ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 11, pp.23221-23224, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IMPACT OF INSECTICIDE MALATHION ON HAEMATOLOGICAL ALTERATIONS OF FRESHWATER FISH LABEO ROHITA

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ARTICLE INFO

Article History:
Received 14th August, 2015
Received in revised form
16th September, 2015
Accepted 11th October, 2015
Published online 30th November, 2015

Key words:

Fish Labeo rohita,
Insecticide, Malathion,
Haematology, Parameters.

ABSTRACT

The Insecticide is common pollutants of freshwater ecosystems where they induce adverse effects on the aquatic biota. Fish, Labeo rohita is an important carp species in Tamil Nadu region having good nutritional values. Fishes living in close association with may accumulate heavy metals. In the present investigation, the toxic effects of insecticide malathion LC50 0.25 ppm on the total RBC, WBC and Hb in the fish, Labeo rohita were estimated. In the present study effect of malathion on RBC and Hb were found to be high ranges mean values at 10%, low values of hematological parameters were found to be 30% SLC, when compared to control. Total erythrocytes of Labeo rohita showed a significant decreasing tendency at 10 and 30% sublethal concentrations when compared to control. The effect of malathion on WBC was found to be high ranges mean values at 30%, low values of hematological parameters were found to be 10% SLC, when compared to control. The leucocytes number of Labeo rohita was increased with the increasing concentrations of insecticide malathion.

Citation: Kumaresan, T. 2015. "Impact of insecticide malathion on haematological alterations of freshwater fish labeo rohita", *International Journal of Current Research*, 7, (11), 23221-23224.

INTRODUCTION

Toxicity of malathion is an organophosphorous insecticide widely used in agriculture and houses for the control of diseases vectors. It is a major source of environment poisoning in developing countries (WHO, 2003). Toxicological tests have shown that malathion affected central nervous system, immune system, adrenal gland, liver and blood. Once malathion is introduced into the environment, it may cause serious intimidation to the aquatic organisms and is notorious to cause severe metabolic disturbances in non-target species like fish and fresh-water mussels (USEPA, 2005). Pesticides in the aquatic environment can negatively affect the ecosystem. Although the aquatic environment is not the actual target of such pesticide, but the widespread use of them had led to some serious problems including toxic residues in grass and toxicity of non-target organisms such as mammals, birds and fish (Saeed *et al.,* 2012; Shankar *et al.,* 2013). Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition which is affected by the water pollution. Alterations in biochemical components as response to environmental stress are authenticated by Arockia and Mitton, (2006) in *Oreochromis mossambicus*. 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). 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). 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. Fish are widely used to evaluate the health of aquatic systems and the histopathological changes in liver of fish can serve as valuable biomarkers of malathion pollution (Dutta *et al.*, 1993). The haematological parameters like haemoglobin, haematocrit, blood cell counts, glycemia and ion concentrations can be used to find physiological response of a contaminated environment (Dethloff *et al.*, 2001). Previous haematological study of nutritional effects, infectious diseases and pollutants brought knowledge that erythrocytes are the major and reliable indicators of various sources of stress (O'neal and Weirich, 2001).

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Table 1. The haematological parameters of fish *Labeo rohita* under two sublethal concentrations of insecticide malathion

Days	Exposure	RBC ($10^6/mm^3$)	WBC ($10^3/mm^3$)	Hb (g/100ml)
5 days	Control	1.67 ± 0.16	39.79 ± 0.48	5.21 ± 0.21
	10% SLC	1.68 ± 0.14	41.55 ± 0.38	4.71 ± 0.46
	30% SLC	1.44 ± 0.13	42.63 ± 0.64	4.04 ± 0.35
10days	Control	1.66 ± 0.26	39.63 ± 0.39	5.25 ± 0.06
	10% SLC	1.39 ± 0.08	43.82 ± 0.49	4.22 ± 0.56
	30% SLC	1.25 ± 0.07	44.68 ± 0.42	3.58 ± 0.33
15 days	Control	1.70 ± 0.13	39.73± 0.42	5.22 ± 0.06
	10% SLC	1.28 ± 0.07	45.75 ± 0.59	3.38 ± 0.28
	30% SLC	1.18 ± 0.07	46.53 ± 0.40	3.11 ± 0.41

Values are mean ± SD – or + indicate present decrease or increase over control

In the present study, tested variations of haematological changes in carp (*Labeo rohita*) were exposed to insecticide malathion.

MATERIALS AND METHODS

Fish, *Labeo rohita* was collected from Chidambaram area and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy, fish having equal size (length 10 to 15 cm) and weight (100 to 200 g) were used for experimentation. Stock solution of insecticide malathion was prepared by dissolving appropriate amount of salt in distilled water. The physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA (1998). Batches of 10 healthy fishes were exposed to different concentrations of malathion to calculate the medium lethal concentration LC_{50} value (0.25 ppm) using probit analysis Finney method (1971). The fishes (Four groups) were exposed to the two sublethal concentrations ($1/10^{th}$ and $1/30^{th}$ mg/L) of copper for 5, 10 and 15 days respectively. Another group was maintained as control. Fish was collected and gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA, an anticoagulant (6% Ethylene diamine tetra acetic acid). The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), a method devised by Yokayama (1974) and later modified by Christensen *et al*., (1978) was followed, White Blood Corpuscle Count (WBC) and Haemoglobin count (HB) the Sahlis Hellige method was followed for haemoglobin determination.

RESULTS

Insecticide malathion caused 50% mortality of fish *Labeo rohita* at 96 hours was 0.25 ppm. The LC_{50} values of malathion for 24, 48, 72 and 96 hours were 0.22, 0.23, 0.24 and 0.25 ppm respectively. The toxic effects of malathion on the haematological parameters of *Labeo rohita* such as number of red blood corpuscles (RBC), white blood corpuscles (WBC) and haemoglobin content (Hb). The observations were made at the end of exposure periods (5, 10 and 15 days) to calculate the percentage of increase and decrease of different haematological parameters. Total erythrocytes of *Labeo rohita* showed a significant decreasing tendency at 10 and 30% sublethal concentrations when compared to control.

A marked decrease of 1.68, 1.39 and 1.28 ($10^6/mm^3$) values were recorded in the RBC in 10% sublethal concentration at 5, 10 and 15 days. The values were 1.44, 1.25 and 1.18 for 5, 10 and 15 days at 30% sublethal concentration and control RBC values were noted 1.67, 1.66 and 1.70 ($10^6/mm^3$) in the periods of 5, 10 and 15 days (Table 1 and Fig. 1).

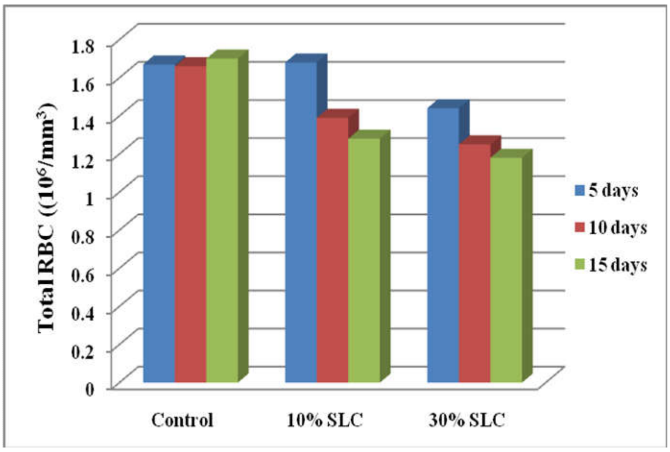

Fig. 1. Total RBC of fish *Labeo rohita* under sublethal concentrations of malathion

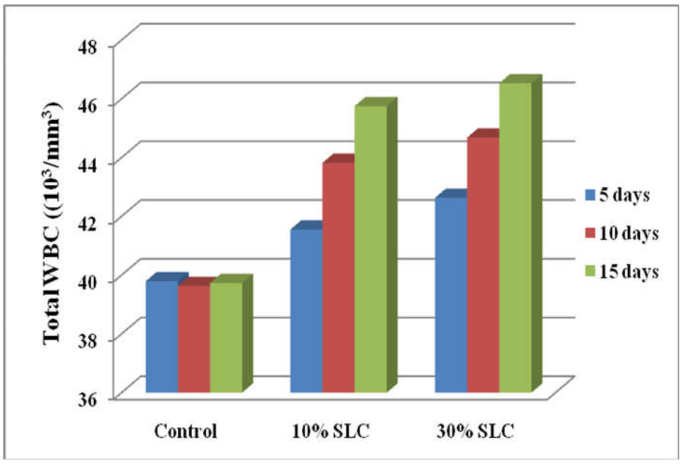

Fig. 2. Total WBC of *Labeo rohita* under sublethal concentrations of malathion

The leucocytes number of *Labeo rohita* was increased with the increasing concentrations of malathion (Table 1 and Fig. 2) when compared to control. The control WBC values were noted 39.79, 39.63 and 39.73 ($10^3/mm^3$) in the periods of 5, 10 and 15 days. The increase in the number of leucocytes was found to be 41.55, 43.82 and 45.75 ($10^3/mm^3$) in 10% sub lethal concentration for 5, 10 and 15 days. The values were recorded 42.63, 44.68 and 46.53 ($10^3/mm^3$) for 5, 10 and 15 days at 30% sublethal concentration. The haemoglobin content was measured from *Labeo rohita* in 10 and 30% sublethal concentrations showed decreasing trend with a significant reduction of malathion, when compared to control. The decrease in the haemoglobin amount was found to be 4.71, 4.22 and 3.38 (g/100ml) at 5, 10 and 15 days exposure at 10% sublethal concentration. The values were noted 4.04, 3.38 and 3.11 (g/100ml) for 5, 10 and 15 days at 30% sublethal concentration. The control haemoglobin values were recorded 5.21, 5.25 and 5.22 (g/100ml) in the periods of 5, 10 and 15 days (Table 1 and Fig. 3).

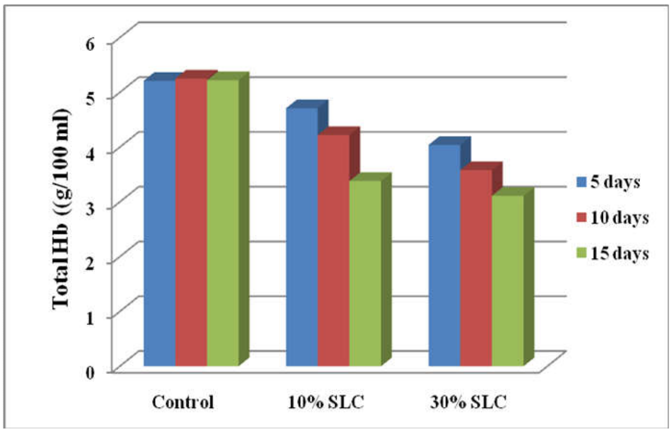

Fig. 3. Total Hb of fish *Labeo rohita* under sublethal concentrations of malathion

DISCUSSION

Hematological indices are very important parameters for the evaluation of fish physiological status under metallic stress. The changes in blood indices and their peculiarities depend on the concentrations of heavy metals and duration of exposure of fish to them. Hematological indices are of different sensitivity to various environmental factors and chemicals. Gill *et al.,* (1991) on the other hand suggested that the fish experience respiratory difficulty when they confront toxic environment and try to compensate for the reduced oxygen uptake at the gill surface by increasing the level of blood constituents concerned with oxygen uptake and delivery. However, a prolonged exposure exhausts the haematopoietic potential revealed by lowered RBC count and haemoglobin. An anaemic condition is generally indicated by the tendency of lower RBC count and haemoglobin content as seen in fishes exposed to environmental pollution (Ramesh, 2001). In the fish *Channa punctatus* exposed to malathion haemoglobin percentage decreased significantly. This indicates that malathion caused anaemia. This may be due to a decreased rate of production of red blood cells or an increased loss of these cells. The results showed that malathion treatment inflicted a drastic reduction in the total count of RBC's. The reduction was dosage dependent. However, the number of WBCs was deviating significantly from normal values. The significant decrease in the WBC count may be due to a generalised stress response (Ruparelia *et al*., 1990).

White blood cells play a Genotoxicity Induced by Malathion in *Channa punctatus* major role in the defence mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Leucocyte count showed greater and quite different pattern change due to malathion exposure when compared with the erythrocyte levels of the control group. Blood of all experimental groups contained higher concentrations of leucocytes than those of controls. An increase in lymphocyte number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindag, 2005). Gill and Pant, (1985) have reported that the stimulation of the immune system causes an increase in lymphocytes due to injury or tissue damage. Increase in the total leucocyte count has been attributed to several factors like increase in thrombocytes, lymphocytes or squeezing of WBC in peripheral blood (Agarwal and Srivastava, 1980). Increase in the TLC could be due to stimulated lymphopoiesis and/or enhanced release of lympohocytes from lymphomyeloid tissues as has been expressed by Das and Mukherjee (2000). The WBC showed greatest sensitivity to changes in the environment and the most important of leucocytes were lymphocytes. Leucocytosis which may be directly proportional to the severity of the causative stress condition may attribute to an increase in leucocyte mobilization. Further, the increase in WBC in the present study count could be attributed as an adaptive value of fishes under insecticide stress.

At last the indication of the earlier study is that both the organophosphates are toxic to fishes, however, malathion is more toxic as compared to parathion due to its active constituents. Among three fishes, *Catla catla* is most sensitive to toxic stress. Reduction of TEC may be suggestive of an appreciable decline in the hematopoiesis leading to various types of anaemia eg. Poikilocythemic, microcytic and haemolytic anemia. Increase in TLC is recorded probably due to thrombocytosis, lymphocytosis or leucopoiesis and/or enhanced release of lymphocytes from the lymphoid tissue under the effect of toxic compounds (Lalit Pathak *et al*., 2013). The haemoglobin level in *Labeo rohita* exposed to 96hrs LC_{50} concentration of chromium for 24 and 96 hrs. The haemoglobin level were observed decreasing trend when compared to control (Vutkuru *et al.,* 1996). The haemoglobin percent were appreciably declined in *Labeo rohita* exposed to chromium reflecting the anemic state of the fish which could be possibly due to iron deficiency and its consequent decreased utilization for haemoglobin synthesis. Similar findings on *labeo rohita*, which also reported hypochromic microlytic anemia under lead chloride stress (Janardhana Reddy *et al.,* 1998). A significant decrease in haemaglobin in the fresh water fish *Channa punctatus* from polluted waters can definitely be related to the pollution due to slaughter house wasters (Iqbal *et al.,* 1997). Jeyapriya *et al.,* (2013) was reported a significant decrease in the Hb, when the fresh water fish, *Catla catla* were exposed to the pesticide, monocrotophos. A similar finding was reported by Naveed Abdul *et al.* (2010), Aliakbar Hedayati and Zahra Ghaffari, (2013). In the present study, total RBC and Hb of *Labeo rohita* showed a significant decreasing tendency at 10 and 30% sublethal concentrations when compared to control. The leucocytes number of *Labeo rohita* was increased with the increasing concentrations of insecticide malathion.

Conclusion

The present study indicates that presence of 10% and 30% sublethal concentrations of malathion in the water is toxic to fishes and alters the hematological aspects of the fish. The results indicate that the usage of the malathion in the agriculture fields may be a threat to aquatic fauna and flora as well as humans. The results obtained clearly indicated that the haematopoietic organs of fish were adversely affected by malathion. The use of pesticide in the field may be a threat to human health and fauna and flora of the environment.

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