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# ACUTE TOXICITY OF MATRINE CONTAINING BIOPESTICIDE KETHRIN ON A FRESHWATER FISH, *Labeo rohita* (HAMILTON)

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## ABSTRACT

**The objective of this study was to determine the toxicity of biopesticide Matrine (Kethrin, Brand name) on the freshwater fish *Labeo rohita.* Fishes were exposed to various concentrations of botanical insecticide matrine for 96 h and the percent mortality was recorded. The 96h $LC_{50}$ value determined by Finney's Probit Analysis Method was found to be 21.68 ppm. Behavioural patterns were observed critically during the whole experiment. The test fish exhibited erratic swimming, increased surfacing, decreased rate of opercular movement, reduced agility and inability to maintain normal posture and balance with increasing exposure time.**

**KEYWORDS :** Toxicity, Matrine, Kethrin, *Labeo rohita*, $LC_{50}$

In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of pest control that minimize this damage, there has been extensive research on pest control by substances from plants. One of the most promising natural compounds is matrine, an alkaloid extracted from the roots of sophora flavescens. S.flavescens is an evergreen shrub growing upto 1-1.5m. The plant prefers sandy, loamy and clay soils and requires well drained soil. Its roots have been found to contain many alkaloids including matrine and its oxide, oxymatrine. Characterization of alkaloids in Sophora flavescens Ait was reported by (Liu, 2011).Antifeedent activity and acute and residues toxicity of alkaloids from Sophora flavescens against formosan subterranean termites was reported by (Henderson , 2007).

Recently plant based pesticides are popularised due to their high efficiency, broad spectrum, low toxicity, non-residue and green protectin to environment. However, many botanical pesticides have been found to be toxic to non-target organisms where they induce marked alterations in experimental animals (Mahboob et al., 1998; Anjaneyulu et al., 1999; Mondal et al., 2007). Pesticides are major cause of concern for aquatic environment. They are carried into aquatic ecosystem by surface runoff from sites of application where they enter the organisms through food webs and also through contact in water. Therefore, the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides.

Fishes are highly sensitive to the alterations in the quality of water. So they are considered as good indicators of aquatic pollution. *Labeo rohita* was selected as the test species because of its wide distribution and high food value. So the present study was aimed to determine the 96h $LC_{50}$ value and the behavioural response of a freshwater fish *Labeo rohita* to a biopesticide Kethrin.

## MATERIALS AND METHODS

Kethrin (Manufactured by Ezzy Bio Sciences Pvt. Ltd., Khasra no. 90/1, Gram Meharja, Khargone, M.P.) was used in this study. It contains a minimum of 0.5% EC (500ppm) of matrine, obtained from the roots of shrub sophora flavescens.

*Labeo rohita* weighing 50±2g and average length of 12cm were collected from the Patra Fish Farm, Berkhedi, Bhopal, Madhya pradesh. The fishes were acclimatized to the laboratory conditions for 15 days. They were fed daily with commercial fish pellets.Water was renewed after every 24hrs. Physio-chemical characteristics of water were determined and maintained.

### Procedure

The experiments were conducted in a series of glass aquariums filled with 60 litre tap water. The stock solution was prepared and the required quantity of matrine was drawn from this stock solution to find out the $LC_{50}$ value for 96 h. Different concentrations were prepared and for each concentration a control was maintained. Ten

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acclimatized fishes of uniform size were exposed to each concentration. Preliminary tests were carried out to find out the median lethal concentration ($LC_{50}$) of the fish to matrine for 96h by Probit Analysis Method. The control and the exposed fish were aerated frequently to prevent hypoxic condition of the medium.The control and matrine exposed fish were kept under continuous observation during the experimental period. Feeding to fishes was stopped during the experiment. Behaviour of the test fishes was observed and the dead fishes were removed and recorded from time to time during 96 hr exposure period. The water in the containers was changed every 24 hr and a constant concentration of matrine was maintained during the period of exposure.

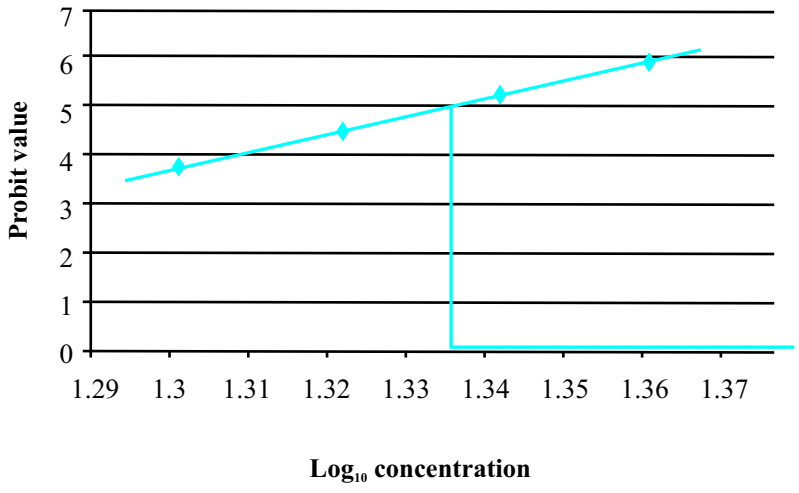
## OBSERVATION AND RESULTS

The maximum concentration at which zero percent mortality and minimum concentration at which 100% mortality of *Labeo rohita* were observed was at 19 ppm and 24 ppm respectively. The determination of 96h $LC_{50}$ value of matrine (Kethrin) to *Labeo rohita* was found to be 21.68 ppm by Finney's Probit Analysis Method,(1971) and is depicted in the table,1 and graph,1 below.

In this study, *Labeo rohita* was subjected to various concentrations of Kethrin and its behavioral changes were observed. The behavioral and the swimming patterns of the fish were normal in case of control group and there was no mortality. After the exposure of fishes to Kethrin, various behavioral changes were observed. First the schooling of fishes starts disrupting and then abnormal swimming behavior increases. The fishes were observed to hit the aquarium walls. The opercular movement initially increases and then decreases with rising toxicant concentration in the exposed fishes. Vertical and downward swimming patterns were observed. Loss of balance increased and the color of the fish were observed to get lighter with an increased secretion of mucus. Surfacing frequency and gulping of surface water with occasional coughing increases remarkably in exposed fishes. Defecation was increased and more fecal matter was found at the bottom of the

**Table1:Shows the relationship between the conc. of kethrin vs mortality of fish, *Labeo rohita***

| Conc. (mg/L) | $Log_{10}$ Conc. | Total No. | No. Dead | %Mor-tality | Probit. |
|---|---|---|---|---|---|
| Control | | 10 | 0 | 0 | - |
| 19 | 1.2788 | 10 | 0 | 0 | - |
| 20 | 1.3010 | 10 | 1 | 10 | 3.72 |
| 21 | 1.3222 | 10 | 3 | 30 | 4.48 |
| 22 | 1.3424 | 10 | 6 | 60 | 5.25 |
| 23 | 1.3617 | 10 | 8 | 80 | 5.84 |
| 24 | 1.3802 | 10 | 10 | 100 | - |




**Graph1: The graph showing linear curve between probit mortality of fish against $log_{10}$ concentration in *L. rohita* on exposure to Kethrin**

aquarium than control. Finally due to complete loss of balance, fishes sank to bottom with their ventral side facing upwards. After 96h exposure of pesticide to fishes, at 24ppm hundred percent mortality was observed.

## DISCUSSION

The acute toxicity values of several biopesticide products for different fish species have been reported earlier by many workers. Das et al., (2002) have studied the acute toxicity of neem in the fingerlings of Indian major carps i.e., *Labeo rohita, Catla catla* and *Cirrhinus mrigala* and the 96h $LC_{50}$ values were found to be 2.36, 2.04 and 2.78ppm respectively. Hassanein et al., (2007) reported the 96h $LC_{50}$ value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm. In the present study, the 96h $LC_{50}$ value of matrine (Kethrin) on the *Labeo rohita* was found to be 21.68ppm. The variation in the $LC_{50}$ values is due to its dependence upon various factors viz., sensitivity to the toxicant, its concentration and duration of exposure; type and size of the test animal and so on.

Behavioral changes are the most sensitive indication of potential toxic effects. Impact of different pesticides on the behavior of *Labeo rohita* have been studied by various workers (Marigoudar et al., 2009; Anita et al., 2010; Nagaraju et al., 2011).

Fishes exhibited a number of behavioral changes when they were exposed to different concentrations. The opercular movement of fishes initially increases and then gradually decreases. Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Abnormal swimming and loss of balance was caused by the decifiency in nervous and muscular coordination which may be due accumulation of acetylcholine in synaptic and neuromuscular junctions (Rao et al., 2005). A thick coat of mucus was observed all over the body of the fish, making the fish slimier. The fish were swimming with the belly upwards and in zig zag motion. There were also erratic and parallel movements observed in the fish, indicating loss of equilibrium while in control, the fish was swimming normally without loss of equilibrium. .

Botanical pesticides are presently used against different pest species in the agricultural farms and gardens but their effect on non-target organisms can not be ruled out. So, these botanical pesticides should also be used cautiously like synthetic pesticides.

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