The Bioscan 8(3): 1111-1114, 2013 (Supplement on Toxicology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF SUBLETHAL CONCENTRATION OF MALATHION ON HEMATOLOGICAL PARAMETERS OF *MONOPTERUS CUCHIA* (HAMILTON- BUCHANAN)

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KEYWORDS
Malathion
Hematological parameter
IUCN
Metabolism

Received on :
17.05.2013

Accepted on :
26.08.2013

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ABSTRACT
Impact of pesticides used in agricultural operations on the environment as well biotic community as a whole is a global concern particularly in developing countries like India. The present study encompasses impact of sub lethal concentration of a commonly used OP pesticide 'Malathion' on certain hematological parameters of *Monopterus cuchia* (Ham- Buch) popularly known as 'mud eel' under laboratory condition. The study revealed significant decrease in Total Erythrocyte Count (TEC) in treated fish upon exposure to sub lethal dose of Malathion with a mean value of (2.02 ± 0.2) 10^6/mm^3 in comparison to the control (2.49 ± 0.2) 10^6/mm^3. In addition to that, significant increase in leucocytes count in treated fish with a mean value of 2.57 ± 0.5 10^6/mm^3 in comparison to control (2.00 ± 0.5) 10^6/mm^3 indicated ailing condition of the fish. Significant difference in percentage of Eosinophil, Neutrophils, Lymphocytes, Monocytes in blood samples of controlled and treated fish indicates the enormity of the impact of the pesticide on the hematological parameters of the fish. Although immediate mortality was not recorded, the findings indicated that prolonged exposure may have severe impact on the life processes of the species.

INTRODUCTION

Extensive use of chemicals is in vogue in present day agriculture for enhancing crop productivity per unit area to meet the demand of food for the growing population of the world, particularly in developing countries like India. Among the different chemicals used in modern agricultural systems, pesticides are unique toxic substances, as these are deliberately applied or added to suppress or eliminate some components of the food chain of crop field ecosystem, commonly known as pests, to safeguard the crop from being damaged or feed upon. However, while doing so, the toxic chemicals affect other non target components of the ecosystem and pollute the soil, air and water. More so, when the pesticides enter the aquatic ecosystem through direct application or run off from agricultural operation they often cause adverse impact on the aquatic biota including fish (Kumar, 1994). One of the very commonly used pesticides in India is 'malathion' an organophosphorus pesticide. The toxic effects of the organo phosphorus components are the results of their ability to inhibit the enzyme Acetyl chlorine esterase (ACHE) which in turn disrupts the neural transmission through binding of the ACHE with 'Malatoxin', the oxygen analog of Malathion (O'Brian *et al.,* 1974). Fish species, inhabiting the paddy fields, swamps, canals and derelicts that are directly linked with paddy field are more susceptible to exposure to such chemicals. Fresh water eel *Monopterus cuchia* (Ham Buch) commonly known as "Mud eel" is one such species which needs immediate attention in this aspect. Earlier study conducted by Baruah *et al*. (2008) on behavioral response of the fish to different concentration of the pesticides indicated that this species is highly sensitive to exposure to malathion even in very low concentration leading to weak and ailing health condition. Hematological parameters reflect health status of fish and have been used for detecting disease, dietary deficiency and environmental stress (Conroy 1972; Srivastava, 1979). A study of hematological parameter is therefore necessary to assess the extent of damage caused by any toxic material. *M cuchia* has very high demand among the ethnic population of the NE region of India for its unique taste and therapeutic value and is collected from its natural habitats like beels, swamps and paddy fields by traditional method. The species have this special nature of burrowing in mud throughout its life. The natural population of this species is apparently on a decreasing trend during the recent years. The conservational status of the species is reported to be lower risk near threatened as per IUCN based CAMP report (1998). Increasing demand for the species has been observed during recent years as a major component of diet of anemic and weak people for the popular belief that consumption of this species increase hemoglobin count of blood and enhance physical strength in ailing person. In addition to its importance as food fish, the species is known to have ornamental value for its peculiar snakelike body shape and movement. The species is enlisted as one of the 58 fish species currently being exported as ornamental fish species from NE Region of India. Vulnerability of this species to pesticide

exposure due to its habits of mud dwelling as well as habit of living in paddy field swamps etc., it is important to assess the impact of different pesticides on the health and population status of the species. In view of the above present study was conducted on the toxic affect of malathion on some hematological parameters like total blood cell count, Differential Count of leucocytes (DLC)of *M. cuchia,* under exposure to sub lethal concentration of Malathion.

MATERIALS AND METHODS

M. cuchia of average length (60-68 cm) and weight (180-250 gm), procured from the local wet-lands of Bahona area, Jorhat, Assam were used during this study as test animals. The fish were acclimatized to the controlled condition for 5 days period to treatment. The concentration of malathion 50 EC is kept at sublethal level (0.006 ml/l) determined on the basis of bioassay trial. The test fishes (Total no. 30) were kept in laboratory conditions in 2 glass aquaria separately each filled with 10 liters pond water treated with 0.006 ml malathion 50 EC/l water for 25 days. One group of test fish (10 no.) placed in fresh water without pesticide served as the control group. The acclimatized fishes were not fed during the course of the experiment (Delala *et al*., 1980). The pond water was changed on alternate days and the concentration of pesticide was maintained. In the treated and control fishes, the blood was collected from the lateral line of experimental fishes by inserting a heparinized microsyringe. The following methods were used for the different blood parameters.

I. Total Erythrocyte count (RBC, $10^6/mm^3$): The total erythrocytes count was estimated by the dilution chamber technique using Hayem's fluid as diluent (Benjamin, 1985).

II. Total leucocytes count (WBC $10^3/mm^3$): Total leucocytes count was estimated by the standard dilution technique using diluting fluid (4% glacial acetic acid and two drops of gentian) (Talib and Khurana, 1995).

III. Differential count of leucocytes (DLC): Differential count of blood smears prepared by staining with Giemsa stain were made under 100X by using, and counting was done by the Meander method. Hundred cells were counted and tabulated viz. Neutrophils, Eosinophils, Basophils, lymphocytes and monocytes.

IV. The total number of differential leucocytes present was calculated as per the formula. DLC = Number of types cells/Total number of white blood corpuscles × 100

V. Haemoglobin (Hb%): Haemoglobin was estimated by Shalis and Haematin method as per modified by Talib and Khurana (1995).

RESULTS AND DISCUSSION

Treatment with sub lethal concentration of Malathion (0.006 ml/l) has not indicated any immediate behavioral response as well as mortality. However, the fish become lethargic and subsequently become still upon exposure to prolonged period. Mortality has not been recorded in any of the treatments during the period of investigation.

Hematological investigations carried out by taking blood samples from control and treated fishes revealed that the Total Erythrocyte Count (TEC) had been reduced considerably in treated specimen with an average TEC ranging from $(1.00 \pm 0.3)10^6/mm^3$ to $(2.00 \pm 0.2)\ 10^6/mm^3$ and a mean $2.02 \pm 0.2\ 10^6/mm^3$ while in the control specimen it was $(2.20 \pm 0.5)10^6/mm^3$ to $(3.20 \pm 0.5)\ 10^6/mm^3$ and a mean $2.49 \pm 0.2\ 10^6/mm^3$. The difference was significant at 1% level. On the other hand there was significant increase in Total Leukocyte Count (TLC) (Table 1). Significant differences in the TEC and TLC records of treated and controlled group indicated severity of

Table 1: Impact of malathion (0.006 mL/L) on the different blood parameters of *Monopterus cuchia*

Group No. and No. of fish	Fish group treatment period (days)	Normal control period (days)	TEC $10^6/mm^3$		TLC $10^3/mm^3$		Hb%	
			Control	Treated	Control	Treated	Control	Treated
1 (n=8)	5	5	3.20±5	2.00±0.2	2.80±0.2	2.00±0.1	18±4	17.0±0.5
2 (n=8)	10	10	2.60±0.3	1.60±0.5	2.00±0.5	1.80±0.5	16±5.5	12.5±1.5
3 (n=8)	15	15	2.25±0.3	1.00±0.3	1.80±0.1	1.50±0.5	12±2.5	9.2±2.5
4 (n=5)	20	20	2.20±0.5	1.50±0.3	1.80±0.1	1.55±0.5	10±1.5	7.0±3.0
5 (n=5)	25	25	2.20±0.5	1.60±0.4	1.60±.05	1.00±0.5	8±0.5	5.5±0.5
Mean			2.49±0.2	2.02±0.2	2.00±.05	2.57±0.5	12.8±1.5	12.64±1.5
't' - value between control and treated			5.875**		4.924**		0.266NS	

Table 2: Effect of malathion (0.006 mL/L) on the DLC of *Monopterus cuchia*

Group No. and No. of fish	Fish group treatment period (days)	Normal control period (days)	Eosinophil %		Neutrophils %		Basophils %		Lymphocytes %		Monocytes %	
			Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1 (n=8)	5	5	4±0.5	3±0.5	45±5.5	36±6.0	2±0.5	2±0.5	55±7.5	60±10.0	19±2.0	27±4.5
2 (n=8)	10	10	4±1.2	3±0.5	35±7.0	25±4.0	1.5±1.5	2±0.5	47±2.5	58±5.0	16±1.5	25±3.5
3 (n=8)	15	15	3±0.1	2±1.0	31±8.0	22±3.0	1±1.5	1±0.5	45±3.0	53±3.5	20±1.5	27±2.5
4 (n=8)	20	20	2±0.5	1±0.2	32±9.0	22±2.0	1±1.5	1±0.5	50±9.5	58±3.5	18±1.5	23±3.5
5 (n=8)	25	25	2±0.5	1±0.1	30±5.0	23±1.5	1±1.5	1±0.5	52±0.11	55±2.5	18±2.5	20±4.5
Mean			3±0.2	1±0.01	34.6±5.5	25.6±0.2	1±1.5	1.2±3.0	49.8±7.5	56.8±0.7	18.2±2.5	24.4±3.0
't' - value between control and treated			3.207**		16.432**		0.535NS		5.078**		4.966*	

* - Significant at 5% level of significance, ** - Significant at 1% level of significance, NS - Non-significant

impact of the pesticides on the hematological parameters of the test fish. There was a distinct reduction in percentage of Haemoglobin (Hb%) also in between treated and control fish, which was however not significant statistically.

Blood analysis forms a useful tool for determining physiological and pathological conditions of the fishes. *M. cuchia* exposed to sub lethal concentrations of malathion at laboratory conditions resulted in a significant decrease in RBCs count leading to anaemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopeotic organs. Similar reports have been made by Sampath *et al*. (1998) on *Oreochromis mossambicus* after exposure to zinc and copper. Srivastava (1979) have shown that exposure to sub lethal concentration of lade produced haemolytic anaemic condition leading to the lysis of erythrocytes, Hb content, RBC count and PCV values, resulting in hypochronic anemia due to deficiency of iron and decreased utilization for Hb synthesis.

In the present study on *M. cuchia* exposed to sub lethal concentration of malathion, significant reduction in RBC count was recorded causing macrocytic anaemia. While the DLC revealed decrease in percentage Eosinophil and neutrophils, there was significant increase in lymphocytes and monocyte count (Table 2). The increased leucocytes count in treated fishes may be due to the inclusion of thrombocytes in the WBC population. Although the difference in mean haemoglobin percentage is not significant between the treated and controlled groups, yet the difference reflects the ailing condition of the test group.

The anaemic condition recorded in the present study could be due to the destruction of mature RBC by the effect of pesticide as well as inhibition of erythrocyte production. Such a decrease in RBC and anaemic response was earlier observed by Koundinya and Ramamurthy (1979) in *Sarotherodan mossambicus* and *Tilapia mossambicus* after exposure to lethal concentration of a similar pesticide Sumithion, Lal *et al*. (1986) recorded similar findings in *H. fossilis* exposed to malathion. In the present study it was apparent that the reduction in the number of RBC and Hb content had hampered oxidative metabolism in the test fish. Since the Hb and RBCs are oxygen carrying devices, the quantitative decrease in their levels might have lead to the derangement of the oxidative metabolism with a decrease in cellular activities with respiratory potential. The weak and lethargic stage of the treated fishes might be the reason of the reduction in the oxygen level in the body.

The neutrophil increase in fish exposed to malathion may be due to tissue damage. Mahajan and Dheer (1979) and Sampath *et al*. (1998) reported that neutrophils showed great sensitivity to changes in the environment and increase in the nutriphil number the important indicators of distressed condition. The increase in lymphocytes and monocytes suggests that these fish become stimulated under pesticide stress to fight against the pollutants in the environment. However, in the differential count there was a gradual decline in the number of lymphocytes in fish exposed to malathion for a longer duration (20 days and 25 days). This significant finding may be due to continuous stress. The present observation is in accordance with Mc Leay (1973) who suggested that continuous stress might lead to a decrease in the number of circulating lymphocytes.

The results in the present study indicated that prolong exposure to Malathion has lead to significant increase in the total number of leucocytes and while the RBC count, Hb concentration gradually declined as time went by. This phenomenon suggests that exposure to malathion even at sub lethal dose cause anaemic and macrocytic changes in *M. cuchia* which becomes severe upon prolong exposure.

The findings indicate that malathion 50 EC has harmful effects on the non-target organisms like *M.cuchia* at a very low concentration, leading to serious impact on the hematological parameters of the infected fish. Although there was no immediate mortality of the test fishes, such weak health status would lead to secondary disease infection, low growth, low metabolism, low reproductive development and will impact on the overall population status of the species in the natural resources. Hence, increasing necessity has been realized to take step for controlling application of chemical pesticides in agricultural operation and strategy for alternative (biological or organic) pest control measures may be adopted in paddy field to safeguard the aquatic biota.

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