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33. TO DETERMINE THE ACUTE TOXICITY PESTICIDE OF MALATHION AND METABOLIC ACTIVITIES AND RESPIRATORY RESPONSES IN FRESHWATER FISH LABEO ROHITA.

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ABSTRACT

The water pollution of aquatic environment with wide array of pesticides has become a menace to the aquatic flora and fauna. The organophosphorus pesticide malathion has been widely used in agriculture for several crops such as paddy, cotton and vegetables to control serious insects and mites. These chemicals cause severe damage to aquatic ecosystem especially to fishes. The main aim and objective of present study is to evaluate the impact on protein and glycogen in fingerlings of *Labeo rohita* an economically important Indian major carp. The 96 hrs LC50 value of malathion for *L.rohita* was 9.0 ml/L. No mortality was observed below the concentration of 7.5 ml/L. The changes in biochemical parameters such as protein and glycogen are important to indicate the susceptibility of organs system to pollutants by altering their function. In the present investigation the protein and glycogen contents of muscles, gills, liver, kidney and brain of *L.rohita* were analysed. Fish reared in control group registered high protein and glycogen contents when compared with malathion treated groups during 24, 48, 72 and 96 hrs respectively. The similar results were obtained in the oxygen consumption of *L.rohita* during 24, 48, 72 and 96 hrs respectively. It is concluded that malathion concentrations (low and high) is very toxic and causes alterations in vital organs of fish *L. rohita*.

KEYWORDS : water pollution, pesticides, fish, **INTRODUCTION**

The pollution of rivers and streams are caused mainly due to chemical contaminant. It has become one of the most critical environmental problems. The industrial and agricultural problems throughout the world in general and in India due to alarming rise in human population. It also caused tremendous environmental contamination. The aquatic environment is affected by various types of toxicants. Particularly pesticides used in agriculture are one of the major sources of water pollution. The

widespread use of pesticides not only brought adverse influence on agro-ecosystems but also caused alternation in physiological processes of non-target organisms. In many countries large-scale mortality of fishes has been recorded due to pesticides in water bodies as pollutants. The toxicity study is essential to find out limit and safe concentration of toxicants. Malathion is a non-systemic wide spectrum organophosphate insecticide. It was one of the organophosphate insecticides developed in 1950. Malathion is used for agricultural and non-agricultural purposes. One of the main factors causing pollution of the environment is irrational use of organophosphorous insecticides (A1-Haj et al., 2005). It is a major source of environment poisoning in developing countries (WHO,2003). Among the organophosphorous pesticides malathion is considered relatively safe for use in mammals. However, impurities in commercial formulations are potent inhibitors of carboxylesterases, allowing dramatic increase in malathion formation (Buratti et al., 2005). The present work has been carried out to study the lethal concentration of 50% (LC50) of pesticides, preferably malathion on the fresh water fish *L. rohita*.

MATERIALS AND METHODS FISH ACCLIMATIZATION

Almost equal sized fish *L. rohita* were brought from the local fish farm, Tirunelveli district of Tamil Nadu, India, and immediately transported to laboratory. These fishes were observed for any pathological symptoms and then placed in 0.1% potassium permanganate (KMnO₄) for two minutes so as to avoid any dermal infection. Then fishes were washed with water and acclimatized to laboratory conditions for three weeks during which they were regularly fed with dried pellets of 20% protein diet. The water used was clear and unchlorinated. Salinity, temperature, pH, dissolved oxygen content and water hardness of water were averaged to 0.25±0.1 ppt, 22.5±0.5°C, 7.9±0.1, 4.71±0.15ml-1 and 179±5.10 mgCaCO₃l-1, respectively.

Active and healthy *L.rohita* (2.97g:5cm) were chosen from acclimation tank, food supply was withdrawn 25 hrs prior to experimentation. A commercial grade of pesticide malathion (50% emulsified concentration, EC) was used for bioassay test. An acute toxicity (LC50) test by static renewal bioassay method was conducted to determine the toxicity of malathion in the *L.rohita* which was exposed to various concentration of malathion for 96 hrs and the pesticide was procured from the local market at Tuticorin, Tamil Nadu, India. The required quantity of malathion was drawn directly from this emulsified concentration using a variable micropipette.

For LC50 calculation, active fish (2.97±1.50g) were chosen and sorted out into seven groups

each consisting of 10 fishes. Test animals were exposed (in circular plastic trough of 25l capacity) to 0, of malathion, mortality was recorded for every 24 hrs and dead fishes were removed when observed, every time noting the number of fish deaths at each concentration up to 96 hrs. Triplicates were maintained simultaneously. The LC50 were determined / estimated with 95% confidence limit for malathion for 96 hrs by probit analysis (FINNEY, 1971). The concentration at which 50% survival / mortality occurred in malathion treated fishes was taken as the median lethal concentration (LC50) for 96 hrs which was 9.0 ml/L. One tenth of the LC50 value (9.0 ml/L) was taken for the sub lethal studies according to S Prague (1973).

RESULTS:

The 96 hrs LC50 value of malathion for *L.rohita* was 9.0 ml/L. No mortality was observed below the concentration of 7.5 ml/L. However the concentration of 7.5ml/L and above were observed to be toxic. The upper and lower 95% confidence limits were found to be 1.96 and 1.92, respectively. The **value of slope was 1.12. (Table 1).**

Table 1: Effect of malathion concentrations on per cent mortality in *Labeo rohita* exposed for 96 hr. Lethal concentration, slope function and 95% confidence limits are expressed in (µl/L)

Concentration of malathion	D e a d / tested	Mortality(%)	Lethal concentration(µl/L)			S l o p e function(S)	95% confidence limit	
			Slope function(S)	95% confidence limit			Lower	Upper
			16%	50%	84%			
7.5	1/10	10						
8.0	2/10	20						
8.5	3/10	30						
9.0	5/10	50				1.12		
9.5	7/10	70	7.8	9.0	9.8		1.92	1.96
10.0	10/10	100						

Table 2: Glycogen content in various tissues of *Labeo rohita* exposed sublethal concentrations of malathion.

Tissue	Control	Experimental Periods			
		24	48	72	96
Muscles	1.35± 0.21	1.10± 0.01	0.90± 0.06	0.43± 0.09	0.21± 0.87
Gills	0.4±00.32	0.33±0.08	0.29±0.12	0.21± 0.006	0.09±0.48
Liver	0.63±0.19	0.55±0.32	0.43±0.02	0.31±0.12	0.16±0.09
Kidney	0.60±0.14	0.54±1.04	0.43±0.009	0.31±0.23	0.19±0.11
Brain	1.02±0.42	0.98±0.54	0.76±0.06	0.45±1.07	0.28±0.10

Table 3 : Oxygen consumption *Labeo rohita* exposed sublethal concentrations of malathion

Rate of oxygen consumption (mg 2 g-1 hr-1)	Experimental Periods				
	Control	24	48	72	96
		0.20±0.02	0.29±0.22	0.47±0.05	0.53±0.04

The biochemical changes in both protein and glycogen against to the pesticides of malathion in experimental fish *L.rohita* were provided (Table 2). The changes in the biochemical constituents in the of the *L.rohita* exposed to malathion at different exposure were observed in the present study. The proteins contents were found to be significantly reduced in different tissues of malathion exposed fish. Similarly the results obtained showed that glycogen contents were found to be significantly reduced in different tissues of malathion exposed *L.rohita*, it indicates may be excess pollution which induced toxicity. In the present investigation fish reared in control group registered high protein and glycogen contents when compared with malathion treated groups. The similar results were obtained in the oxygen consumption of *L.rohita* during 24, 48, 72 and 96 hrs respectively (Table 3).

DISCUSSION

During behavioural manifestation, the *L.rohita* showed normal behavior such as well-coordinated with active movements, static equilibrium, active swimming, normal gill movement, free gulping of air at the surface water, horizontal hanging in the water with natural body color and zero mortality were observed in control group. But in the toxic environment fishes exhibited irregular, fishes frequently coming to the surface of water, loss of equilibrium, erratic and darting swimming movements, vertical hanging, increased opercular movements, rapid gill movement, fading of their body color and excess mucus secretion all over body and restlessness. Finally the fish sank to the bottom with their least opercular movement and died with their mouth open.

In the present study during acute toxicity test, the fishes exhibited several abnormal behavioural responses such as erratic and darting swimming movements and loss of equilibrium. They slowly become lethargic, hyperexcited, restless and secreted excess mucus all over their bodies. Opercular movements increased initially in all exposure periods but decreased later steadily in the sub lethal exposure periods. Borah and Yadav (1995) observed that, opercular movements are increased. loss of equilibrium, erratic swimming and jerky movement and mucous secretion all over the body were in *Heteropneustes fossilis* after exposure to rigor and endosulfan pesticides. Santhakuamr and Balaji (2000) also observed this phenomenon in *Anabas* test studies after exposure to monocrotophos. Fishes are the excellent models for monitoring environmental contamination in aquatic system. Many authors investigated that

pesticide toxicity induced respiratory distress in fishes. Natarajan (1981) found reduction in oxygen consumption in *Channa punctatus* exposed to organophosphate pesticide. Similarly Boradbury et al., (1986) stated that the rate of oxygen consumption decreased in *Cirrhinus mrigala* may be due to internal action of pesticide. Variation in oxygen consumption is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. Similarly Khillare and Wagh (1987) also found that rate of oxygen consumption reduced in the fish *Barbus stigma* when exposed to malathion and nuvan. In addition to Verma and Dalela (1975) observed that reduction of oxygen consumption of fish might be due to perched solids present in the effluents which cause mechanical injuries to fish and disturb the osmotic regulation. And also several authors reported that the disturbance in oxidative metabolism leads alternation in whole oxygen consumption in different species of fish exposed to pesticides.

CONCLUSION

In conclusion, the present work indicates that Malathion causes considerable changes in the intermediary metabolism of the fish *L.rohita*. The cause for these alterations appears to be the result of high energy demands in order to survive from the stressful condition from the toxicants. Hence it may leads to decrease the glycogen and protein from various tissues of the test fish.

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