CONSEQUENCES OF FIPRONIL EXPOSURE ON IMMUNOLOGICAL AND HEALTH STATUS OF CULTURED *OREOCHROMIS NILOTICUS*

Tamer S. Imam*, Abdelhakeem El-Murr**
and Yasser AbdElhakim**

**Departments of Forensic Medicine and Toxicology, ** Department of Fish Diseases and Management, Faculty of Vet. Medicine, Zagazig University.*

Correspondence authors*:* Yasser Abd Elhakim
Lecturer of Fish Disease and Management,
E-mail*:*Dryasserhakim@yahoo.com
Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt.
Tel\: 00201024410724

Received 9/ 6/ 2015 *Accepted 28/ 7/ 2015*

Abstract

The current experiment was carried out to evaluate the effects of different concentrations of the insecticide fipronil on the immune response and health condition of Nile tilapia fish (*Oreochromis niloticus*). For this purpose, immunological, biochemical and hematological parameters were assayed. Two hundred and forty *O.niloticus* fish were randomly assigned into four groups in triplicates: a control group and three fipronil treated groups in which fish exposed to $^1/_3$ of 96 hrs LC_{50} for 4 days, $^1/_{10}$ and $^1/_{20}$ of 96 hrs LC_{50} of fipronil for 10 weeks respectively. The mortality rate in fish exposed to $^1/_3$ LC_{50} for 4 days was 53%; meanwhile it was 21% and 8 % in those exposed to $^1/_{10}$ and $^1/_{20}$ of LC_{50} of fipronil for 10 weeks. Nervous manifestations, pale gills, congestion and hemorrhages of different internal organs were observed. There was a significant decrease in erythrocytic count, total leukocytic count, PCV% and Hb content. Also, reduced serum IgM level and lysozyme activity with a concurrent increase in the serum nitric oxide level were recorded. Obvious significant increase in serum AST, ALT, urea, creatinine and cortisol levels in all fipronil exposed groups when compared with control group. In addition, liver, gills and skin tissues revealed marked histopathological alterations.

Key words: Fipronil, *O. niloticus*, IgM, Lysozyme activity**.**

INTRODUCTION

In recent years, freshwater ecosystems are the most vulnerable systems worldwide which suffer from a harsh loss of biodiversity (Geist, 2011). The

various threats in which freshwater ecosystems faced include climate alteration, nutrient swings, acidification, habitat loss, exploitation and biological invasions. Besides, chemical contamination that is considered a substantial factor which established by indiscriminate and common use of pesticides, results in the excess inflow of toxic chemicals into the aquatic ecosystem (Kalavathy *et al.*, 2001*)*, thus, it becomes hazardous to the aquatic life (Barbieri, 2009 and Schäfer *et al.*, 2011).

Contamination of water with large amounts of pesticides leads to fish mortality or starvation by destruction of food organisms. Moreover, many toxicants have been shown affecting the growth parameters and reproduction, with evidence of tissue damage (*Srivastav et al., 2002*).

Fipronil is a new broad spectrum phenylpyrazole insecticide that is identified by the United States Environmental Protection Agency (USEPA) and is used as an alternative to organophosphate compounds (US EPA, 2002b and Chiovarou and Siewicki, 2008). Recently, fipronil is gaining a considerable attention where as it is highly effective against various insects and pests of crops, notably rice insects, trips and termites using a minute concentration of it (Mulrooney *et al.*, 1998), owing to its lipophilicity and persistency properties. It has also non-agricultural applications, including control of veterinary pests (Jennings *et al.*, 2002). Fipronil is highly toxic for crustaceans, insects and zooplankton as well as bees, termites, rabbits, the fringe-toed lizard and certain groups of gallinaceous birds. It is also highly toxic to many fish. Moreover, its toxicity is varied within different species. Conversely, the substance is relatively innocuous to passerines, wild fowl and earthworms .There is evidence that fipronil and some of its degrades may bio accumulate particularly in fish (Tingle *et al.*, 2003).

Due to the high consumption of Nile tilapia by humans and considering the insecticides used in agriculture practice, the possible toxic effects of these products in fish tissues for commercial interest have become of a great concern. So**,** the present study was carried out to evaluate the consequences of short and long term exposure to different concentrations of fipronil on health

and immune response of *O.niloticus* through the measurement of some immunological, biochemical and hematological parameters in addition to histopathological examination.

MATERIALS AND METHODS

Fish:

Two hundred and forty of live *O. niloticus* (35 ± 2.0 g) were employed in the present study at spring. Fish were obtained from private fish farm in Abbassa, Sharkia Province. Fish were apparently healthy and free from any skin lesions or external parasites, were kept in fully prepared glass aquaria, each aquarium (80 X 30 X 40cm) provided with aerator and thermostatically controlled heater and filled with clean and dechlorinated water. Fish were acclimated for two weeks to the laboratory environment before the start of the experiments. They were fed on basal diet containing crude protein 30%. The amount of feed (on dry matter basis) given daily to fish was 5% of body weight and the fish were fed three times daily. During all experimental period, the water parameters were maintained as follows: temperature 25.5± 2ºC, pH 6.4 ± 0.2, dissolved oxygen 5.1± 2.0 mg/L, non-ionized ammonia 0.8±0.01 µg/L and nitrite 0.06±0.01 mg/L.

Chemicals:

Fipronil 20% SC was obtained from Yongnong Biosciences Co., Ltd. China.

Experimental protocol:

Fish were divided into four triplicated groups (20 fish per aquarium) .The 1st group kept as a control. 2nd group was exposed to 0.014 mg/l ($^1/_3$ of 96hrs LC_{50}) for 4 days. 3rd group was exposed to 0.0042mg/l ($^1/_{10}$ of 96hrs LC_{50}) for 10 weeks. 4th group was exposed to 0.002 mg/l ($^1/_{20}$ of 96hrs LC_{50}) for 10 weeks.

The recorded 96hrs LC_{50} of fipronil for *O. niloticus* was 0.042 mg/l according to USEPA, (2011).

The experimental fish were observed daily, the clinical signs and postmortem lesions of the affected fish and the mortality rate were recorded according to Noga (1996).

Samples collection:

At the end of experiment the blood samples were collected from caudal blood vessels of control and fipronil exposed groups, where it classified into 2 parts. 1st part allowed to clot overnight at room temperature, then centrifuged at 3000 rpm for 10 min for separation of serum which stored at -20°C for biochemical uses, while the other part was collected in heparinized tubes and immediately used for hematological analysis.

After fish scarification, specimens from liver, gills and skin from all groups were kept in neutral buffered formalin for histopathological examination.

Hematological analysis:

Erythrocytes (RBCs) and leukocytes (WBCs) counts were carried out according to the method described by Natt and Herric (1952), hematocrit packed cell volume (PCV) was measured according to Jain (1986) while hemoglobin concentration (Hb) was done according to acid hematin method using farstab heamometer as rapid collection using sahlis method. The obtained hemoglobin values were adjusted according to equation of Larsen (1964).

Immunological biomarkers:

Serum lysozyme activity was ascertained by the turbidometric assay Schultz (1987) .The serum nitric oxide level was assessed as described by Rajarman (1998).Using Griess reagent. Immunoglobulin M (IgM) was determined using ELISA Kit, Catalog No.CSB-E12045Fh. According to the techniques described by Laemmli (1970).

Biochemical analysis:

Serum aspartate amino transferase (AST), serum alanine aminotransferases (ALT) were measured according to (Reitman and Frankel (1957*)*, serum cortisol, urea and creatinine level were quantified according to Foster and Dunn *(*1974); Henry *(*1974) and Patton and Crouch, (1977) respectively.

Histopathological examination:

Specimens from the liver, gills and skin were fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with Hematoxylin and Eosin (H and E) dyes (Bancroft and Gamble, 2008) and then eventually examined microscopically.

Statistical analysis:

Data were expressed as mean ± SE. The data were analyzed for a statistical significance between groups with an analysis of variance (one-way ANOVA) with the SPSS 16.0 computer program (SPSS) followed by Duncan's multiple range test. *P*-Values <0.05 were considered statistically significant (SAS, 1996).

RESULTS AND DISCUSSION

Clinical signs and postmortem lesions:

Pesticides are widely studied in the aquatic ecotoxicology where large amounts are used in agriculture and livestock in the whole world. Fish exposure to fipronil in 2nd group ($^{1}/_{3}$ LC_{50} 96hrs for 4 days) exhibited nervous and sluggish movements with no response to tested reflexes. The body was covered with a dense layer of mucus. Moreover, gills appeared pale with excessive mucous secretion. Postmortem examination showed congestion of all internal organs with distended gall bladder. The observed nervous manifestations may be attributed to its mode of action which including the disruption of the normal

nerve function by targeting the gamma-aminobutyric acid type and blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system which lead to muscle exhaustion and shutting down of C.N.S (**Kidd and James, 1991).**

Regarding effect on health , the mortality rate of fish exposed to $^{1}/_{3}$ LC_{50} for 4 days demonstrated 53% mortality rate, nevertheless, it was 21 and 8 % in fish exposed to $^{1}/_{10}$ and $^{1}/_{20}$ LC_{50} of fipronil for 10 weeks respectively. These results were in agreement with **Colliot *et al.* (1992**) who mentioned that fipronil caused mortality to many fish species like rainbow trout and to bluegill sunfish with 96 hrs LC_{50} of 0.246 mg /L and 0.083 mg /1 respectively. This may be due to the high toxic effect of fipronil in high doses and short period.

Hematological evaluation:

There was a significant decrease ($P < 0.05$) in erythrocytic count in fipronil treated groups compared with control (Table 1). These results are in accordance with that obtained by Ghisi *et al.,* (2011) who not surprised that the lowest recorded concentration of fipronil 0.0002 mg/L causes erythrocyte injury in silver catfish, *Rhamdiaquelen* due to detrimental effect of fipronil on erythrocytes synthesis.

Hemoglobin content and total leukocytic count in fipronil exposed groups showed a significant decrease ($P < 0.05$) compared with the control group. This might be due to the fast oxidation of hemoglobin to methemoglobin or release of oxygen radical due to the toxic effect and oxidative stress induced by fipronil as observed by Clasen *et al*. (2012). These results were compatible with that obtained by Gupta *et al*. (2012) who found that exposure of *caprinus carpio* fry to sub lethal dose ($1/_{10}$ LC_{50}) of fipronil for 45 days resulted in significant decrease in erythocytic count, total leucocytic count and Hb%, however these results were differed from those reported by Gupta *et al.* (2013) and Gill and Dumka (2013). The latter mentioned that neither hemoglobin concentration nor total erthrocytic count was affected when buffalo calves were exposed to

fipronil at dose level 0.5 mg/kg body weight per day. This disagreement most probably will be due to the species difference.

Table 1. The effect on some hematological parameters of *O.niloticus* exposed to different concentrations of fipronil for various durations comparing with control group (Mean±SE).

Groups	Parameters			
	RBCs (10^6/µL)	**PCV** (%)	**Hb** (g/dL)	**TLC** (10^3/µL)
Control	1.30^{a} ±0.003	21.0^{a} ±0.30	4.76^{a} ±0.08	36.98^{a} ±0.07
2nd group	0.36^{b} ±0.02	19.2^{ab} ±0.50	4.90^{a} ±0.10	29.35^{b} ±1.50
3rd group	0.38^{b} ±0.02	17.8^{b} ±0.30	4.30^{b} ±0.13	27.57^{b} ±1.50
4th group	0.30^{c} ±0.01	14.8^{c} ±1.15	4.24^{b} ±0.04	30.96^{b} ±1.00

Means within the same column bearing different superscripts are significant at $p \leq 0.05$.

Effects of fipronil on immunological biomarkers:

Table 2 revealed that *O.niloticus* exposed to different concentrations of fipronil for various durations showed significant increase ($P < 0.05$) in the serum level of nitric oxide with concurrent significant reduced($P < 0.05$) serum lysozyme activity and IgM level in fipronil treated groups in comparison with control group. These results were similar to Gupta *et al.* (2013) who proved the negative effect of fipronil on immune response of fish through decreased serum level of lysozyme and nitrobluetetrazolium(NBT) in *Cyprinuscarpio* fry after exposure to sub lethal dose($1/_{10}$ LC_{50} for 96 hr) of fipronil for 45 days. Also similar results were obtained by Clasen *et al.* (2012) who found that exposure of *Cyprinus carpio* to 0.65mg/l fipronil for 7, 30 and 90 days cause changes in the antioxidant profile and elevation of oxidative stress parameters and subsequently altered immune status in different tissues of common carp.

Table 2. The effect on some immunological parameters of *O.niloticus* exposed to different concentrations of fipronil for various durations comparing with control group (Mean±SE).

Groups	Parameters		
	Nitric oxide µg /ml	Lysozyme µg /ml	Ig M (g/L)
Control	4.22 ±0.06 [a]	319.00 ±1.00 [a]	21.52 ± 0.33 [a]
2nd group	4.31 ±0.04 [b]	280.60 ±1.02 [b]	15.22 ±0.07 [d]
3rd group	4.92 ±0.03 [b]	260.40 ±1.72 [b]	19.22 ± 0.01 [b]
4th group	5.33 ±0. 08 [c]	300.00 ±2.62 [ab]	16.43 ± 0.18 [c]

Means within the same column bearing different superscripts are significant at $p \leq 0.05$.

Biochemical parameters:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver specific enzymes and they are more sensitive measures of hepatotoxicity and histophathalogic changes (Rao, 2006C). Morowati (1997) clarified that, the elevation of ALT activity appears to reflect an acute hepatic disease more specifically than using AST values.

Exposure of *O.niloticus* to fipronil resulted in significant increase in serum level of ALT and AST (Table 3). Like many toxic chemicals, fipronil has been well known to affect metabolic enzyme profile and thus can alter the physiological and biochemical responses of aquatic organisms (USEPA, 2011). Aguiar*et et al*. (2004) attributed the increase observed in the liver AST to mitochondrial membrane damage. While, Arshad *et al*. (2007) revealed that, the raised level in liver AST may be due to enzyme induction as a result of insecticide stress or due to the adverse effect of the insecticide on the oxidation by Kreb's cycle. Moreover, Ramaswamy *et al.* (1999) added that, fish adaptively increase the activity levels of tissue AST and ALT to meet the energy demand under pesticide stress, possibly by promoting gluconeogenesis. Thus, the increased serum level of AST and ALT in this study could be owed to the stress effect of fipronil as an insecticide.

There was increase in the serum level of urea, creatinine and cortisol (Table 3). Gupta *et al.* (2012) observed an elevation in serum level of cortisol of *Cyprinus carpio* fry after exposure to 0.0428 mg/l fipronil equivalent to ($1/_{10}$ LC_{50} for 96 hr) for 45 days. The elevated level of urea and creatinine may be attributed to alteration of detoxifying power of kidney caused by fipronil.

Table 3. The effect on serum level of some biochemical parameters of *O.niloticus* exposed to different concentrations of fipronil for various durations comparing with control group (Mean±SE).

Group / Parameters	1 Control	2 2nd group	3 3rd group	4 4th group
Creatinine (mg / DL)	0.22 ± 0.12^{c}	0.45 ± 0.14^{a}	0.39 ± 0.02^{b}	0.38 ± 0.15^{ab}
Urea (mg/dl)	$12.10+0.11^{b}$	$22+0.15^{c}$	$19+0.011^{d}$	$17+0.02^{ab}$
ALT (µ/ml)	$12+0.57^{d}$	$19+0.57^{a}$	$17+0.57^{b}$	$15+0.57^{c}$
AST (µ/m l)	17 ± 0.21^{d}	44 ± 0.23^{a}	34 ± 0.06^{b}	29 ± 0.24^{c}
Cortisol (µg/DL)	0.80 ± 0.16^{b}	2 ± 0.12^{a}	1.5 ± 0.03^{ab}	1.4 ± 0.07^{ab}

Means within the same row bearing different superscripts are significant at $p \leq 0.05$.

Histopathological examination:

Regarding histopathological results, light microscopical examination of liver, gills and skin of the control group showed no microscopical abnormalities (figure 1A, 2A and 3A). In the 2nd group; liver showed focal areas of necrosis infiltrated with numerous lymphocytes (Fig. 1 B). Severe congestion in the hepatic blood vessels and sinusoids and hemorrhages among the hepatic cells were seen. Diffuse hydropic degenerations and vacuolations in the hepatocytes were identified (Fig. 1 C). While the liver of the 3rd group; showed severe congestion of the hepatic blood vessels and hemorrhages (Fig. 1 D). Periportal coagulative necrosis was visualized besides extensive necrosis in the adjacent pancreatic acini. Few eosinophilic hyalinized globules (Mallory bodies) were seen in the cytoplasm of some vacuolated hepatocytes (Fig. 1 E). The liver of 4th group; showed mild to moderate fatty change, hydropic degeneration and

individual hepatocyte necrosis (Fig. 1 F). Severe congestion and hemorrhages were seen alongside few round cells infiltrations were detected in the portal areas and interstitial tissue (Fig. 1 G). These results matched with that obtained by Melo (2004), who recorded increased foci of necrosis in the liver of silver catfish after 48 and 72 hours of exposure to fipronil. After 96 hours of exposure, the author describes cells indistinguishable contour, presence necrosis focus, in addition of damaged blood vessels, vacuolization of the cytosol and the presence of an unknown material strongly eosin stained in the cytoplasm of the hepatocytes. Blood leukocyte infiltration and congestion were detected in addition to melanomacrophage were found in various locations between the hepatocytes as well as pyknotic nuclei cells.

Gills of intoxicated groups with fipronil showing various histopathological changes. Gills of 2nd group; showed focal necrosis and sloughing in the covering epithelium of the secondary lamellae with intense lymphocytes infiltrations (Fig. 2 B). In some cases, focal epithelial proliferations and fusion were seen at the base of gill filaments besides severe congestion of the lamellar blood capillaries and edema (Fig. 2 C). Scarce lymphocytes together with a significant number of EGC were detected at the base of gill filaments. Mucinous degeneration in the lining epithelium of the gill rackers was observed besides edema, extravasated erythrocytes and EGCs infiltration in the submucosa (Fig. 2 D). The gills of 3rdgroup; revealed extensive hyperplasia of the covering epithelium in the interlamellar spaces, followed by fusion of the lamellae and clubbing of such filaments (Fig. 2 E). Sometimes, the gill filaments were focally necrotic and infiltrated with lymphocytes. Congestion of ellipsoids and capillaries was observed as well as focal hemorrhages (Fig. 2 F). The gills of 4thgroup; showed mild hyperplasia in the epithelium of the secondary lamellae and congestion (Fig. 2 G). Comparable results were declared by Ghisi (2010) who researched the effects of the phenyl pyrazole fipronil in the gills of the silver catfish after 60 days of intoxication in the sublethal concentrations 0.05, 0.10and 0.23µg/L. The latter described hyperplasia, lamellar fusion and aneurysms in all treated groups that

can impair the gill function. However, we consider the injuries of low severity and possible regression if the source of stress is eliminated, since the concentration of fipronil used was very low. Lamellar fusion is a nature of defense mechanism to protect the epithelium of the lamella from direct contact with toxic agents (Ojha, 1999). Skin of 2nd group; showed intact epidermis with severe proliferation of epidermal cells, spongiosis and hydropic degeneration (Fig. 3 B). The underlying dermis and hypodermis revealed Zenker's necrosis and inflammation. The latter was represented by numerous round cells infiltrations and few extravasated erythrocytes (Fig. 3 C). Edema and numerous melanin-carrying were rarely separated the epidermis from the dermis. Sometimes, the epidermis was focally eroded. Skin of the third group; revealed increased of mucous cells and slight vacuolations of the epidermal cells. The dermis particularly at the subepithelial zone showed congested capillaries, edema and aggregations of the leukocytes and melanin-carrying cells (Fig. 3 D). Sometimes, erosions in the epidermis were noticed with destructed or desquamated epithelium. The underlying muscles showed edema, hyaline degeneration and infiltrated with few lymphocytes (Fig. 3 E).The skin of 4th group showed intact epidermis with activation of melanomacrophages in the dermis (Fig. 3 F). Edema and inflammation were rarely detected.

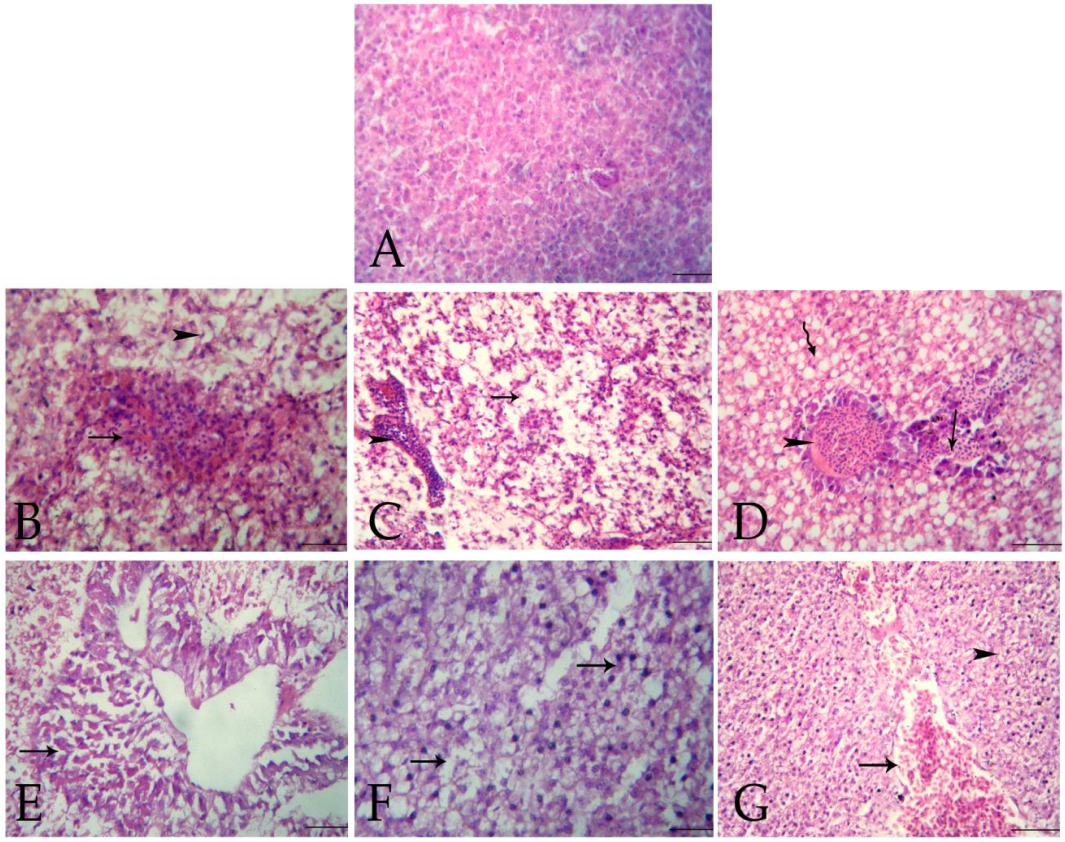

Figure (1 :(A ؛Section of control *O.niloticus* liver showing normal hepatocyte and sinusoidal architectures ،HandE) Bar = 100 µm.(

B and **C**; Section of *O.niloticus* liver of second group showing, **B;** focal area of necrosis infiltrated with numerous lymphocytes and few erythrocytes (arrow) and severe hydropic degeneration (arrowhead), **C;** diffuse hydropic degenerations and vacuolations in the hepatocytes (arrow), and lymphocytes infiltrations in the portal area (arrowhead), HandE (Bar = 100 µm).

D and **E;** Section of *O.niloticus* liver of third group, showing,**D;**severe congestion of the hepatic blood vessels (arrowhead), hemorrhages (arrow) and diffuse fatty change (irregular arrow), **E;** coagulative necrosis in the pancreatic acini (arrow), HandE (Bar = 100 µm).

F and G; Section of *O.niloticus* liver of fourth group showing, **F;** moderate fatty change and hydropic degeneration (arrows), **G;** severe congestion and hemorrhages (arrow) besides hydropic degeneration of hepatocytes (arrowhead), HandE (Bar = 100 µm).

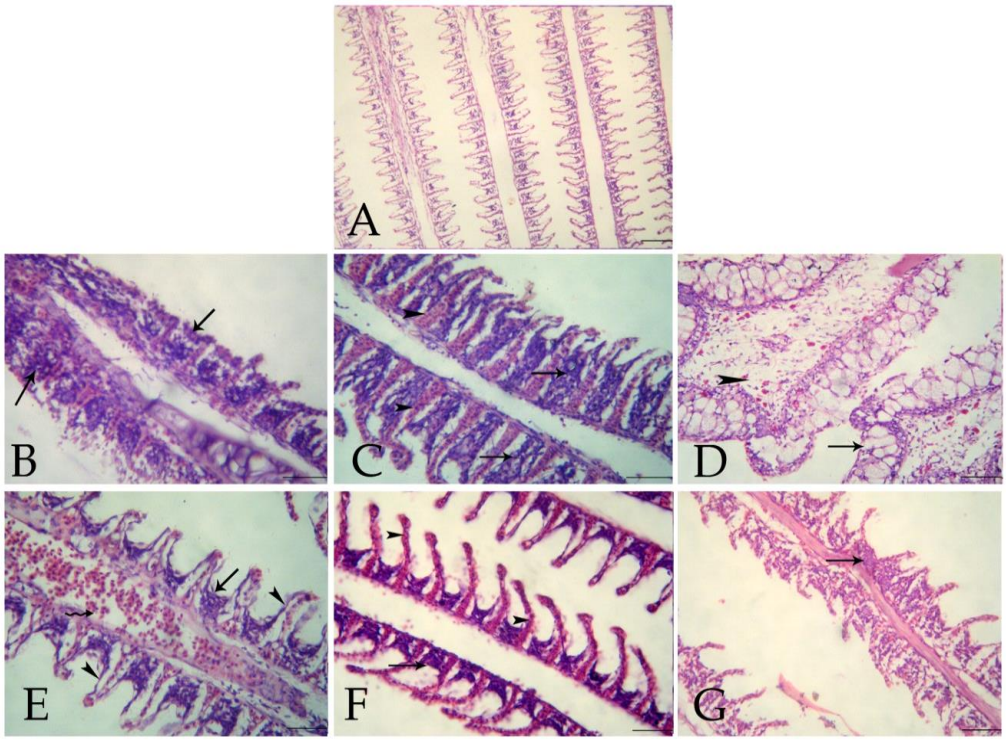

Figure (2): A; Section of control *O.niloticus* gills showing normal filaments and lining epithelium, HandE (Bar = 100 µm).

B, C and **D:** Section of *O.niloticus* gills of second group showing, **B;** focal necrosis and sloughing in the covering epithelium of the secondary lamellae with intense lymphocytes infiltrations (arrows), **C;** epithelial proliferations and fused at the base of gill filaments (arrows) besides severe congestion of the lamellar blood capillaries (arrowheads), **D;**mucinous degeneration in the lining epithelium (arrow) and edema and EGCs infiltration in the sub mucosa of gill racker (arrowhead), HandE (Bar = 100 µm).

E and **F;**Section of *O.niloticus* gills of third group showing, **E;** hyperplasia of the covering epithelium in the interlamellar spaces, followed by fusion of the lamellae (arrow), congestion (arrowheads) and hemorrhages (irregular arrow), **F;**severe congestion and focal hemorrhages (arrowhead) besides the hyperplasia in the lining epithelium (arrow), HandE (Bar = 100 µm).

G; Section of *O.niloticus* gills of fourth group showing mild hyperplasia in the epithelium of the secondary lamellae and congestion (arrow), HandE (Bar = 100 µm).

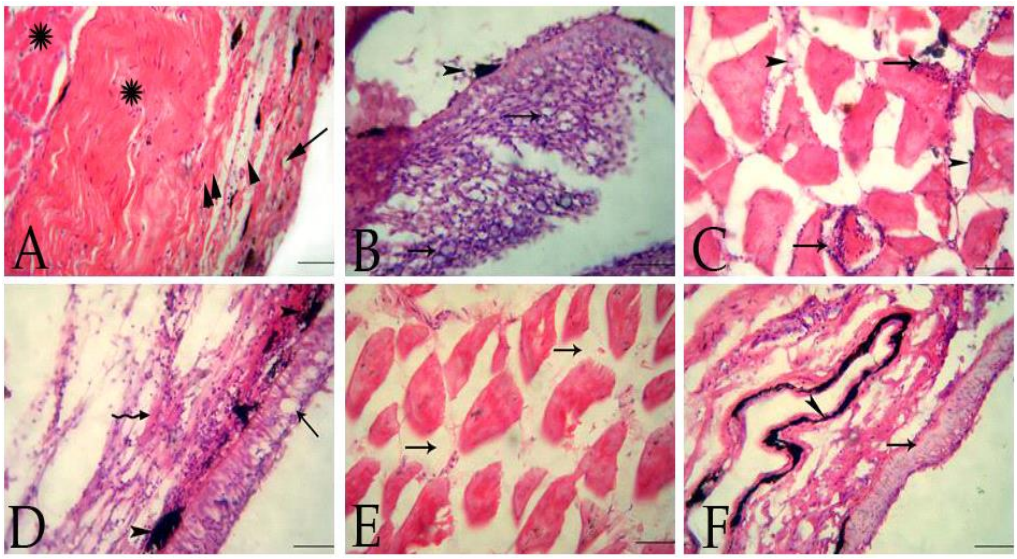

Figure (3): A; Section of control *O.niloticus* skin showing normal epidermis (arrow), dermis (arrow head) and dermal skeletal muscles (star), HandE (Bar = 100 µm).

B and C; Section of *O.niloticus* skin of second group showing, **B;** intact epidermis with severe proliferation of epidermal cells, spongiosis and hydropic degeneration (arrows) besides few melanin carrying cells (arrowhead),**C;**Zenker's necrosis infiltrated with numerous round cells (arrows) and edema (arrowheads), HandE (Bar = 100 µm).

D and E; Section of *O.niloticus* skin of third group showing, **D;**increased of mucous cells (arrow) and slight vacuolations of the epidermal cells. Edema and aggregations of the leukocytes (irregular arrow) and melanin-carrying cells (arrowhead) were seen in the dermis, **E;**edema and focal hyaline degeneration in the skeletal muscles (arrows), HandE (Bar = 100 µm).

F; Section of *O.niloticus* skin of fourth group showing intact epidermis (arrow) with activation of melanomacrophages in the dermis (arrowhead), HandE (Bar = 100 µm).

REFERENCES

Ajarman, V.R.; B.J. Nonnecke; S.T. Franklin; D.C. Hammell and R.L. Horst, 1998. Effect of vitamin A and E supply on nitric oxide production by blood mononuclear leuckocytes from neonatal calves fed milk replacers. J. Dairy Sci., 81: 3278-3285.

Arshad, N.; G. Shabbir; S. Aleem and M. Arshad, 2007. GOT is one of the enzymes, which gives valuable diagnostic information for a number of disease conditions. *Asian J. Exp. Sci*., 21 (2), 239-24.

Bancroft, J.D. and M.Gamble, 2008. Theory and Practice of Histological Techniques. 5th ed., Churchill Livingstone. New York, London, Philadelphia, 281-285.

Barbieri, E., 2009. Effect of 2, 4- Dherbicide(2,4-dichlorophenoxyaceticacid) on Oxygen consumption and ammonium excretion of juveniles of Geophagusbrasiliensis (QuoyandGaimard,1824) (Osteichthyes, Cichlidae). Ecotoxicology, 18 (1): 55–60.

Chiovarou, E.D. and T.C. Siewicki, 2008. Comparison of storm intensity and application timing on modeled transport and fate of six contaminants. Sci Total Environ. 389: 87–100. doi: 10.1016/j.scitotenv.2007.08.029.

Clasen, B.; V.L. Loro; R. Cattaneo; B. Moraes; T. Lópes, L.A. De Avila; R. Zanella; G.B. Reimche and B. Baldisserotto, 2012. Effects of the commercial formulation containing fipronil on the non-target organism Cyprinuscarpio: Implications for rice fish cultivation. Ecotoxicology and Environmental Safety, 77: 45–51.

Colliot, F.; K.A. Kukorowski; D.W. Hawkins and D.A. Roberts, 1992. Fipronil: a new soil and foliar broad spectrum insecticide. Brighton Crop Protection Conference-Pests and Diseases, 2 (1): 29–34.

De Aguiar, L.; G. Moraes; I. Avilez; A. Altran and C. Correa, 2004. Metabolical effects of Folidol 600 on the neotropical freshwater fish matrinxã, Bryconcephalus. Eviron. Res., 95 (2): 224-230.

Eitman, S.R. and S. Frankel, 1957. A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol., 28: 56-63.

Environmental Protection Agency, 2011 (U.S.E.P.A.). Fipronil Summary Document Registration Review: Initial Docket June 2011 EPA-HQ-OPP-2011-0448, Washington, D. C. http://www. Regulations. gov/>documentDetail;D=EPA-HQOPP-2011-0448-0003. Accessed 30 July 2012.

Foster, L. and R. Dunn, 1974. Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clin. Chem*., 20: 365- 368.

Geist, J., 2011. Integrative fresh water ecology and biodiversity conservation .Ecol Indic., 11: 1507–16.

Ghisi, D.C.; W.A. Ramsdorf; M. Vinícius; M. Ferraro; M.I.M. Almeida; C.A.D.O. Ribeiro and M.M. Cestari, 2011. Evaluation of genotoxicity in Rhamdiaquelen (Pisces, Siluriformes) after sub-chronic contamination with Fipronil. Environ Monit Assess., 180: 589–599.

Ghisi, N.C., 2010. Avaliação genotóxica em *Rhamdia quelen* após contaminação subcrônica com fipronil. Thesis, Pós-Graduação em Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba.

Gill, K.K. and V.K. Dumka, 2013. Haematological alterations induced by subchronic oral exposure of buffalo calves to fipronil and fluoride. Research report Fluoride, 46 (2): 65–72

Gupta, S.K. ; A.K. Pal; N.P. Sahu; N. Saharan; S.C. Mandal; C.P. Rakash; M.S. Akhtar and A. K.Prusty, 2012. Dietary microbial levan ameliorates

stress and augments immunity in Cyprinuscarpio fry (Linnaeus, 1758) exposed to sublethal toxicity of Fipronil. *Aquaculture Research*, 1–14.

Gupta, S.K.; A.K. Pal; N.P. Sahu; N. Saharan; M.S. Akhtar and S. Kumar, 2013. Haemato-biochemical Responses in Cyprinuscarpio (Linnaeus, 1758) Fry Exposed to Sub-lethal Concentration of a Phenylpyrazole Insecticide, Fipronil. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. DOI 10.1007/s40011-013-0201-y.

Henry, T.J., 1974. Determination of serum creatinine. Clin. Chem. Principles and Techniques, 2nd Ed-Harper and Row publisher. New York.

Jackson, D.; C.B. Cornell; B. Luukinen; K. Buhl and D. Stone, 2009. Fipronil Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services.http://npic.orst.edu/factsheets/fiptech.pdf

Jain, N., 1986. Schalm's Veterinary Heamatology. 4th Ed., Lea and Fibiger, Philadelphia, USA, 66-67.

Jennings, K.A.; A.R.J. Keller; B.H. Atieh; R.B. Doss and R.C. Gupta, 2002. Human exposure to fipronil from dogs treated with Frontoline, Vet. Hum. Toxicol. 44(5): 301-303.

Kalavathy, K.; A.A. Sivakumar and R. Chandran, 2001. Toxic effect of the pesticide Dimethoate on the fish Sarotherodonmossambicus. Journal of Ecological Research and bioconservation, 2: 27-32.

Kidd, H.and D. James, 1991. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK.

Laemmli, U.K., 1970. Structural proteins during the assembly of the head of bacteriophage T4.Nature, 227(15):680-685.

Larsen, H., 1964. Comparison of various methods of haemoglobin determination of catfish blood. Progressive fish Culturist. 26 (1): 11-15.

Melo, G.C., 2004. Sublethal effects of organophosphate folido l600 action in the liver of the fish freshwater catfish quelen (QuoyandGaimard, 1824): An anal-216 New Advances and Contributions to Fish Biologyise histopathology. Thesis. UFPR, Curitiba, Pr, 2004. Dissertation (Master in Cell and Molecular Biology) - Graduate Program in Cellular and Molecular Biology, Division of Biological Sciences, Federal University of Paraná, Curitiba.

Morowati, M., 1997. Inhalation toxicity studies of thimet (phorate) in mall swiss albino mouse, *Musmusculus*: *I. Hepatotoxicity. Environ. Pollut*, 96 (3): 283-288.

Mulrooney, J.E.; D.A. Wolfenbarger; K.D. Howard; D. Goli and D. Goli, 1998. Efficacy of ultra-low volume and high volume applications of fipronil against the boll weevil. Journal of Cotton Science, 2: 110–116.

Natt, M. and K. Herric, 1952.A new diluent for counting the red and white cells of chickens. Poult. Sci., 31: 335.

Noga, E.J., 1996. Fish diseases: Diagnosis and treatment Mosby- year book, Inc., St. Louis, Missouri. 367.

Ojha, J., 1999. Fish gills: Potential indicators of ecodegradation of aquatic environments. In A. K. Mittal, F. B. Eddy, and J. S. Dattamunshi (Eds.), Water/air transition in biology, 18: 263–279. New York: Science.

Patton, C.J. and S.R. Crouch, 1977.Enzymatic determination of urea.Anal. Chem., 49: 466-469.

Ramaswamy, M.; P. Thangave and N. Selvam, 1999. Glutamic oxaloacetic transaminase(GOT) and glutamic pyruvic transaminase (GPT) enzyme activities in different tissues of *Sarotherodon mossambicus* (Peters) exposed to a carbamate pesticide, carbaryl. Pestic. Sci., 55: 1217-1221

Rao, J.V., 2006c. Toxic effects of novel organophosphorous insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, Oreochromismossambicus.Pestic.Biochem. Physiol., 86: 79-84.-.

SAS (Statistical Analysis System) Institute, Inc., 1996. The statistical analysis system for windows 6. 12, Ed. Cary, N C. USA.

Schäfer, R.B.; P.C. Vonderohe; R. Kühne; G. Schüürmann and M. Liess, 2011. Occurrence and toxicity Of 31 organic pollutants in large rivers of north Germany over adecade (1994 to 2004). *Environ. Sci. Technol.*, 45: 6167–74.

Schultz, A., 1987. Methods in clinical chemistry. The C.V. Mosby Co. St. Louis, 742-746.

Srivastav, A.K.; S.K. Srivastava; D. Mishra and S.K. Srivastav, 2002. Ultimobranchial Gland of Freshwater Catfish, Heteropneustesfossilis in response to Deltamethrin Treatment. BulletinEnvironmental Contamination Toxicology, 68: 584-591.

Tingle, C.C.; J.A. Rother; C.F. Dewhurst; S. Lauer; and W.J. King, 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Rev Environ ContamToxicol., 176: 1-66.

Tomlin, C.D.S., 2006. The Pesticide Manual, A World Compendium, 14th ed.; British Crop Protection Council: Hampshire, England, 462-464.

U.S.E.P.A., 1996. New Pesticide Fact Sheet -Fipronil; EPA 737-F-96-005; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office Washington, DC, 1-10.

U.S.E.P.A., 2002b. Interim Reregistration Eligibility Decision for Chlorpyrifos. Prevention, Pesticides and Toxic Substances, EPA 738-R-01-007, Washington, DC.

Van Der Oost, R.; J. Beber and N.P. Vermeulen, 2003. Fish bioaccumulation and biormarkers in environmental risk assessment: a review. Environ. Toxicol.Pharmacol., 13: 57–149.

عواقب التعرض للفيبرونيل على الاستجابه المناعيه والحاله الصحيه في أسماك البلطي النيلي المستزرعه

تامر سعيد امام*، عبد الحكيم ابراهيم عبدالرحمن، ياسر عبد الحكيم****

قسم الطب الشرعى والسموم و قسم أمراض ورعاية الاسماك**، كلية الطب البيطرى – جامعة الزقازيق – مصر*

الملخص العربي

أجريت تلك الدراسة لتقييم الأثارالناجمة عن التعرض لتركيزات مختلفة من المبيد الحشري الفيبرونيل ولفترات مختلفة علي أسماك البلطي النيلي وذلك من خلال دراسة تأثير الفيبرونيل علي بعض القياسات الكيميائية والمناعية والدمويه بالاضافة الي التغيرات الهستوباثولوجية فى أنسجة الكبد والخياشيم والجلد. أستخدم لهذا الغرض عدد ٢٤٠ من اسماك البلطى النيلى بمتوسط وزن ١±٣٥ جم . تم تقسيم الاسماك الى أربع مجموعات. المجموعة الأولى أعتبرت كمجموعة ضابطة أما الثلاث مجموعات الأخرى تم تعرضها الى ٣/١ من الجرعة نصف المميتة في ٩٦ ساعة لمدة أربعة أيام وايضا تعريضها الي ١٠/١ و ٢٠/١ من الجرعة نصف المميتة في ٩٦ ساعة لمدة عشرة أسابيع على التوالى وقد أظهرت النتائج الي أن تعرض اسماك البلطى النيلى الى الفيبرونيل قد تسببت فى معدل نفوق وصل ألى ٥٣ %، ٢١%، ٨% فى المجموعة المعرضة للفيبرونيل الى٣/١ من الجرعة نصف المميتة في ٩٦ ساعة لمدة أربعة أيام ، ١٠/١ و ٢٠/١ من الجرعة نصف المميتة في ٩٦ ساعة لمدة عشرة أسابيع على التوالى بالاضافة الى وجود احتقان وأنزفة فى معظم الاحشاء الداخلية.كما أظهرت النتائج أن هناك أنخفاض معنوى فى عدد خلايا الدم الحمراء ، وخلايا الدم البيضاء ، والهيماتوكريت و الهيموجلوبين .ولوحظ أيضا ارتفاع معنوى فى انزيمات الكبد والبولينا والكرياتينين وهرمون الكورتيزول مصحوبا بانخفاض معنوى فى مستوى الجاماجلوبيولين والليسوزيم وارتفاع معنوى في مستوى أوكسيد النيتريك فى كل المجموعات المعرضة للفيبرونيل مقارنة بالمجموعة الضابطة.كما أسفر الفحص الهستوباثولوجى عن وجود تغيرات نسيجية مرضية في كل من أنسجة الكبد والخياشم والجلد مقارنة بالمجموعة الضابطة.