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EFFECT OF ADDING *PENICILLIUM ROQUEFORTI* SPORES AND *ALPHA TOCOPHEROL* ON LEAD TOXICITY IN CULTURED NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

BARAKAT, M. [1]; EL–GOHARY, M.S. [2] and AMANY, M. DIAB [3]
[1] Biochemistry Unit, Animal Health Research Institute- Kafr El-Sheikh, Branch.
[2] Fish Diseases Unit, Animal Health Research Institute- Kafr El-Sheikh, Branch
[3] Aquatic Microbiology Faculty of Aquatic and Fisheries Sciences – Kafr El-Sheikh University.

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ABSTRACT

This study was designed to investigate, evaluate and compare the detoxifying efficiency of *Penicillium roqueforti* spores and *Alpha-Tocopherol* dietary supplementation in case of sublethal exposure of Nile tilapia (*Oreochromis niloticus*) to Lead. A total of 100 fish *O. niloticus* were divided into five equal groups. Group one, untreated control free aquarium water, was fed on basal diet lead acetate (ninety mg/L) was added to the aquarium water to the other four groups. Group (2), lead acetate control positive fed on basal diet, group (3) fed on diet supplemented with (9×10^2 CFU/kg feed) of *Penicillium roqueforti*, group (4) fed on diet supplemented with *Alpha-Tocopherol* (300mg/kg feed), group (5) fed on diet supplemented with *Penicillium roqueforti* spores in addition to *Alpha-Tocopherol* as (9×10^2 CFU/kg feed and 300mg/kg feed) respectively. Nile tilapia fed at 3% body weight per day for 10 weeks. Results of Pb intoxicated control positive group showed no characteristic clinical signs with presence of some postmortem and histopathological changes. Moreover serum analysis showed significant decreasing in growth hormone (GH), Calcium (Ca), phosphorus, Serum bactericidal activity and Serum lysozyme activity with increasing the mortality rates after challenging with *Aeromonas hydrophila*. Lead residues in blood, musculatures, gills, kidney and liver indicated that lowest Lead residue was recorded in musculatures but the highest residues were recorded in gills in all groups. Supplementation of *Penicillium roqueforti* spores and *Alpha-Tocopherol* improves the adverse effect of Lead in 3rd, 4th and 5th groups. Best detoxification results were in 5th group. It could be concluded that inclusions of 9×10^2 CFU /kg feed *Penicillium roqueforti* spores in addition to 300mg/kg feed *Alpha-Tocopherol* in *O.niloticus* diets could reduce Lead adverse effects to the favor of fish health, immunity and minimizing the Pb residues specially in fish musculatures.

Keywords: *Oreochromis niloticus*, Lead acetate, *Penicillium roqueforti*, *Alpha-Tocopherol*.

INTRODUCTION

The heavy metals effects on environment and human health are of great interest, especially aquatic products (Uluozlu *et al*., 2007). Some of these metals, as Cd and Pb, are toxic even at low concentrations to living organisms (Stephen *et al.*, 2000). The effects of Lead on the environment is usually addressed and highlighted in respection to its effects on human, including loss of coordination, mental retardation and learning dysfunction (Olaifa *et al*., 2003).

Anthropogenic and natural sources release heavy metals into aquatic ecosystem continuously. In freshwaters heavy metals cause serious problem because their bioaccumulation, long persistence, biomagnifications in the food chain and their toxicity to the living organisms. Fish, are considered as heavy metal pollution indicators. Lead is heavy metal which has been used in many ways including smelting, mining, refining, battery manufacturing, gasoline, electrical wiring, painting, making of stained glass and ceramic glazing. Lead has non-degradable nature so it gets into the environment moreover eventually enters the man and animal's blood stream. Lead is accumulated in soft tissues as brain, nervous system, liver and kidneys. In fishes, Lead accumulated in various tissues (Linde *et al*., 2004) causing alterations in hematological and biochemical parameters (Ates *et al.*, 2008) and changing physiological, behavioral parameters and genetic. Fish is the most susceptible aquatic inhabitants to heavy metals pollution or contamination (Sidra and Sumera, 2012).

Heavy metals are chemical stressors and disease development will reflect the interactions between stressors, the host and the disease causing the situation. Moreover, Suppression of immune system and immune response may result from several pollutants including heavy metals which provide

Corresponding author: Dr. BARAKAT, M.
E-mail address: mmbarakat2003@gmail.com
Present address: Biochemistry Unit, Animal Health Research Institute- Kafr El-Sheikh, Branch

opportunities for many pathogens entering, but up-till now the heavy metals effects on the immune response and immune system are not fully understood (Storelli *et al*., 2002).

Recently microbial systems as fungi, algae and bacteria have been explored for their role in heavy metals removal from polluted environments. One form of this bioremediation is Mycoremediation where fungi are used to degrade or sequester environmental contaminants (Dugal and Gangawane, 2012). Fungi biomasses are known to be heavy metals tolerantes (Gavrilesca, 2004). Fungi offer the advantage of having cell wall material that shows excellent metal-binding properties (Gupta *et al*., 2000). *Penicillium* and *Aspergillus* isolates were the most heavy metals tolerants and exhibited strong growth, often exceeding the isolates grown in agar medium without heavy metals that after studying of thirty-six micro-organisms, of fungi and yeasts strains that were isolated from sites in Tangier, Morocco contaminated with heavy metal for screening the resistance of these fungi to heavy metals (Cr, Pb, Zn and Cu) (Ezzouhri *et al*., 2009).

Alpha-Tocopherol has an important role in antioxidant activity and immune response enhancement; it gives the protection to the body cells from the adverse effects of free radicals that produced during several stressors and normal cell activity (John *et al.*, 2001). *Alpha-Tocopherol* maintains the cell membrane structure integrity of the important immune cells causing enhancement of fish immunity as well as antioxidants activity (Montero *et al*., 2001; Puangkaew *et al*., 2004). El-Shebly (2009) reported that *Oreochromis niloticus* had protected against Pb-induced oxidative stress by vitamin E.

The present work was conducted to investigate the adverse effects of single sub-lethal dose of Lead on *Oreochromis niloticus* and evaluate the best protective effect of *Penicillium roqueforti* spores and/or *Alpha-Tocopherol* dietary supplements in reduction Lead drastic effects.

MATERIALS AND METHODS

1. Fish and experimental design: A total number of 100 apparently healthy *O. niloticus* with average body weight of 50±10 g/fish were obtained from a private fish farm at Kafrelsheikh governorate transported alive to the laboratory of Animal health research institute at Kafrelsheikh and kept in glass aquaria. Fish were acclimated for one week; the health status was examined throughout the acclimation period. During the acclimation fish fed on the pelleted basic diet only contained 30% protein twice daily. The aquaria were supplied with chlorine free tap water according to Innes, (1966). The aquaria were aerated continuously by electric pump and held at 25±2^{o}C and half of the water was changed daily with respect to constant Lead acetate concentration as 90 mg/l in Lead treated groups. Fish were randomly divided equally to five experimental groups. *Penicillium roqueforti* spores and *Alph-Tocopherol* dietary supplements were used and mixed thoroughly with the prepared basal fish diet during its preparation.

Table 1: Outline of the experimental design.

Group	No	Lead acetate*	Diet
1	20	0.0 mg/l	Basal diet **
2	20	90 mg/l	Basal diet
3	20	90 mg/l	Basal diet + (9 x10^2 CFU /kg feed) of *Penicillium roqueforti****
4	20	90 mg/l	Basal diet + (300 mg/kg feed) of *Alpha-Tocopherol* *****
5	20	90 mg/l	Basal diet + (9 x10^2 CFU /kg feed) of *Penicillium roqueforti* + (300 mg/kg feed) *of Alpha-Tocopherol*

* Lead acetate: Delta Company, Egypt. **Basal diet (Tocopherol free)
***Penicillium roqueforti*: Total Viable Count 9×10^2 CFU. Kindly Provided by *Prof. Dr. Riad Khalil,* Department of Fish and Avian, Faculity of Vet., Med., Alex. Univ.
***** *Alpha-Tocopherol*: BASF Company, Egypt, Batch no. 131076.

2. Clinical sings and postmortem lesions of fish: During the duration of experiment clinical signs and post mortem examination of ***Oreochromis niloticus*** were performed according to *Austin and Austin (1987)* and *Plumb and Bowser (1982).*

3. Blood collection: At end of the 4th and the 8th week of the experiment, 2ml blood sample from each fish from the caudal vessels were collected from 3 fish from each group according to (Hawak *et al*., 1965). One ml of blood was collected with syringe containing anticoagulant (Heparin) and used for detection of Lead in blood by atomic absorption spectrophotometric method according to (Jonsson *et al*., 2012) while other blood samples used for serum collection (Lied *et al*., 1975). Determination of

growth hormone was done according to (Kates and Albericio, 2000) and (Nakane and Kawaoi, 1974). Determination of phosphorus was determined by the colorimetric method according to (APHA, 1976) and calcium was determined by atomic absorption spectrophotometer according to (AOAC, 1984). Serum bactericidal activity was detected according to (Rainger and Rowley, 1993). Serum lysozyme activity was measured with the turbidimetric method described by Engstad *et al.* (1992). The result was expressed as one unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min.

4. Detection of Lead residues in Muscular tissues, gills, kidney and liver: At the 4^{th} and 8^{th} weeks of the experiment dorsolateral musculature, gills, kidney and liver were rapidly removed from the fish and were stored in plastic container at -20°C for Lead residues analysis. Samples prepared as a wet digestion procedure (Mason, 1991), then Atomic absorption spectrophotometric method was used for the determination of Lead as described in Perkin Elmer catalogue of atomic absorption model 2380, U.S.A (1982).

5. Challenge test: At the 9^{th} week ten fish from each group were bacteriologically examined and determined to be free from bacterial infection, then they were I/p injected with 0.2ml/fish of culture suspension of pathogenic *Aeromonas hydrophila* previously adjusted to 10^5. Specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of observation (one week) according to Soliman (1988).

6. Histopathological examination: At the end of the experiment specimens from different parts of liver, kidneys and gills were immediately fixed in 10% neutral buffered formalin, dehydrated cleared and then stained according to Bancroft *et al.* (2013).

7. Statistical analysis: The data were statistically analyzed by one way Anova test by S.P.S.S, (1997) program.

RESULTS

Clinically the experimentally Lead intoxicated *Oreochromis niloticus* (2^{nd} group) showed, no characteristic clinical signs externally comparing to the control negative (Lead free) group. Postmortem examination revealed the presence of edematous fluid, yellowish enlarged liver, enlarged gall bladder, congested spleen, kidneys and gills comparing to control negative group (Photo:1 A, B). Regarding to 3^{rd}, 4^{th}, 5^{th} groups externally were apparently healthy but Postmortem examination revealed to relatively enlarge pale liver, enlarged gall bladder and spleen comparing to the control negative group.

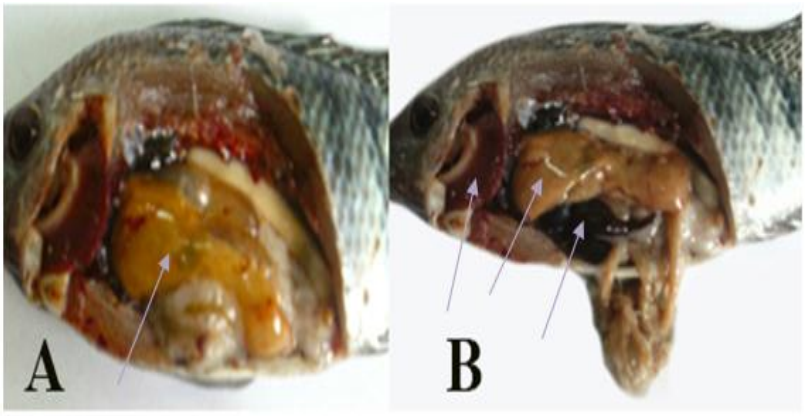


Photo (1): A- Experimentally Lead intoxicated *O. niloticus* shows, presence of edematous fluid and yellowish enlarged liver.

B- Experimentally Lead intoxicated *O. niloticus* shows, yellowish enlarged liver and enlarged congested spleen, kidney and gills.

The analysis of growth hormone, calcium and phosphorus levels in 2^{nd} group (control positive) showed significant reduction comparing to other groups moreover this reduction increased by increasing the period of exposure to Lead (8^{th} weeks). Highest levels of calcium and phosphorus levels were in control negative (untreated) group followed by 5^{th} group followed by 3^{th} group then 4^{rd} group respectively but best results of growth hormone levels were in 5^{th} group followed by 3^{th} group followed by 4^{rd} group then control negative (untreated) group respectively (Table 2) and Fig. (1, 2 and 3).

Table 2: Effect of different treatments on growth hormone, calcium and phosphorus levels of (*Oreochromis niloticus*) during experimental period (n=3).

	Groups	N	Growth Hormone (ng/mL)	Calcium (mg/L)	Phosphorus (mg/L)
4th week	G1	3	6.59 ±0.17 c	3.03+0.04 a	2.23+0.04 a
	G2	3	2.5 ±0.21 d	1.61+0.06 e	1.49+0.01e
	G3	3	14.53 ±0.29 a	2.14+0.03 c	1.85+0.03 c
	G4	3	8.12 ±0.07 b	1.86+0.03 d	1.55+0.02d
	G5	3	14.61 ±0.31a	2.83+0.03 b	2.19+0.01b
8th week	G1	3	7.99+0.05 b	3.45+0.07 a	2.55+0.06 a
	G2	3	1.69+0.022d	0.67+0.03 e	0.76+0.03 e
	G3	3	9.66+0.23 a	1.25+0.03 d	1.05+0.02d
	G4	3	6.42+0.23 c	1.71+0.03 c	1.35+0.01c
	G5	3	10.02+0.05 a	2.58+0.1 b	2.06+0.02b

Means with different small litters in the same column at the same period are significantly different at ($p \leq 0.05$).

Examination of Lead residues in blood, gills tissues, liver, kidney and Musculatures indicated that significant increasing in 2nd group (control positive) in comparing to other groups and the control negative group. Best results were in control negative group followed by group 5th then 4th group then 3rd group (Table 3) and Fig. (4, 5, 6, 7 and 8). Lowest Lead residues were recorded in musculatures but the highest residues were recorded in gills in all groups.

Table 3: Effect of different treatments on Lead residues (µg /g) wet weight) of the examined samples of blood, gills, liver, kidney and Muscular tissues of (*Oreochromis niloticus*) during experimental period (n=3).

	Groups	N	Blood	Gills	Liver	Kidneys	Musculature
4th week	G1	3	0.614+0.01e	0.74+0.03 e	0.021+0.001e	0.53+0.02 e	0.003+0.001e
	G2	3	6.27+0.14 a	7.43+0.08 a	3.16+0.03 a	6.27+0.02 a	1.35+0.27a
	G3	3	3.17+0.03 c	3.34+0.04 c	1.82+0.04 c	3.4+0.14 c	0.75+0.02c
	G4	3	3.82+0.07 b	4.0+0.04 b	1.88+0.05 b	3.5+0.07 b	0.83+0.02b
	G5	3	2.01+0.01d	2.01+0.01d	1.71+0.01d	2.46+0.09 d	0.56+0.04 d
8th week	G1	3	0.15+0.02 e	0.11+0.01e	0.01+0.001e	0.002+0.001e	0.001+0.001e
	G2	3	6.99+0.02 a	7.6+0.31a	3.96+0.07 a	5.86+0.15 a	1.87+0.06 a
	G3	3	3.71+0.09 b	4.36+0.26 b	2.38+0.03 b	3.64+0.17 b	0.85+0.03 b
	G4	3	3.26+0.1 c	3.47+0.21 c	1.73+0.09 c	3.26+0.07 c	0.71+0.014 c
	G5	3	1.3+0.14 d	1.3+0.06 d	0.55+0.04 d	1.17+0.07 d	0.23+0.02 d

Means with different small litters in the same column at the same period are significantly different at ($p \leq 0.05$).

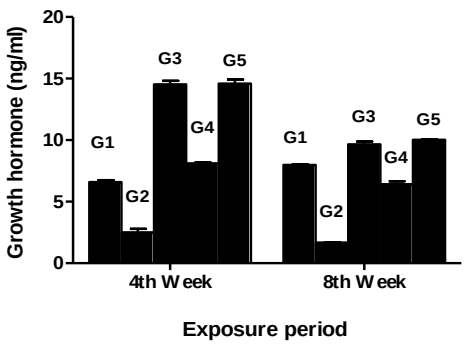
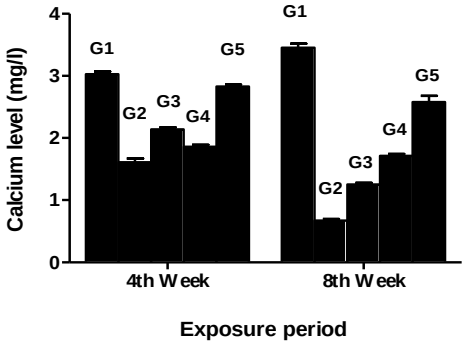


Fig. (1)



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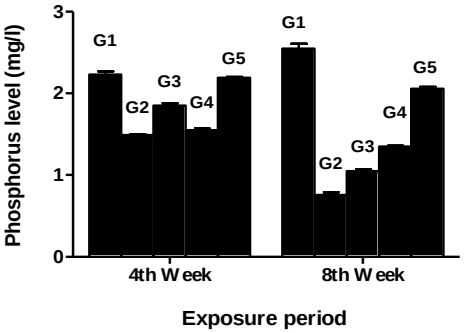

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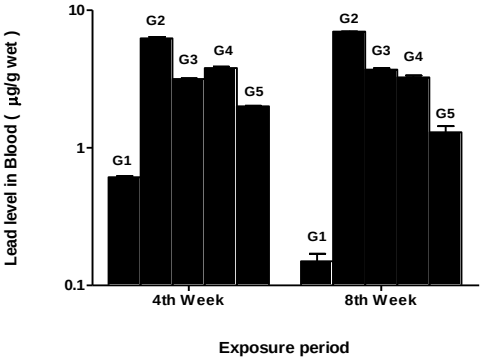

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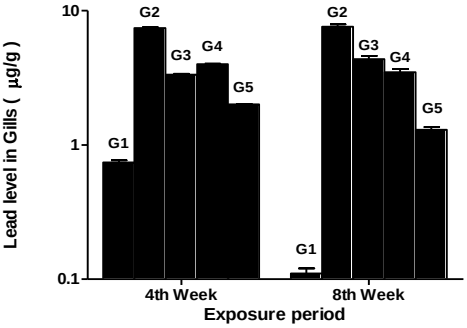

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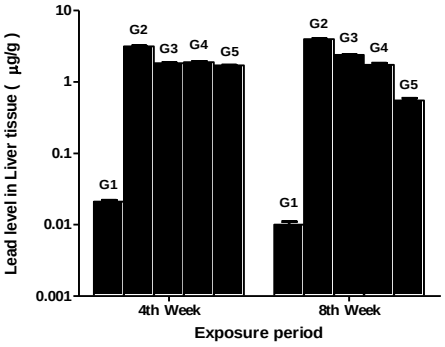

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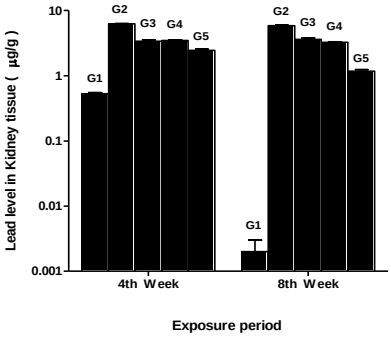

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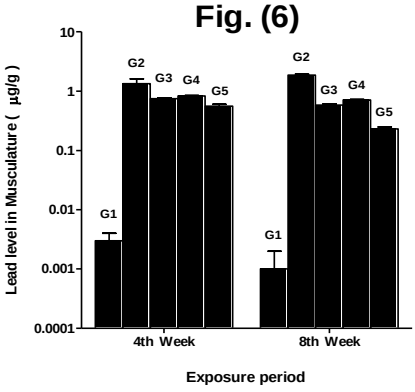


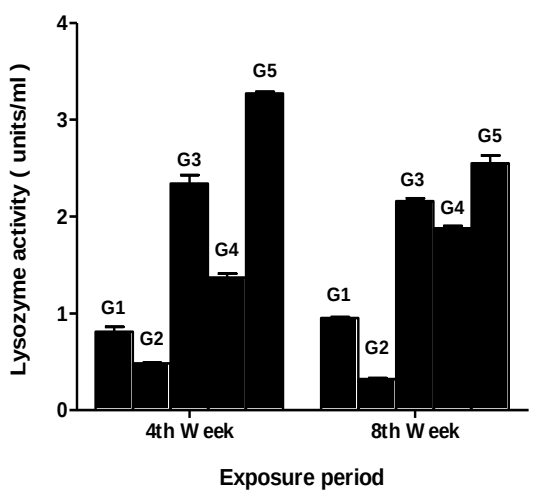
Fig. (8)

Concerning to the serum bactericidal activity as well as serum lysozyme activity significantly decreased in the lead-intoxicated control positive group (2nd group) compared with control negative group especially with increasing the time of exposure to lead acetate at the 8th week. Best detoxification results were in groups fed on diet contain (9 x10^2 CFU /kg feed) of *Penicillium Roquefort* + (300 mg/kg feed) of *Alpha-Tocopherol* although the presence of 90 mg/l lead acetate (Table 4) and Fig. (9 and 10).

Table 4: Effects of different treatments on serum lysozyme and bactericidal activity of (*Oreochromis niloticus*) during the experimental period (n=3).

	Groups	N	Lysozyme activity(units/ml)	Bactericidal activity survives index (SI)
4th week	G1	3	0.81+0.05 d	4.67+0.05 c
	G2	3	0.48+0.01 e	3.71+0.13 d
	G3	3	2.34+0.09 b	6.27+0.02 b
	G4	3	1.37+0.04 c	5.25+0.04 c
	G5	3	3.27+0.02 a	7.42+0.02 a
8th week	G1	3	0.95+0.01 d	5.09+0.01 b
	G2	3	0.32+0.01e	2.28+0.03 d
	G3	3	2.16+0.03 b	5.18+0.02 b
	G4	3	1.88+0.02 c	4.31+0.05 c
	G5	3	2.55+0.08 a	6.15+0.02 a

Means with different small letters in the same column at the same period are significantly different at ($p \leq 0.05$).



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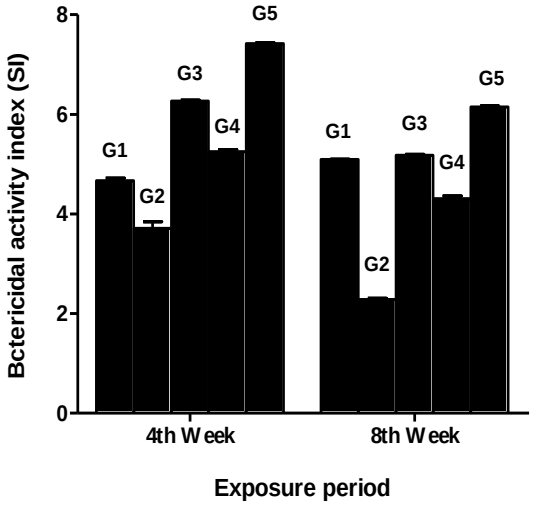

Fig. (10)

Mortalities of Lead acetate intoxicated *Oreochromis niloticus* challenged with *Aeromonas hydrophila* were significantly higher in control positive group than all treated groups and the control negative group (Table 5).

Table 5: Effects of different treatments on mortality percent of *Oreochromis niloticus* after challenge with *Aeromonas hydrophila* (n=10).

Group	N	Mortalities		Protected	
		Number of fish	%	Number of fish	%
G1	10	0	0	10	100
G2	10	4	40	6	60
G3	10	2	20	8	80
G4	10	3	30	7	70
G5	10	2	20	8	80

Means with different small litters in the same column at the same period are significantly different at ($p \leq 0.05$).

The histopathological examination of group received Lead acetate only (control positive) showed slight pathological alteration where, Gills showed cellular hypeplasia and congestion, telangiectasis at the tips of secondary lamellae and extensive apoptosis i.e. the cells die and are sloughed (Photo: 2 A, B, C). Liver showed congestion in hepatic blood vessels and sinusoid, with necrotic changes and melanomacrophage infiltration (Photo: 3 A, B). Kidneys revealed degenerative changes in glomerular epithelium with inflammatory cells infiltration (Photo: 3C). The histopathological examination of 3rd and 4th groups revealed slight degenerative changes in the examined tissues. More-over best results were in the 5th group that received *Penicillium roqueforti* and *Tocopherol* as gills only showed, mild congestion (Photo: 3D). Liver showed slight modulation as in (Photo: 4).

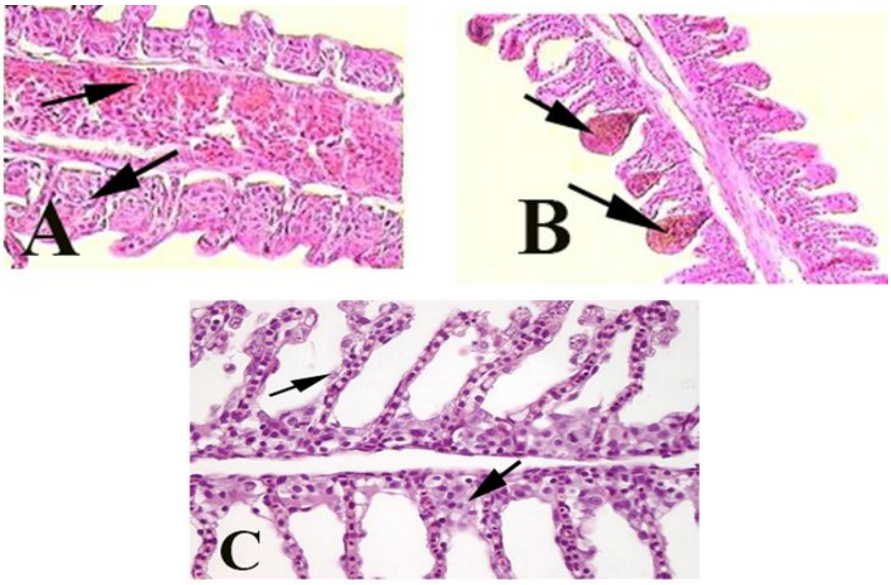

Photo 2: A- Gill of experimentally Lead intoxicated *O. niloticus* shows epithelial hyperplasia and blood congestion (arrows) (stain H&E X 200).
B- Gill of experimentally Lead intoxicated *O. niloticus* shows telangiectasis at the tips of secondary lamellae (arrows) (stain H&E X 200).
C- Gill of experimentally Lead intoxicated *O. niloticus* displaying extensive sloughing of epithelia linning (arrows) (H &E X 200).

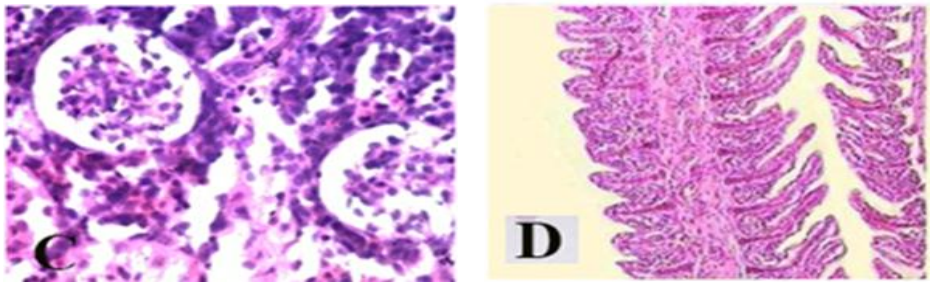

Photo 3: A- Liver of experimentally Lead intoxicated *O. niloticus* shows blood congestion (stain H&E X 200).
B- Liver of experimentally Lead intoxicated *O. niloticus* shows melanomacrophages aggregate close to a bile duct (arrows) (stain H&E X 200).
C- Kidney of experimentally Lead intoxicated *O. niloticus* shows degenerative changes in glomerular epithelium with inflammatory cells infiltration (H &E X 200).
D- Gills of experimentally Lead intoxicated *O. niloticus* treated with *Penicillium roqueforti* and *Alpha-Tocopherol* shows slight hyperplasia and partial fusion of some lamellae (stain H&E 200).

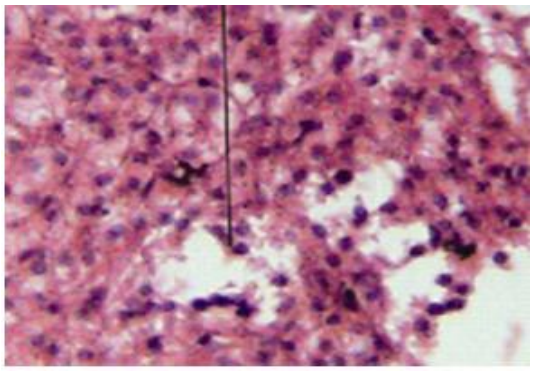

Photo 4: Liver of experimentally Lead intoxicated *O. niloticus* treated with *Penicillium roqueforti* and *Alpha-Tocopherol* shows minute focal areas of necrosis (stain H&E X 200).

DISCUSSION

Heavy metals contamination of natural aquatic receptors and sediments is a major environmental problem (Srivastava and Thakur, 2006). Lead (Pb^{+2}) is one of heavy metals which found in the environment and causes several adverse effects. Pb^{+2} can change the physiological activities as well as causes histopathological changes of different organs in fish Marwa Salah *et al*. (2013).

Lead intoxicated fish groups (2^{nd}, 3^{rd}, 4^{th} and 5^{th}) showed no characteristic symptoms externally comparing to the control negative (lead free) group and this is may be related to the cumulative and choronic nature of heavy metals adverse effects. Postmortem examination of 2^{nd} (control positive) group revealed the presence of edematous fluid, yellowish enlarged liver, enlarged gall bladder and spleen. Congestion of spleen, kidneys and gills was also recorded in comparing to control negative group. Regarding to 3^{rd}, 4^{th}, 5^{th} groups Postmortem examination revealed relatively enlarged pale liver, enlarged gall bladder and spleen comparing to the control negative group. These findings may be related to that gills, liver and kidneys are the main tissues of heavy metal (Pb) uptake and accumulation (Gbem *et al*., 2001), Patnaik *et al*. (2011) and Luszczek-Trojnar *et al*. (2013). Moreover gill is an organ having a large surface as wellas separates fish blood from water and is very sensitive to changes in concentrations of heavy metals, temperature, pH and etc. in fish environment. For this reason gill tissue is a good indicators for water pollution (Koca *et al*., 2007). Liver plays a major role in the metabolism of lead; lead causes adverse effects to liver cells because liver is one of the major organs of lead storage, biotransformation as well as detoxification (Yomn and Mariam 2011). The observed Postmortem examination results (2^{nd} group) were confirmed by Histopathological picture in liver, gills and kidney which coordinate with results obtained in gills of experimentally lead acetate intoxicated Silver Sailfin Molly by (Yomn and Mariam 2011) in addition to Doaa and Hanan (2013) and Marwa Salah *et al*. (2013) in the histological structure of gills and liver and in the histological structure of gills, liver and kidneys of *o.niloticus* experimentally intoxicated by lead acetate respectively. More-over nearly similar histopathological findings were reported by Al-Balawi *et al*. (2013) in gills, liver and kidneys of *Clarias gariepinus* experimentally intoxicated by lead acetate and Sirimongkolvorakul *et al*. (2012) in gills and liver of experimentally intoxicated *Puntius altus* by Lead Nitrate [$Pb(NO_3)_2$], respectively.

The analysis of growth hormone (GH) levels revealed significant reduction in 2^{nd} group (control positive) in comparing to other groups similar finding were observed by El-Shebly (2009) in lead (Pb) intoxication *Oreochromis niloticus*. Moreover this reduction increased by increasing the period of exposure to lead (8^{th} week) this may be related to the cumulative and choronic nature of heavy metals adverse effects. Best results of growth hormone levels were in 5^{th} group followed by 3^{rd} group followed by 4^{th} group then control negative (untreated) group respectively and this is may be related to the synergism between *Penicillium roqueforti* and alpha *Alpha-Tocopherol* as Several fungi are able to mineralize pollutant compounds by their non-specific highly oxidative ligninolytic enzymes (Bergsten *et al*., 2009). More-over *Alpha-Tocopherol* may act as useful tool for minimizing the lead toxic effects by its potent antioxidant activity El-Shebly (2009) in lead (Pb) intoxicated *Oreochromis niloticus* (El-Shebly, 2002 and Apigail *et al*., 2003), recorded also, GH inhibition to heavy metals in the environmental samples.

The present study indicated significant reduction in calcium and phosphorus levels in all lead acetate treated *O.niloticus* groups in comparison to control negative group. These results are similar to those observed by Ajai *et al*. (2013) in the freshwater catfish, *Heteropneustes fossilis*. That was subjected to164.4 mg/L (0.2 of 96 h LC_{50}) of lead nitrate for long-term experiment. Rogers *et al.* (2003) reported also hypocalcemia in lead-exposed rainbow trout. Generally divalent metals such as of zinc, lead and

cadmium are considered calcium antagonists Kosai *et al*. (2011). The hypocalcemia observed in fish exposed to lead may be attributed to impairment of net electrolyte influx at the gill or impairment of renal function. Gills degeneration may affect the ionic permeability in addition to cause decreasing of the blood ionic levels. Tubular necrosis may be other possible cause for the observed hypocalcemia and hypophosphatemia (Ajai *et al*., 2013) they stated that, this may be also due to ionoregulatory toxicity induced by lead, particularly the Ca^{2+} homeostasis disturbance is not exclusively a branchial phenomenon, but is in part a result of ionoregulatory mechanisms disruption at the kidney. The observed results are confirmed by the gill and kidneys histopathological picture previously discussed.

Concerning effect of different treatments on lead residues of the examined samples of blood, gills tissues, liver, kidney and Muscular tissues of *Oreochromis niloticus* results revealed to that lowest lead residues were recorded in musculatures but the highest residues were recorded in gills These findings were similar to those obtained by (Al-Balawi *et al*. 2013) in *Clarias gariepinus* experimentally intoxicated by lead acetate. Moreover these results were in agreement with Bahnasawy *et al.* (2011) in their examination of the heavy metals in the water and fish in Lake Manzala they found that, the lowest lead residues concentrations were recorded in musculatures but the highest concentrations were in gills. Similar results were recorded by Yılmaz, (2005) in fish muscles of grey mullet (*Mugil cephalus*) and Sea bream (*Sparus aurata*). Best results were recorded in the 5th group followed by 3rd then 4th group. This is may be related to *Penicillium roqueforti* adsorption and mineralization to pollutant compounds through their non-specific highly oxidative ligninolytic enzymes (Bergsten *et al*., 2009) and its ability for removal of heavy metals from contaminated environments (Dugal and Gangawane 2012).

The non-specific immune parameters are considered useful tools for determining the fish health status and evaluation of the immunemodulatory substances for fish farming as pollution markers and diseases resistances (Sahoo *et al.*, 2005). The present study revealed decreasing lysozyme activity as well as Bactericidal activity during the experimental period in control positive group (2nd group) in compare with control negative group moreover this decreasing level increased in the 8th week more than in 4th week. These results were supported by El-Boshy and Taha (2011) in investigation of Mercuric chloride effect on the immunological parameters of Nile Tilapia. The best results were in 5th group and this is may be related to the synergism between *Penicillium roqueforti* and *Alpha-Tocopherol*, where *Alpha-Tocopherol* has a very important role in immunity enhancement *(*John *et al.,* 2001*)*. In addition *Penicillium roqueforti* may act as probiotic so improve nonspecific immune response and so improve lysozyme activity as well as bactericidal activity this is may be supported by Taoka *et al.* (2006) who showed that viable probiotics supplemented to Japanese flounder *(Paralichthys olivaceus)*, increased nonspecific immune response, that was determined by parameters as bactericidal activity, lysozyme activity and neutrophil migration, which improved fish resistance to infection.

Concerning mortalities of lead stressed *O. niloticus* challenged with *Aeromonas hydrophila* were significantly lower in all treated groups than the control positive group. These results supported by Omima Aboud (2010*)* in *Oreochromis niloticus* challenged with *Ps. Fluorescence* to evaluated lead, mercury and cadmium effects on both cellular and humeral immune response. Moreover low mortalities may attributed to the cumulative and chronic nature of lead toxicity and /or the lead acetate dose was sub-lethal dose similarly Pandey *et al*. (2000) concluded that dose- and dose-time-dependent increases the rate of mortality in response to mercuric toxicity in fish.

The best detoxifying results were achieved by the mixing *Penicillium roqueforti* with alpha-*tocopherol*. The observed detoxifying results may be related either to heavy metals removing ability of *Penicillium roqueforti* from contaminated environments (Dugal and Gangawane 2012) as Several fungi are able to mineralize pollutant compounds by their non- specific highly oxidative ligninolytic enzymes (Bergsten *et al*., 2009) and / or that vitamins ability to mobilize lead into the urine (Guluzar *et al*., 2006) as well as *Alpha-Tocopherol* provide protection against oxidative stress resulted from Pb toxicity (*Seok et al*., 2007 and El-Gaml *et al.*, 2015).

CONCLUSION

Addition of *Penicillium roqueforti* plus *Alpha-Tocopherol* as feed supplements in *O.niloticus* diet in lead contaminated areas could have positive impacte on fish health by improving productivity (growth) through modulation of GH, Ca and phosphorus. Moreover improving fish immunity and survivability by reducing the hazard effect of Pb, it improve the external fish picture, growth and minimizing the Pb residues especially in fish musculatures.

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تأثير إضافه حويصلات البنسيليوم ريكفورت وألفا-توكوفيرول على سميه الرصاص في أسماك البلطي النيلي المستزرع

محمد السيد عبد الجيد بركات ، محمد سيد أحمد الجوهري ، أماني مختار دياب

Email: mmbarakat2003@gmail.com Assiut University web-site: www.aun.edu.eg

صممت هذه التجربه لدراسة التأثير السام لجرعه الرصاص (خلات الرصاص) الغير مميته على أسماك البلطي النيلي وكذلك تأثير إضافة حويصلات البنسيليوم ريكفورت وألفا-توكوفيرول على عليقه الاسماك كمواد مضاده لسميه الرصاص. تم احضار ١٠٠ سمكه من اسماك البلطي النيلي بمتوسط وزن ٥٠±١٠جم لكل سمكه وتم تقسيمها إلى خمسة مجموعات متساويه: المجموعه الأولى تركت كمجموعه ضابطه بدون معاملات، المجموعه الثانية تم إضافه ٩٠مجم من خلات الرصاص/ لتر مياه في أحواض التربيه، المجموعه الثالثه تم إضافه ٩٠مجم من خلات الرصاص/ لتر مياه في أحواض التربيه مع إضافة ٩×١٠^{٢} CFU/Kg من حويصلات البنسيليوم ريكفورت على العليقة، المجموعه الرابعه تم إضافه ٩٠مجم من خلات الرصاص/ لتر مياه في أحواض التربيه مع إضافه ٣٠٠مجم/كجم من الفا توكوفيرول على العليقة، المجموعة الخامسة تم إضافه ٩٠مجم من خلات الرصاص/ لتر مياه في أحواض التربيه مع إضافة ٩×١٠^{٢} CFU/Kg من حويصلات البنسيليوم ريكفورت مع ٣٠٠مجم/كجم من الفا توكوفيرول على العليقة. تمت تغذية مجموعات الاسماك يوميا لمدة ١٠ أسابيع بنسبه ٣% من وزن الاسماك. لم تظهر المجموعه الثانيه اي أعراض ظاهرية على الرغم من وجود تغيرات تشريحيه وهستوباثولوجيه. كما أضهرت تحاليل مصل الدم انخفاض معنوي في هرمون النمو، نسب الكالسيوم، الفوسفور، وزيادة نشاط activity and Serum lysozyme activity bactericidal وفي المقابل زيادة في معدلات الوفيات بعد العدوى بميكروب الاريموناس هيدروفيلا. كما تم دراسه متبقيات الرصاص في الدم والعضلات والخياشيم والكلى والكبد. ووجد ان اضافة حويصلات البنسيليوم ريكفورت وألفا-توكوفيرول يحسن التأثير السلبي لسميه الرصاص في المجموعات (٣، ٤ و ٥) وكانت افضل النتائج في المجموعه الخامسه. نخلص من هذه النتائج أن إضافه حويصلات البنسيليوم ريكفورت وألفا-توكوفيرول لعلائق الاسماك يقلل من التأثير السام للرصاص كما انه يقلل من الأثر التراكمي للرصاص وخصوصا في عضلات الاسماك.