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Use of humic acid and Yucca extract as a benefactor on water quality and their impact on some hematological and histological parameters of *Oreochromis niloticus*

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ABSTRACT

Adding natural product such as Humic acid (Humabol) and Yucca schidigera extract to enhance water quality under scarce water exchange regiment; has numerus effects on both fish environment and health. Yucca showed significant reduction on NH_3 and NO_2 levels, while humic acid showed significant reduction on orthophosphate and total phosphorus compared to negative control; in addition to maintaining acceptable growth rate under designed experimental conditions. Furthermore, the hematological study showed that, Yucca and humic had positive effect on Red cell indices values such as, HCT, MCV, MCH, MCHC as well as WBCs total count and the proportion of the NEU, MONO, ESO and LYM of the treated treatments. Also, the histological study of gills, liver, stomach and intestine highlighted the tissue protective effect manifested by Yucca and humic.

INTRODUCTION

Yucca schidigera, a native plant of the southwestern USA and Mexico, has aroused interest in livestock and poultry farms operations due to its ability to absorb ammonium (Headon and Walsh 1993). *Yucca schidigera* has high levels of steroidal saponins and polyphenolics (Cheeke *et al*. 2006). The extract of *Yucca* has a potential to modulate nitrogen metabolism and reduce ammonia excretion (Sarkar 1999; Kelly & Kohler 2003).

Y. schidigera extract could be used in aquaculture in the same way as it has been done in terrestrial animal, in the literature *Y. schidigera* extract has been used as an additive in fish feeds in order to improve nitrogen / protein metabolism and thus reduce ammonium excretion (Francis, (2001), Kelly & Kohler 2003, El Saidy and Gaber 2004b and Gaber, 2006).

There have been numerous studies on the use of *Y. schidigera* in feeds for various fish species, such as, Nile tilapia *O. niloticus* (Gaber 2006) and hybrid tilapia *O. niloticus x O.mossambicus* (Kelly & Kohler 2003) and channel catfish (Kelly & Kohler 2003).

Johnston *et al*. (1982) suggested that surface components of *Y. schidigera* extract could aid in nutrient absorption. *Y. schidigera* extract has been used successfully to control the accumulation of ammonium in animal housing, as well as to reduce the concentration of ammonium in water, faecal odor in animal excrement (Cheeke 2000, Hristov *et al*. 1999, Killeen *et al*. 1998, and Wallace *et al*. 1994) and improve both survival and performance of cultured animals (Sarkar 1999a, Sarkar, (1999b).

(Kelly and Kohler 2003, Santacruz-Reyes and Chien 2010) revealed that *Y. shidigera* can reduce ammonia buildup produced by biogenic sources, hydrogen sulfide and other compounds affecting fish health in both fresh and seawater.

Humic substances are the most ubiquitous carbon substance on the surface of the earth, found in almost every drop of water and in almost all soils. Humic substances are the most widely distributed organic products of biosynthesis on the face of the earth (Tan, 2003).

Humic acid is one of the major components of humic substances which are dark brown and major constituents of soil organic matter. Humic acid contributes to soil chemical and physical quality and acts as precursor of some fossil fuels. They can be found in peat, coal, many upland streams and ocean water. It arises by the microbial degradation of bio-molecules (lipids, proteins, carbohydrates and lignin) dispersed in the environment after the death of living cells (Marinsky *et al*., 1995).

Gressler *et al*., 2016; Shin *et al*., 2016 and Sinha *et al*., 2014, 2013 and Ching *et al*., 2009, highlighted that; aquatic organisms exposure to excessive unionized ammonium (NH_3); led to increase of blood and tissue ammonia concentration, which subsequently manifest numerous health problems to fish such as; high mortality, growth and reproductive performance reduction, hyperactivity, tissue erosion and degeneration, immunosuppression, as well as, neurotoxicity, oxidative stress, oxygen supplement impairment, convulsions and coma.

Hence, the present study aims to evaluate the effect of Humic acid (Humabol) and *Yucca schidigera* extract on improving the quality of fish environment in light of the limited water exchange, preserve the life of the fish and keep them alive with the possibility of obtaining acceptable growth if possible, the effect of ammonia stress on blood parameters such as, hematocrit, erythrocyte count, leukocyte count. In addition to, histological investigation to gills, liver and kidney for detecting stress related alterations.

MATERIALS AND METHODS

This work was conducted in Fish Production Branch, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, to investigate the effect of Humic acid (Humabol) and *Yucca schidigera* extract on water quality, blood parameters and histology of Tilapia (*Oreochromis niloticus*).

An experimental design of three treatments and two replicas was carried out. A total of 180 *Oreochromis niloticus* (L.) of about (51 g ± 0.02) initial average weight were randomly allotted in the six concrete ponds (2 m^3 / each), with thirty fish / pond. All the three treatments were tested using a commercial diet (3 mm 30 % protein floating pellets; from Alleraqua Egypt). (T_1) was a negative control treatment, (T_2) commercial natural liquid extract of *Y. schidigera* of concentration of 1 mg / m^3 (Egyvit) and (T_3) (0.2 % Humic acid / kg feed (Salem and Shehab El- Din (2010) were administrated in water for 93days. During the experimental period, fish were fed the commercial diet at (08:00 and 14:00 h) six days a week at a rate of 2.5 % of tilapia biomass and one day starvation till the end of the experimental period.

The experimental ponds were supplied with fresh dechlorinated water every 15 days and no aeration were applied throughout the experimental period.

At the experiment wrap-up, five fish from each treatment were homogenized and frozen.

Water quality

Water temperature and dissolved oxygen (oxygen meter WPA 20 Scientific Instrument) were measured daily at 7 a. m, according to APHA (1992). Water samples were taken every 2 weeks to determine pH using glass electrode pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA), total ammonia concentration was measured by HACH comparison apparatus using HACK kits (Hach Co., Loveland, Colorado, USA). The percentages of unionized ammonia (NH_3) calculated from multiplying the total ammonia value by the appropriate factor according to the following equation NH_3-N = A / 100 x total ammonia (Boyd 1995), Nitrate was measured by phenoldisulphonic acid method, using spectrophotometer (model Milton Roy 21D), at wavelength of 410 nm, nitrite-nitrogen was measured by diazodyzing method; using spectrophotometer (model Milton Roy 21D) at wave length of 543 nm, total alkalinity (mg / l as $CaCO_3$) was measured by titration against sulfuric acid, after adding methyl orange (M.O) as an indicator, total hardness (mg / l as $CaCO_3$) was measured by titration against Ethylene diamine tetra acetic acid (EDTA) after adding ammonium buffer solution, and erochrom black T, as an indicator, total nitrogen, total phosphorus after the samples have been digested using persulfate digestion method, the concentration of total phosphorus was measured using the spectrophotometer (model, WPA Linton Cambridge UK) at wave length of 880 nm and orthophosphate using spectrophotometer (model Milton Roy 21D) at wave length of 880 nm according to (APHA,1985).

Blood sampling

At the end of the experiment; twelve *O. niloticus* from each treatment were randomly taken and anaesthetized. Blood was collected from each individual trough caudal venous puncture with the use of 1ml syringe washed with K_2EDTA; the sampling tubes contained K_2EDTA as anticoagulant **(**Faggio *et al.*, 2014).

Automatic haematological and biochemical analysis

Hematological parameters were performed using automated blood testing machine auto-haematology analyzer Rayto model RT7200 to determine some haematological parameters Such as: haemoglobin (Hb), Packed cell volume (HCT or PCV), Red blood cell (RBC), White blood cell (WBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin content (MCHC) were investigated.

Histological Investigation

Six fish were taken dissected from each treatment to obtain the gills, liver, stomach and intestine and fixed in Davidson's modified solution Fournie *et al.*, 2000; later on, samples transverse sections were cut and stained with hematoxylin and eosin protocol. The examined tissue samples per treatment were 3 organs x 3 replications x 5 examined fields in each slide for abnormalities detection by a light microscope and were photographed by a fluorescence microscope Leica DM2500 Germany.

Growth parameters

Average daily gain (ADG)

Daily gain was estimated according to the following formula:

$$ADG = (Wt_2 - wt_1)/ t$$

Where: wt_1 = first fish weight in grams.

wt_2 = following fish weight in grams.

t= period in day.

Statistical analysis

The data were analyzed by using the GLM procedure with One-way analysis of variance (SAS, 2009), Differences among means were tested for significance according to Duncan's multiple rang test (Duncan, 1955). The following model was used to analyze the obtained data:

Yij=u+Ti+eij

Where:

Yij =observation.

U = the overall mean.

Ti= the effect of treatment.

eij= random error.

RESULTS AND DISCUSSION

Water quality parameters

The average water temperature obtained in the current experiment (27.35 - 30.85°C) is suitable for all physical, chemical and biological processes in pond water as described by Boyd (1979). (Balarin, 1988), the performance of tilapia was very good when the temperature ranged between 25 and 30°C. The optimum temperature for natural growth, reproduction and tilapia growth is about 25 and 32 ° C, (Chervinski, 1982). In the present study, DO readings were between (0.765 to 1.35 mg/L) from week 2 till the end of the experimental period which considered very low concentrations of oxygen for tilapia fish according to Riche and Garling (2003), the preferred DO for optimum growth of tilapia is higher than 5 mg / L. (Tsadik and Kutty, 1987). On the lower limit, Ross (2002) observed that 3 mg / L DO concentration should be the minimum for optimum growth of tilapia, The pH values in the current study ranged from 6.83 to 7.54, which were in the safe and preferred range for the conservation of live fish and the growth of tilapia according to Ellis (1973), who showed that water with pH values of about 6.5 to 9 at dawn it is most suitable for fish production and acidic and alkaline death points for fish approximately 4 and 11 pH respectively. Yucca extract in treatment 2 significantly reduced NH_3 compared with the control and humic treatment Fig. (4).

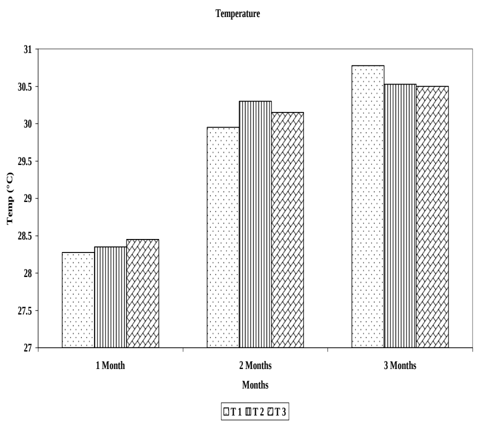

Fig. 1: Changes in water temperature °C in the different treatments

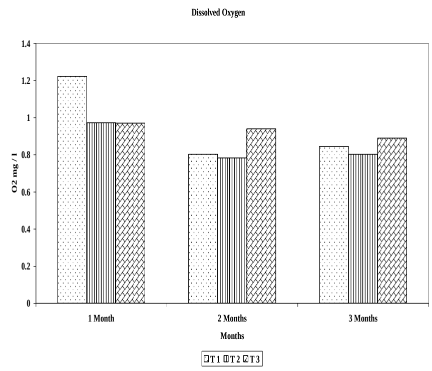

Fig. 2: Effect of different treatments on water D.O levels (mg / l).

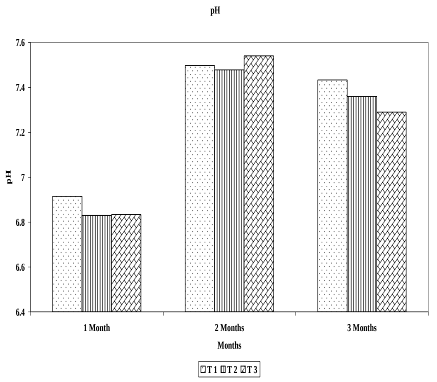

Fig. 3: pH Values of the treatments during the experiment.

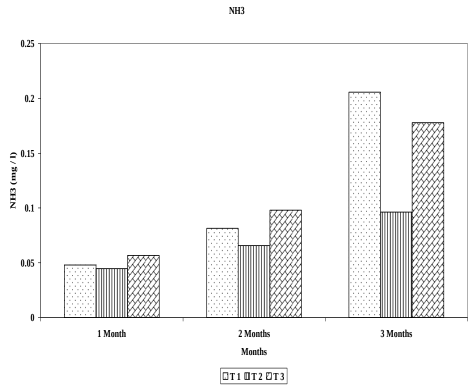

Fig. 4: The concentrations of water NH_3-N

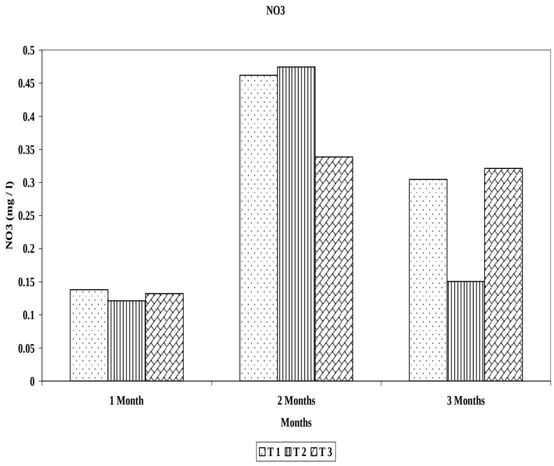

Fig. 5: Changes in water (NO_3-N) values (mg / l) during the experimental period

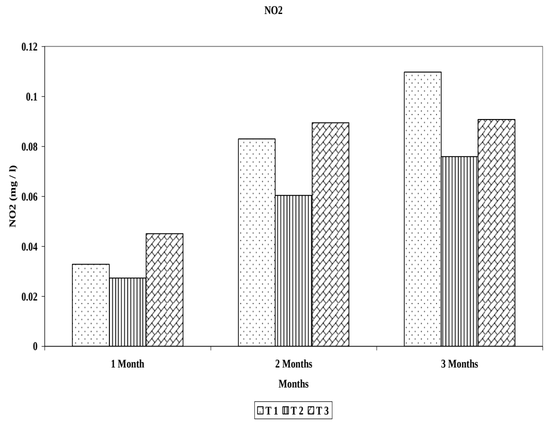

Fig. 6: Changes in (NO_2-N) values (mg / l) during the experiment

Jacques and Bastien (1989) stated that the use of *Y. schidigera* extract contribute in decreasing ammonia accumulation by increasing bacterial use of ammonia and acting as a urease inhibitor. The results in these study are agree with those of Headon and Dawson (1990) and El-Saidy, and Gaber, (2004) who reported that reduction of ammonia could be due to either binding of ammonia with some fraction of *Y. shidigera* or by conversion of ammonia to another compound. The water of treatment 2 showed increase nitrate levels as ammonia levels declined, subsequently, nitrite concentrations decreased. Data of Figs (7 and 8) revealed that the highest value of orthophosphate and total phosphorus were acquired by the control treatment followed by yucca and humic treatments, humic acid has the capability of reducing the negative effect of high dosage of phosphorus (P) in treatments. Humic acid acts as supplier and storehouse P for algae and phytoplankton in aquatic ecosystem (Bakhsh, 2001).

Ayuso *et al*. (1996) reported that the addition of humic acid to fish water positively affects the availability of P and micronutrients by increasing uptake rates of these nutrients by algae. From Figs (9 and 10) it was noticed that humic treatment gave insignificant increase in total hardness and total alkalinity than the other two treatments ($P < 0.05$). These results are in agreement with results who obtained by (Mounes, 2015), who noted that treatment without humic acid gave the lower values in total hardness (102.08 vs 150.15 mg / L) and total alkalinity (137.03 vs 144.43 mg / L) compared with treatment with humic acid.

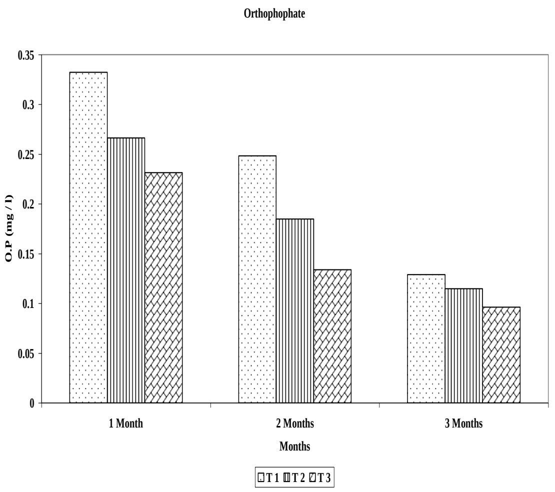

Fig. 7: Change in water orthophosphate concentrations (mg / l)

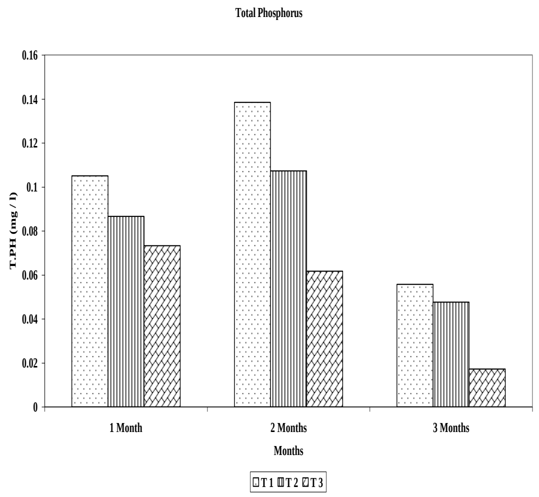

Fig. 8: Mean concentrations of water total phosphorus (mg / l)

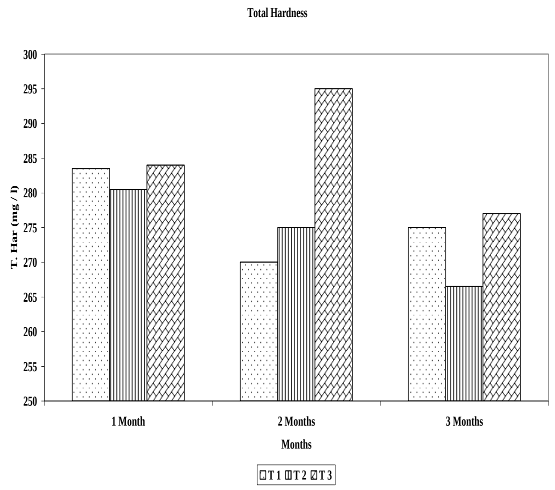

Fig. 9: Changes in water Total Hardness values (mg / l) during the experiment

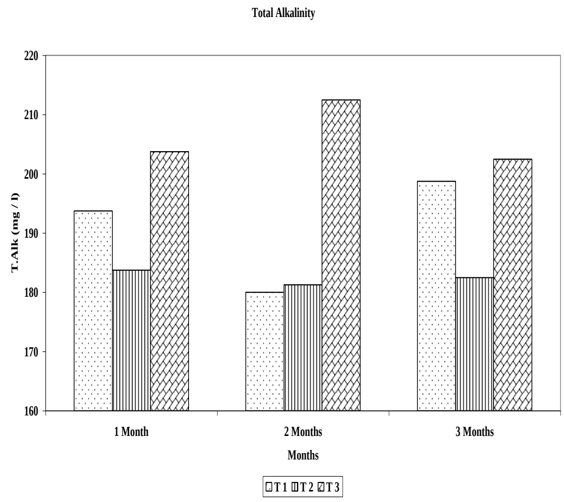

Fig. 10: Total Alkalinity of the treatments during the experiment

Growth Parameters

Growth responses in this experiment was acceptable in all treatments, considering that the main objective of this experiment was to preserve the life of the fish and keep them alive with the possibility of obtaining acceptable growth if possible. The means of fish body weight for the experimental treatments are presented in Table (1). The data clearly indicated that T_1 have the highest final body weight and average daily gain (89.91 ± 0.2 and 0.41 ± 0.1 g / fish) while the lowest one (75.79 ± 0.2 and 0.26 ± 0.1 g / fish) was recorded for T_3. These results are in disagreement with results who obtained by (Kelly & Kohler 2003), who stated that channel catfish fed diets containing the yucca extract improved growth compared with the control diet.

Table 1: Growth performance parameters of *O. niloticus* in different treatments

	Treatment 1	**Treatment 2**	**Treatment 3**
Initial Body Weight (g / fish)	50.86	51.55	51.2
Final Body Weight (g / fish)	$89.81^{a} \pm 0.2$	$83.04^{ab} \pm 0.2$	$75.79^{b} \pm 0.2$
Average Daily Gain (g / fish)	$0.41^{a} \pm 0.1$	$0.33^{b} \pm 0.1$	$0.26^{c} \pm 0.1$

Haematological and biochemical analysis

The obtained haematological data showed that yucca treatment obtained the highest RBCs count followed by humic treatment and control Fig. (11). The treated treatments humic and yucca hemoglobin value compared to control Fig. (12); both RBCs and HB values were statistically not significant, while the parameters related to Red cell indices values such as, HCT, (MCV) define the size and hemoglobin content (MCH, MCHC) of red blood cells were in favor of treated treatments as shown in Figs. (11 to 13). The WBCs total count of the treated treatments had elevated in humic and yucca treatments respectively as shown in Fig. (15). whereas, the proportion of the NEU, MONO, ESO and LYM showed significant difference among treatments except for the latter as illustrated in Figs. (15 & 16).

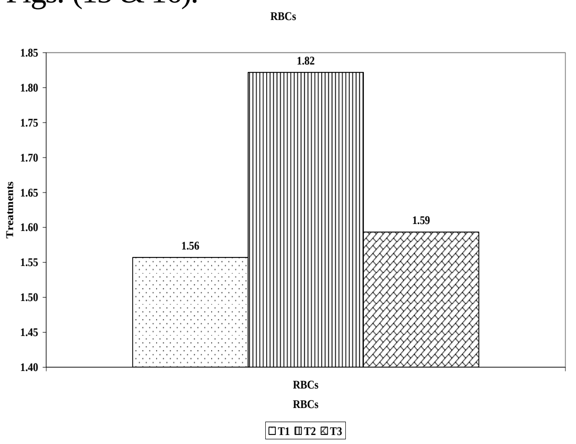

Fig. 11: Illustrates red blood corpuscles RBCs count among different treatments

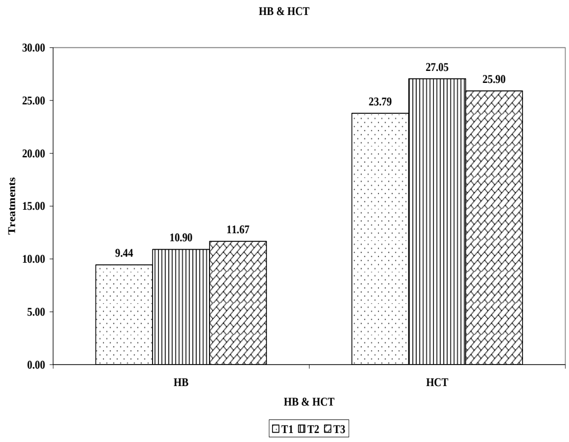

Fig. 12: Showing hemoglobin (Hbg/dl) and HCT values among treated treatments

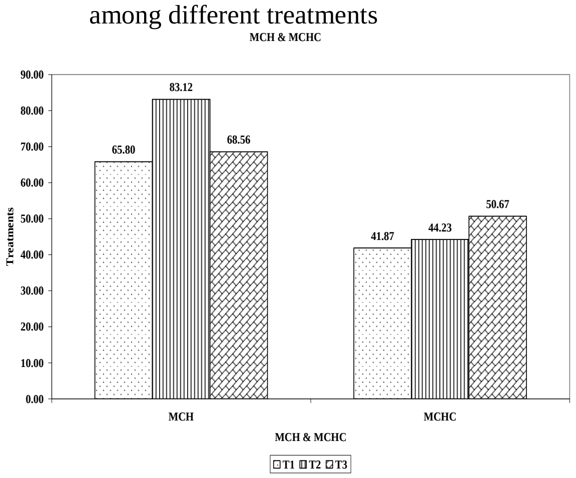

Fig. 13: MCH & MCHC values measured by pictogram (pg) among treated treatments

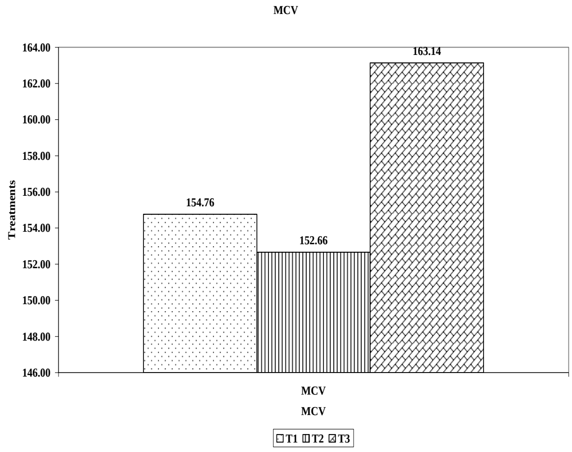

Fig. 14: MCV values measured by among treated treatments

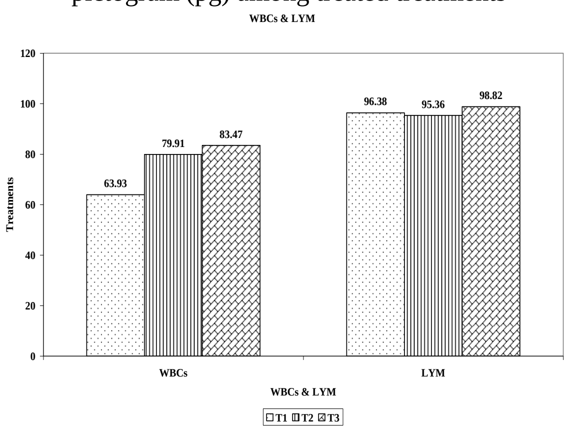

Fig. 15: Illustrates WBCs & LYM for all treatments

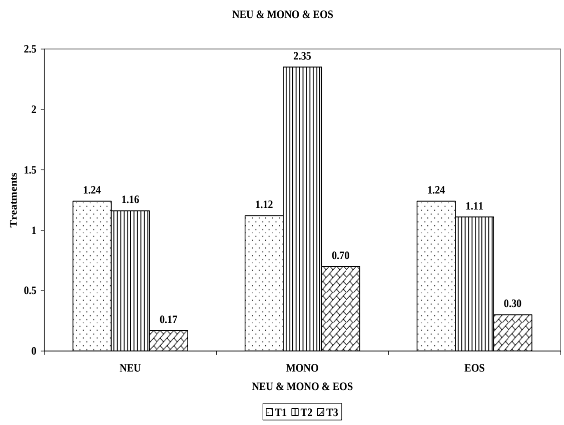

Fig. 16: Showed NEU & MONO and EOS

Ammonia prolonged exposure especially NH_3 could cause multifactorial pathogenesis to multiple organs such as gills, liver, kidney, spleen and thyroid in fish, crustaceans and mollusks El-Sherif and El-Feky, 2008.

This result was in agreement with Shin *et al*., 2016 and Kim *et al*., 2017; who stated that, high ammonia levels affect both Rockfish *Sebastes schlegelii*, sablefish *Anoplopoma fimbria* by induced the following effects; hemato-somatic index and hematological parameters, such as RBCs count, WBCs count, Hb and Ht Furthermore, MCV, MCH.

In addition to, the findings of EL-Sherif and El-Feky, 2008 and Abbas 2006, who stated that there was significant change in Nile tilapia and common carp Hct and HB values with the increase of NH_3 concentration in water.

Histological Investigation

There were signs of disfiguration, hemorrhage and shorter main filaments and secondary lamellae in the vast majority of the control individuals gill tissues compared to treated treatments. Additionally, they manifested other signs such as, reduction in mucus secretion, hyperplasia, telangiectases, degeneration of secondary lamellae and outflow of liquids as illustrated in Fig. (17).

Meanwhile, both treated treatments showed intact gill tissue with longer main and secondary filaments as well as heavier mucus coating for the gills epithelial tissue which was more pronounced in yucca treatment. hence Sinha *et al*., 2014 and Benli *et al*., 2008; stated that mucous may play an important role in excessive mucus production, gill epithelium thickening. Da Costa *et al*., 2015 also stated that, humic influence gill epithelium function by altering membrane permeability, enhance ion uptake.

The histological examination of intestinal tissue of the control treatment showed large intestinal lumen with shorter intestinal villi compared to the treated treatments which showed small intestinal lumen and taller villi especially in yucca treatment and there were no pronounced symptoms Fig. (18). Moreover, macrophages were present in the control intestinal sections which indicated immune response to inflammation, slight degeneration, vacuolization and fibrosis were present.

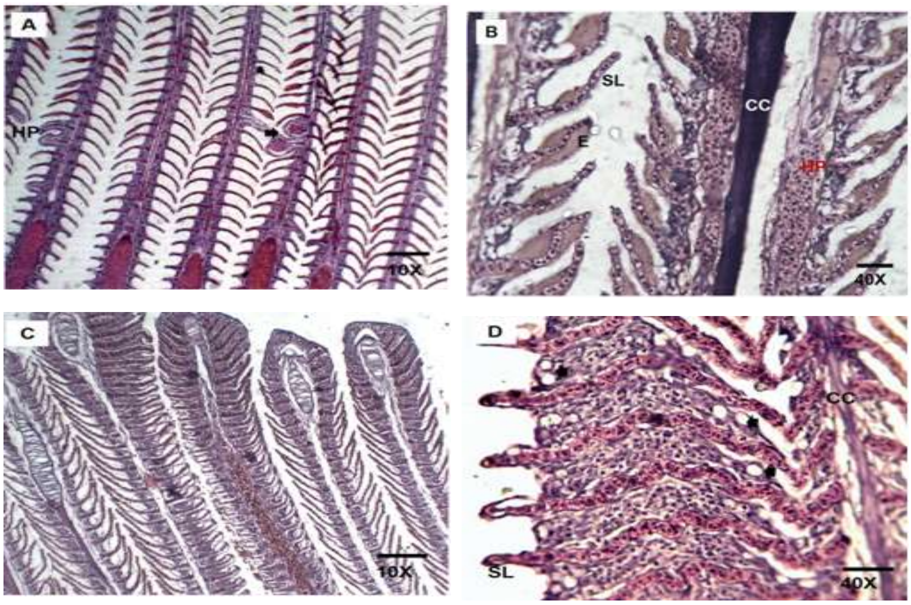

Fig. 17: Cross section in tilapia Gills showing the difference in size between control (a, b) and treated treatments (c, d). The magnified field in (B) showing chondrocytes (CC), Effusion (E), Hyperplasia (HP), secondary lamella (SL), telangiectases black arrow; while (D) shows the thick mucus cover on secondary lamella and mucus cell proliferation (MC)

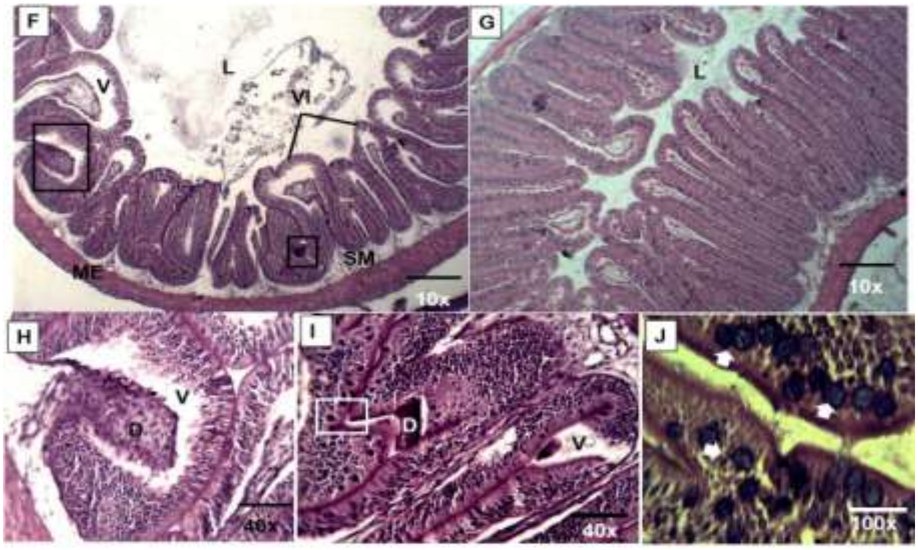

Fig. 18: Cross section in tilapia intestinal canal showing the difference in (L) lumen and (Vi) villi size between control (a) and (b) treated treatments which shows no changes in intestine folds. The magnified fields in C, D and E showing (d) degeneration and (v) vacuolization of intestinal villi and the arrows refers to microphages presences control specimens.

During samples taking the control treatment showed friable liver tissue. Histological examination of hepatic tissue section showed degenerated and vacuolated hepatic cells in control, while the treated treatments showed intact hepatic membrane structure and centric nuclei, however, there were rare occurrences of degeneration and vacuolization in humic treatment compared to the control in the same field of vision Fig. (19).

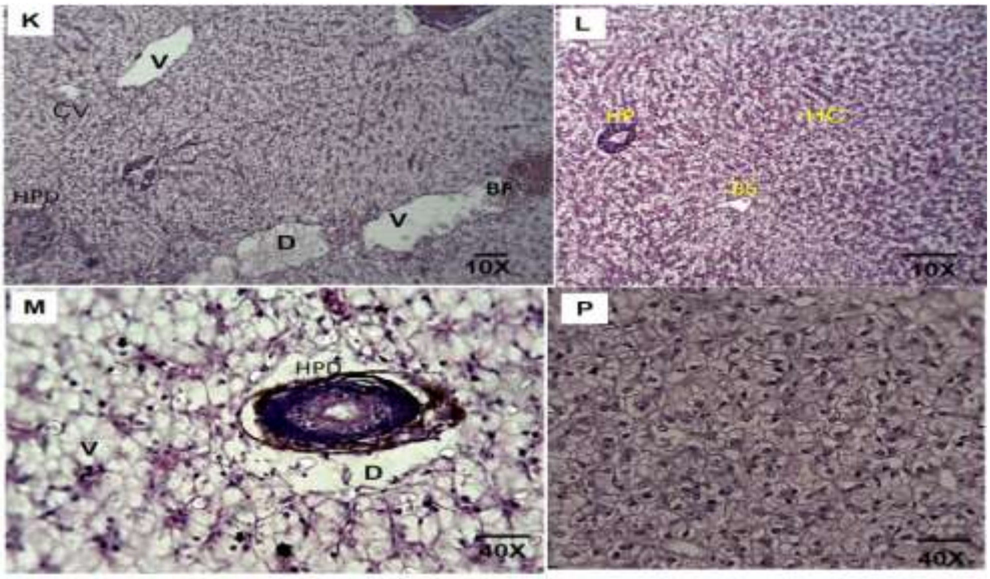

Fig. 19: Cross section in tilapia liver showing the difference in hepatic structure between control (f) and treated treatment s (G). The magnified fields in H highlighting the degeneration in Hepatopancreas (HP) and arrows shows the displacement of nucleus. section (I) shows hepatic cells with normal structures. Central vein (CV), Hepatic cells (HC), Hepatopancreas (HP), degenerative Hepatopancreas (HPD), Blood sinus (BS), blood infiltration (BF).

Kidney tissue in the control showed signs of degeneration of kidney glomerulus, dissociation tissue and liquid outflow while treated treatments with humic or yucca extract showed no significant abnormalities (Fig. 20).

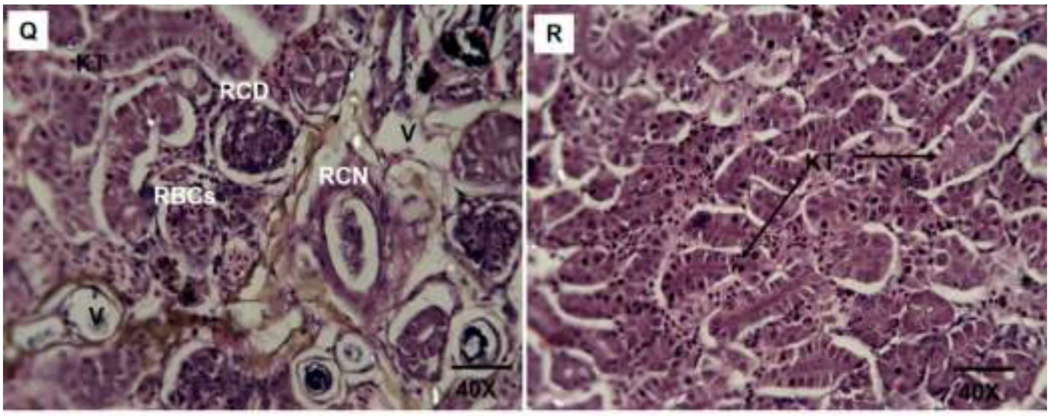

Fig. 20: Cross section in tilapia kidney showing striking differences between J control and K treated treatments; The control showing signs of Renal corpuscle degeneration (RCD), Renal corpuscle necrosis (RCN) dissociation of kidney structures,while, treated treatments show slight dissociation of kidney structures with good shape kidney tubules (KT).

Intestine of control treatment showed the presence of macrophages, which indicated immune response to inflammation, which came in agreement with the findings of Rodrigues *et al*. (2010); who recorded the same action as response to inflammation in the intestinal mucosa. Our histological findings in control liver and kidney were supported by the findings of Benli *et al*. (2008); who stated that, ammonia causes liver glycogen to vacuolate due to energy malfunction and degenerative vacuolization and focal necrosis were present. While, Abbas (2006) found that, there was significant increase of liver and kidney tissue necrosis or dysfunction in common carp that agrees with or findings in control treatment histological sections.

CONCLUSION

It was noted from the results obtained that the yucca extract had the greatest effect on the elimination of harmful nitrogen compounds, which had the best effect on improving water quality and thus the environment surrounding fish, while the humic acid had the greatest effect on the availability of phosphorus compounds to benefit. Both yucca and humic acid reduced the effect of ammonia stress, so it is recommended to use both of them to improve water quality in the coming water poverty in the near future.

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ARABIC SUMMARY

استخدام حامض الهيوميك ومستخلص اليوكا كمحسن لجودة المياه وتأثيرهما على بعض القياسات الدموية والنسيجية لأسماك البلطي النيلي

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إضافة منتجات طبيعية مثل حمض الهيوميك (هيومابول) ومستخلص اليوكا شيديجرا لتحسين جودة المياه تحت ظروف تغيير المياه الشحيحه؛ له آثار عديدة على كل من بيئة الأسماك وصحتها .أظهر مستخلص اليوكا انخفاضًا ملحوظًا في مستويات الأمونيا و النيتريت، في حين أظهر حمض الهيوميك انخفاضًا ملحوظًا في الفوسفور الذائب والفوسفور الكلي مقارنة بالمجموعة الضابطة؛ بالإضافة إلى الحفاظ على نمو مقبول في ظل الظروف التجريبية. علاوة على ذلك، أوضحت دراسة الدم أن اليوكا و حمض الهيوميك كان لهما تأثير إيجابي على قيم مؤشرات خلايا الدم الحمراء مثل HCT و MCV و MCHو MCHC بالإضافة إلى إجمالي عدد خلايا الدم البيضاء ونسب أنوع كرات الدم البيضاء NEU و MONO و ESOو LYM في المجموعات التجريبية. كذلك سلطت الدراسة النسيجية الضوء على وضوح التأثير الوقائي لكلا من اليوكا وحمض الهيوميك على أنسجة الخياشيم والكبد والمعدة والأمعاء.