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Integrated Comparative Impacts of Using Dietary Supplementation Plant Wastes (*Opuntia Ficus-Indica, Moringa Oleifera* and *Telfairia Occidentalis*) on Hemato-Biochemical Blood Status of *Oreochromis Niloticus* Exposed to Mercury Toxicity.

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ABSTRACT

The present study was conducted to investigate the role of using the plant-based wastes as a supplementary diet in improving the hemato-biochemical blood status of *Oreochromis niloticus* and resistance against mercuric chloride toxicity (0.08 mg/l). Fish were distributed (10 fish/40-liter aquarium) into 8 groups. Control group and $MC_{0.08}$ group were fed on a commercial diet; $MC/OFI_{5\%}$ & $MC/OFI_{10\%}$ fed on a commercial diet supplemented with 5% and 10% *Opuntia ficus-indica*; $MC/MO_{5\%}$ & $MC/MO_{10\%}$ fed on a diet supplemented with 5% and 10% *Moringa Oleifera*; and $MC/TO_{5\%}$ & $MC/TO_{10\%}$ fed on diet supplemented with 5% and 10% *Telfairia occidentalis*. The experiment extended for 60 days in triplicates.

The obtained results showed a significant increase (P<0.05) in WBCs, MCH, MCHC, glucose, cholesterol, uric acid, triglyceride, creatinine, and total bilirubin in $MC_{0.08\ group\ compared}$ to the control group. And a significant decrease in RBCs, HGB, MCV, HCT, protein, albumin, and globulin was noticed. Alteration in levels of GOT and GPT was also observed due to the toxic metal exposure. All diet supplementations improved the tested blood parameters (RBCs, HGB, MCV, HCT, protein, albumin, and globulin) compared to $MC_{0.08}$ group. A reduction was noticed in WBCs, MCH, MCHC, glucose, cholesterol, uric acid, triglyceride, total bilirubin, creatinine, GOT, and GPT. The degree of improvement among the three tested supplementations was arranged as *Moringa Oleifera* followed by *Opuntia ficus-indica* and *Telfairia occidentalis*. It was found that $TO_{10\%}$ can be efficient in enhancing fish health and blood characteristics. The dose 10% of MO and OFI were not effective and had no additional benefit. Our findings showed that the use of plant-based wastes as food supplements can improve blood properties and increase the ability of fish to resist the effects of toxins. The use of these materials is particularly promising because they are inexpensive and easily available.

INTRODUCTION

Fish can be exposed to high loads of heavy metals due to increased pollution in water bodies (Amachree *et al*., 2014; Beheary & El-Matary, 2018). Elevated levels of toxic metals have been reported from areas experiencing increasing settlement, traffic and agricultural activities. The levels of non-essential trace elements in fish are important because fish is an important source of food for the general human population; fish from freshwater bodies receiving industrial effluents have been reported to be unfit for human consumption because of high tissue levels of some trace metals (Atobatele & Olutona, 2015; Kortei *et al*., 2020).

Mercury is one of the most dangerous toxic metals, where the fate and behavior of the metal in the environment depended on its chemical form (Looi *et al*., 2016). Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and the metal may be highly toxic and corrosive once absorbed into the blood stream (Moniruzzaman *et al*., 2017). The most toxic form is methyl-mercury (MeHg), including reduced neuronal development and immunodeficiency (Renieri *et al*., 2019). Long-term exposure to both organic and inorganic mercury is deleterious for health including the kidney (Rana *et al*., 2018). It reduces the function of tight junction protein in the kidney and perturbs cellular permeability. According to Kawedia *et al*. (2008), Hg decreases transepithelial electrical resistance (TER) and facilitates the phosphorylation of tight junction protein, occludin via a protein kinase A (PKA) dependent mechanism (Rana *et al*., 2018). The ecotoxicology and environmental fate of mercury has been extensively studied (Boening, 2000; Scheuhammer *et al*., 2007; Riva-Murray *et al*., 2011; and Driscoll *et al*., 2013).

Cultured fish are susceptible to a wide range of toxins and pathogens which may cause a wide range of diseases and subsequent economic losses in farmed fish (Austin & Austin, 2007). The search for natural alternatives to eliminate heavy metal toxicity and to improve the performance of fish is a new trend in research. Several studies have been conducted on the possibility of using a plant–based diet supplement to improve the immunity of fish and increase the degree of resistance to toxins and diseases (Yin *et al*., 2006; Awad & Austin, 2010; Dada & Oviawe 2011; Harikrishnan *et al*., 2011; Park & Choi, 2012; Arulvasu *et al*., 2013; and Awad *et al*., 2013).

Moringa oleifera, a tree belonging to family Moringaceae, has remediation properties and is highly effective in treating malnutrition and several diseases, Therefore, it is called "the miracle tree" (Mbokane & Moyo, 2018). A variety of essential phytochemicals are existed in *Moringa* leaves, pods and seeds such as vitamins A, C, E, carotenoids, phenolics and flavonoids (Moyo *et al*., 2012). It has also been reported that *Moringa* possesses several nutrients including protein, calcium, magnesium, potassium and iron (Foidl *et al*., 2001 and Rockwood *et al*., 2013). The World Health Organization (WHO) is promoting *M. oleifera* as a food supplement against malnutrition due to its valuable nutritional profile. The plant is also used as potential anti-oxidant, anti-cancer, anti-inflammatory, ant-diabetic and anti-microbial agent, which explains the widespread use in animal feeding in recent years (Hlophe & Moyo, 2014; Al-husnan & Alkahtani, 2016 and Gopalakrishnan *et al*., 2016).

Opuntia ficus-indica (Family Cactaceae) is among the most important medicinal plants (Abdel-Hameed *et al*., 2014) because of the existence of antioxidants (Angulo-Bejarano *et al*., 2014), nutrition signification (Jana, 2012), fatty acids (Matthaus & Ozcan, 2011; Khatabi *et al*., 2013 and Cejudo-Bastante *et al*., 2014). The plant can be used in river water and wastewater decontamination through both adsorption and coagulation-flocculation processes (Nharingo & Moyo, 2016).

Fluted pumpkin (*Telfairia occidentalis*, family Curcubitaceae) contains vitamin C, alkaloids and flavonoids, with high antioxidant properties. Many studies have shown that antioxidants present in the aforementioned species can enhance fish performance either directly or indirectly (Dada, 2016).

In most studies using plant residues as a dietary supplement to increase fish resistance to diseases and pollutants, attention has been paid to studying the haematological indices, blood biochemical properties and immunity attributes. The pattern of these parameters is used as an indication to the treatment effectiveness in disease resistance (Mbokane & Moyo, 2018). *Oreochromis niloticus* is one of the most commonly cultured freshwater fish species in Egypt. It has been reported to be susceptible to metal poisoning and diseases in intensive farming systems (Oner *et al*., 2008). In this study, *O. niloticus* has been selected as a test organism for its great aquaculture and commercial value. The present work aims to explore the effects of mercury on hematological and biochemical blood indices of Nile tilapia (*O. niloticus*) and the ameliorating effect of dietary supplementation with *Moringa oleifera*, *Opuntia ficus-indica*, and *Telfairia occidentalis*.

MATERIALS AND METHODS

Fish:

The experiment was held in El-serw fish farm. It was established on a plot of lake Manzala which was reclaimed for agriculture in 1952 for experimental purposes and to compensate partially the deficiency of animal protein in Egypt. Healthy specimens of Nile tilapia (*O. niloticus*) with total body weight of 45-50 g were obtained from El-Serw fish farm. Fish were treated with 0.1% $KMNO_4$ to remove any fungal infection. Group of 10 fishes were kept in glass aquaria loaded with 40 liter of de-chlorinated tap water and gently aerated for two weeks to acclimate. Fish were fed twice a day at a rate of 3% of their biomass. The aquarium was kept at water temperature of 25 ± 1.02 °C, pH of 6.9 ± 0.1 and dissolved oxygen of 7.4 ± 0.34 mg/l.

Supplementary diet preparation:

The plant based wastes of *Moringa oleifera* (MO), *Opuntia ficus-indica* (OFI), *Telfairia occidentalis* (TO) were repeatedly washed with tape water to remove soluble and dirty particles and finally rinsed with deionized water. It was cut into strips of 1 cm width, then dried in the sun for two weeks before drying in the oven at 60 °C for 48 h. Dry matter was then ground into fine particles by using commercial blender, sieved to the fine homogenous particle size. Plant based wastes of 5% and 10% were added to the fish diet. The components were blended thoroughly and formed into pellets using a pellet machine. The pellets were dried in the air at room temperature (27 °C) for 24 h and stored at 4 °C until use.

Experimental design and diets:

The acclimatized fish were randomly assigned to two major groups. The first group consisted of three replicates and received only the traditional fish diet (control) and the second group consisted of 21 replicates and received fish diet plus 0.08 mg/l Hg. The second group was also divided into seven subgroups (three replicates each): either not receiving any dietary supplement or supplemented with the powder of *Moringa oleifera (MO)*, *Opuntia ficus-indica* (OFI) or *Telfairia occidentalis* (TO) at a dose of 5% or 10% of the fish diet **(Go, *et al*., 2007; Dada, 2016;** and **Mbokane *et al*, 2018).** The dose of Hg was calculated as 27% of the LC_{50} for *O. niloticus* which was estimated by **El-Matary *et al*., (2019)** to be 0.3 mg/l. Each replicate was a glass aquarium with 40 liter capacity and contained 10 homogenous fishes. Water of the aquarium was completely renewed every 48 h. throughout the experimental period (60 days), the fish were monitored for clinical signs, post-mortem lesions, and mortalities.

Haematological and biochemical analysis:

Blood samples were collected at the end of the experimental period from the caudal vessels in Eppendorf tubes. Haematological variables included white blood cell count (WBC), hemoglobin concentration (Hb), haematocrit (Hct), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was analyzed by using a Mindary BC-2800 Auto Hematology Blood Analyzer. Serum was obtained from blood samples by centrifugation at 3000 rpm for 15 min. The biochemical variables of the serum included cholesterol, triglyceride, albumin, globulin, creatinine, total bilirubin, protein, uric acid, glucose, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) concentrations and were determined by using a Roche Hetachi Cobas C311.

Statistical analysis:

Data are expressed as mean ± standard error (SEM). Data was subjected to one-way ANOVA using SPSS version 22. Mean separation was performed using Duncan's multiple range test at P<0.05.

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RESULTS

Throughout the feeding period, fish from all experimental groups looked healthy and dose related mortalities were not observed.

The effects of $HgCl_2$ and dietary supplement treatments on the hematological parameters of *O. niloticus* after 60 days of experiment are shown in Table (1). In the treated $MC_{0.08}$ group, a significant increase was observed in the count of WBCs by 70.99% than the control group. This value tended to be reduced in all treatments that used plant-based waste as a dietary supplement by (3.97 - 21.30%).

Table 1. Effect of $HgCl_2$ and dietary supplement treatments on the hematological parameters of *O. niloticus* after 60 days of experiment. (Values expressed as mean of three replications ± standard error)

Variables	Treatment groups							
	Control	$MC_{0.08}$	$MC/MO_{5\%}$	$MC/MO_{10\%}$	$MC/OFI_{5\%}$	$MC/OFI_{10\%}$	$MC/TO_{5\%}$	$MC/TO_{10\%}$
RBC (10^6/ml)	$2.7±0.95^{a}$	$0.7±0.48^{e}$	$1.2±0.63^{cd}$	$1.1±0.61^{d}$	$1.4±0.68^{c}$	$1.3±0.66^{cd}$	$1.9±0.80^{b}$	$1.7±0.75^{b}$
WBC (10^3/ml)	$16.2±0.23^{e}$	$27.7±0.12^{a}$	$22±0.58^{d}$	$24.3±0.29^{c}$	$21.8±0.46^{d}$	$23.9±0.46^{c}$	$25.6±0.17^{b}$	$26.6±0.29^{b}$
Hb (g/dl)	$7.2±0.23^{a}$	$4.8±0.12^{gh}$	$5.6±0.12^{de}$	$5.2±0.12^{fg}$	$5.3±0.12^{ef}$	$5.9±0.17^{cd}$	$6.5±0.17^{b}$	$6.2±0.12^{bc}$
Hct (%)	$39.28±0.21^{a}$	$24.89±0.51^{e}$	$28.54±0.44^{c}$	$26.12±0.27^{d}$	$25.02±0.15^{e}$	$29.76±0.31^{b}$	$25.29±0.18^{de}$	$29.13±0.10^{bc}$
MCV (Fi)	$144.1±5.43^{de}$	$133.7±0.29^{f}$	$159.7±0.17^{b}$	$149±0.40^{cd}$	$171.9±0.23^{a}$	$150.2±0.29^{c}$	$138.6±0.87^{ef}$	$167.5±0.81^{a}$
MCH (Pg)	$41.8±0.29^{b}$	$68.9±0.35^{a}$	$39.6±0.40^{c}$	$38.8±0.35^{c}$	$37.9±0.12^{d}$	$37.2±0.23^{d}$	$34.6±0.23^{f}$	$35.6±0.17^{e}$
MCHC (g/dl)	$29±1.15^{b}$	$52±1.73^{a}$	$25±1.15^{bc}$	$26±1.73^{bc}$	$22±1.15^{c}$	$25±2.31^{bc}$	$29±2.31^{b}$	$21±1.15^{c}$

Different letters indicate significant differences between treatments (P < 0.05)
RBC: red blood cell counts; WBC: white blood cell counts; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration. $MC_{0.08}$ ($HgCl_2$ = 0.08 mg/l); $MC/MO_{5\%}$ (diet supplemented with 5% *Moringa Oleifera)*; $MC/MO_{10\%}$ (diet supplemented with 10%

Moringa Oleifera); MC/OFI$_{5\%}$ (diet supplemented with 5% *Opuntia ficus-indica*); MC/OFI$_{10\%}$ (diet supplemented with 10% *Opuntia ficus-indica)*; MC/TO$_{5\%}$ (diet supplemented with 5% *Telfairia occidentalis*); MC/TO$_{10\%}$ (diet supplemented with 10% *Telfairia occidentalis*).

The count of red blood cells (RBCs) decreased sharply in the treated MC$_{0.08}$ group by 74.07% when compared to the control group. The count values were enhanced greatly in all dietary supplement treatments. Hemoglobin (Hb) and hematocrit (Hct) levels were also reduced in the treated MC$_{0.08}$ group by 33.33% and 36.63%, respectively, than the control. These levels were enhanced and increased in the other treatments by (8.33- 35.41%) for Hemoglobin and (0.52- 19.56%) for hematocrit.

A decrease in MCV values in the treated MC$_{0.08}$ group by 7.21% was observed, as compared to the control group. MCV values increased in all the dietary supplement treatments by (3.66 -28.57%) more than the treated MC$_{0.08}$ group. MCH and MCHC levels showed an increase in the treated MC$_{0.08}$ group by 64.83 % and 79.31%, respectively compared to the control, while the other treatments showed reduction in MCH values by (42.53 - 49.78 %) and MCHC by (44.23- 59.62%).

The result showed that the tested blood protein constituents (total protein, albumin, and globulin) were significantly reduced in treated MC$_{0.08}$ group compared to the control group by 39.3%, 38.57%, and 41.53%, respectively ($P<0.05$). In all treatments that used plant-based waste as a dietary supplement, an increase in protein values with range of (2.93-71.26%), and albumin (4.30- 41.43%), while globulin values reached 2.12 fold more than in $HgCl_2$ group (Figure 1).

Lipid and glucose pictures are illustrated in Figure 2. Triglycerides levels increased in MC$_{0.08}$ by twofold compared to the control, while the TG levels in the other treatments decreased by (15.25-59.45%) when compared to MC$_{0.08}$. However, it remained higher than in the control group, except for the TO$_{5\%}$ group. The levels of cholesterol in MC$_{0.08}$ increased by 47.54% than the control, and these levels tended to decrease significantly in the other groups (6.11- 47.22%) and (15.24-59.45%). The blood concentration of glucose significantly increased in treated MC$_{0.08}$ group by three fold than the control group, the levels significantly decreased in the other dietary supplement treatments by (40.57- 86.23%).

The liver function is illustrated in Figure 3. Total bilirubin levels decreased in all treatments used dietary supplement plant-based wastes in a range of (13.28- 54.69%) than MC$_{0.08}$ group, which increased than the control by 9.40%. Concentrations of Glutamic oxaloacetic transaminase (GOT) and Glutamic pyruvic transaminase (GPT) showed an increase in fish treated with MC$_{0.08}$. Significant increase than the control was noticed in GOT (97.58%) and GPT (19.16%). The values of blood enzymes decreased in the all supplementary plant treatments in a range of (4.08- 51.83% for GOT) and (1.26 - 44.72% for GPT) than the treated MC$_{0.08}$ group.

The kidney function is illustrated in Figure 4. Uric acid increased in the treated MC$_{0.08}$ group by 93.87% than control, and the levels in the other treatments have been greatly reduced in a range of (56.31- 88.94%). Mercuric chloride increased creatinine levels by 20.83% than the control. But the levels began to decrease with range of (6.8- 41.37%) in other treatments, except for MC/TO$_{5\%}$ (0%).

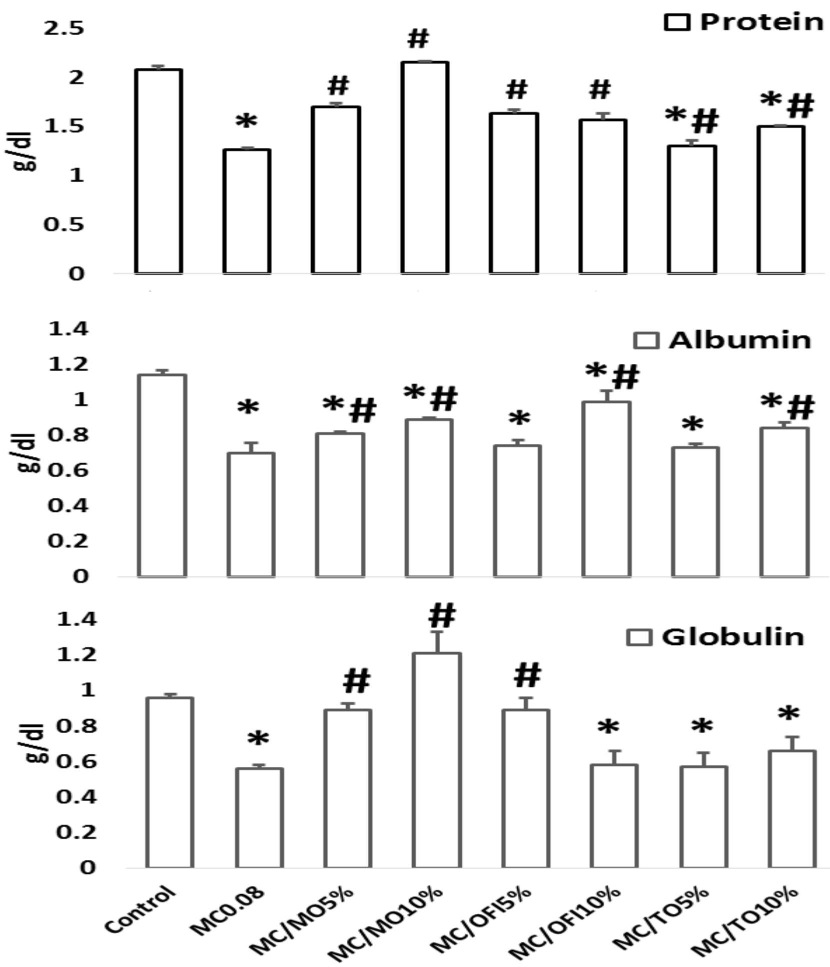

Figure 1. Effect of $HgCl_2$ and dietary supplement treatments on the blood protein picture in *O. niloticus* after 60 days of experiment.

$MC_{0.08}$ ($HgCl_2$ = 0.08 mg/l); $MC/MO_{5\%}$ (diet supplemented with 5% *Moringa Oleifera)*; $MC/MO_{10\%}$ (diet supplemented with 10% *Moringa Oleifera*); $MC/OFI_{5\%}$ (diet supplemented with 5% *Opuntia ficus-indica*); $MC/OFI_{10\%}$ (diet supplemented with 10% *Opuntia ficus-indica)*; $MC/TO_{5\%}$ (diet supplemented with 5% *Telfairia occidentalis*); $MC/TO_{10\%}$ (diet supplemented with 10% *Telfairia occidentalis*). Statistical analysis: * denotes statistically different from the control value, and # denotes statistically different from the $MC_{0.08.}$

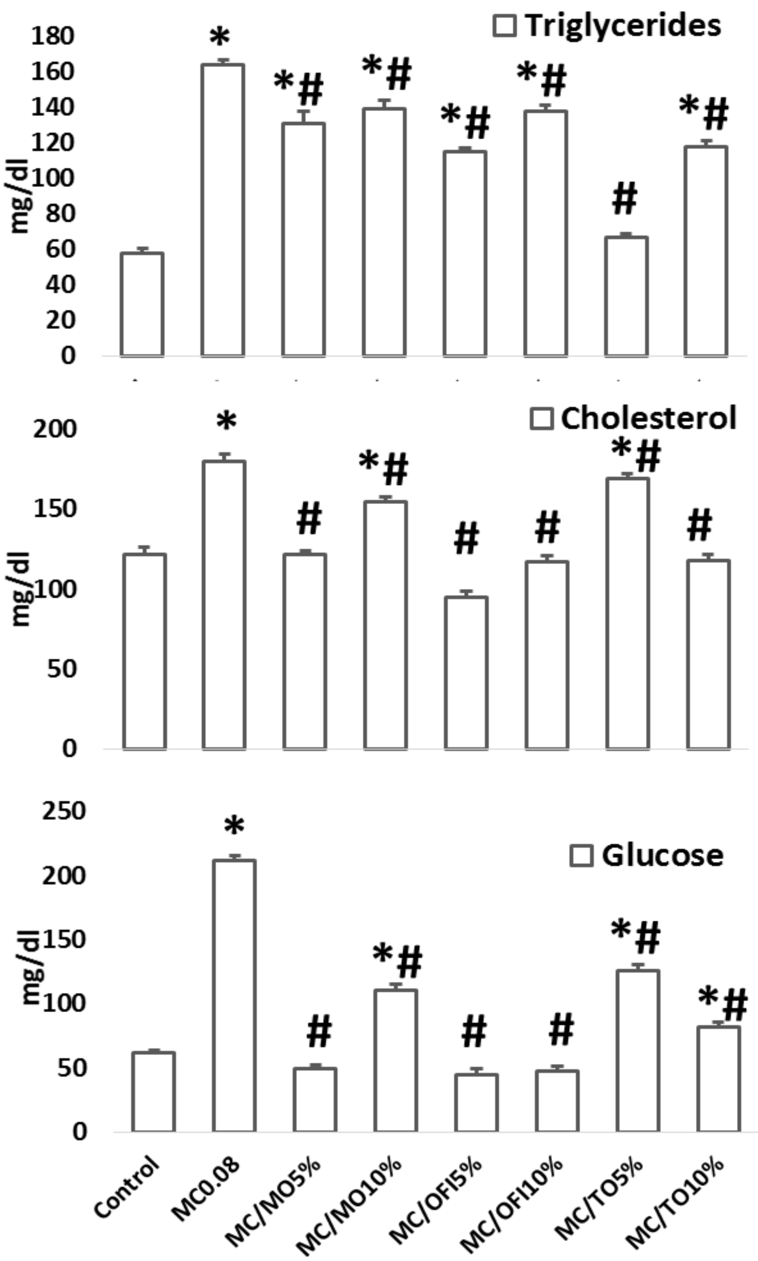

Figure 2. Effect of $HgCl_2$ and dietary supplement treatments on the blood lipid and glucose levels in *O. niloticus* after 60 days of experiment. (Abbreviations as in Figure 1)

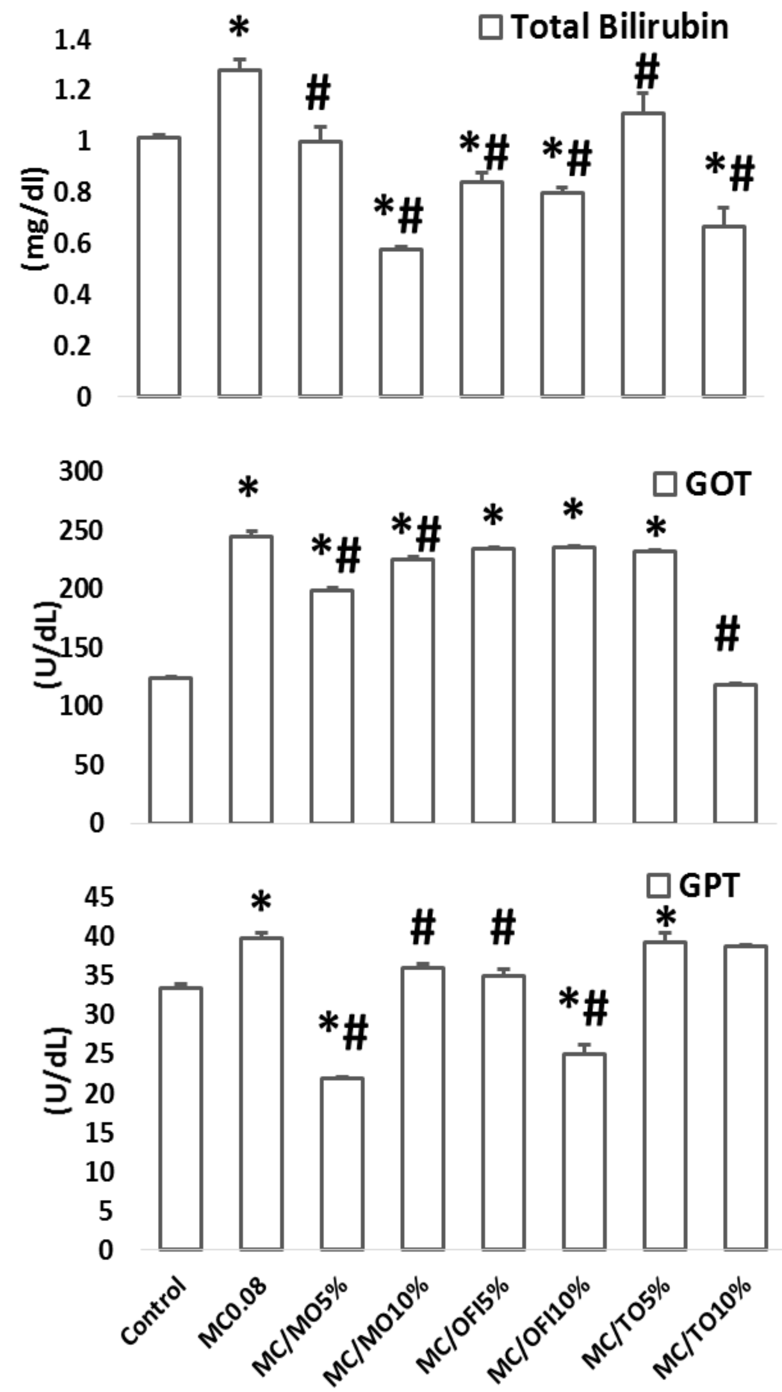

Figure 3. Effect of $HgCl_2$ and dietary supplement treatments on the liver function of *O. niloticus* after 60 days of experiment. (Abbreviations as in Figure 1)

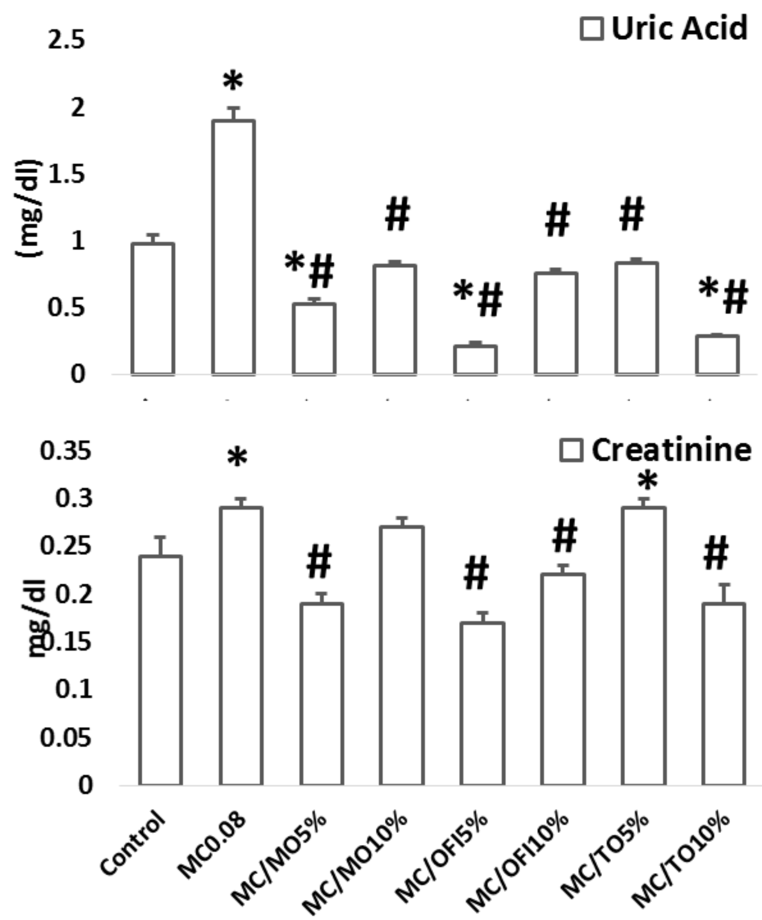

Figure 4. Effect of $HgCl_2$ and dietary supplement treatments on the kidney function of *O. niloticus* after 60 days of experiment. (Abbreviations as in Figure 1.)

DISCUSSION

Changes in the hematological parameters are used to monitor the nutritional, physiological and pathological status in fish (Maita, 2007; Malczyk & Branfireun, 2015; and Mbokane & Moyo, 2018). In the present study, a remarkable decrease was detected in levels of RBCs, HB, HCT and MCV as well as an increase in WBCs count, MCH and MCHC due to exposure to mercuric chloride. The decline in RBC counts in fish under stress situations might result from the severe kidney injuries and inhibition of RBC manufacture (Kavitha *et al*., 2010; Li *et al*., 2011; Saravanan *et al*., 2011; and Hedayati & Ghaffari, 2013). The significant decrease in hemoglobin concentration of fish can be either due to increased rate of destruction of hemoglobin or due to decreased rate of synthesis of hemoglobin. A decline in RBCs count, hemoglobin and hematocrit levels can reveal an anemic situation (Banavathu & Mude, 2017). The expansion of WBCs, MCH and MCHC may be due to generalized immune response and a protective response to stress caused by metals (Sakamoto *et al*., 2015). our findings agree with some reports (Allin & Wilson, 2000; Shah & Altindag 2005;

Chowdhury *et al*., 2004; Witeska, 2004; Heyes *et al*., 2006; Ates *et al*., 2008; and Saravanan *et al*., 2011), while others were in opposed point of view (Carvalho & Fernandes 2006).

In the present study, the values of protein, globulin and albumin decreased in $MC_{0.08}$ compared to the control. Liver is considered the main organ in the synthesis of albumin as the main protein in plasma (Bratkič *et al*., 2018). An increase in protein metabolism under severe stress, a decline in protein synthesis due to liver injuries and less protein reabsorption by injured kidneys could be referred to as a reason for the decline of total protein (Dias *et al*., 2016). Besides, loss of appetite due to toxic stresses could be another reason for the decline of total protein level in serum (Olsvik *et al*., 2013). Several reports indicated a decline of total protein and albumin in blood serum of animals which have been exposed to toxin (Sarica *et al*., 2005; Miklavcˇicˇ *et al*, 2011; Mutlu *et al*., 2015; and Li *et al*., 2018), others are not in accordance with these reports (Yan *et al*, 2010; Malczyk & Branfireun, 2015;and Abdel-Tawwab *et al*., 2015).

Environmental Toxins (heavy metals, pesticides) increased glucose levels in the blood, for this reason, blood glucose can be used as an indicator of environmental stress (Almeida *et al*., 2001) the current findings showed increased levels of blood glucose due to exposure to mercuric chloride, this may be due to liver and kidney injury and lack of nutrition because of the cessation of carbohydrate metabolism which accelerates the process of liver glycogen breakdown to cover the body's energy needs (John, 2007; Hedayati & Ghaffari 2013; Mutlu *et al*., 2015; and Banavathu & Mude, 2017). Our results indicate that the levels of cholesterol increased in fish exposed to mercuric chloride compared to control. Cholesterol increases may due to renal and hepatic failure. Our results are supported by other studies showed an increase in cholesterol concentration in fish exposed to metals (John, 2007; Scheuhammer *et al*., 2015 and Jadán-Piedra *et al*., 2018). Other studies have been of different opinion (Agrahari *et al*., 2007).

The obtained results indicated high levels of triglycerides content in MC treatment compared to control. Increased levels were due to hepatitis and lack of ability to store glycogen (Yang & Chen, 2003). It provides energy to the body, as it used as an important indicator to evaluate the metabolism of lipids and the state of nutrition in the body. Similarly, Yang & Chen (2003) indicated an increase in serum triglyceride levels considerably in *C. carpio* exposed to gallium (Ga) compared to control. Total bilirubin reached the liver by engaged to albumin and globulin in the serum through filtration by the kidney. As, bilirubin is a predominant bile pigment found in the circulation in the Nile tilapia derived from disruption of hemoglobin (Folmar, 1993). Serum bilirubin levels could be changed with liver disorder (Chen *et al*., 2004) and mycotoxin effects (Van Vuren *et al*., 1994). There is a relation between exposure to heavy metals and kidney disease as, uric acid recorded high levels due to fish exposure to mercuric chloride in the current study. This refers to a kidney failure which may be due to the inhibition of uric acid and other nitrogenous compounds secretion processes which cause the precipitation of uric acid in the blood (Mutlu *et al*., 2015). In the present study, the serum total bilirubin, uric acid, and creatinine levels in the exposed groups to mercuric chloride were significantly higher than those in the control group. Several studies agreed with our results and reported high serum creatinine, triglyceride, and uric acid levels after exposure of *O. niloticus* to copper (Chen *et al*., 2004; and Mutlu *et al*., 2015), pesticides (Jain, 1999), and other heavy metals and nitrate (Adham et al., 2002). While, an opposite findings was reported by Abdel-Tawwab *et al*., (2015) who worked on zinc toxicity on biochemical status of common carp, (Cyprinus carpio L.) The levels of Serum glutamic oxaloacetic transaminase (sGOT) and serum glutamic pyruvic transaminase (sGPT) is used to assess fish health (Agrahari *et al*., 2007). Higher amounts of these enzymes in the blood may indicate damage to liver and kidney tissues. The data obtained showed remarkable increases in both enzymes after fish were exposed to mercuric chloride. The activities of these enzymes imply that the liver and kidney were affected (Van der *et al*., 2003; and Palanivelu *et al*., 2005). Several reports recorded an increase in these enzymes after exposure to toxins (John., 2007, Agrahari *et al*., 2007; and Kavya *et al*., 2016).

The use of plant-based wastes as an alternative to chemical treatments of toxins in aquaculture is one of the most prevalent concerns at present (Olusola *et al*., 2013; and Reverter *et al*., 2014). In this regard, the current study used different types of plant residues as a partially dietary supplement (5 and 10% / kg commercial fish diet) to study their health effects on fish exposed to Hg and to show their ability in improving fish blood properties and enzymes efficiency. In the current study, there were important observations: the enhancement in the levels of hematological variables (RBCs, Hb, Ht, MCV) and a significant reduction in (WBCs count, MCH and MCHC) after the dietary replacement of *O. niloticus*. All plant residue treatments enhanced the biochemical blood constituents (protein, globulin and albumin). These findings were in accordance with Dügenci *et al*. (2003) and Alinezhad *et al*. (2017) who reported that the serum total protein levels increased significantly after feeding fish with various herbal extracts. Also, Xu *et al*., (2014) reported that exogenous alanyl-glutamine dipeptide (AGD) supplements can improve serum biochemical index (albumin and globulin) in young mirror carp *C. carpio*. On contrary, Abdel-Tawwab *et al*. (2010 & 2015), Abdel-Tawwab (2015) and Ahmad *et al*. (2011) reported a decrease of total protein after feeding on dietary green tea (*Camellia sinensis L*.), roasted coffee powder, and cinnamon (*Cinnamomum zeylanicum*). The levels of blood glucose, Cholesterol, triglycerides, total bilirubin, uric acid, creatinine, GOT and GPT showed a significant decrease in all supplementary diet treatments compared to $MC_{0.08}$ group. Several reports deals with our results (Agrahari *et al*., 2007; Amachree *et al*., 2014; Xu *et al*., 2014; and Alinezhad *et al*., 2017), although Adel *et al*., (2015) reported no changes even after dietary supplementation with peppermint, and Li *et al*., (2018) found that dietary AGD supplement could not alleviates the damage to the liver caused by ammonia toxicity.

When comparing the efficacy and economic benefit of using two doses of plant-based wastes as partially supplementary diets, it was found that; $MO_{10\%}$ enhanced protein levels by 25.53%, albumin by 10.2%, and globulin by 35.9 % than $MO_{5\%}$. A reduction in MCH (2%) and total bilirubin values (42%) also have been observed. while $MO_{10\%}$ was not effective in changing the rest blood variables. $OFI_{10\%}$ enhanced Hb values by 11.32%, Hct by 18.94%, protein by 3.5%, and albumin by 34.5% when compared with $OFI_{5\%}$. And a reduction in MCH, total bilirubin and GPT values by 1.84%, 4.7% and 28.5 % respectively were obtained. $TO_{10\%}$ enhanced the levels of blood indices more than $TO_{5\%}$ as: Ht (15.18%), MCV (20.85%), protein (15.6%), albumin (15%), and globulin (15.7%). While, it reduced values of MCHC by 27.5%,, uric acid by 65 %, glucose by34.92%, cholesterol by 30.1%, creatinine by 34.48%, total bilirubin by 39.6%, GOT by 49.13%,, and GPT by1.2%, than $TO_{5\%}$. Generally, the dose 10% of MO and OFI were not effective and had no additional benefit. On the contrary, $TO_{10\%}$ can be efficient in enhancing fish health and blood characteristics.

The degree of improvement among the three treatment materials used can be concluded as: protein, globulin, total bilirubin and GPT levels takes the following order: *Moringa Oleifera*, MO > *Opuntia ficus-indica*, OFI > *Telfairia occidentalis*, TO. WBCs, Ht, MCV, albumin, glucose, cholesterol, and creatinine take the following order: *Opuntia ficus-indica,* OFI > *Moringa Oleifera*, MO > *Telfairia occidentalis*, TO. While GOT ordered as: (*Telfairia occidentalis*, TO > *Moringa Oleifera*, MO > *Opuntia ficus-indica,* OFI). RBCs, Hb, MCH and triglycerides were as: *Telfairia occidentalis*, TO > *Opuntia ficus-indica,* OFI > *Moringa Oleifera*, MO. MCHC and uric acid were arranged as: *Opuntia ficus-indica,* OFI > *Telfairia occidentalis*, TO > *Moringa Oleifera*, MO.

MCHC and uric acid were arranged as: OFI > TO > MO. These findings indicate that the best dietary supplementation caused changes and improvements in the hematological profile, and biochemical blood indices in *O. niloticus* was *Moringa Oleifera* followed by *Opuntia ficus-indica* and *Telfairia occidentalis*. The obtained findings are in accordance with Mbokane & Moyo (2018) who indicated that, immunity was enhanced in fish fed with *M. oleifera*. This enhancement is attributed to the presence of biologically active compounds with immune-stimulatory properties. The phyto-chemistry of the *M. oleifera* revealed high levels of total polyphenol, total phenols, total flavonoids, carotenoids, vitamins C and E (Brilhante *et al* 2017). Previous studies have shown that, *Opuntia ficus-indica* contains polyphenols, anti-oxidants, anti-inflammatory agents (Butera *et al*., 2002; Kuti, 2004; and Yang *et al*., 2008) in addition to the medical and animal breeding applications (Ayadi *et al*., 2009). *Telfairia occidentalis* has quite number of antioxidants such as phenols. Therefore, a high possibility that *T. occidentalis* can promote health and increase growth and haematological parameters in animals (Olorunfemi *et al*. 2005; Fasuyi and Nonyerem 2007) and in Nile tilapia fish *O. niloticus* (Dada & Abiodun 2014; and Dada & Fagbohun 2018).

Finally, this study showed that *M. oleifera*, *Opuntia ficus-indica* and *Telfairia occidentalis* possesses therapeutic Properties that can improve the health of *O. niloticus* with different degrees. Carefully selected doses can be beneficial for fish health and alleviate health-related problems. Although there are many different medicinal plant species with medicinal activities, the use of these materials are particularly promising because they are inexpensive and easily available.

CONCLUSION

In this study we concluded that exposure to mercuric chloride could cause a significant destruction to hematological and biochemical characteristics and affect the fish health of *O. niloticus*. An improvement in the hematological profile, and biochemical blood indices in *O. niloticus* were observed plant-based waste was used as diet supplements. *Moringa Oleifera* residues were the most effective one in improving the fish blood profile. It is recommended to use a dose of 5% of *Moringa Oleifera* and *Opuntia ficus-indica* as fish diet supplementation. On the contrary, as the doses of *Telfairia occidentalis* increased, more enhancement in blood characteristics and fish health might be implemented.

REFERENCES

Abdel-Hameed, E.S.S.; Nagaty, M.A.; Salman, M.S. and Bazaid, S.A. (2014). Phytochemicals, nutritionals and antioxidant properties of two prickly pear Cactus cultivars (*Opuntia ficus-indica* Mill.) growing in Taif, KSA. Food Chem. 160: 31-38. http:// dx.doi.org/10.1016/j.foodchem.2014.03.060.

Abdel-Tawwab, M. (2015). The use of American ginseng (*Panax quinquefolium*) in practical diets for Nile tilapia (*Oreochromis niloticus*): resistance to waterborne copper toxicity. Aquac. Res. 46: 1001–1006.

Abdel-Tawwab, M.; Ahmad, M.H.; Seden, M.E. and Sakr, S.F. (2010). Use of green tea, Camellia sinensis L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against Aeromonas hydrophila infection. Journal of the World Aquaculture Society, 41: 203-213.

Abdel-Tawwab, M.; Sharafeldin, K.M.; Mosaad, M.N.M. and Ismaiel, N.E.M. (2015). Coffee bean in common carp, *Cyprinus carpio* L. diets: Effect on growth performance, biochemical status, and resistance to waterborne zinc toxicity. Aquaculture, 448: 207–213. http://dx.doi.org/10.1016/j.aquaculture.2015.06.010

Adel, M.; Safari R.; Pourgholam, R.; Zorriehzahra J. and Esteban, M.A. (2015). Dietary peppermint (*Mentha piperita*) extracts promote growth performance and increase the main humoral immune parameters (both at mucosal and systemic level) of Caspian brown trout (*Salmo trutta caspius Kessler*, 1877). Fish & Shellfish Immunology, 47: 623-629.

Adham, K.G.; Ibrahim, H.M.; Hamed, S.S. and Saleh, R.A. (2002). Blood chemistry of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757). Aquatic Ecology, 36: 549-557.

Agrahari, S.; Pandey, K.C. and Gopal, K. (2007). Biochemical alteration induced by monocrotophos in the blood plasma of Wsh, *Channa punctatus* (Bloch). Pesticide Biochemistry and Physiology 88: 268–272. doi:10.1016/j.pestbp.2007.01.001.

Ahmad, M.A.; El Mesallamy, A.M.D.; Samir, F. and Zahran, F. (2011). Effect of cinnamon (*Cinnamomum zeylanicum*) on growth performance, feed utilization, whole-body composition, and resistance to *Aeromonas hydrophila* in Nile tilapia. J. Appl. Aquac .23: 289–298.

Al-husnan, L.A. and Alkahtani, M.D.F. (2016). Impact of Moringa aqueous extract on pathogenic bacteria and fungi in vitro. Annals of Agricultural Science 61: 247–250. http://dx.doi.org/10.1016/j.aoas.2016.06.003.

Alinezhad, S.; Faridi, M.; Falahatkar, B.; Nabizadeh, R. and Davoodi, D. (2017). Effects of nanostructured zeolite and aflatoxin B1 in growth performance, immune parameters and pathological conditions of rainbow trout *Oncorhynchus mykiss* Fish & Shellfish Immunology, 70: 648-655. https://doi.org/10.1016/j.fsi.2017.08.021.

Allin, C.J. and Wilson, R.W. (2000). Effects of Pre-Acclimation to Aluminium on the Physiology and Swimming Behaviour of Juvenile Rainbow Trout (*Oncorhynchus Mykiss*) during a Pulsed Exposure, *Aquatic Toxicology* 51 (2):213-224.

Almeida, J.A.; Novelli, E.L.B.; Dal-Pai Silva, M. and AlvesJunior, R. (2001). Environmental cadmium exposure and metabolic responses of the Nile tilapia *Oreochromis niloticus*. Environ. Pollut, 114: 169-175.

Amachree, D.; Moody, A.J. and Handy, R.D. (2014). Comparison of intermittent and continuous exposures to inorganic mercury in the mussel, *Mytilus edulis*: Accumulation and sub-lethal physiological effects. Ecotoxicology and Environmental Safety, 109: 133–142. http://dx.doi.org/10.1016/j.ecoenv.2014.07.025.

Angulo-Bejarano, P.I.; Martínez-Cruz, O. and Paredes-Lopez, O. (2014). Phytochemical content, nutraceutical potential and biotechnological applications of an ancient Mexican plant: nopal (*Opuntia ficus-indica*). Curr. Nutr. Food Sci. 10: 196-217.

Arulvasu, C.; Mani, K.; Chandhirasekar, D.; Prabhu, D. and Sivagnanam, S. (2013). Effect of dietary administration of *Zingiber officinale* on growth, survival and immune response of Indian major carp, Catla catla (Ham.), Int. J. Pharm. Pharmaceut. Sci. 5: 108–115.

Ates, B.; Orun, I.; Talas; Z. S.; Durmaz, G. and Yilmaz, I. (2008). Effects of Sodium Selenite on Some Bio-chemical and Hematological Parameters of Rainbow Trout (*Oncorhynchus Mykiss Walbaum*, 1792) Exposed to Pb2+ and Cu2+. Fish Physiology and Biochemistry, 34: 53-59.

Atobatele, O.E. and Olutona, G.O. (2015). Distribution of three non-essential trace metals (Cadmium, Mercury and Lead) in the organs of fish from Aiba Reservoir, Iwo, Nigeria. *Toxicology reports*, *2*: 896-903.

Austin, B. and Austin, D.A. (2007). Bacterial Fish Pathogens: Disease in Farmed and Wild Fish, fourth ed., Springer-Praxis, Godalming.

Awad, E. and Austin, B. (2010). Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis. 33: 413–420.

Awad, E.; Austin, D. and Lyndon A.R. (2013). Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture 388: 193–197.

Ayadi, M.A.; Abdelmaksoud, W.; Ennouri, M. and Attia, H. (2009). Cladodes from *Opuntia ficus-indica* as a source of dietary fiber: effect on dough characteristics and cake making. Ind Crops Prod 30(1): 40–47

Banavathu, S.N. and Mude, J. (2017). Mercuric Chloride ($HgCl_2$) Induced Hematological Changes In Fresh Water Fish *Labeo Rohita* (Hamilton). Int. J. Adv. Res. 5 (6): 1922-1928. http://dx.doi.org/10.21474/IJAR01/4623.

Beheary, M.S. and El-Matary, F.A. (2018). Bioaccumulation of Heavy Metals and Implications Associated with Consumption of the Thinlip Mullet (*Liza ramada*) Collected from Sites of Varying Salinity. Asian Journal of Fisheries and Aquatic Research, 2(2): 1-15; Article no.AJFAR.44449, DOI: 10.9734/AJFAR/2018/44449.

Boening, D.W. (2000). Ecological effects, transport, and fate of mercury: a general review. Chemosphere, 40: 1335–1351.

Bratkič, A.; Tinta, T.; Koron, N.; Guevara, S. R.; Begu, E.; Barkay, T.; Horvat, M.; Falnoga, I. and Faganeli, J. (2018). Mercury transformations in a coastal water column (Gulf of

Trieste, northern Adriatic Sea). Marine Chemistry 200: 57–67. https://doi.org/10.1016/j.marchem.2018.01.001.

Brilhante, R.S.N.; Sales, J.A.; Pereira, V.S.; Castelo-Branco, D.S.C.M.; Cordeiro, R.A.; Sampaio, C.M.; Paiva, M.A.N. and Rocha, M.F.G. (2017). Research advances on the multiple uses of Moringa oleifera: a sustainable alternative for socially neglected population. Asian Pac J Trop Med 10: 621–630. http://dx.doi.org/10.1016/j.apjtm.2017.07.002.

Butera, D.; Tesoriere, L.; di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A.M.; Kohen, R. and Livrea, M.A. (2002). Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extract and reducing properties of its betalains: Betanin and indicaxanthin. J. Agric. Food Chem. 50: 6895–6901.

Carvalho, C. S. and Fernandes, M. N. (2006). Effect of Temperature on Copper Toxicity and Hematological Responses in the Neotrophical Fish, Prochilodus Scrofa at Low and High pH. Aquaculture 251: 109-117.

Cejudo-Bastante, M.J.; Chaalal, M.; Louaileche, H.; Parrado, J. and Heredia, F.J. (2014). Betalain profile, phenolic content, and color characterization of different parts and varieties of Opuntia ficus-indica. Journal of agricultural and food chemistry, 62(33): 8491-8499.. http:// dx.doi.org/10.1021/jf502465g.

Chen, C.Y.; Wooster, G.A. and Bowser, P.R. (2004). Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulphate. Aquaculture, 239: 421-443. doi:10.1016/j.aquaculture.2004.05.033.

Chowdhury, M.J.; Pane, E.F. and Wood, C.M. (2004). Physiological Effects of Dietary Cadmium Acclimation and Waterborne Cadmium Challenge in Rainbow Trout : Respiratory, Ionoregulatory, and Stress Parameters," Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 139: 163-173.

Dada, A.A. (2016). Use of fluted pumpkin (Telfairia occidentalis) leaf powder as feed additive in African catfish (Clarias gariepinus) fingerlings. Journal of Applied Animal Research, 45(1): 566-569. DOI: 10.1080/09712119.2016.1233108

Dada, A.A. and Abiodun, A.D. (2014). Effect of dietary fluted pumpkin (*Telfairia occidentalis*) extract on growth performance, body composition and haematological parameters of Nile tilapia (*Oreochromis niloticus* Linnaeus). Journal of Fisheries, Volume 2 Issue 3 Pages: 203-208. dx.doi.org/10.17017/jfish.v2i3.2014.47.

Dada, A.A. and Fagbohun, A.E. (2018). Effects of Dietary Supplementation of Date palm (*Phoenix dactylifera* L.) Seed on Milt Quality of African Catfish (*Clarias gariepinus*) Broodstocks. Journal of Agricultural Sciences, 13 (2): 153-161. http://dx.doi.org/10.4038/jas.v13i2.8339.

Dada, A.A.; and Oviawe, N.E. (2011). The use of bitter kola *Garcinia kola* dry seed powder as a natural growth-promoting agent for African sharptooth catfish *Clarias gariepinus* fingerlings. Af. J Aquat. Sci., 36(1): 97–100.

Dias, D.; Bessa, J.; Guimara˜es, S.; Soares, M.E.; Bastos M. L. and Teixeira, H.M. (2016). Inorganic mercury intoxication: A case report. Forensic Science International 259: e20–e24. http://dx.doi.org/10.1016/j.forsciint.2015.12.021.

Driscoll, C.T.; Mason, R.P.; Chan, H.M.; Jacob, D.J. and Pirrone, N. (2013). Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol., 47: 4967-4983.

Dügenci, S.K.; Arda, N. and Candan, A. (2003). Some medicinal plants as immunostimulant for fish. J. Ethnopharmacol. 88: 99–106.

El-Matary, F.A.; Beheary, M.S.; Barakat, R.O. and Elgamal, A. E. H. A. (2019). Remediation of toxic effect of Mercuric Chloride on the Nile Tilapia, Oreochromis niloticus using Selenium. Egyptian Journal of Aquatic Biology and Fisheries, 23(2): 109-121.

Fasuyi, A.O. and Nonyerem, A.D. (2007). Biochemical, nutritional and haematological implications of *Telfairia occidentalis* leaf meal as protein supplement in broiler starter diets. African Journal of Biotechnology 6: 1055-1063.

Foidl, N.; Makkar, H.P.S. and Becker K. (2001). The potential of Moringa oleifera for agricultural and industrial uses. *The miracle tree: The multiple attributes of Moringa*, 45-76.

Folmar, L.C. (1993). Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effect. Environ. Toxicol. Chem, 12: 337-375. doi:10.1002/etc.5620120216.

Go, G.M.; Satoh, S. and Oh, S.L. (2007). Effects of the dietary supplementation of fermented cactus fruit (Opuntia ficus-indica) fluid on the growth of red sea bream, Pagrus major. *한국양식학회지*, *20*(1), 1-6.

Gopalakrishnan, L.; Doriyaa, K. and Kumara, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application Lakshmipriya. Food Science and Human Wellness 5: 49–56. http://dx.doi.org/10.1016/j.fshw.2016.04.001.

Harikrishnan, R.; Balasundaram, C. and Heo, M.S. (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish, Aquaculture 317: 1–15.

Hedayati, A. and Ghaffari, Z. (2013). Effect of mercuric chloride on some hematological, biochemical parameters in silver carp (Hypophthalmichthys molitrix). International Journal of Veterinary Medicine: Research & Reports, 1-11. DOI: 10.5171/2013.183410.

Heyes, A.; Mason, R. P.; Kim E.H. and Sunderland, E. (2006). Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. Marine chemistry, 102(1-2): 134-147.

Hlophe, S.N. and Moyo, N.A.G. (2014). A comparative study on the use of *Pennisetum clandestinum* and *Moringa oleifera* as protein sources in the diet of the herbivorous Tilapia rendalli, Aquacult. Int. 22: 1245–1262.

Jadán-Piedra, C.; Vélez, D. and Devesa, V. (2018). In vitro evaluation of dietary compounds to reduce mercury bioavailability In vitro evaluation of dietary compounds to reduce mercury bioavailability. Food Chemistry, 248: 353–359. https://doi.org/10.1016/j.foodchem.2017.12.012

Jain, S.K. (1999). Protective role of zeolite on short- and long- term lead toxicity in the teleost fish *Heteropnenstes fossilis*. Chemosphore, 39: 247-251. doi:10.1016/S0045-6535(99)00106-X.

Jana, S. (2012). Nutraceutical and functional properties of Cactus pear (*Opuntia spp*.) and its utilization for food applications. J. Eng. Res. Stud. 3: 60-66.

John, P. J. (2007). Alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to Metasystox and Sevin. Fish Physiol Biochem, 33: 15-20. DOI 10.1007/s10695-006-9112-7.

Kavitha, C.; Malarvizhi, A.; Kumaran, S. S. and Ramesh, M. (2010). Toxicological Effects of Arsenate Exposure on Hematological, Biochemical and Liver Transaminases Activity in an Indian Major Carp, *Catla Catla*. Food and Chemical Toxicology 48: 2848-2854.

Kavya, K. S.; Jadesh, M. and Kulkarni, R. S. (2016). Hematology and serum biochemical changes in response to change in saline concentration in fresh water fish *Notopterus notopterus*. World Scientific News 32: 49-60.

Kawedia, J. D.; Jiang, M.; Kulkarni, A.; Waechter, H. E.; Matlin, K. S.; Pauletti, G. M. and Menon, A.G. (2008). The protein kinase A pathway contributes to Hg2+-induced alterations in phosphorylation and subcellular distribution of occludin associated with increased tight junction permeability of salivary epithelial cell monolayers. *Journal of Pharmacology and Experimental Therapeutics*, *326*(3): 829-837.

Khatabi, O.; Hanine, H.; Elothmani, D. and Hasib, A. (2013). Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (Opuntia ficus indica). *Arabian Journal of Chemistry*, *9*: S278-S281.

Kortei, N. K.; Heymann, M. E.; Essuman, E. K.; Kpodo, F. M.; Akonor, P. T.; Lokpo, S. Y.; ... and Tettey, C. (2020). Health risk assessment and levels of toxic metals in fishes (*Oreochromis noliticus* and *Clarias anguillaris*) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety. Toxicology Reports, 7: 360-369.

Kuti, J.O. (2004). Antioxidant compounds from four *Opuntia cactus* pear fruit varieties. Food Chem. 85: 527–533.

Li, C.; Zhang, M.; Li, M.; Zhang, Q.; Qian, Y. and Wang, R. (2018). Effect of dietary alanyl-glutamine dipeptide against chronic ammonia stress induced hyperammonemia in the juvenile yellow catfish (*Pelteobagrus fulvidraco*). Comparative Biochemistry and Physiology, Part C 213: 55–61. https://doi.org/10.1016/j.cbpc.2018.08.001.

Li, Z. H.; Velisek, J.; Zlabek, V.; Grabic, R.; Machova, J.; Kolarova, J.; Li, P. and Randak, T. (2011). Chronic Toxicity of Verapamil on Juvenile Rainbow Trout (*Oncorhynchus Mykiss*): Effects on Morphological Indices, Hematological Parameters and Antioxidant Responses. *Journal of Hazardous Materials* 185: 870-880.

Looi, L.J.; Ahmad, Z.A.; Hazzeman, H.; Fatimah, M.Y. and Zailina, H. (2016). The levels of mercury, methylmercury and selenium and the selenium health benefit value in grey-eel catfish (*Plotosus canius*) and giant mudskipper (*Periophthalmodon schlosseri*) from the Strait of Malacca. Chemosphere;152: 265-73.

Maita, M. (2007). Fish Health Assessment. Dietary Supplements for the Health and Quality of Cultured Fish, CAB International, Washington, 2007: 10–34.

Malczyk, E.A. and Branfireun, B. A. (2015). Mercury in sediment, water, and fish in a managed tropical wetland-lake ecosystem. Science of the Total Environment 524: 260-268. http://dx.doi.org/10.1016/j.scitotenv.2015.04.015.

Matthaus, B. and Ozcan, M.M. (2011). Habitat effects on yield, fatty acid composition and tocopherol contents of prickly pear *(Opuntia ficus-indica L.)* seed oils. Sci. Hortic. 131: 95-98. *http://dx.doi.org/10.1016/j.scienta.2011.09.027*.

Mbokane, E.M. and Moyo, N.A.G. (2018). Alterations of haemato-biochemical parameters pre and post-challenge with *Aeromonas hydrophila* and survival of *Oreochromis mossambicus* fed *Moringa oleifera*-based diets. Fish and Shellfish Immunology, 83: 213–222. https://doi.org/10.1016/j.fsi.2018.09.017.

Miklavcˇicˇ, A.; Cuderman, P.; Mazej, D.; Tratnik, J. S.; Krsnik, M.; Planinˇsek, P.; Osredkar, J. and Horvat, M. (2011). Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women. Environmental research, 111(8): 1201-1207.

Moniruzzaman, M.; Lee, J-H.; Lee J-H.; Won, S.; Damusaru J. H. and Bai S. C. (2017). Interactive effect of dietary vitamin E and inorganic mercury on growth performance and bioaccumulation of mercury in juvenile olive flounder, *Paralichthys olivaceus* treated with mercuric chloride. Animal Nutrition 3: 276-283. http://dx.doi.org/10.1016/ j.aninu.2017.07.001.

Moyo, B.; Oyedemi, S.; Masika, P.J. and Muchenje, V. (2012). Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzymatic activity of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. Meat science, 91(4): 441-447.

Mutlu, E.; Aydın, S. and Kutlu, B. (2015). Alterations of Growth Performance and Blood Chemistry in Nile Tilapia (*Oreochromis Nuoticus*) Affected by Copper Sulfate in Long-Term Exposure. Turkish Journal of Fisheries and Aquatic Sciences 15: 487-493. DOI: 10.4194/1303-2712-v15_2_35.

Nharingo, T. and Moyo, M. (2016). Application of *Opuntia ficus-indica* in bioremediation of wastewaters. A critical review. Journal of Environmental Management 166: 55-72. http://dx.doi.org/10.1016/j.jenvman.2015.10.005.

Olorunfemi, A.E.; Arnold, C.I.; Emmanuel, O.; Nkaima, N. and Akeem, A. (2005). Hypoglycaemic activity of *Telfairia occidentalis* in rats. Journal of Pharmaceutical and Bioresources 2: 36-42.

Olsvik, P. A.; Lindgren, M. and Maage. A. (2013). Mercury contamination in deep-water fish: transcriptional responses in tusk (Brosme brosme) from a fjord gradient. Aquatic toxicology, 144: 172-185.

Olusola, S.E.; Emikpe, B.O. and Oleifa, F.E. (2013). The potentials of medicine plants extracts as bio-antimicrobial in aquaculture, Int. J. Med. Aromat. Plants 3: 404-412.

Oner, M., Atli G. and Canli, M. (2008). Changes in serum biochemical parameters of freshwater fish Oreochromis niloticus following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Environmental Toxicology and Chemistry: An International Journal, 27(2): 360-366.

Palanivelu, V.; Vijayavel, K.; Ezhilarasibalasubramanian, S. and Balasubramanian, M.P. (2005). Influence of insecticidal derivative (Cartap Hydrochloride) from the marine polychaete on certain enzyme systems of the freshwater fish *Oreochromis mossambicus*. J. Environ. Biol. 26: 191–196.

Park, K. and Choi, S. (2012). The effect of mistletoe, Viscum album coloratum, extract on innate immune response of Nile tilapia (*Oreochromis niloticus*), Fish Shellfish Immunol. 32: 1016–1021.

Rana, M. N.; Tangpong, J. and Rahman, M. M. (2018). Toxicodynamics of lead, cadmium, mercury and arsenic-induced kidney toxicity and treatment strategy: a mini review. *Toxicology reports*, *5*: 704-713.

Renieri, E. A.; Safenkova, I. V.; Alegakis, A. K.; Slutskaya, E. S.; Kokaraki, V.; Kentouri, M.; ... and Tsatsakis, A. M. (2019). Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: Risk evaluation for consumers. *Food and chemical toxicology*, *124*: 439-449.

Reverter, M.; Bontemps, N.; Lecchinia, D.; Banaigs, B. Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives, Aquaculture 433: 50-61.

Riva-Murray, K.; Chasar, L.C.; Bradley, P.M.; Burns, D.A.; Brigham, M.E.; Smith, M.J. and Abrahamsen, T.A. (2011). Spatial patterns of mercury in macro invertebrates and fishes from streams of two contrasting forested land scapes in the eastern United States. Ecotoxicology 20: 1530–1542.

Rockwood, J.L.; Anderson, B.G. and Casamatta, D.A. (2013). Potential uses of Moringaoleifera and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to under-served indigenous populations, Int. J. Phytothearpy Res. 3: 61–71.

Sakamoto, M.; Itai T.; Yasutake, A.; Iwasaki T.; Yasunaga, G.; Fujise, Y.; Nakamura, M.; Murata, K.; Chan, H.M.; Domingo, J.L. and Marumoto, M. (2015). Mercury speciation and selenium in toothed-whale muscles. Environmental research, 143: 55-61.

Saravanan, M.; Karthika, S.; Malarvizhi, A. and Ramesh, M. (2011). Ecotoxicological Impacts of Clofibric Acid and Diclofenac in Common Carp (*Cyprinus Carpio*) Fingerlings: Hematological, biochemical, Ionoregulatory and Enzymological Responses. Journal of Hazardous Materials 195: 188-194.

Sarica, J.; Amyot, M.; Hare, L.; Blanchfield, P. and Bodaly, R.A. (Drew), Hintelmann, H., & Lucotte, M., (2005). Mercury transfer from fish carcasses to scavengers in boreal lakes: the use of stable isotopes of mercury. Environmental Pollution 134: 13–22. doi:10.1016/j.envpol.2004.07.020.

Scheuhammer, A.; Braune, B.; Chan, H.M.; Frouin, H.; Krey A.; Letcher, R.; Loseto, L.; Noël, M.; Ostertag, S.; Ross, P. and Wayland, M. (2015). Recent progress on our understanding of the biological effects of mercury in fish and wildlife in the Canadian Arctic. Science of the Total Environment, 509: 91-103.

Scheuhammer, A.M.; Meyer, M.W.; Sandheinrich, M.B. and Murray, M.W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. AMBIO: a Journal of the Human Environment, 36(1): 12-19..

Shah, S.L. and Altindag, A. (2004). Hematological parameters of tench (Tinca tinca L.) after acute and chronic exposure to lethal and sublethal mercury treatments. *Bulletin of environmental contamination and toxicology*, *73*(5): 911-918.

Van der, R.O.; Jonny, B. and Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment, a review, Environ. Toxicol. Pharmacol. 13: 57–149.

Van Vuren, H.J.; Van der Merwe, M. and Du Preez, H.H. (1994). The effect of copper on the blood chemistry of Clarias gariepinus (Clariidae). Ecotoxicol. Environ. Safety, 29: 187-199. doi: 10.1016/0147-6513(94)90019-1.

Witeska, M. (2004). The Effect of Toxic Chemicals of Blood Cell Morphology in Fish, *Fresenius*. Environ. Bull. 13 (12): 1379-1384.

Xu, H., Zhu, Q.; Wang, C.-A.; Zhao, Z.-G.; Luo, L.; Wang, L.-S.; Li, J.-N. and Xu, Q. Y. (2014). Effect of dietary alanyl-glutamine supplementation on growth performance, development of intestinal tract, antioxidant status and plasma non-specific immunity of young mirror carp (*Cyprinus carpio L*.). J. Northeast. Agric. Univ. 21: 37–46.

Yan, H.; Rustadbakken, A.; Yao, H.; Larssen, T.; Feng, X.; Liu, T.; Shang, L. and Haugen, T. O. (2010). Total mercury in wild fish in Guizhou reservoirs, China. Journal of Environmental Sciences, 22`(8): 1129–1136. DOI: 10.1016/S1001-0742(09)60228-X.

Yang, J. L. and Chen, H. C. (2003). Effects of gallium on common carp (Cyprinus carpio): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere, 53(8): 877-882.

Yang, N.; Zhao, M.; Zhu, B.; Yang, B.; Chen, C.; Cui, C. and Jiang, Y. (2008). Anti-diabetic effects of polysaccharides from Opuntia monacantha cladode in normal and streptozotocin-induced diabetic rats. Innovative food science & emerging technologies, 9(4): 570-574.

Yin, G.; Jeney, G.; Racz, T.; Xu, P.; Jun, X. and Jeney, Z. (2006). Effect of two Chinese herbs *(Astragalus radix and Scutellaria radix)* on non-specific immune response of tilapia, Oreochromis niloticus. Aquaculture, 253(1-4): 39-47.

الملخص العربى

دراسة مقارنة لتأثير استخدام المخلفات النباتية (التين الشوكي، المورينجا، القرع العسلي) كمكملات غذائية على كيماء الدم في سمك البلطي النيلي المعرض لسمية الزئبق

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أجريت الدراسة الحالية لبحث دور استخدام المخلفات النباتية كنظام غذائي تكميلي في تحسين حالة الدم البيوكيميائية ومقاومة سمية كلوريد الزئبق (0,08 مجم / لتر) لسمك البلطي النيلي. تم توزيع الأسماك (10 أسماك لكل حوض سعة 40 لتر ماء) وذلك في 8 مجموعات. تم تغذية المجموعة الحاكمة (control) ومجموعة كلوريد الزئبق ($MC_{0.08}$) بتركيز (0,08 مجم / لتر) على نظام غذائي بدون أي اضافات. اما مجموعات كلوريد الزئبق التي تم معاملتها بمخلفات النباتات فقد تم تغذيتها على نظام غذائي كالتالي: مجموعات كلوريد الزئبق & $C/OFI_{5\%}$ ($MC/OFI_{10\%}$) تم تغذيتها على نظام غذائي بإضافة مخلفات التين الشوكي بتركيزات 5% و10% لكل كيلو جرام علف، مجموعات كلوريد الزئبق ($MC/MO_{5\%}$ & $MC/MO_{10\%}$) تم تغذيتها على نظام غذائي بإضافة مخلفات المورينجا بتركيزات 5% و10% لكل كيلو جرام علف، مجموعات كلوريد الزئبق ($MC/TO_{5\%}$ & $MC/TO_{10\%}$) تم تغذيتها على نظام غذائي بإضافة مخلفات القرع العسلي بتركيزات 5% و10% لكل كيلو جرام علف. استمرت التجربة لمدة 60 يومًا في ثلاث مكررات لكل مجموعة.

أظهرت النتائج وجود زيادة معنوية ($P < 0.05$) في عدد خلايا الدم البيضاء (WBCs)، صبغة كريات الدم الحمراء (MCH)، تركيز صبغة كريات الدم الحمراء (MCHC)، الجلوكوز، الكوليسترول، حمض اليوريك، الدهون الثلاثية، الكرياتينين، والبيليروبين الكلي في مجموعة كلوريد الزئبق ($MC_{0.08}$) مقارنة بالمجموعة الحاكمة (control). كما لوحظ انخفاض معنوي في عدد كرات الدم الحمراء (RBCs)، هيموجلوبين (HGB) ، حجم كريات الدم الحمراء (MCV)، هيماتوكريت (HCT)، البروتين، الألبومين، والجلوبيولين مقارنة بالمجموعة الحاكمة (control). كما لوحظ ايضا تغير في مستويات انزيمات الكبد (GOT و GPT) بسبب التعرض لسمية الزئبق. أدى استخدام المخلفات النباتية كنظام غذائي تكميلي في تحسين معاملات الدم التي تم اختبارها (عدد كرات الدم الحمراء (RBCs)، هيموغلوبين (HGB)، حجم كريات الدم الحمراء (MCV)، هيماتوكريت (HCT)، البروتين، الألبومين، والجلوبيولين) مقارنةً بمجموعة كلوريد الزئبق ($MC_{0.08}$). كما لوحظ انخفاض في عدد خلايا الدم البيضاء (WBCs)، صبغة كريات الدم الحمراء (MCH)، تركيز صبغة كريات الدم الحمراء (MCHC)، الجلوكوز، الكوليسترول، حمض اليوريك، الدهون الثلاثية، البيليروبين الكلي، الكرياتينين، GOT، GPT مقارنةً بمجموعة كلوريد الزئبق ($MC_{0.08}$). تم ترتيب درجة التحسن بين المكملات الثلاثة المختبرة كالتالي: المورينجا يليها التين الشوكي ثم القرع العسلي. وقد وجد أن مجموعة كلوريد الزئبق ($MC/TO_{10\%}$) والتي تم تغذيتها على نظام غذائي بإضافة مخلفات القرع العسلي بتركيز (10% لكل كيلو جرام علف) كانت اكثر فاعلية في تحسين صحة الأسماك وخصائص الدم. ولم تكن الجرعات المماثلة في المجموعات الاخرى فعالة ولم يكن لها فائدة إضافية. وأظهرت النتائج التي توصلنا إليها أن استخدام المخلفات النباتية كمكملات غذائية تضاف الى اعلاف الاسماك بتركيزات معينة يمكن أن تحسن من خصائص الدم وتزيد من قدرة الأسماك على مقاومة آثار السموم. كما يعد استخدام هذه المواد واعدًا بشكل خاص لأنها رخيصة الثمن ومتاحة وسهلة التطبيق.