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ALTERATION OF HAEMATOLOCIAL PARAMETERS OF "ZEOL FISH"- *CLARIAS BATRACHUS* EXPOSED TO MALATHION

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Abstract: *Clarias batrachus,* locally called zeol fish, reared in fresh water on protein diet were exposed to 5% (8.77 mg/l active ingredient) and 10% (17.74 mg/l active ingredient) concentrations of malathion at 24 h intervals, for three weeks in cement tanks containing 580 liters of water with a constant flow (1.5/1 min) of aerated dechlorinated tap water with no recirculation under natural light. Weekly two cc venous blood was drawn from each sample to determine the haematolocial parameters using EDTA (ethylenediamine tetraacetic acid) as an anticoagulant. The findings of this study showed that malathion in different concentrations have some diverse effects on the haematological parameters *of Clarias batrachus*. Exposer to10% concentration of malathion was found to have a lethal effect on RBC, thrombocyte, neutrophil and Hb concentration which caused death of the fish with the sign of hypochromic microcytic anemia. Exposer to 5% concentration, on the other hand, showed leucopoenia due to decreased leucocyte counts. Haemoglobin (Hb) concentrations and values of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and differential count of WBC levels showed decreased values at 5% concentration of malathion. Significant decrease ($P<0.05$) in Hb and concentration and MCH were observed during the exposer to the pesticide malathion. No significant difference ($P>0.05$) was observed in the levels of RBC, MCHC, PCV, MCV and WBC.

Key words: Zeol Fish, *Clarias batrachus*, malathion, haematology, anemia and leucopoenia.

INTRODUCTION

Synthetic carbaryal insecticides like sevin, pyrethroid insecticides cypermethrin, deltamethrin, fevalerate and sumithion are used to protect many fruits, vegetable, pulses and field crops against a wide spectrum of fungal diseases and pests. They are also used for seed treatment of cereal grains, cotton, tomatoes etc., for the control of undesirable insects in order to increase agricultural production and in fish culture for the eradication of unwanted insects grown as a result of fertilizer used in aquatic media. According to Armella *et al.* (1987) those insecticides have harmful effects on aquatic environment and organisms.

Haematological parameters reflect the state of fish under stress more quickly than other commonly measured parameters, as they respond quickly to changes in environmental conditions (Alkinson and Judd 1978, Haque *et al.* 2006). For monitoring stress responses, predicting systematic relationships and the physiological adaptations of animals, haematological parameters have been widely used for the description of general health of fish (Blaxhall 1972). Haematological alterations in some fish exposed to various toxicants, viz. *Cyprinus carpio* to cypermethrin and fenvalerate (Mughal *et al*. 1993), *Tilapia mossambica* to cadmium chloride (Aziz and Shakoori1993), *Cenopharyngodon idella* to fenvalerate (Mughal *et al*. 1993) and *Heteropneustis fossilis* to deltamethrin (Kumar *et al*. 1999) have been investigated.

Malathion kills a wide range of non target species and extremely toxic to bees, moderately toxic to bird species and moderately to highly toxic to aquatic organisms including fish. Its leaching properties can contaminate the ground water as well as pond water. Malathion and its oxygen analog malaoxon both are carcinogenic and have been linked with increased incidence of leukemia in mammals. Malathion's chronic health hazards include suspected mutagen and teratogen, delayed neurotoxin, allergic reactions, behavioral changes, ulcer, eye damage, abnormal brain waves and immuno-suppression.

Clarias batrachus a potential cultivable fish with high economic and nutritional values needs relevant information about different culture procedures. So, present investigation aims to determine the effects of malathion on the haematological parameters of *C. batrachus* in order to monitor the general health of the fish species.

MATERIAL AND METHODS

A group of 30 fishes with an average weight of 140 ±14 g, reared in natural pond water at a fish farm, located at Gazipur, Dhaka were transferred to aquarium fish rearing facility at zoological garden of Dhaka university. They were acclimatized for a week during April 2000. The fish were exposed to 5% (8.77 mg/l active ingredient) and 10% (17.74 mg/l active ingredient) concentrations of malathion at 24 h intervals, for three weeks in rectangular glass tanks (100 cc x 100 cm) containing 580 liter water with constant pump aerated flow (1.5/l min) of dechlorinated tap water with no recirculation under natural light. Ten fishes were placed in each two treatment tanks and rest 10 were treated as control without exposure to malathion.

The temperature range, dissolved oxygen, pH levels and total hardness, $CaCo_3$ were record as 18.5-32°C, 8-9 ppm, 7.8 and 102 mg, respectively. During the period the fishes were on protein diet (75% fish meal + 10% rice barn + 10% oil cake + 5% vitamins).

The treatment of 10% malathion could not be completed due to the death of all the fishes within two days. At the end of each week, four fishes from 5% malathion exposure tank and four fishes from control tanks were taken out for blood collection and released again into the tanks. Collection was done following Shakoori *et al.* (1996).

Approximately two cc venous blood using EDTA (ethylene diaminete traacetic acid) as anticoagulant was drawn from each sample for the estimation of haematological parameters, viz. Red Blood Cell (RBC) count, Total White Blood Cell (WBC) count and Haemoblobin (Hb) concentration. For the estimation of the above parameters the Packed Cell Volume (PVC) method described by Blaxahall and Daisley (1978) was followed. The erythrocytic characteristic like values of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) were calculated according to Reddy and Bashamohideen (1989). The results are presented as means ± SD. Difference between parameters were analyzed by one way analysis of variance (ANOVA), and significant means were subjected to a multiple comparison test (Duncan's Multiple Range Test) at P=0.05 level.

RESULTS AND DISCUSSION

The blood parameter values, namely Hb, MCH, MCV, PCV and WBC counts showed decreasing trend and RBC count showed mild increasing trend after exposure to man-made organophosphate anticholinesterase, non-systemic wide spectrum insecticide malathion (Table 1). No significant ($P > 0.05$) changes were observed in the estimated parameters of the control fish. A significant ($P < 0.05$) decrease in Hb concentration ($F = 5.16$) and MCH ($F = 3.89$) were observed in the fish samples exposed to 5% malathion, which is incisive of normochromic anemia. No significant ($P > 0.05$) difference was observed in the counts of RBC ($F = 1.22$), WBC ($F = 2.09$), PCV ($F = 1.45$), MCV ($F = 2.40$) and MCHC ($F = 1.41$).

RBC count without significant differences contradicts with the findings of *Oonchorynchis mykiss* exposed to cypermethrin (Shakoori 1996); *Cenopharyngodon idella* exposed to fenvalerate and *Heteropneustis fossilis* exposed to deltamethrin (Kumar *et al.* 1999). On the other hand Koundinya and Ramamurthi (1979) reported decline in RBC of *Oreochromis mossombica* after exposure to sumithion and sevin. Reddy and Bashamohideen (1989) observed decreased RBC count in *Cyprinus carpio* after 48 h exposure to cypermethrin; and Santakumar *et al.* (1999) in *Anabas testudineus* exposed to azodir.

Significant decrease in Hb concentration after exposure to 5% malathion, may be due to impaired oxygen supply to various tissues, resulting in slow metabolic rate and low energy production (Ahmad *et al.* 1955), or may be due to increased in metabolic rate, which may have led to decrease Hb concentration level (Reddy and Bashamohideen 1989). Decreased Hb concentration level in

Table 1. Showing changes in the hematological parameters of zeol fish *Clarius batrachus* exposed to 5% malthion at 24 h interval for three weeks. Superscripts in a row with different letters represent significant differences ($P < 0.05$). Each value is the mean ± SD of 4 individual observations. The values in the parentheses are percentage changes over the control.

Haematological parameters	Alteration in haematological parameters for three weeks (Mean ± SD)					
	1st week		2nd week		3rd week	
	Treated	Control	Treated	Control	Treated	Control
RBC (10^6/mm^3	0.65 ± 0.01^a (-9.72)	1.17 ± 0.05^a	0.69 ± 0.01^a (-41.02)	0.77 ± 0.02^a	0.70 ± 0.01^a (-9.09)	0.72 ± 0.04^a
Hb (g/100 ml)	4.90 ± 0.24^a (-19.54)	6.27 ± 0.44^a	$4\ 28 \pm 1.28^b$ (-31.73)	6.82 ± 0.44^a	3.65 ± 1.14^{ab} (-46.48)	6.09 ± 0.20^a
PCV (%)	35.25 ± 3.20^a (- 20.89)	44.75 ± 3.21^a	38.25 ± 12.65^a (- 14.52)	45.20 ± 12.71^a	38.75 ± 9.35^a (- 14.26)	44.56 ± 3.18^a
(MCV (μm^3)*	542.30 ± 49.11^a (-12.37)	382.97 ± 22.84^a	544.37 ± 164.13^a (-42.50)	587.01 ± 34.77^a	553.57 ± 132.73^a (-5.79)	618.88 ± 46.54^a
MCH (pg)*	75.38 ± 10.29^a (-10.87)	53.58 ± 16.22^a	62.02 ± 20.37^b (-15.75)	88.57 ± 10.31^a	$52.14 \pm 14.49\ ^{ab}$ (-41.13)	84.58 ± 11.16^a
MCHC (g per 100 ml)*	13.90 ± 2.46^a	14.01 ± 2.36^a (-1.57)	11.18 ± 7.38^a	15.08 ± 1.94^a (-20.19)	9.41 ± 2.10^a	13.66 ± 2.06^a (-37.60)
WBC (10^4/mm^3)	6.26 ± 0.12^a (- 16.20)	7.46 ± 1.06^a	6.68 ± 0.13^a (- 10.45)	7.42 ± 1.08^a	6.51 ± 0.12^a (- 12.26)	7.47 ± 1.04^a

*MCV = [PCV (%)/RBC (10^6)] x 10 μm^3; MCH = [Hb (g)/RBC (10^6)] x 10 pg; MCHC = [Hb (g)/PCV (%)] x 100 g per 100 mlRBC: Red blood corpuscle count; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin; WBC: White blood cell count.

various fishes exposed to insecticides was reported, viz. *Heteropneustis fossilis* exposed to deltamethrin (Kumar *et al.* 1999); *Channa punctatus* to malathion (Pendey *et al.* 1981) and *Anabas testudineus* to metasystox (Natarajan 1981).

Malathion exposure of *C. batrachus* during the present investigation showed no significant decline in packed cell valume (PCV), but decline in PCV value was observed in danitol and fenvalerate treated *Cenopharyngodon idella* (Shakoori *et al.* 1996) and malathion exposed to *C. puctatus* (Pendey *et al.* 1981, and Geol and Garg 1980), whereas, the increased value of PCV was recorded in *Tilapia mossambica* exposed to cadmium chloride (Aziz and Shakoori 1993).

In spite of decrease in RBC count, a decline in PCV may show the extent of shrinking cell size due to insecticide intoxication (Ahmad *et al.*1995). Decline PCV and MCV values in malathion treated *C. batrachus* might have interfered with the normal physiology of RBC which ultimately leads an anemic condition of the fish. RBC indices like MCV and MCHC declined during prolonged exposure to malathion. The same results were recorded in *C. punctatus* treated with metasystox (Natarajan 1981); with 2,3,'4-tetraminazo-benzine methyle parathion and dinicthonate (Anees 1978). A significant decline in MCH ($F = 3.89$; $P < 0.05$) along with MCV and MCHC levels are indicative of hypochromic microcytic anemia. Which is in lieu with domino effect of fenvalerate treated *Cenopharyngodon idella* (Shakoori *et al.*1996).

In the present investigation decrease in WBC count contradicts with the significant increase in leucocyte count in cardmium exposed *Anabas testudineus* (Saravanan and Natarajan 1991). Bimodal respiration in metasystox treated *Channa striatus* with leucocytosis was reported by Natarajan (1981).

Significant alteration in the haemotological parameters of malathion treated *C. batrachus* results in hypochromic microcytic anemia and leucopenia, which are the prerequisite of immune failure and impaired growth with high mortality. Therefore, use of insecticide in cropland and in aquaculture for the eradication of unwanted insects should be restricted to prevent possible contamination by leaching into the aquatic environment to save and protect the non target aquatic organisms from these kind of toxicity.

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