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TOXIC EFFECT OF SELECTED PESTICIDES ON AN ENDEMIC LOACH *LEPIDOCEPHALICHTHYS IRRORATA* (FAM. COBITIDAE)

W. Vidyarani *et al.*

Pesticides

LC_{50}

L. irrorata

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W. VIDYARANI*, H. SUNITA AND S. SANAYAIMA
Department of Life Sciences, Manipur University,
Canchipur-795 003

ABSTRACT

Toxic effect of some commonly used pesticides like Endosulfan chlorpyrifos,Quinalphos, Dimethoate, Cypermethrin, Fenvalerate have been studied to determine the LC_{50} at 24 hr; 48hr; 72hr, 96hr, on an endemic loach *L. irrorata*., Hora. The results showed that the Organochlorine compound endosulfan is highly toxic to fish than the other Organophosphate and Pyrethroid pesticide compounds. The sequence of toxicity is in the order given as endosulfan > Cypermethrin > Quinalphos > chlorpyrifos > Dimethoate > Fenvalerate.

***Corresponding author**

INTRODUCTION

Fish serve as consumers of small organism and food chain continues to higher organisms. They contribute to economy of many nations and provided recreational and psychological value to the naturalists, sports, enthusiast and aquarist. They also play important role directly or indirectly in the protection of heritage and life of human beings. The threats and the integrity of the environment and germplasm resource pose serious concern.

In recent years human intervention have brought major changes in the aquatic ecosystem. One of the important such intervention is that of pesticides. The pesticides are used to enhance agricultural production however their deleterious effects are often noticed in non target organisms like the fish. Reports revealed that the fishery resources in some region of India have dwindled considerably and some of the fish species are of the wane (Kurup *et al.*, 1990). Reports on the toxic effects of pesticide like phosphamidon on different fish species are available (Toor and Kaur, 1974; Rita Kumari and Nair, 1978; Choudhuri *et al.,* 1984; Gopalakrishnan, 1990; Govindan *et al.*, 1994).

The term pesticide encompasses a group of chemical compounds used to eliminate or control of pests. Pesticides have economic and public health benefits and have been used over the years for the control of vector borne diseases such as malaria, rocky mountain fever, and domestic pest (Jacob *et al.,* 1982).

The registration and regulatory requirements concerning pesticides are governed under the Federal Insecticides, Fungicides and Rodenticides Act (FIFRA). Interpretation of acute toxicity and data base for 410 chemicals and 166 species of freshwater animals are recorded in the resources publication of U.S. Dept. of interior fish and wild life service Washington DC. (Mayer and Eller, 1986). The pesticides have accumulative residual effect on the organisms. The design of toxicity test essentially incorporates the following five basic components: selection of test organism, selection of a response to measure exposure period, test duration and series of doses to treat. Doses are described as lethal doses (LD) or lethal concentration (LC) where the response measured is mortality. Construction of the cumulative dose response curve enables us to identify doses that affect a specific percent of the exposed population. It means that LD_{50} or LC_{50} is the dosage lethal to 50 percent of the test organism or one may chose to identify a less hazardous dose such as LD_{10} or LD_{01}. The route of exposure also determines how much of the chemical substance enters or absorbs into the test animal and which organs are initially exposed.

The pollution of rivers, streams and larger water bodies like lakes with chemical contaminant in the form of industrial effluents and pesticides has become one of the most critical environmental problems of the century. There is growing concern worldwide over the indiscriminate use of such chemicals resulting in environmental pollution and toxicity risks to aquatic organisms (Khan, 1996). In aquaculture, organophosphate, organochlorine and pyrethroid pesticides are used to control agricultural pests as well as parasites in fish. During rainy season, these pesticides are accumulated to the pool of water bodies and poses the risks of survivability and reproduction capacity. Taking into the importance of the above facts, the expected toxicity on an endemic fish of Manipur (*Lepidocephalichthys irrorata* have been selected as the test animal to observe the responses of pesticides like endosulfan (Organochlorine compound), Chlorpyrifos, Quinalphos, Dimethoate (Organophosphate compound), and Cypermethrin, Fenvalerate (Pyrethroid compounds) which are the commonly used pesticides in the agricultural fields of Manipur.

MATERIALS AND METHODS

Toxicity of selected pesticides were observed under room temperature in the laboratory. The fish were brought from the market and acclimatized in the aquarium for one week in the laboratory. The animals were fed with dried prawn powder and multivitamins once a day. Pond water (pH -7.4) was used for acclimatization

and experiment giving a change on alternate day. Prior to the commencement of the experiment length and weight of the fishes were taken and grouped into six of five males and five females in each group per

aquaria. The sex of the animals ranged from size 4.5cm to 4.9cm in length and 0.30g to 0.53g in weight. For the formulation of the test concentration, pilot experiment was conducted and range of concentration selected was such that it resulted into zero to total mortality. The formula used to prepare the pesticide solution is as follows:

$$\text{No. of mL for required volume} = \frac{\text{reqd.ppm x reqd.volume}}{\text{Stock solution}}$$

The volume of the aquaria were maintained at 3L for each container. One aquarium with pond water was kept for each series of experiment as control. Observations were made at 24 hr,48hr,72hr, 96hr. Behavioural changes of the fishes after introduction into the different pesticide concentration were determined through probit analysis using the software SPSS for Window 98. The structural formulas of the different pesticides are given in Table1.

RESULT AND DISCUSSION

The LC_{50} values of the six pesticides on *L. irrorata* at 24 hr, 48hr, 72hr. and 96hr. are given in table 2. At the time of introduction of the fishes into the different concentration of the pesticides, almost all the fishes showed inactive behavior through slow swimming, lying of the body by the side at the bottom of the aquarium. However, they gain their activity after five hours of exposure. Prior to death the fishes showed sluggish and

Table 1: The different concentration of pesticides used are

Name of pesticides		Selected concentration$_S$
1.	Endosulfan	0.0000000069ppm,0.0000000075ppm,0.0000000081ppm,0.0000000087ppm and 0.0000000093ppm
2.	Chlorpyrifos	0.007ppm,0.009ppm,0.011ppm,0.013ppm and 0.015ppm
3.	Quinalphos	0.0010ppm,0.0015ppm,0.0020ppm, 0.0025ppm and 0.0030ppm
4.	Dimethoate	0.025ppm,0.027ppm,0.029ppm,0.031ppm and 0.033ppm
5.	Cypermethrin	0.0001ppm,0.0003ppm,0.0005ppm,0.0007ppm and 0.0009ppm
6.	Fenvalerate	0.02ppm,0.04ppm, 0.06ppm,0.08ppm and 0.10ppm

imbalance movement and they sink into the bottom of the aquarium. The reddening colour of the gills could be observed externally through the operculum which might be due to haemorrhage in the gills.
The pooled data after analysis through probit and the calculated LC_{50} indicates a decreasing toxicity order of the pesticides as follows:

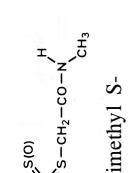
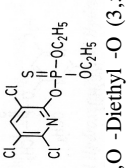
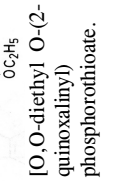
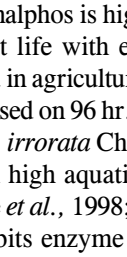
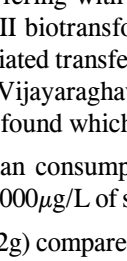
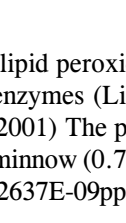
Endosulfan > Cypermethrin > Quinnalphos > Chlorpyrifos > Dimethoate Fenvalerate.

The environmental destruction caused by anthropogenic factor lead to further destruction of aquatic fauna and especially the fishes. Endosulfan is an synthetic organochlorine pesticide and act on the nervous system through interference with cation exchange across the nervous cell membrane resulting in hyperactivity of the nerves. The rate of degradation is slow and the bioaccumulation in food chain is very high leading to the eventual discontinuance of their use (Phillip *et al.*, 2000). In mice the primary site of organochlorine storage

Table : 3 The LC_{50} values of six pesticides on *L. irrorata* at 24 hr, 48 hr, 72 hr and 96 hr are as follows, (Conc. in ppm).

Chemicals	24hr-LC_{50}	48hr-LC_{50}	72hr-LC_{50}	96hr-LC_{50}
Endosulfan	0.0000000079	0.0000000072	0.0000000069	0.0000000066
Fenvalerate	0.18670	0.11326	0.09422	0.07760
Cypermethrin	0.00092	0.00068	0.00035	0.00034
Dimethoate	0.05557	0.05557	0.02978	0.02883
Chlorpyrifos	0.01251	0.00549	0.00510	——
Quinalphos	0.00277	0.00192	0.000137	——

Table 2: Structural formulas of the pesticides

Endosulfan	Chlorpyrifos	Quinalphos	Cypermethrin	Fenvalerate	Dimethoate
					
6,7,8,9,10 Hexachloro 1,5,Sa, 6,9,9a-hexahydro-6-9-methano-2-4-3-benzodioixathiepin -3-oxide	O,O -Diethyl -O (3,5,6-trichloro-2- Pyridyne) phosphoro - thioate	[O,O-diethyl O-(2-quinoxalinyl) phosphorothioate.	cyno-(3-phenoxyphenyl-3)-2-2-dimethyl-cyclopropane carboxylate	Cyano-(3-phenoxyphenyl)-methyl 4-chloro-±-(1-methylethyl) benzeneacetate	O,O-Dimethyl S-(N-methylcarbamoyl) methyl phosphoro-dithioate

is adipose tissue. It is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by monooxygenase systems which causes oxidative stress (Kitchin, 1984).

Free radicals generated during oxidative stress causes lipid peroxidation of cell membrane which in turn prevents antioxidant enzymes (Liang *et al.,* 1922; Kurutus and Tuncer, 2000, Kurutus *et al.,* 2001) The present dose applied is based on 96 hr LC_{50} 1.4mg/L of feather minnow (0.7g) and endosulfan is found to be very toxic to *L. irrorata* (-6.622637E-09ppm).

Dimethoate is applied to kill mites and insects systematically with contact toxicity. They act on animals by

interfering with the activities of cholinesterase and can undergo phase I and II biotransformation reactions including oxidation, hydrolytic GSH-mediated transfer and conjugation reactions. In *Clarius batrachus* (Begum and Vijayaraghavan, 1996) poisoning of dimethoate with low protein value was found which is not fit for

human consumption. The present dose applied is based on 96 hr LC_{50} 111.000μg/L of sheepshead minnow

(0.52g) compared to 0.02883ppm in *L. irrorata*.

Quinalphos is highly active against biting and sucking insects and due to its short life with easy detoxification in animal tissue they are still widely used in agriculture and aquaculture. The doses employed in this experiment is based on 96 hr. LC_{50}. 0.22mg/l of *Labeo rohita* compared to 0.00192 ppm in *L. irrorata* Chlorpyrifos is a broad spectrum organophosphate insecticide with high aquatic toxicity specially to fresh water fish and invertebrates (Foe *et al.,* 1998; Baily *et al.,* 1997). The action is mediated through contact, inhibits enzyme acetylcholinesterase. The preparation of chlorpyrifos is based on the 96 hr. LC_{50}-7.17 μg/L of Guppy (3.5±1.0cm@ and, 2.0±1.0cm B and). In *L. irrorata,* the 48hr. LC_{50} of Chlorpyrifos is 0.00549.

Fenvalerate is a broad spectrum contact synthetic pyrethroid insecticide. Metabolic studies have shown that

the fishes have lower capacity to metabolise and eliminate pyrethroids (Glieckman *et al.,* 1982). Generally crustaceans, molluscs and fishes are more sensitive to fenvalerate followed by amphibians, reptiles, birds and mammals (Lenden *et al.*, 1979. Mc Leese *et al.,* 1980). The referral concentration has been based on the 48hr LC_{50} 25μg/l in *Cyprinodon macularis* (4-5cm) and *Tilapia mossambica* 48hrs, LC_{50} 200 μg/L (5-10cm). In *L. irrorata*, the 48hr. LC_{50} is 0.11326ppm.

Cypermethrin is the most active pyrethroid contact pesticide and is used for the control of ectoparasites of cattle. The action of the pyrethroids is on the voltage- dependent sodium channels in nerve. They have greater insecticidal activity with lower mammalian toxicity than the organophosphates, carbamates and organochlorine insecticides. The toxicity of cypermethrin in laboratory tests 96 hr LC_{50} is 0.4-2.8μg/L for fish and 96 hr LC_{50} in aquatic invertebrates ranges 0.01-5 μg/l (Stephanson, 1982; Sarkar *et al.*, 2005). The preparation used is based on 96 hr. LC_{50} -0.139ppm/l in *Labeo rohita* and *Nile Tilapia, Tilapia nilotica* LC_{50} 4.00μg/L (0.3-1.49g).

Result of the investigation showed that endosulfan is more toxic to fish than the other pesticides. It is conclusive that organochlorine compound (endosulfan) is highly toxic than other organophosphate and pyrethroid compounds in *L. irrorata*.

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