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ASSESSMENT OF THE HAZARDOUS EFFECT OF FUNGICIDE DITHANE ON *CLARIAS lazera* (CATFISH) INCLUDING HAEMATOLOGICAL, BIOCHEMICAL, AND IMMUNOLOGICAL PARAMETERS

[69]

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ABSTRACT

The aim of present work was to study the toxicity of (mancozeb)Dithane fungicide on fish *Claras lazera* (catfish) and consequently to human beings.The fishes were exposed to Dithane in dose of 0.5 ppm /L (equivalent of 1/10 of LD50)for 30 days. Different Haematological, Biochemical, Bacteriological, and Immunological parameters were assessed. The results showed significant increase in Blood level of Sodium (Na), Potassium (K), Cortisol, Urea, Creatinine, Glucose, Insulin as well as Aspartate Amino Transferase (AST) and Alanine Amino Transferease (ALT)in blood. However there was a decrease in blood level of Iron and IgM, accompanied by decrease in Haemoglobin (HB), Macrocytic hypochromic anemia (R.B.Cs) count, Packed cell volume (PCV) which was observed in fish in 7, 15, 30, days after exposure to Dithane. The Haemogram shows reticulocytosis and increase in mean corpuscular volume (MCV). Dithane produces metabolic stress and cell damage with malfunction of haemopoetic system. Microbiological examination revealed a presence of pathogenic bacteria mainly *E. coli*, *Flavabacterium*, and *Staphylococcus aureus*. It was concluded that in catfish reared on low dietary carbohydrate (CHO) diet there was hyperglycemia due to increase in cortisol hormone. However immunological results revealed decrease in the level of IgM in blood; a loss of scales and petichial haemorrhage in parts of skin was observed. Ascitic and erosion due to complication of bacterial infection, was also accorded.

Key words: Dithane (mancozeb), Haematological change, Biochemical change Microbial changes, Immunoglobulin M (IgM)

INTRODUCTION

Environmental toxicology is the study of how ecological systems, their structure, dynamics and function are affected by pollutants. A developing subfield of environmental toxicology is ecotoxicology, in which special concern is placed on the release of toxic pollutants

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into the environment, especially into aquatic systems, by focusing on how these toxicants may become distributed within food chains and by measuring the toxic responses made within a particular ecosystem to such pollutants. These are by nature interdisplinary fields drawing their knowledge from ecology, organic chemistry, molecular biology, genetics, soil science, mathematics and so on. The main source of pollution of water bodies with pesticides is in the melt waters, rainwaters and underground waters. Pesticides, may reach water bodies through the air at the time of their application to objects located near by insecticides.

Insecticides are applied to water to prevent the development of aquatic phase or blood sucking insects. Pesticides reach water sources subsequently through the trophic chains and cycles of various substances. As a result of circulation of pesticides in a water body, they sometimes accumulate in fish, silt bottom, zooplankton, algae and aquatic plants. Fish had long been regarded as a highly desirable food due to its content of high quality animal protein, its calcium and phosphorus content as well as its generous supply of vitamins.

Organochlorinated pesticides accumulate in fish mainly in the visceral fat, whereas 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 **Dummer *et al* (1991), Barton and Iwama (1991), Clealand *et al* (1998), Bennett and Wolke (1987), and Mona *et al* (2003)**.

Compared to the controls some of the behavioral toxicity of fish showed decreased sheltering and increased horizontal displacements, burst swimming, buccal movements, and antagonistic interactions. The aim of this study is to detrmine the effect of low CHO diet on *Claras lazar*a fish, exposed to Dithane (0.5ppm) as fungicide, on some hematological and clinicopathological, microbiological and immunological parameters.

MATERIAL AND METHODS

Materials

Dithane was obtained from Central Agriculture Pesticide Laboratory, Dokki, Cairo. Dithane M.45 (mancozeb) 80% wp {[1,2-ethanediylbis(carbamodithioato)] (2-)} manganese mixture with{[1,2-ethanediylbis (carbamodithioato)](2-)} zinc.

$$[\text{-SCS NH CH}_2\ \text{CH}_2\ \text{NH CSS M}\square\text{n}_{\text{r}}](\text{Zn})_y$$

Experimental Conditions

Claras lazara fish (150-200 gram /each) were obtained from the river Nile Rashid branch, at El-Kanater El-Khairiya. Fish were acclimatized to laboratory condition one week before

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treatment 115L glass aquaria with a flow system and dechlorinated tap water. Static conditions with water renewal every 3 days were used. Fishes were fed once a day at 10.00 a.m. Three groups 15 fishes each were used. The first group was maintained under the same condition but free from any toxicants, and kept on a balanced diet (Table, 1) that meets its requirements from nutrients as described by **Dixon and Hilton (1981)** and was used as control group. The second group was kept on low carbohydrate diet but free from any toxicants, and maintained on a balanced diet that meets its requirements from nutrients as described by **Dixon and Hilton (1981) Hilton and Dixon (1987) and Roberts (1989).** The third group was fed on a mixture of low CHO diet, the constituents of the diet used in the experiment are presented in (Table, 1) and it was exposed to Dithane (0.5 ppm) within 30 days. The diet ingredients included, dissolved oxygen, temperature, pH, ammonia and nitrite, in water tanks. The quality of water was measured daily, while water alkalinity, hardness, carbon dioxide, sodium, potassium and chlorides were analyzed before and after each water renewal using commercial kits of Biochringer, France, as shown in (Table, 2).

Blood Sampling

Blood samples were taken after 7, 15, 30 days. The fish were anaesthetized by 1/1000 aqueous solution of Ms 222 and bled from the caudal vein. Blood samples were taken with heparinized micrhaematocrite tube. The tubes were centrifuged at 3000 r.p.m. for vit. A, 8000U; vit. E, 21U; vit. K, 4mg, vit. B2 3.6mg; niacin 20mg; choline chloride 160mg; pantothenic acid 7mg; vit. B12, 5ug; Mn, 70mg; Zn, 60mg; Fe 20mg; Cu, 2mg; I, 1mg; CO, 0.2mg. 10 min. Serum was separated and stored at 20˚C until used. Tested kits supplied from biomerieux (France) were used for determination of the activity of serum Alanine Amino Transferease (ALT) and Aspartate Amino Transferase (AST) as described by **Reitman and Frankel, (1957).** Serum glucose was assessed according to **Trinder, (1969).** Enzymatic determination of urea was done according to Patton and Insulin was estimated by radioimmunoassay method using coat A Insulin **(Patton and Crouch 1988).** Kits were obtained from Diagnostic Corporation Co. (DPC) 57700 west 96th street, LosAngeles, U.S.A. **(Pickering and Duston, 1983).**

Haematocrite value was carried out by using microhaematocrite capillary tubes centrifuged at 1200 r.p.m. for 5 min. Mean corpuscular volume (MCV). Reticulocytic count was done according to **Drabkin (1946).** Serum cortisol level was determined using radio-immunoassay technique according to the method of **Pickering and Pottinger (1983**). Serum iron **was** determined using atomic absorption according to **Joseph and Roger (1979) and Anderson, (1990).** Values of sodium and potassium in serum were determined by flamephotometer according to the method described by **Silversmith (1965).** Serum creatinine was measured according to **Bartels *et al* (1972).** Enzymatic determination of urea was done according to Patton and Insulin was estimated by radioimmunoassay method using coat A. Insulin **(Patton and Crouch, 1988).** Kits obtained from Diagnostic Corporation 57700 west 96th street, LosAngeles, U.S.A. **(Pickering and Duston 1983).**

Bacterial isolation

Aseptic swabs from the skin, gills, base of fins and blood of tested fish were cultivated on blood agar, MacConky agar, Nutrient agar, TSA, Nutrient broth and peptone water and Sabaroud's dextrose agar. Inoculated media were incubated at 37ºC for 48 hour. Bacterial isolates were identified by examination of the colony morphology and biochemical characteristic described by **Bastawrows and Amal (1999). Koneman *et al* (1994) and Palumbo *et al* (1985) and Nagae *et al* (1993)**. Bacteria were detected by

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Table 1. Ingredients and proximate chemical composition of diets used in the present experiments

Diets 2	Diets 1 (control)	Ingredients
30	30	Fish meal
10	8	Meat meal
3	1	Bone meal
4	3	Skimmed milk
7	5	Soybean
20	20	Wheat Bran
5	20	Wheat flour
15	10	Yeast
4	1	Cod liver oil
2	2	Mineral & vitamin premix

Proximate chemical composition

38.89	35.87	Crude protein (C.P) g %
2415.4	2297.21	Metabolizable energy / Kg
2.86	2.78	Ether extract (E.E.) g %
4.27	3.91	Crude fiber (C.F.) g %
10.25	8.735	Ash g %
3.99	3.094	Calcium (Ca) mg %
2.53	2.069	Phosphorous (Ph) mg %
2.29	2.105	Lysine mg %
0.613	0.562	Methionine mg %

*Mineral and vitamin premix per/kg pelleted food.

Table 2. Water quality characteristics in tanks , Initial conditions values are mean ± SE

6.40±0.1	PH
20°C±0.8	Temperature ° C
0.022±0.04	Nitrates mg/l
0.0015±0.004	Un ionised amonia (mg / l)
4.03±0.5	Carbonic dioxine (mg / l)
32.8±2.8	Alkalinity (mg / l)
4.54±0.53	Permanganate oxidabole matter (mg / l)
34.6±0.1	Hardness (mg /.l)
8.4±0.6	Chlorides (mg)
0.12±0.007	Potassium (mg / l)
5.68±0.01	Sodium (mg / l)

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counting colonies using surface spread plate technique according to quantitative method described by **Palumbo *et al* (1985).**

Measurement of serum immunoglobulin M (IgM) -IgM determination

The serum IgM was measured according to **Fuda *et al* (1991).** Double antibody sandwich Elisa according to the method of **Matsubara *et al* (1985)** for determination of IgM was done.

Statistical analysis

The obtained data were statistically subjected according to **Duncan (1955), Steel and Torrie (1980).**

RESULTS

Haematological results

Regarding heamatotoxic effect of dithane, the blood analysis of fish revealed significant decrease in R.B.Cs count, HB% and P.C.V. whereas there was significant increase in mean corpuscular volume M.C.V. than control ($P \leq 0.01$) 36.67 versus 31.00, respectively. However there was insignificant change in reticulocytic count compared to control values (Table, 3).

Concerning periods of exposure, the values of R.B.C.s, HB% and P.C.V. after exposure to dithane for 7 days were significantly higher ($P \leq 0.01$) than the values after exposure for 15 and 30 days respectively. However, exposure for 30 days showed the lowest values (Table, 3). While the M.C.V. and reticulocyte % values after 30 days exposure were significantly higher ($P \leq 0.01$) values than exposure for other periods.

The changes in the treated groups for R.B.Cs, HB%, P.C.V. values start to decrease after exposure from 15 up to 30 days respectively ($P \leq 0.01$) (Table 3). On the other hand, the change in M.C.V. and reticulocyte % values of the treatment after 30 days of exposure showed highly significant increase than other periods. Concerning periods of exposure treatments interaction in (Table 3), there were non significant difference between treatment and control under 7 days, while there was significant difference between control and treatment under the other two periods for R.B.Cs and M.C.V. parameters, whereas HB, P.C.V. and reticulocyte % were significantly different between control and treatment under all period experiment.

Clinico pathological results

Regarding treatment effect (Table, 4), there was high significant increase in treatment values of AST, ALT., urea, creatinine, Na, K, Cortisol, Glucose and Insulin ($P \leq 0.01$) compared to control values. On the other hand, iron level

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values for treated group showed significant lower values ($P \leq 0.01$) than the control as shown in Table (4). Concerning period of exposure, the highest significant values of AST, ALT, Urea, Creatinine, Na , K, Cortisol, Glucose and Insulin were attained after 15 and 30 days exposure, ($P \leq 0.01$) while iron values showed high significant decrease after 30 days exposure compared with 7 days and 15 days.

Concerning periods of exposure by treatment interaction (Table, 4), the treated groups were significantly higher than the control regardless the period of exposure except iron which decreased.. On the other hand, the treatment for 30 days of ALT, Urea, Creatinine, Na, K, Cortisol, Glucose and Insulin showed the highest significant increase compared to other groups.

Bacteriological results

At the end of experimental period there was difference on the bacterial counts on the different organs of the fish e.g. mainly external surface, kidneys, livers, and gills in all kinds of detected bacteria (Table, 5).

Immunological results: were shown in (Table, 6). The control values of IgM were significantly higher than mean treated groups ($P \leq 0.01$).However comparison between the values after different periods and the same trend for interaction between treatments and periods of exposure showed insignificant change. The period by treatment interaction for IgM was statistically insignificant ($P \geq 0.05$).

DISCUSSION

Several investigators reported that the application of Dithane a dithiocarbamate compound has been used extensively as fungicide for control of wide range of fungi **(Worthing, 1983 and 1987).** Therefore human and animals may be subjected to such compound via food, water and air and this could affect their health as demonstrated by **Yamanaka *et al* (1993)**. In the present study, the toxicity of Dithane was evaluated in *Claras lazera* (catfish) because the fishes were exposed to water pollution by insecticide and fungicide, also fish had long been regarded as a highly desirable food due to its content of high quality animal protein, its calcium and phosphorus content as well as its generous supply of vitamins.

As resulted from hematological examination, iron level decreases also R.B.Cs count, Hb % and P.C.V. with the resulting anemia may be attributed to one consistent effect of Cortisol which causes reduction in the R.B.Cs, Hb%, P.C.V.% 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. Also concerning the haemogram it was showed macrocytic hypochromic anemia characterized by significant decrease in R.B.Cs., Hb, P.C.V. and increase in M.C.V. Such animal picture might by attributed to the observed reticulocytosis in fish specially in 15 and 30 days. Such findings are in agreement with those of (**Roberts 1989, Lall (1991)** and **Mona *et al* (2003).** The clinicopathological showed an increase in hepatic enzymes, AST, ALT. The liver is the primary organ of detoxification as well as a major site for detoxification reaction which was due to exposure of dithane in water. Such result agree with **Dummer *et al* (1991), Yalow and Bawman (1983)** who observed that aquatic pollution with heavy metals cause immunosuppression and contribute to outbreaks of infections, and bacterial diseases in fish. It could be concluded that dithane can affect fish after 7 days but there is some complications with this pesticide. The clinicopathological results of increased

serum level of sodium and potassium concentrations, may be attributed to kidney impairment, where the

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Table 5. Bacterial isolates recovered from fish treated with Dithane (0.5 ppm)

Gill	Liver	Kidney	External surface	*Bacterial Strain*
3×2^3	10×3^3	2×7^3	4×10^3	*E-coli*
3×4^6	4×10^3	7×10^2	3×10^2	*Staphyloccus pyogenes*
2×5^3	1×10^6	2×10^3	2×10^3	*Flavobacterium sp.*

Table 6. IGM mean values of *Claras lazara* exposed to Dithane (0.5 ppm) and periods

* IgM Parameters	Periods						Mean Overall of all periods	
	7 days		15 days		30 days			
	Diet free	Diet with dithane	Diet free	Diet with dithane	Diet free	Diet With dithane	Diet free	Diet With dithane
IgM mean value	0.80± 0.15 [a]	0.78± 0.50 [a]	0.80± 0.15 [a]	0.72± 0.55 [a]	0.80± 0.15 [a]	0.65± 0.45 [a]	0.80± 0.15[a]	0.73± 0.64[b]
Mean of all treatments	0.79±0.12[a]		0.76±0.12[a]		0.74±0.13[a]			

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Means having different superscripts within the same raw are significantly different at $P \leq 0.05$
* IgM : Immunoglobulin M

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kidney is the normal pathway for Na and K. This may explain the main cause for elevation of the serum creatinine and urea in the treated groups. This confirms the previous reports recorded by **Neish and Hughes (1980)**. Also one of the reasons for hyperinsulinaemia was that low CHO diet with dithane causes significant increase of cortisol level which may be due to activation of hypothalamus pituitary internal axis, inducing a significant increase in cortisol level. These results coincide with those observed by **Vargut and Studnieka, (1994)** who observed that serum cortisol increased linearly in salminid fish fed on 5% CHO diet. Microbiologically the results were confirmed by the previous reports of **Neish and Hughes (1980),** who stated that hyphae of orgamisms may invade the deep tissue of the fish and penetrate the vital organs such as kidneys, liver, and even the central nervous system.

Such results also agree with that of **Osfor *et al* (1991 and 1998),** who demonstrated that the kidneys were the main organs of localization of the lesions, even they reported that yeast cells of the fungus occurred in rat's kidney fed on food diets contaminated with moulds for 30 days. Immunological decrease in level of IgM may be explained by the fact that production of lymphocytes in fish is apparently in the head of kidney, gut-associated tissues and spleen. However only the IgM antibody class has been found not to destroy antigen-bearing invaders. They instead inactivate antigens and mark them for destruction by macrophages and complement **Jurd, (1985) Willoughy and Pickering (1977).**

From the results obtained in this study, it was evident that there was a decrease in antibody titer in vaccinated group of fish exposed to the tested pesticides.

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تقييم تأثير المبيد الفطري ديثاين على سمك القراميط وتقييم فحص الدم والمعايير البيوكيماوية والمناعية

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2- المركز القومي للبحوث - الدقي - القاهرة - مصر

الغرض من هذا البحث تقييم السمية للمب… الفطري ديثاين على سمك القراميط… عرضت الأسماك للمبيد بجرع… مقدارها 0.5ppm ، وأجريت دراسات على… مختلف صور الدم والمعايير البيوكيمي… والمناعية بعد التعرض لمدد مختلفة 7… أيام 15 يوم و30 يوم.

وقد أظهرت الدراسات وجود نقص ف… نسبة الهيموجلوبين وكرات الدم الحمرا… وحجم الخلايا المضغوطة (P.C.V) خلال… التجربة بينما حدثت زيادة في نس… Reticulocytes ومتوسط حجم الخلايا الحمرا… (M.C.V) ، بينما أظهرت الدراسا… البيوكيماوية زيادة في معدل الأنزيما… الكبدية ALT & AST وكذلك مستو… الصوديوم والبوتاسيوم والكولستير… والأنسولين والجلوكوز والبولينا والكرياتي… في الدم ، في حين أن الدراسا… البكتيرية والأجسام ضد البكتيريا الممرض… (*E. coli, Flavobacterium* and *Staphyloccus aureus*) في الأسماك تح… التجربة حدث نقص في مستوى IgM ف… الدم كما أثبتت الدراسات المناعية.

وعموماً فقد حدث للأسماك سلوك… للقشور وتجمعات مع نزيف في بعض… أجزاء من الجلد كذلك استسقاء في البطن… وتقرحات

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