Biochemical, Clinicophathlogical and Microbial Changes in Clarias Gariepinus Exposed to Pesticide Malathion and Climate Changees

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Abstract: The effect of Malathion on Biochemical changes in catfish (Clarias gariepinus) after exposure to Malathion 4.5 mg/l for 98 hours and high temperature 30◦. The obtained results showed significant increase in cooper, sodium, cortisol, urea as well as ALT and AST. It was concluded that Malathion produces metabolic stress, cell damage with malfunction of haemopoeitic system. The microbiological examination revealed presence of E-coli, Acromonas Sp, Vibrio. We can conclude that in fish reared on low CHO diet there was hyperglycemia due to increase in insulin and cortisol hormone. (Macrocytic hypochromic anemia was observed in fish in 38H and 98H treatment of Malathion. The hemogram shows increase in MCV and decrease of HB%, PCV and RBC's count. There is decrease in IgM. There was petichial haemorage in some part of skin, ascites and erosion due to complications of bacterial infections and there is vertebral column curvature syndrome. [Report and Opinion, 2009;1(6):6-11]. (ISSN 1545-4570).

Key words: Malathion Biochemical changes, Haematological changes, Microbial changes, IgM.

1. Introduction

The organophosphorous insecticide malathion (Fig. 1) is used to control pests, which attack many economic crops, (Anderson,1990). In Egypt, the Ministry of Agriculture recommended the use of malathion against pests which attack vegetable crops, ornamental plants, medicinal and aromatic plants and for protection of stored grains. Malathion is also used to control different mosquito and fly species, household insects, animal ectoparasites and human head and body lice (Roberts, 1989). The wide use of malathion is attributed to its relatively low mammalian toxicity. But like DDT and other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than the product label would lead one to believe.

Shown to be mutagenic, a possible carcinogen, implicated in vision loss, causing myriad negative health effects in human and animal studies, damaging to nontarget organisms, and containing highly toxic impurities, malathion has a legacy of serious problems (Cabello *et, al.* 2001).

According to a report by the Washington, D.C. based group, *World Resources Institute* (WRI), many pesticides appear to be increasing the incidence of infections, pneumonia, ear infections, and tuberculosis. The three pesticides listed as causing this problem were DDT, malathion, and the pesticide aldicarb (Breener, 1992).

The environmental protection agency (EPA) has been stating for years that they would require more detailed tests for chemical effects upon the immune and nervous system. However, to date, these requirements have not been implemented. Perhaps the biggest unknown risk from malathion is its potential to increase risk of contracting bacteria or viral infections such as encephalitis, this paradoxical situation arises since exposure to malathion can weaken a person's immune system (Giri *et, al*. 2001).

Other effects of malathion for which there is no research, but seriously needed include its ability to cause: Learning disabilities, short term memory damage, increase risk of allergies (Cabello *et, al.* 2001).

Research has accumulated which indicates that nutritional factors can significantly modify the host response to environmental toxicants. Correction of malnutrition can clearly mitigate the effects of many toxicants; however, evidence is mounting that supraphysiologic doses of nutrients (nutritional supplements) can further lessen toxicity. The possibility that nutrition could be implemented as a secondary

prevention strategy on a public health scale raises important ethical and policy issues. Nutritional strategies can lessen, but not abolish, toxic effects; moreover, they require dissemination and compliance, which are unlikely to be fully effective (Hu *et, al.*1995).

Malathion accumulate in fish mainly in the visceral fat, where as the gills and muscles retain a lower amount subsequently, with an increase in fat consumption. For example, at the time of migration and hibernation, pesticides may enter the more sensitive organs and induce poisoning (Bruno and Stamps, 1987), (Barton and Iwama, 1991), (Bennett and Wolke, 1987) (Pickering and Duston, 1983). It has been presumed for decades that environmental pollutants especially pesticides can affect one or more of the immunological functions in the fish. It is almost common knowledge cat fish frequently then become more susceptible to various diseases given the extreme variety of pesticides used (Vergut and Studnicka, 1994), (Areechon and Plumb, 2000). Andreson, 1990 suggested a decreased disease resistance in fish exposed to various pesticides. There is so little is known about how pesticides affect the immune systems of fishes (Cabello *et, al.* 2001).

CH_3 O P - S – CH – CO_2– C_2H_5
CH_3 O CH_2 – CO_2 – C_2H_5

Fig. 1: "Malathion"

The chemical name is : S-1 2 bis (ethoxycaronyl) ethyl 0,0-dimethyl-phoshordithioate (IUPAC).
-Trade names : Malathion, Cythion, Fyfanon and Calmathion (Royal Society of Chemistry, 1993).

The present study discusses the effect of low CHO diet on cat fish, which also exposed to Malathion (4.5 mg/l) as pesticide for 98H. Some microbiological and clincopathological parameters were interpreted.

2-Material and methods

Experimental condition: Catfish (50 – 60 gram/each) were obtained from River Nile Rashid branch, El-Kanater El-Khyria. Fish were acclimatized to laboratory conditions one week before infection in 115 L. glass aquaria with a flow system and dechlorinated tap water. Two groups of fishes were used.

The 1st group (15 fishes) was maintained kept on low carbohydrate diet but free from any toxicants, and kept on a balanced diet that meets its requirements from nutrients as described by (Robberts, 1989).

The 2nd group (15 fishes) were kept under the low CHO diet but were exposed to Malathion (4.5 mg/l) during 98 H hours (Areechon and plumb 1990). The diet ingredient is shown in (Table 1).

Copper, dissolved oxygen, temperature, pH, ammonia and nitrites were analyzed daily, while water alkalinity, hardness carbon dioxide, sodium, potassium and chlorides were analyzed before and after each water renewal using commercial kits of Bohringer, France, as shown in (Table 2). Malathion was obtained from National Institute of Pesticide, Dokki, Cairo. The 98h LC50 of Malathion for channel cat fish determined in separate study was 9.65 mg (Areechon and Plumb, 1990).

Table (1): Ingredients and proximate chemical composition of diets used in experiments.

Ingredients	Diets I (control)	Diets 2
Fish meal	30	30
Meat meal	8	10
Bone meal	1	3
Skimmed milk	3	4
Soybean	5	7
Wheat bran	20	20
Wheat flour	20	5
Yeast	10	15
God liver oil	1	4
Minenral & Vitamin* premix	**2**	2

Proximate chemical composition:

Crude protein (CP) %	35.87	38.89
Metabolizable	2297.21	2415.4
energy/kg	2.78	2.86
Ether extract (EE) %	3.91	4.27
Grude fiber (CF) %	8.735	10.25
Ash %	3.094	3.99
Calcium (Ca) %	2.069	2.53
Phosphorous (Ph) %	2.105	2.29
Lysine %	0.562	0.613
Methionine %		

*** Mineral and vitamin premix per/kg of pelleted food :**

Vit.A, 8000 U; Vit. D, 9001, Vit. E 21 U, vit. K, 4 mg; Vit. B2 3.6 mg; niacin 20 mg choline chloride, 160 mg; pantothenic acid, 7 mg; pyridoxine, 0.2 mg; Vit. B 12, 5 ug; Mn, 70 mg, Zn 60 mg, Fe 20 mg, Cu 2 mg, I 1 mg, Co 0.2 mg.

Blood sampling :

Blood samples were taken after 24h, 38h, 98h. The fish were anaesthetized by 1/1000 aqueous solution of Ms 222 and bled from the caudal vein. Blood samples were taken with heparinized microhaematocrit

tube. The tubes were centrifuged at 3000 r.p.m. for 10 min. Serum was separated and stored at 20°C until used.

Table (2) : Water quality characteristics in tanks. Initial conditions values are mean ± SE.

pH	5.40 ± 0.1
Temperature °C	18°C ± 0.904
Nitrates mg/l	0.020 ± 0.04
Un ionized ammonia (mg/l)	0.0014 ± 0.004
Carbonic dioxide (mg/l)	4.1 ± 0.5
Alkalinity (mg/l)	31.8 ± 2.8
Permanganate oxidabole matter (mg/l)	3.54 ± 0.53
Hardness (mg/l)	34.6 ± 0.1
Chlorides (mg)	8.4 ± 0.6
Potassium (mg/l)	0.12 ± 0.007
Sodium (mg/l)	5.68 ± 0.01

Tested kits supplied form biomerieux (France) were used for determination of the activity of serum glutamic pyruvic transaminase (ALT) and glutamic oxaloacetic transaminase (AST) as described by (Reitman and Frankel, 1957).Serum glucose was assessed according to (Trinder, 1969). Haematocrit value was carried out by using micro-haematocrite capillary tubes centrifuged at 1200 r.p.m. for 5 min. mean corpuscular volume (MCV). Reticulocytis count according to (Drabkin, 1946). Serum cortisol level was determined using radioimmunoassay technique according to the method of (Pickering and Pottinger, 1983). Serum iron were determined using atomic absorption according to (Barham *et al*., 1972). Values of sodium and potassium in serum were determined by flame photometer according to method described by (Silversmit, 1965). Serum creatinine was measured according to (Bartels *et al*., 1972). Enzymatic determination of urea was done according to (Patton and Crouch, 1988). Insulin was estimated by radioimmunoassay method using oat. A Cout insulin Kits obtained from Diagnostic Corporation (DPC) west 96th street, Los Ageles U.S.A. (Pickering and Duston, 1983).

Bacterial Isolation:

Aseptic swabs from the skingills, base of fins and blood of tested fish were cultivated on blood agar, MacConky agar, Nutrient agar, TSA, Nutrient broth, and peptone water (Oxoid and Difco).

Inoculated media were incubated at 37°C for 48 hours. Bacterial isolates were identified by examination of the colony morphology and biochemical characteristic described by (Nagae *et al*., 1993). Bacteria were detected by accounting colonies using surface spread plate technique according to quantitative method described by (Bruno & Stamps 1987).

Measurement of serum immunoglobulin M (IgM) :

IgM determination :

The serum IgM was measured according to (Fuda *et al.* 1991).

Preparation of antisera :

Antisera of cat fish was prepared by immunizing rabbits as described by (Hara, 1976).

(Cat fish) IgM antibody :

The procedure for labeling antibody of fragment with enzyme was performed according to the method of (Nagae *et al.*, 1993).

Elisa assay procedure :

Assays were carried out in 96 well polystyrene ELISA microtiter plates (Titertex, Horsham, PA).

Antibody coating :

The micortiter plates were coated with rabbit Anticat fish IgM which was fractionated by DE-52 at a concentration of 40 ug/ml in 0.01 M PBS. A volume of 150 µl was dispensed into each well and incubated for 4 hr at 4°.

Blocking :

After one washing with 200 µl of 0.01 M PBS + 0.1 % Tween 20 per well and two washings with 200 µl of PBS + 1 % thimerosol was added to each well and included for 2hr at room temperature.

Incubation of samples and standards:

After washing as described above 100 µl of sample and standard were placed into the appropriate wells in the microtiterplates and incubated at room temperature.

Incubation with peroxidase labeled antibody:

After washings as described above, each well received 150 µl of peroxidase labeled antibody 1:1600 in PBS-BSA, followed by incubation 12 hr at room temperature.

Enzymatic color reaction:

The plates were washed as described above and 150 µl 0-phenylenediamine (3 mg/ml 0.1 M citric acid-phosphate buffer (pH 5.0) containing 0.02 % H2O2 were added to each well for enzymatic color reaction. The reaction was stopped after 30 min at room temperature by adding 100 µl of 4NHCI. The absorbance at 492 nm was 2250 (Richmond, CA).

Double antibody sandwish Elisa according to the method of (Matsubara *et al.* 1985) for determination of IgM described. After one washing with 200 µl of 0.01

M PBS + 0.1 % Tween 20 per well.

3-Statistical analysis:

The obtained data were statistically subjected to the students't-test (Gad and Weil, 1983).

4-Results :

Experimental exposure to Malathion (4.5 mg/l) revealed that there was a significant increase in the level of serum creatinine ALT, AST, urea, potassium and insulin, were increased in the 24 hours and 38 h and 98 h non-significantly. There was a significantly increase of cortisol, glucose, copper during all times of experiments. Concerning iron there was a significant decrease of iron level (Table 3).

Hematological results in the present work revealed anemia indicated by a significant reduction in RBCs count, HB concentration PCV %, MCV and increase in reticulocyte count especially in 38 h, (Table 4).

Concerning microbiological examination, the results showed that the isolated microorganisms from internal organs (liver and kidneys), gills, and fins were Aeromonas Vibrio Sp and E. coli. (Table 5). With regard to IgM, a significant decrease was observed by exposing cat fish to the pesticide. (Table 3).

There was petichial haemorhage in some part of skin and erosion due to complications of bacterial infection and vertebral column curvature syndrome.

5-Discussion:

It was evident that decrease in the level of CHO with Malathion 4.5 mg/l in fish diet caused a significant increase in glucose level during the experimental period, also insulin level was slightly increased. It is well known that any stress factor such as handling, incubation, anaestesia etc. has been shown to cause hyperglycemia followed by hyperinsulinemia (Yallow and Bawman, 1983).

Low CHO diet with Malathion causes a significant increase of cortisol level which may be due to the activation of hypothalamus, pituitary internal axis. Induced a significant increase in cortisol level, these results coincide with those observed by (Barton and Iwama, 1991), who observed that serum cortisol increased linearly in salminid fish fed on 5% CHO diet.

One consistent effect of cortisol was the reduction in the hemoglobin, PCV % and iron levels, as a result of decrease in appetite in the rainbow trout, or more likely to be the direct result of a catabolic effect, or cortisol of the fish tissues.

This present study revealed that, sodium and potassium concentrations were significantly increased. This retention may be attributable to kidney impairment where the kidney is the normal pass way for Na and K this may explain the main cause for elevation of the serum creatinine and urea in the treated groups.

Marked elevation was noticed in the activity of Asparate Amino Transferase (AST) and Alanine Amino Transferase (ALT). The liver is the primary organ of detoxification as well as a major site for detoxification reaction. Therefore, significant increase in the liver enzymes suggests explanation Malathion affected the liver cells or may be attributed to secondary bacterial infection.

The present results agree with (Bruno & Stamps 1987) they observed that aquatic pollution with heavy metals cause immunosuppression and contribute to outbreaks of infections, and bacterial diseases in fish. We can say that Malathion can affect fish after 24h and there are some complications with this pesticide.

IgM level was determined to find out information about fish immune system, which was previously investigated in different species by many authors as (Matsubara *et al*., 1985) and (Fuda *et al.*, 1991).

There is a significant decrease in IgM level in fish with Malathion, if compared with control groups. Anderson *et al.,* 1982 found a relation between corisol and IgM as when cortisol increased IgM decrease.

IgM is one of the most important factors in the immune factor to neutralize bacteria and render them more susceptible to phagocytosis (Mona S. Zaki *et al.,* 2003). It is well known that in mammals immunoglobulin production is closely related to endocrine status for example thyroid hormone enhances the production of immunoglobulin (Chen, 1980) cortisol intensity suppress immunoglobulins production (Pickering & Pottinger, 1983).

In conclusions Malathion will reduce humoral immune response as detected by decrease of IgM level and cortisol elevation.

6-Akhnowalgment

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7-Refrances

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Table (3): Effect of low CHO diet on some biochemical and hormonal parameters in cat fish exposed to Malathion (4.5 mg/l).

Parameters	Control	24 hours	38 hours	98 hours
AST (U/I)	77.0 ±0.53	80.7 ±0.60	83 ± 0.51	100±0.85**
ALT (U/I)	15.3 ± 0.33	17.4 ±0.24	18.9 ±0.83	23.0±0.83*
Urea (mg/dl)	4.3 ± 0.51	4.9 ± 0.76	4.9 ±0.83	5.1±0.83*
Creatinine (mg/dl)	0.67 ± 0.62	0.69 ± 0.72	0.90±0.51*	1.3±0.53*
Na (Meq/L)	117 ± 1.3	127 ± 2.3*	128 ± 4.5*	142±5.4*
K (Meq/L)	2.58 ± 0.12	3.65 ± 0.13	1.3 ± 0.8	5.00±0.80*
Cortisol (ng/dl)	0.85 ± 0.23	0.88 ± 0.54	0.91 ± 0.39	1.5±0.45**
Glucose (mg/dl)	52 ± 0.59	54.1 ± 0.50	68 ± 0.59	85±0.23**
Insulin (ng)	7.6 ± 0.3	8.5 ± 0.4	11.7 ± 2.4	12.8±0.63**
Copper (mg %)	181 ± 4.0	187 ± 2.3	168 ±1.3	148±0.45**
Iron (mg %)	190 ± 1.26	176 ± 5.2	168 ± 3.3	151±4.3*
IgM Ng/MI	0.85 ± 1.32	0.78 ± 1.20	0.63 ± 0.60	0.65±0.44*

*P < 0.01 **P < 0.05

Table (4): Effect of low CHO diet on hematological parameters in fish exposed to malathion (4.5 mg/l) for 98 H.

Time Groups	**Parameters**				
	RBCs (106/mm3)	HB gm/dl	P.V.C.(%)	MCV Fl.	Reticulocyte %
Control	3.2±0.34	7.7 ±033	8.7 ± 0.33	31± 0.51	1.21±0.2
24 hours	3.3±0.34	8.6 ± 0.10	8.6 ± 0.11	37 ±0.41	1.92±0.3
38 hours	3.9±0.63	8.7±0.17*	7.87±0.11	38±0.73	2.3±0.4
98 hours	3.7±0.62	8.3±0.28*	6.3±0.37*	39±0.83*	2.4±0.4*

*P < 0.01

Table (5): Bacterial isolates recovered from fish exposed to Malathion (4.5 mg/l).

Bacterial strain	**External surface**	**Kidneys**	**Liver**	**Gills**
E. Coli	2 X 10	3 X 10	2 X 10	1 X 10
Aeromons Sp.	3 X 10	6 X 10	3 X 10	2 X 10
Vibrio/ Sp.	2 X 10	1 X 10	2 X 10	2 X 10

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