH), catalase (CAT), total antioxidants content (TAC) and nitric oxide (iNOS). Furthermore, the MSE treated water decreased behavioral variations, TNF-α, oxidative stress damage (malonaldehyde (MDA)) and hepatic DNA fragmentation. While it increased body weight and improved hepatosomatic indices (HSI). Additionally, normalized hematological variables and biochemical markers serum e.g. aspartate aminotransferase (AST), alanine aminotransferase (ALT), AchE, total protein, albumin and globulin were achieved under the MSE treatment. The obtained results suggest the use of MSE treated wastewater for fish farms as antioxidant and protective medium, especially with the concurrent aggravation of the global crisis of water shortage. We recommend further studies on the use of MSE treated effluent for growing fish in fisheries and aquatic farming.

***Keywords*:** Wastewater; Moringa oleifera; Oxidative stress; Clarias gariepinus;Fish farming.

1. Introduction

The repurposed wastewater is generally composed of household kitchens, laundry, bathroom shower, and other municipal sources (*e.g.*, restaurants, supermarkets, offices and industries) [1]. Thus, reusing this water could impose serious environmental drawbacks to plants and animals due to the hazardous chemicals and pathogenic microorganisms along with solid waste, feces, engine oil, grease [1]. A member of the *Moringaceae* family with a large geographic distribution is *Moringa oleifera*, particularly in Asian and African countries [2, 3]. Usually, the leaves, fruits, roots and flowers of *M. oleifera* are consumed as vegetables[2]. Phytochemical research indicated the abundance of potassium, calcium, phosphorus, iron, vitamins A and D, β-carotene, flavonoids, vitamin C, vitamin E, polyphenol oxidase, ascorbic acid, oxidase, and catalase in their leaves [4]. *M. oleifera* has been also demonstrated as anti-tumor, antioxidant and hepatoprotective agent [5, 6].

M. oleifera has been proposed as cardio-protective due to the unique phenolic component which inhibits oxidative cardiac cell damage by increasing oxidative stress defense enzymes, avoiding lipid membrane peroxidation [7]. Recently, *M. oleifera* seeds extract (MSE) has been recommended as a viable tool for enhancing water quality as well as an effective disinfection agent for treating raw wastewater [3, 8, 9].

Around 50% of the world's fish production comes from aquaculture, which greatly contributes to human food resources at a quicker rate. In order to meet the increasing demand for food-based fish, it is

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predicted that an additional 27 million tonnes of fisheries products must be produced worldwide by 2030 [10]. However, the use of recycled wastewater in the production of fish could threat the human health and aquatic organisms by introducing infections and emerging contaminants [1, 11,12]. It was stated that pathogenic microorganisms, not physical or chemical pollutants, are the causative agent for most outbreaks of food-related sickness [1]. The possibility of disease transmission exists if fecal coliform is present in fish meant for human consumption [1].

In fact, numerous attempts have been made to use medicinal plant leaf extracts to aid fish that dwell in polluted ecosystems with antioxidant protection and detoxification [13], to boost their health and growth [14]. Feeding Nile tilapia (*Oreochromisniloticus*) on raw *M. oleifera* leaf meal by replacing 10% of their diet with fish meal-based dietary protein had no negative effects on their ability to grow [15]. The addition of *M. oleifera* leaf diet to20 g/kg of soybean protein in high-end carp (*Cyprinus carpio*) diets have no adverse effects on growth and digestibility [16].

Previous studies indicated that exposure of *C. gariepinus* to heavy metals, inorganic and organic contaminants increased levels of reactive oxygen species followed by consecutive induction in H_2O_2, MDA and lipid peroxidation) [17, 18]. As a result, the high level of oxidative stress induced response of antioxidant defense system. Which include antioxidant enzymes such as superoxide dimutase (SOD), catalase (CAT), and glutathione peroxidase (GP_X) and antioxidant metabolites such as vitamins A, C, flavonoids, and carotenoids [19].Thus, the addition of exogenous antioxidants into catfish diet can alleviate oxidative stress [4, 20]. The present investigation aims to (1) explore the possibly of growing catfish (*Clarias gariepinus*) in MSE treated effluent for long time. (2) to evaluate catfish growth performance, behavior (3) to intensively study the physiological and biochemical responses (e.g., hepatosomatic indices (HSI), hepatic DNA fragmentation, hematological biochemical variables) following catfish growth in the treated effluent.

2. Materials and methods:

2.1. Fish rearing and Experimental design

Before the experiment began, 150 immature channel catfish of both sexes with average weights of 100±5 g were kept in a nursery plastic aquarium for a week to acclimate [21]. The rearing pools are small size with dimensions of 1.3 m×1.5 m ×0.3 m. The pH level was maintained at 7.6±0.4 and the growing media temperature was set at22°C to 23°C. An air pump was used to provide oxygen to growth media at 88–95% saturation level. Fish were maintained at14L: 10D light: dark cycles. The commercial meal was supplied to the fish at a rate of 3% of body weight/day. The experimental study was conducted at Biochemistry Department, College of Veterinary Medicine, Beni-Suef University. Mature fishes were divided into 3 groups each comprised of 50 fishes, were reared separately in 500 liters water media for one month using 3 different propagation media (1) Control group: (dechlorinated water), (2) Sewage effluent water group, (3) *Moringa* seeds treated effluent (MSE) group, *Moringa* seeds were extracted by water according to the method of Amin, *et al* [3] at the concentration of 400 mg/l which has the most potent effect in the treatment process [3]. The water was changed daily.

2.2. Survival and mortality percentages, behavioral alterations and growth response

Fish behavior changes were monitored throughout the experiment and results were recorded regularly according to College of Veterinary Medicine, Beni-Suef University.

The Survival and mortality percentages were calculated using the following equation:

$$\textbf{Survival } \% = \frac{\boldsymbol{C-T}}{\boldsymbol{C}} \times \mathbf{100}$$

Where C and T are the numbers of catfishes at control and effluent treatment conditions

Growth performance was estimated as follows:

$$\textbf{Weight gain} = \textbf{W2} - \textbf{W1}$$

Where W1 and W2 are the starting and ultimate weights, respectively.

2.3. Serum sampling

At the end of the experiment (1 month), fish were caught using a net, and blood was extracted from the caudal peduncle. The blood was then transferred to clean, dry centrifuge tubes, left for 15 minutes to allow for clotting, and centrifuged for 15 minutes at 3000 rpm. Finally, the serum was separated and collectedin sterile tubes, then stored in a deep freezer forbiochemical analysis.The fish were sacrificed. The liver was then gently taken out and weighed.

2.4. Hepatosomatic Indices:

Hepatosomatic indices of the liver were then determined according to the method described by Jenkins [22] and Adams, *et al* [23] using the formula:

$$\textbf{Hepatosomatic indices} = \frac{\textbf{Weight of liver}}{\textbf{Weight of fish}} \times \mathbf{100}$$

2.5. Hematological parameters:

Blood was immediatelytransferred todry and clean tube with EDTA solution for measuring hemoglobin (Hb), red blood cells (RBCs) and white

blood cells (WBCs).WBCs and RBCs were counted by a haemocytometer according to the method of Kanaeu [24]. Hematocrite in blood (PCV%) was determined as packed cells volume by using micro hematocrite tubes method as described by Dacie and Lewis [25]. The Haemoglobin (Hb) concentration was immediately measured using method of Vankampen [26].

2.6. Biochemical markers:

Using reagent kits bought from Biosystem S.A., the activities of the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed using the Gella, *et al* [27] method. The serum total protein and albumin were measured using biochemical kits obtained from Bio-Diagnostic Co. (Cairo, Egypt) using the procedures proposed by Gornal, *et al* [28] and Doumas, *et al* [29], respectively. The amount of serum globulin was calculated by deducting the albumin value from the total protein value of the same sample.

2.6.1. Cortisol and acetylcholine esterase (AChE):

Cortisol and acetylcholine esterase (AChE) kits were purchased from Gamma Trade Co. (Cairo, Egypt) and evaluated using the approaches outlined by Foster and Dunn [30] and Knedel and Böttger [31], respectively.

2.6.2. Serum tumor necrosis factor-α (TNF-α):

The sandwich enzyme immunoassay was utilized to measure the serum levels of tumor necrosis factor (TNF-α) using kits donated by R&D systems (USA). This technique was developed using the accepted guidelines and steps from Croft, *et al* [32].

2.7. Oxidative stress and antioxidants activities:

Glutathione (GSH) and malondialdehyde (MDA) kits were obtained fromBio-Diagnostic Co. (Cairo, Egypt) and were quantified in accordance with Beulter [33] and Satoh [34] techniques, respectively. Catalase (CAT), total antioxidant capacity (TAC) kits were received from Bio-Diagnostic Co. (Cairo, Egypt) and tested using techniques outlined by Aebi [35] and Koracevic, *et al* [36], respectively. A spectrophotometer (Photometer 5010, BM Germany) and commercial test kits (Biodiagnostics, Egypt) were used to quantify the levels of serum nitric oxide (NO) according to Montgomery and Dymock [37]

2.8. Hepatic DNA fragmentation:

The amount of hepatic DNA fragmentation was measured using a spectrophotometer (Micro-lab 200; Vital Scientific, Dieren, The Netherlands) at 575 or 600 nm against a reagent blank in accordance with the procedure outlined by Kurita-Ochiai, *et al* [38]. The formula used to calculate the percentage of fragmented DNA is,

$$\%\ \mathbf{offragmentedDNA} = \frac{\mathbf{fragmented\ DNA}}{\mathbf{fragmented\ DNA\ +\ intact\ DNA}} \times \mathbf{100}$$

2.9. Statistical analysis:

The data were statistically evaluated using the one way ANOVA test and reported as mean± SEM using the SPSS statistical programs. T-tests were performed to compare the initial and final condition factors; Regression models were used to calculate how much the weight of each fish changed over time.

3. Results:

3.1. Growth response and behavior of *Clarias gariepinus* grown under different water conditions:

As demonstrated in Table 1, the mortality percent of fish grown in wastewater recorded the highest mortality percent by94%, while the mortality percent of fish left in treated wastewater with *moringa* seeds extract was only 4%.

Table 1:

Survival and mortality percentages of *Clarias gariepinus* grown in control conditions, wastewater effluent or in *moringa* seed treated wastewater for a month

Groups	No of fish	Cumulative mortality	Mortality %	*Survival %*
Control	50 ± 0	0 ± 0	0 ± 0	100 ± 0
Eff water	50 ± 0	47 ± 2	94 ± 2	6 ± 2
Eff water + MSE	50 ± 0	2 ± 1	4 ± 1	96 ± 1

All expressed results are mean of 3 independent replicates ± SD. Different superscript letters indicate a significant difference among treatments at $P < 0.05$.

Fish in the control group exhibited typical activities, including vigorous feeding, coordinated body movements, and extreme sensitivity to small stimuli. In order to analyze altered fish behavior among different groups, control fish behavior was used as the baseline. In comparison to the control group, wastewater significantly increased the behavioral changes that fish displayed such as opercula movement, loss of balance, air gulping, skin pigmentation, and mucus secretion. Fish in treated effluent water, however, only showed a modest variation in opercula movement and air gulping as compared to control group. Table 2 includes a list of those outcomes.

Table 2:
Behavioral alterations of *Clarias gariepinus* grown in control conditions, wastewater effluent or in *moringa* seed treated wastewater for a month

Groups	Opercula movement	Loss of balance	Air gulping	Skin coloration	Mucus secretion
Control	–	–	–	–	–
Eff water	+++	+++	+++	+++	+++
Eff water + MSE	+	–	+	–	–

All expressed values aremean of 3 independent replicates ± SD. Different superscript letters indicate a significant difference among treatments at $P < 0.05$

Data in Table 3 revealed that wastewater significantly decreased weight of fish (90 g) along with time of the experiment (30 days). However, groups of catfish that grown in effluent water treated with MSE had a comparable weight value to those of the control group (160 g). As anticipated the highest fish weight was observed in control group by (180 g).

Table 3:
Changes in the weight of *Clarias gariepinus* fish grown in control conditions, wastewater effluent or in *moringa* seed treated wastewater for a month

Groups	Weight (g) at 0 day	Weight (g) at 30th day
Control	100 ± 10	180 ± 20^{a}
Eff water	100 ± 10	90 ± 15^{b}
Eff water +MSE	100 ± 10	160 ± 10^{c}

All expressed values aremean of 3 independent replicates ± SD. Different superscript letters indicate a significant difference among treatments at $P < 0.05$.

3.2. Hepatosomatic indices and hematological parameters of *Clarias gariepinus* grown under different water conditions:

The HSI values in Table 4 reveal substantial differences between the control conditions (2.2), the fish grown in the wastewater (6.1), and the treated effluent water with MSE (2.3).

Table 4:
Changes in hepatosomatic indices (HSI) of *Clarias gariepinus* fish grown in control conditions, wastewater effluent or in *moringa* seed treated wastewater for a month

Groups	HSI at 0 day	HSI at 30th day
Control	2.2 ± 0.01	2.22 ± 0.01^{a}
Eff water	2.21 ± 0.01	6.1 ± 0.02^{b}
Eff water +MSE	2.2 ± 0.02	2.31 ± 0.02^{a}

All values are mean of 3 independent replicates ± SD. Different superscript letters indicate a significant difference among treatments at $P < 0.05$

The haematological parameters are listed in Table 5. Notably, application of wastewater significantly decreased the erythrocytes count (RBCs) of *C. gariepinus* fish by 4.32 ×10^{12} /L when compared with control group (8.2 ×10^{12} /L). While using MSE treated wastewater showed a comparable value of RBCs counts to control conditions (8 ×10^{12} /L).

Similarly, application of wastewater significantly decreased the haemoglobin (Hb) concentration to 5.2 g/dl when compared to the control group (9.21 g/dl). Whilst *C. Gariepinus* group grown in MSE-treated wastewater had a level of 9 g/dl haemoglobin, showing a similar value to control condition. The results also showed that the number of white blood cells (WBCs) were considerably decreased in the wastewater group (6000) compared to the control group (11200×10^6/L). Whereas the WBCs number of MSE- treated wastewater was (11212×10^6 /L). Moreover, the hematocrit value (PCV %) was substantially lower in the wastewater group (20.2 %) when compared to control group (40 %). However, a comparable PCV% was found in the group kept in MSE-treated wastewater was 35.21%.

Table 5:
Hematological parameters of *Clarias gariepinus* fish grown in control conditions, wastewater effluent or in *moringa* seed-treated wastewater for a month

Groups	WBCs (×10^6 /L)	PCV %	RBCs (×10^{12}/L)	Hb (g/dl)
Control	11200 ± 201[a]	40 ± 1.2[a]	8.2 ± 0.98[a]	9.21 ± 0.23[a]
Eff water	6000 ± 150[b]	20.21 ± 0.65[b]	4.32 ±0.25[b]	5.2 ± 0.23[b]
Eff water + MSE	11212 ± 100[a]	35.21 ± 1.4[a]	8 ± 0.14[a]	9 ± 0.21[a]

All values are mean of 3 independent replicates ± SD. Different superscript letters denote a significant difference among treatments at $P < 0.05$.

3.3. Biochemical response of *Clarias gariepinus* grown under different water conditions

The biochemical parameters imply the physiological and biochemical status of organism and indicate either it grown under normal or stressful condition. Data in Table 6 revealed that fish exposed to wastewater had significantly higher serum levels of the liver enzymes ALT, AST and cortisol levels by 40 U/L, 120 U/L and 91 µg/ dl, respectively compared to control conditions 20 and 50 U/L and 15 µg/dl, respectively. Obviously the MSE-treated water had comparable values to control conditions.

Whereas, *C. gariepinus* fish grown in MSE-treated wastewater showed a non-significant changes in the levels of serum total protein, albumin, globulin, and AChE (6.42, 4.0, 2.42 g/dl and 392.21 U/L) compared to those of control conditions (6.6, 4.1, 2.5 g/dl, 400 U/L).

Table 6:
Changes of biochemical parameters of *Clarias gariepinus* fish grown in control conditions, wastewater effluent or in *moringa* seed-treated wastewater for a month

Parameter	Control	Eff water	Eff water + MSE
ALT (U/L)	20 ± 0.01[a]	40 ± 1.22[b]	23.2 ± 0.02[a]
AST (U/L)	50 ± 0.45[a]	120 ± 0.24[b]	51.05 ± 1.06[a]
Cortisol (µg/ dl)	15 ± 0.028[a]	91 ± 1.07[b]	17.12 ± 0.02[a]
Acetylcholineesterase (U/L)	400 ± 3.12[a]	203 ± 1.87[b]	392.21 ± 5.21[a]
Albumin (g/dl)	4.1 ± 0.03[a]	2.25 ± 0.02[b]	4.0 ± 0.12[a]
Globulin (g/dl)	2.5 ± 0.01[a]	1.2 ± 0.001[b]	2.42 ± 0.03[a]
Total protein (g/dl)	6.6 ± 0.01[a]	3.45 ± 0.02[b]	6.42 ± 0.01[a]

All values are mean of 3 independent replicates ± SD. Different superscript letters denote a significant difference among treatments at $P< 0.05$

Data in Fig 1A demonstrates that tumor necrosis factor TNF-α measurements offish serum from the effluent group were considerably higher (25.98 U/ml serum) than those from the control group (22.11 U/ml serum). Whilst, MSE-treated sewage showed the lowest TNF-α by 3.8 U/ml serum. Meanwhile, the level of oxidative stress marker (MDA) in fish serum of the effluent group were substantially higher (58.7 U/ml serum) than those in the control group (25.26 U/ml serum). On contrast, the MDA level of the fish group of the MSE-treated effluent (31.59 U/ml serum) was significantly lower than those of effluent group (Fig 1B).As shown in Fig 1C, the GSH activities of catfish were significantly lower in sewage effluent (7.9 mg/ml serum) compared to the control group (54.14 mg/ml serum), while they were significantly higher in sewage effluent treated with MSE (46.06 mg/ml serum). The catalase enzyme activity (CAT) level in fish serum from the effluent group was significantly higher (32.61 U/ml serum) than in fish serum from the control group (14.03 U/ml serum), While the CAT activity in the MSE-treated sewage was 17.55 U/ml serum (Fig 1D). In a similar line with previous results, the total antioxidant contents TAC of catfish was much lower in sewage effluent (12.67 mg/ml serum) compared to the control group (86.62 mg/ml serum). However, MSE-treated effluent markedly increased TAC by 73.69 mg/ml serum (Fig 1E). Water effluent considerably increased the catfish's iNOS level (9.08mg/ml serum) when compared to the control group (2.63 mg/ml serum) andMSE-treated effluent (4.47 mg /ml serum) as represented in (Fig 1F).

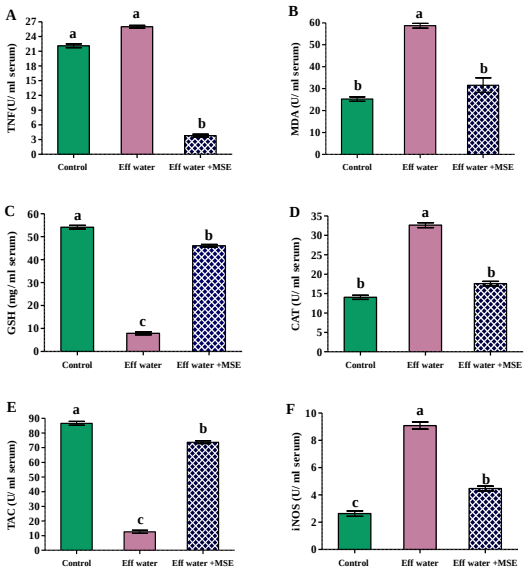

Figure 1: Effects of wastewater and MSE- treated wastewater on the biochemical parameters of *Clariasgariepinus* serum (A) tumor necrosis factor TNF-α, (B) malondialdehyde (MDA), (C)glutathione (GSH) activity, (D) catalase activity (CAT), (E)total antioxidant contents (TAC) and (F) nitric oxide (iNOS) level.

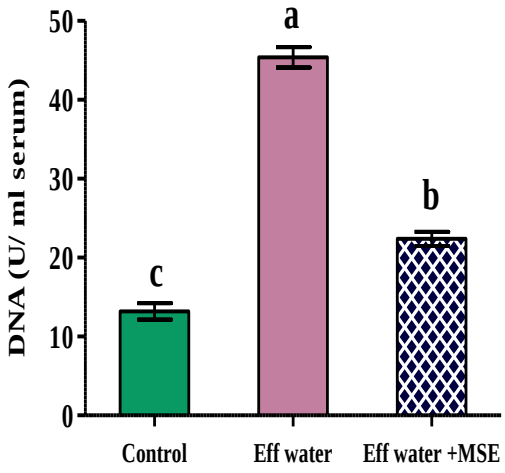

Figure 2: Effects of wastewater and treated wastewater with MSE on the percentage of DNA fragmentation in catfish serum.

Figure 2 shows that the percentage of DNA fragmentation in fish serum ofthe effluent group (45.39 U/ml serum) was markedly higher than those of the control group (13.18 U/ml serum) and MSE- treated effluent (22.39 U/ml serum). Overall, the use of raw water effluent for growing *C. gariepinus* had detrimental effects on the all biochemical parameters including tumor necrosis factor TNF-α, DNA fragmentation and considerably increased lipid peroxidation (MDA level) and oxidative stress enzymes CAT). However, using MSE-treated water had comparable normal effect like control condition.

4. Discussion

Our research indicated that the mortality percent of fish kept in EFF group was 94%, these results are consistent with Kaur and Dua [39] and McCallum, *et al* [40]. The physicochemical and microbiological findings of a prior research by Amin, *et al* [3] proposed that the degradation of water quality was caused by the discharge of hazardous effluents from various industries and domestic sewage from Byad-Elarab, Beni-Suef, Egypt. However, the mortality percent of fish kept in MSE-treated effluent group was just 4%, indicating that the MSE treatment process was effective and the water quality was appropriate and could be a secure source for fish farming.

Our investigation also revealed that the behavioral alterations of the wastewater group mentioned earlier (opercula movement, loss of balance, air gulping, skin pigmentation, and mucus secretion) were dramatically increased, our results are run concurrently with Kaur and Dua [39]. Compared to the control group, effluent water significantly decreased fish weights,this because of the

chemical composition of effluents from wastewater treatment plants (WWTPs), which may have an impact on fish weight [41, 42].

Fishesof MSE-treated effluentshowed increased weights similar to the control group, this finding is consistent with Hussain, *et al* [43]. According to Foild, *et al* [44] and Compaoré, *et al* [45], *M. oleifera* seeds are a good source of proteins, lipids, and crude fibers. Hence, these nutrients may be a good source for the weight gain. Gad, *et al* [46] stated that the HSI is a helpful biomarker to identify potentially hazardous consequences of environmental stressors. Our current research revealed that fish that were kept in EFF group had a greater HSI values that were much higher than those of the control group, but fish that were kept in EFF+MSE had normal HSI readings that were similar to control condition.

Theresults of the current investigation show that fish exposed to sewage had significantly lower leucocyte counts. The toxic effect of water effluent was evidenced by haematopoietic system malfunction of fishes [4], as seen by the drop in white blood cell count.Fish exposed to effluent water also experienced significant reductions in RBCs count, Ht values and Hb concentrations. Hamed and El-Sayed [4] noted that this decrease might be explained by the inhibition of erythropoiesis and haemosynthesis or an increase in the rate of erythrocyte apoptosis in the haematopoietic organ.

In contrast, the haematological values of EFF+MSE group are within normal limits. This may be due to the phenolic compounds in *M. oleifera* seed can bind to the erythrocyte membranes, protecting them. Our results imply that *M. oleifera* has an antihaemolytic effect on catfish.

Fish illnesses, liver, muscle, and gill damage are brought on by pollution, and are frequently diagnosed using liver enzymes (ALT, AST and ALP) [47]. Due to pollution and other physical variables, glucose and cortisol are frequently utilized as stress markers in fish [48 – 50]. Our research indicated that fish grown in effluent water had elevated ALT and AST activity as well as cortisol content; these findings are consistent with Zahran, *et al* [18]. On the other hand, EFF+MSE group showed normal rates in the former metrics, proving that *M. oleifera* has hepatoprotective characteristics. These results are also in line with Hamza [51] and Hamed and El-Sayed [4].

Proteins are thought to be a useful tool for stresses, and aquatic pollution's detrimental effects on fish [52]. Fish that were placed in EFF group had significantly lower amounts of total protein (hypoproteinaemia), albumin, and globulin than the control group. The decrease in serum total protein may be due to the fish's stress reaction as it adapts to its new surroundings [53]. Albumin levels may have dropped because blood viscosity has decreased [54]. Also, since the liver was unable to produce enough globulins for immunological activities, the drop in globulin concentration in the blood of wastewater-exposed fish may be a sign of diminished immunity in the body [55]. Our results are supported by previous finding of Hamed and El-Sayed [4] where, *M. oleifera* triggered hepatoprotective effects in catfish against the decline in liver synthetic function by increasing serum total protein and albumin levels in comparison to EFF group.

In the current study, the pro-inflammatory mediators, NO and TNF- levels in EFF group was increased when compared to control group, these findings are consistent with Zahran, *et al* [18]. NO, which is produced by nitric oxide synthases, is a crucial element of the immune system and possesses antibacterial activity (NOS). The most significant isoform of NOS, inducible NOS (iNOS), is up-regulated in inflammatory circumstances, increasing NO levels and enabling effective defense against invading microorganisms [56]. Contrarily, EFF+MSE group displayed a normal range of iNOS. The generation of pro-inflammatory mediators by RAW macrophages, particularly iNOS, TNF-α and NO was found to be considerably reduced by isothiocyanates derived from *M. oleifera* leaves [57].

Results also revealed that, EFF group showed a marked decrease in the activity of the AChE enzyme, this inhibition of the enzyme could be attributable to the oxidative stress produced by wastewater exposure, which resulted in neurotoxicity. However, those that were left in EFF+MSE group exhibited a normal range of AChE activity, indicating that *M. oleifera* has neuroprotective properties [58].

It has been discovered that exposure to untreated sewage water causes considerable changes in the protein and oxidative stress markers of aquatic organisms in response to contaminants [59, 60].

MDA is used as a marker of membrane oxidation phospholipids by lipid peroxidation [61]. The increase in MDA level may be due to excessive ROS generation, which damages cell membranes owing to antioxidant enzyme deficiency [62, 63]. Fish living in EFF group, in the instant examination, showed rose in MDA content when contrasted to fish living in control group. These rulings are consistent with those of Benassi, *et al* [64] and Arojojoye and Adeosun [65]. In regard to fish placed in EFF+MSE group, MDA level was decreased than that of EFF group, these data are in agreement with Mohammed, *et al* [66].

GSH serves as a major non-protein thiol in living organisms and is essential for the body's antioxidant defense system [67]. In most cells, it protects cell entities from reactive oxygen and nitrogen species and detoxifying electrophilic endogenous and external and transmits environmental signals to the cellular transcription machinery [68]. A considerable increase in the GSH level in the kidney, liver, and gills of African catfish (*C. gariepinus*) taken from the Ogun River. Fish with a pro-oxidant/antioxidant imbalance may experience oxidative stress [69]. Our current results indicated that fish placed in EFF+MSE group GSH level was higher than that of effluent water, these data are in agreement with Mohammed, *et al* [66]. The observed decrease in GSH level of EFF group when compared to control group was in agreement with Benassi, *et al* [64] and Arojojoye and Adeosun [65].

In an aquatic environment, organisms exposed to pollutants that promote the generation of ROS that can exhaust antioxidant enzymes and result in oxidative stress experience higher levels of oxidative damage [65]. Fish of the EFF group had a noticeable increase in CAT activity, which is consistent with Benassi, *et al* [64] and Tabrez and Ahmad [70]. This may be attributed to high free radicals found in effluent water which consume most of antioxidants in animals, decreasing the level of TAC. Fish placed in EFF+MSE showed higher TAC concentrations and lower CAT activity than those in effluent water. These findings would suggest that MSE treatment of effluent water improved the equilibrium between the oxidant and antioxidant states in animals since *M. o* is a major source of antioxidants [71, 72].

The findings of the current study demonstrated that wastewater stimulates the antioxidant system, and *M. oleifera* serves as a dual-purpose inducer by stimulating the phase-I and phase-II system enzymes that provide the balance of xenobiotic metabolism towards detoxification [66]. In this regard, the fish left in EFF+MSE group displayed normal levels of GSH, TAC, MDA, and CAT, these findings concur with those of Hamed, and El-Sayed [4].

Oxidative stress may result in DNA damage, enzymatic inactivation, and peroxidation of cell components when antioxidant defenses are compromised or overcome[65]. Regarding effluent water, it increased the fragmentation of fish DNA, and this finding is consistent with Benassi, *et al*[64]. This work supports the idea that oxidative stress plays a significant role in genotoxicity [13, 73]. DNA of fish kept in EFF+MSE group revealed a normal range, and these findings are consistent with those of Hamed, and El-Sayed [4]. This may be related to the secondary metabolites of MSE, such as phenolic chemicals, which can improve the qualities of water. Collectively, *M. oleifera* not only improved water quality but also, served as efficient detoxification and biostimulant agent for *C. gariepinus*.

5. Conclusion

Based on our obtained results we concluded that administration of MSE improves the properties and characters of wastewater. The use of MSE in fisheries is a promising beneficial utilization of wastewater especially with the rapid progression of water shortage crisis.

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7. Authors' contributions: Asmaa A. Amin, Khalid S. Hashem did the experiment, analysis of data and writing the manuscript. Hanan A. Soliman helped in the experiment and the analysis of data and shared in the methodology of the experiment and writing processes. Khalid S. Hashem and Hanan A. Soliman made the proofreading.

8. Compliance with ethical standards:
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