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Protective Effects of Humic Acid to Intoxication with Deltamethrin in Nile -Tilapia (*Oreochromis Niloticus*)

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ABSTRACT

The present study was conducted to identify the toxic implications of deltamethrin on *Oreochromis niloticus* with special reference to the protective effect of humic acid as feed additives. A total of 300 *O. niloticus* were divided equally into six groups. Group (1) was the control. Group (2) was fed on a ration containing humic acid at 1% for 30 days. Groups (3&4) were subjected throughout the experiment to deltamethrim at concentration of 0.02 and 0.05 ppm respectively and fed on non-treated ration. Group (5&6) were fed on humates at 1% for one week only then exposed to deltamethrim at 0.02 and 0.05 ppm respectively and fed on non-treated ration. Blood samples were collected from the caudal vein of three fish with three replicates for biochemical analyses at 7, 15 and 30 days of treatment. The 96 hr LC_{50} value of deltamethrin for *O. niloticus* was 0.20 ppm. The acute intoxicated fish exhibited abnormal behavioural and postmortem changes in the form of respiratory distress, loss of appetite, and nervous manifestation with erected pectoral fins. Postmortem changes showed congested internal organs and yellowish ascetic fluid. The use of humates as feed additive improves the general health conditions and immunity of *O. niloticus*. Deltamethrin had bad effect on general health status of fish by decreasing enzymes activities of Alanine amino transferase (ALT) and aspartate amino transferase (AST). Glucose and uric acid levels were increased in deltamethrin treated groups. *O. niloticus* exposed to deltamethrin induced immunosuppression in the form of hypoproteinemia, hypoalbuminemia and a great reduction in both globulin and lysozyme levels. The use of humates as feed additives had protective effect against pollutant as indicated in different measured parameters which maintained to normal values It could be concluded that deltamethrin is highly toxic to fish. Biochemical profiles could be used as early warning for toxicity in fish. In addition to the pre-feeding of immunostimulant to fish will be helpful against toxicity.

Keywords: Humic Acid, Intoxication, Deltamethrin, Nile Tilapia.

INTRODUCTION

The culture of freshwater finfish is a major component of the global aquaculture industry. Nile-tilapia (*Oreochromis niloticus*) is the main popular culture species produced in Egypt. It is source of cheap animal protein.

Aquatic ecosystems may be contaminated by untreated wastes of industrial and agricultural origins. Environmental pollution is becoming critical problems hindering the development and sustainability of aquaculture sector. It has been recognized that environmental pollutants can cause a higher prevalence of disease in natural population of fish and are contributing factors in the development of stress in fish. Pesticides and chemicals used in agriculture may finally inter the aquatic environment and accumulate in the food chain and may cause serious ecological and health problems (Sharma and Ansari, 2011 and Khalili *et al.,* 2012).

In Egypt, the use of pesticides in agriculture has significantly increased during the last decades. The synthetic pyrethroids were introduced to the field of pesticides during 1950's and since this decade, drastic change have been occurred in the synthesis of this type of insecticides (Banaee *et al.,* 2011 and Richterová and Svobodová, 2012). Deltamethrin [((s)-a-cyano-3-phenoxybenzyl (R1-R2) – 3 - (2,2 dibromvinyl) – 2 , 2 dimethylcyclo-propancarboxylate] is one of the most important widely used pyrethroids pesticide and insecticides, since the application of pyrethroid as insecticide and antiparasitary preparations has been accepted on a large scale for agricultural purposes and very markedly increased during last 10–15 years; even though it is already known that this insecticide is highly toxic to fish and various other aquatic organisms (Atta, 2010; Sharma and Ansari, 2011 and Amin and Hashem, 2012).

There is a growing concern to overcome aquatic pollutants because of its detrimental effects on biological life including human beings. The introduction of efficacious immunostimulant can help achieve sustainable aquaculture. Humic substances are formed during the decomposition of organic matter in humus, and are found in many natural environments in which organic materials and microorganisms have been present (Meinelt *et al.,* 2002; Perminova and Hatfield, 2005 and Nakagawa *et al.,* 2009).

This study was aimed to investigate the effect of sublethal concentrations of deltamethrin on *O. niloticus* health; in addition to the potential of humic substance in the control of its toxicity.

MATERIALS AND METHODS

Fish

An apparently healthy *Oreochromis niloticus* (30±1.5gm, 15±2.4cm & 8-months of age) were obtained from El-Abassa fish farm and were transported alive to the Laboratory in plastic bags containing water (1/3) and oxygen (2/3). Fish were kept in well equipped glass aquaria filled with de-chlorinated tap water and continuous aeration using an electric air pumping compressors. All fish were acclimatized for 15 days before the start of the experiment. Fish were fed twice daily with standard commercially prepared pellets at 2% of their body weight. About half of the water was changed daily in all experimental aquaria. Water samples for chemical analysis were monitored twice weekly. In all treatments, dissolved oxygen concentrations ranged from 4.5 to 5.1 mg/L. The water temperature was approximately stable for the experimental duration (23±1^{o}C), pH ranged from 7.5 to 8.2, and free ammonia concentration was less than the critical level (<0.3 mg/l). Total alkalinity and total hardness were ranged from 150 to 180 mg/L as $CaCO_3$, and from 140 to 160 mg/L as $CaCO_3$, respectively. Photoperiod was maintained at 12 h light and 12 h dark.

Determination of the 96 hour half lethal concentration (96 hr LC50)

The 96 hour half lethal concentrations (96 hr LC_{50}) of Butox 5% (deltamethrim 5%) from Arab Company for chemical industries, Egypt; were determined for *O. niloticus* according to Behrens and Karbeur (1953). A hundred of previously acclimatized *O. niloticus* were equally divided into ten groups and distributed in 100 liter glass aquaria filled with dechlorinated tap water containing 0, 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32, 0.36 ppm of deltamethrin, respectively and continuous aeration. In all aquaria, pH was 7.5, the dissolved oxygen concentrations was 5 mg/L. The water temperature was 23^{o}C. Fish were starved during the experiment. Gross mortality in each group was recorded and removed every 8 hr for 96 hr (Table, 1).

The intoxicated fish was observed to clinical and postmortem alterations according to Amlacher (1970).

Medicated ration

The humic acid was kindly obtained from Ibn ElWaled Co., Egypt, sprinkled on dry powdered commercial ration to provide a final concentration of 1% according to Abel-Wahab *et al.* (2012).

Experimental Design

A total of 300 *O. niloticus* were divided equally into six groups. Group (1) was the control. Group (2) was fed on a ration containing humic acid at concentration of 1% for 30 days. Groups (3&4) were subjected throughout the experiment to sublethal concentrations of deltamethrim at concentration of 0.02 and 0.05 ppm (1/10 and 1 / 4 Lc_{50}) respectively and

Table (1): Lc_{50} of deltamethrin in O. niloticus.

Group No.	No. of fish in each group	Conc.	No. of dead fish in each group	a	b	Ab
1	10	0.0	0	0	0.0	0.0
2	10	0.04	0	0.04	0.0	0.000
3	10	0.08	0	0.04	0.5	0.020
4	10	0.12	1	0.04	1	0.040
5	10	0.16	1	0.04	2.5	0.100
6	10	0.20	4	0.04	5	0.200
7	10	0.24	6	0.04	6	0.240
8	10	0.28	6	0.04	6.5	0.260
9	10	0.32	7	0.04	8.5	0.340
10	10	0.36	10	0.04	10	0.400

$\sum ab = 1.6$
$Lc_{50} = 0.36- 1.6 /10 = 0.36–0.16= 0.20$ ul/l.

fed on non-treated ration. Group (5&6) were fed on humates at 1% for one week only then exposed to deltamethrim at concentration of 1/10 and 1/4 Lc_{50} respectively and fed on non-treated ration (Table, 2). All groups were fed twice daily at rate of 2% of their body weight. Dead fish in each group were removed immediately.

Blood samples were collected from the caudal vein of three fish with three replicates for analyses at 7, 15 and 30 days of treatment. Activities of Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957). Serum glucose was determined using glucose kit according to Trinder (1969). Serum uric acid was measured according to Barham and Trinder (1972). Total serum protein was measured according

Table (2): *Summary of the experimental design.*

Groups	Treatment
G1	**Control (non-treated).**
G2	**Fed on humates at 1% of ration for 30 days.**
G3	**Exposed to 0.02ppm of deltamethrin.**
G4	**Exposed to 0.05ppm of deltamethrin.**
G5	**Fed on humates at 1% of for one week only then exposed to 0.02ppm of deltamethrin.**
G6	**Fed on humates at 1% of for one week only then exposed to 0.05ppm of deltamethrin.**

to Henry (1964). Serum albumin concentration was measured as described by Gustafsson, (1976). All kits used were produced by Egyptian American Co. for Laboratory Services, Egypt. Serum globulin was calculated by subtracting the concentration of albumin from that of the total protein (Coles, 1986). Serum lysozyme was determined by turbidometric assay according to Sankaran and Gurnani (1972).

Statistical analysis

The data were statistically analyzed using Duncan's multiple range tests to determine differences in means (Duncan, 1955).

RESULTS

The 96 hr LC_{50} of deltamethrim was recorded to be 0.20 ppm (Table 1). Clinical picture of *O. niloticus* exposed to toxicity was increased mucus secretion, respiratory distress, sluggish movements, restlessness and nervous manifestation with erected pectoral fins. Postmortem changes showed congested internal organs and yellowish ascetic fluid.

The results of the current study were summarized in Tables 3, 4, 5, 6, 7, 8, 9&10. The data presented in (Table 3&4) showed that deltamethrin significantly ($P < 0.05$) reduced serum AST and ALT enzyme activities at all weeks of the experiment in compared to control and humic treated groups. However, there is significant ($P < 0.05$) differences in-between pre-treated fish by humus before exposure to pollutant concerning the relevant enzyme activities at all weeks of exposure.

Table (3)*: Changes in the activity of AST of O. niloticus after different treatment for 30 days.*

Group	Time		
	7 days	15 days	30 days
G1	26.3 ± 1.85[a]	24.79 ± 2.31[a]	23.18 ± 2.21[a]
G2	23.68 ± 1.32[a]	23.4 ± 1.46[ab]	20.38 ± 1.25[ab]
G3	10.43 ± 2.10[b]	9.14 ± 1.26[c]	8.32 ± 1.21[c]
G4	10.05 ± 2.06[b]	10.79 ± 2.18[c]	8.34 ± 1.46 [c]
G5	23.68 ± 1.32[a]	20.51 ± 1.34[ab]	19.4 ± 1.31[ab]
G6	23.68 ± 1.32[a]	18.97 ± 2.03[b]	17.08 ± 1.48[b]

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE

Table (4): ***Changes in the activity of ALT of O. niloticus after different treatment for 30 days.***

Group	Time		
	7 days	15 days	30 days
G1	23.34 ± 1.32[a]	22.98 ± 1.34[a]	22.89 ± 1.38[a]
G2	23.31 ± 1.3[a]	23.42 ± 1.32[a]	23.51 ± 1.22[a]
G3	12.06 ± 1.52[b]	11.80 ± 1.5[b]	11.57 ± 1.42[b]
G4	9.91 ± 1.49[b]	9.60 ± 1.2[b]	9.41 ± 1.15[b]
G5	23.31 ± 1.3[a]	20.89 ± 1.06[a]	20.76 ± 1.80[a]
G6	23.31 ± 1.3[a]	18.78 ± 4.56[a]	20.2 ± 4.37[a]

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE

Table (5): *Changes in the level of glucose in O. niloticus after different treatment for 30 days.*

Group	Time		
	7 days	15 days	30 days
G1	56.6 ± 5.26[b]	55.91 ± 6.4[b]	56.85 ± 5.09[cd]
G2	51.94 ± 5.44[b]	49.49 ± 4.83[b]	46.76 ± 4.91[d]
G3	92.45 ± 8.4 [a]	83.44 ± 17.17 [ab]	94.98 ± 11.90 [ab]
G4	96.64 ± 11.16[a]	100.7 ± 9.45[a]	110.06 ± 5.23[a]
G5	51.94 ± 5.44[b]	69.35 ± 6.819[ab]	71.04 ± 5.66[bcd]
G6	51.94 ± 5.44[b]	77.65 ± 13.38 [ab]	92.08 ± 13.52[bc]

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE

Table (6): Changes in the level of uric acid in O. niloticus after different treatment for 30 days.

Group	Time		
	7 days	15 days	30 days
G1	2.08 ± 0.11^{c}	2.16 ± 0.17^{bc}	2.13 ± 0.16^{bc}
G2	1.79 ± 0.18^{c}	1.77 ± 0.23^{c}	1.67 ± 0.28^{c}
G3	3.29 ± 0.19^{a}	3.37 ± 0.19^{a}	3.45 ± 0.21^{a}
G4	2.82 ± 0.09^{b}	3.47 ± 0.18^{a}	3.52 ± 0.19^{a}
G5	1.79 ± 0.18^{c}	2.27 ± 0.13^{bc}	2.47 ± 0.18^{b}
G6	1.79 ± 0.18^{c}	2.37 ± 0.19^{b}	2.62 ± 0.19^{b}

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE.

The fish exposed to different concentrations of deltamethrin indicated a sharp increase in the glucose values compared to control and humic treated fish (Table 5). However, glucose levels were lower in the pre-fed humate groups than deltamethrin exposed fish and higher than control group.Table (6) illustrated that uric acid values were significantly increased in toxicated fish by deltamethrin, while significantly decrease in humate treated group. In addition the pre-treated fish before exposure to deltamethrin showed lower uric acid values.

Results of Tables (7), (8) and (9) recorded that total protein; albumin and globulin were significantly lowered in deltamethrin exposed fish and gradually decreased by time compared to control and humic treated groups. Table (9) demonstrated that globulin was increased in humates treated group than other groups. Also, the addition of humic substance to the ration then exposed to pesticide improved significantly the estimated parameters as compared to fish groups exposed to pollutant only.

The current investigation showed that the serum lysozyme was no significantly increased in humic treated group in comparison with control (Table, 10). The data revealed that deltamethrin significantly ($P < 0.05$) reduced serum lysozyme at all weeks of the experiment in relation to the control values. The results were more pronounced in higher dose and in the last period of investigation. On the other hand, the pre-feeding of fish on immunostimulant maintained these levels to the normal values.

Table (7): ***Changes in the level of total protein in O. niloticus after different treatment for 30 days.***

Group	Time		
	7 days	**15 days**	**30 days**
G1	4.21 ± 0.27^{a}	4.64 ± 0.38^{a}	4.31 ± 0.44^{a}
G2	4.58 ± 0.24^{a}	4.39 ± 0.23^{a}	4.85 ± 0.25^{ab}
G3	2.98 ± 0.29^{bc}	2.25 ± 0.31^{b}	2.14 ± 0.25^{c}
G4	2.00 ± 0.28^{c}	2.04 ± 0.30^{b}	1.52 ± 0.19^{c}
G5	4.58 ± 0.24^{a}	3.92 ± 0.05^{a}	3.88 ± 0.05^{ab}
G6	4.58 ± 0.24^{a}	3.86 ± 0.08^{a}	3.78 ± 0.08^{bc}

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE

Table (8): ***Changes in the level of albumin in O. niloticus after different treatment at different time.***

Group	Time		
	7 days	**15 days**	**30 days**
G1	$\mathbf{2.87 \pm 0.71^{a}}$	$\mathbf{2.36 \pm 0.51^{a}}$	$\mathbf{2.27 \pm 0.37^{a}}$
G2	$\mathbf{2.08 \pm 0.71^{ab}}$	$\mathbf{1.92 \pm 0.51^{ab}}$	$\mathbf{2.20 \pm 0.37^{a}}$
G3	$\mathbf{1.61 \pm 0.21^{b}}$	$\mathbf{1.31 \pm 0.18^{bc}}$	$\mathbf{1.26 \pm 0.16^{b}}$
G4	$\mathbf{1.12 \pm 0.04^{b}}$	$\mathbf{0.92 \pm 0.11^{c}}$	$\mathbf{0.83 \pm 0.07^{a}}$
G5	$\mathbf{2.08 \pm 0.71^{ab}}$	$\mathbf{1.98 \pm 0.3^{ab}}$	$\mathbf{1.98 \pm 0.01^{a}}$
G6	$\mathbf{2.08 \pm 0.71^{ab}}$	$\mathbf{2.07 \pm 0.09^{ab}}$	$\mathbf{2.03 \pm 0.21^{a}}$

-Means with the same letter in the same column are not significantly different at P<0.0.
Data are represented as Mean ±SE

Table (9): Changes in the level of globulin in O. niloticus after different treatment for 30 days.

Group	Time		
	7 days	**15 days**	**30 days**
G1	1.75 ± 0.33^{ab}	2.1 ± 0.16^{ab}	2.05 ± 0.38^{ab}
G2	2.53 ± 0.23^{a}	2.86 ± 0.34^{a}	2.65 ± 0.13^{a}
G3	1.36 ± 0.22^{bc}	1.05 ± 0.27^{dc}	0.87 ± 0.21^{c}
G4	0.89 ± 0.27^{c}	0.81 ± 0.24^{d}	0.69 ± 0.12^{bc}
G5	2.53 ± 0.23^{a}	1.95 ± 0.32^{b}	1.77 ± 0.10^{b}
G6	2.53 ± 0.23^{a}	1.79 ± 0.17^{bc}	1.76 ± 0.17^{b}

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE

Table (10)*: Changes in the activity of lysozyme in O. niloticus after different treatment for 30 days.*

Group	Time		
	7 days	**15 days**	**30 days**
G1	$\mathbf{0.53 \pm 0.05^{a}}$	$\mathbf{0.51 \pm 0.04^{a}}$	$\mathbf{0.52 \pm 0.05^{a}}$
G2	$\mathbf{0.64 \pm 0.04^{a}}$	$\mathbf{0.63 \pm 0.03^{a}}$	$\mathbf{0.65 \pm 0.03^{a}}$
G3	$\mathbf{0.36 \pm 0.03^{b}}$	$\mathbf{0.34 \pm 0.04^{b}}$	$\mathbf{0.30 \pm 0.03^{b}}$
G4	$\mathbf{0.32 \pm 0.04^{b}}$	$\mathbf{0.23 \pm 0.03^{b}}$	$\mathbf{0.21 \pm 0.03^{c}}$
G5	$\mathbf{0.64 \pm 0.04^{a}}$	$\mathbf{0.58 \pm 0.05^{a}}$	$\mathbf{0.58 \pm 0.04^{ab}}$
G6	$\mathbf{0.64 \pm 0.04^{a}}$	$\mathbf{0.53 \pm 0.04^{a}}$	$\mathbf{0.56 \pm 0.04^{b}}$

-Means with the same letter in the same column are not significantly different at P<0.05.
- Data are represented as Mean ±SE

DISCUSSION

The use of Tilapia for culture is the dominating system and is increasing rapidly. The contamination of ecosystem by pesticides is known to have deleterious effects to physiological and health status of aquatic animals (Banaee *et al.,* 2011 and Hedayati *et al.,* 2012). Deltamethrin is among the most used pyrethroids over the world, because it is less toxic to mammalians and birds than other insecticides. Nevertheless, some studies have reported toxic effects of pyrethroids on non-target organisms (Amin and Hashem, 2012 and Richterová and Svobodová, 2012).

Acute toxicity data for deltamethrin in fish have been published as a report of the World Health Organization (1990) and classified as highly toxic to fish, being in the range of LC50<100 µg/l. The 96 hr LC_{50} of deltamethrim was 0.20 ppm for *O. niloticus*. Acute toxicity of different insecticides is influenced by the age, sex, genetic properties and body size of fish, season, water quality and its physicochemical parameters, and purity and formulation of insecticides (Banaee *et al.,* 2011 and Richterová and Svobodová, 2012). Previous studies indicated the high toxicity of deltamethrin to different fish species. Mestres and Mestres (1992) estimated 96-h fish LC_{50} value as 0.39 mg/L for *Salmo gairdneri*. El-sayed *et al.* (2007) recorded that the 96hLC_{50} value of deltamethrin for monosex tilapia was 14.6 µg/L. Deltamethrin is highly toxic to fish; even minute concentration (0.01 mg/l) of deltamethrin could cause 50% mortality of *Clarias gariepinus* in 24 hr (Datta and Kaviraj, 2003). Atta (2010) noticed that the LC_{50} for *O. niloticus* was proved to be 50 µg/L. Khalili *et al.* (2012) indicated that deltamethrin is highly toxic to Swordtail Fish (*Xiphophorus helleri*) (Lc_{50} 2.87 ppm). Hedayati *et al.* (2012) found that the toxicity of deltamethrin on blue gourami increased with increasing concentration and exposure time (LC_{50} 0.223 ppm). Richterová and Svobodová, (2012) mentioned that the value 96 h LC_{50} was under 10µg/L in fish generally. They added that pyrethroids had been shown to be up to 1000 times more toxic to fish than to mammals and birds.

The intoxicated fish exhibited abnormal behavioural and postmortem changes. Similar pictures were noticed by El-sayed and Saad (2007) and El-sayed *et al.* (2007); Atta (2010); Banaee *et al.* (2011) and Richterová and Svobodová (2012) who mentioned that behavioral changes are the most sensitive indicators of potential toxic effects. The behavioral and the swimming patterns of the fish exposed to different insecticides include

changes in swimming behavior and feeding activities.

Different biochemical markers can be examined to evaluate the exposure or the effects of medicated ration and/or toxicants. They can provide useful information about the health status of fish and can be used as early indicators of stressors. From data listed in Tables, 3, 4, 5, 6, 7, 8, 9&10, it could be noticed that the use of humates as feed additive improved the general health conditions and immunity of *O. niloticus*. Similar observations were noticed by Kodama *et al.* (2007); Kodama *et al.* (2008); Meinelt *et al.* (2008); Nakagawa *et al.* (2009) and Abel-Wahab *et al.* (2012).

On the other hand, the present study provided an evidence that deltamethrin caused alterations in the physiological and biochemical parameters in vital tissues which influences the survival of fish. Metabolic enzymes (ALT and AST) are liver specific enzymes and they are more sensitive measure of hepatotoxicity. The present results demonstrated that deltamethrin significantly decreased the activities of AST and ALT. The liver is the target organ for detoxifying deltamethrin in fishes by influencing the activities of several enzymes (Sharma and Ansari, 2011). These findings are supported by Atta (2010) and Banaee *et al.* (2011) who mentioned that in some cases due to the severity of the damage to tissues, particularly liver, synthesis of many biochemical parameters may reduced significantly in cells, which can decrease some biochemical factors in blood of fish exposed to insecticides.

Deltamethrin treated groups showed hyperglycaemic effect when compared to other groups. This view was in agreement with Nayak *et al.* (2004); El-sayed and Saad (2007); Atta (2010) and Richterová and Svobodová, (2012) who found that reduction in hepatic glycogen accompanied by increased level of plasma glucose is a common reaction of fish against xenobiotic insult followed by metabolic stress . They added that the hyperglycaemic effect after pyrethroid treatment suggests effects on the glycogenesis and glycolytic pathways. Wherein, the long-lasting stress caused by deltamethrin resulted in an increase in the synthesis of adrenocorticotropic hormone and glucagon and decrease in the synthesis of insulin.

The data revealed that deltamethrin exposed groups significantly elevated urea levels in comparison with other groups. These results went hand in hand with those reported by Atta (2010) and Amin and Hashem, (2012) who cited that this elevation may be due to correlation between urea and increased protein catabolism or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production.

The immune system of fish is important for defense against a variety of stressors. *O. niloticus* exposed to deltamethrin caused adverse effects in the form of hypoproteinemia, hypoalbuminemia and a great reduction in both globulin and lysozyme levels. This was in accordance with that reported by El-sayed and Saad (2007); El-sayed *et al.* (2007); Atta (2010); Sharma and Ansari (2011) and Amin and Hashem (2012) who noticed that different insecticides at sub-lethal levels have been recognized as stressors causing immunesuppression in fish. This decrease in plasma protein could be attributed to changes in protein and free amino acids metabolism and their synthesis in liver. Also, the observed decreases in plasma proteins could be attributed to the damaging effect of deltamethrin on liver cells. The exposure to sub-lethal concentrations of insecticides is what probably makes fish vulnerable to infectious diseases because of their immune-depressive effects (Zelikoff *et al*., 2000). Sharma and Ansari (2011) found that the protein responses appeared particularly suitable for measuring stressful levels of pollutants and have long been used as indicators of stress in fish. Fish seem to be deficient in the enzyme system that hydrolyzes pyrethroids (Viran *et al.,* 2003).

The current investigation demonstrated that humates had protective effect against pollutant as indicated in different measured parameters which maintained to normal values. Hall and Mirenda (1991) demonstrated that the toxicity of a polymer to *Daphnia pulex* and the fathead minnow (*Pimephales prometas*) was progressively reduced by the addition of humic acid to the dilution water. The treatment with humic acid in protocol reduces the toxicity of acriflavine to juvenile zebrafish *Danio rerio* (Meinelt *et al.*, 2002). Mézin and Hale (2004) observed that humic acids had no effect on mortality of *Americamysis bahia* for chlorpyrifos or DDT at a salinity of 20%, but greatly reduced the mortality of *Cerio dubia* for both pesticides in freshwater (0%). In the latter case, the effect was proportional to the humic acids concentration. Perminova and Hatfield (2005) mentioned that the biological activity of humates resulting from direct interactions with living organisms through adsorption on cell surface or by penetration into the cell. These chemical-biological interactions provide for increasing interest to remedial uses of humic materials. Osman *et al.* (2009) found that the treatment of fish with 30 and 50 mg/l humic acid significantly reversed the effects of the cadmium toxicity. Abel-Wahab *et al.* (2012) observed that the use of humic acid as feed additive improved nonspecific immune response against toxicity and disease resistance in common carp fish (*Cyprinus carpio*).

In the light of the current investigation being conducted, deltamethrin is highly toxic to fish even in very low concentration. In assessing the toxic effects of pesticides

in aquatic organisms the use of biochemical parameters had been useful in diagnosis. Also, these results suggested that pre-feeding of this eco-friendly immunostimulant material will be protective for fish exposed to environments contaminated with pesticides.

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تأثير حامض الهيوميك في حماية أسماك البلطي النيلي ضد سمية مبيد دلتا مثرين

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اجريت هذه الدراسة لمعرفة التأثير السام لمبيد الدلتا مثرين على أسماك البلطى النيلي مع الأشارة الي دور الهيوميك كأحد منشطات المناعة الطبيعية لوقاية الأسماك من التسمم بالمبيد. تم تعيين الجرعة المميتة للنصف خلال 96 ساعة لمبيد الدلتا مثرين لسمكة البلطى فكانت 0.20 جزء فى المليون.

تمثلت العلامات المرضية والصفة التشريحية أثناء التسمم الحاد فى فقدان فى الشهية واضطرابا فى سلوك الأسماك وزيادة فى إفراز المخاط ووجود أعراض سوء تنفس وأعراض عصبية أوضحت الصفة التشريحية عن وجود تغيرات مرضية فى صورة أحتقان للأعضاء الداخلية مع وجود سائل مائي أصفر في التجويف البرتوني.

تم إجراء هذه الدراسة علي عدد 300 سمكة من أسماك البلطى. تم توزيع الأسماك فى ست مجموعات بالتساوى في أحواض زجاجية حيث تركت المجموعة الأولى (المجموعة الضابطة) للمقارنة. أما المجموعة الثانية فقد غذيت على عليقة تحتوى على حامض الهيوميك بنسبة 1% لمدة 30 يوما. أما المجموعتن الثالثة والرابعة فقد تم تعريضهما لتركيز تحت مميت من مبيد الدلتا مثرين 0.02 و 0.05 جزء فى المليون (1/10و 1/4 نصف الجرعة المميتة) على التوالى. وتم تغذيتهما على عليقة ضابطة. المجموعتان الخامسة والسادسة فقد تم تغذيتهما لمدة أسبوع على عليقة تحتوى على حامض الهيوميك بنسبة 1%, ثم تعريضهما لتركيز تحت مميت من مبيد الدلتا مثرين (1/10و 1/4 نصف الجرعة المميتة) على التوالى مع تغذيتهما على عليقة

ضابطة باقى مدة التجربة. تم الحصول على عدد 3 أسماك عشوائيا بثلاثة مكررات من كل مجموعة عند ايام 7, 15 و 30 يوم من المعاملة للحصول على عينات دم ومصل.

اوضحت الدراسة الى ان خلط الهيومات بالعليقة قد أدى الى تحسن فى الصحة العامة والوظائف المناعية فى أسماك البلطى النيلي. ولقد أحدثت الجرعات تحت المميتة من الدلتا مثرين نقصا ذا دلالة فى نشاط الإنزيمات الناقلة لمجموعات الأمين AST, ALT ، بينما سجلت النتائج ارتفاعا ذا دلالة في مستوى الجلوكوز وحمض اليوريك . أظهرت الدراسة ان لمبيد الدلتا مثرين تأثر سئ للغاية على المناعة حيث قللت بصورة معنوية كلا من مستويات البروتين الكلي والألبيومين والجلوبيولين وانزيم اليزوزيم. اوضحت الدراسة ان التغذية المسبقة علي حامض الهيوميك له تأثير قوى وفعال فى رفع حيوية ومناعة أسماك البلطى النيلى ضد التسمم بمبيد دلتا ميثرين وذلك طبقا للقياسات المعملية.

نستخلص من هذه الدراسة ان مبيد الدلتا مثرين سام جدا لأسماك البلطى النيلى. وان القياسات البيوكيميائية من الممكن استخدامها فى التشخيص المبكر لتسمم الأسماك. كما اننا ننصح باستخدام (الهيوميك) هذه المادة الصديقة للبيئة بعد خلطها بالعليقة لحماية الأسماك من الآثار الضارة للملوثات لتاكيد الحفاظ على ربحية المزارع السمكية.