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Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022)

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Rajarshi Shahu Mahavidyalaya (Autonomous),
Latur- 413531, Maharashtra

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Impact of Dimethoate Toxicity with Special Reference To Histopathological Alteration In Intestine of The Freshwater Fish *Rasbora Daniconius*.

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Abstract:

The acute toxicity bioassay of dimethoate was carried out by the probit analysis method. The 24, 48, 72 and 96 h LC_{50} values were obtained as 11.63, 10.08, 10.54 and 9.136 ppm respectively. In the present investigation the effect of dimethoate on the gill of *Rasbora daniconius* at 96 hours LC_{50} marked histopathological changes found like degenerative effect is evident in the mucosal lining and villi of the intestine, loss of intestinal epithelilal layer and shape, hypertrophy of epithelial cells, swelling, fusion of villi due to excessive hypertrophies, swelling in lamina propria, ultimately leading to the rupture of villi at tip and cracked clay aperance in columner epithelium.

Key Words: *Rasbora daniconius*, dimethoate, LC_{50}, Intestine.

Introduction:

The toxicity studies are especially useful in determining the sensititive species of an ecosystem that can be used as i9ndicator species, for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration, LC_{50} or median tolerance limit (Vasait and Patil, 2005). In aquatic toxicology, the traditional LC_{50} test is often used to measure the potential risk are valid only for the species that are tested and the specific conditions used (Sivakumar *et.al*. 2006). Toxicity to aquatic biota is significantly influenced by abiotic factors such as hardness, temperature, pH and salinity.

Histology is an important tool for determining the action of any toxicant at tissue level, providing data concerning tissue damage (Sprague, 1973).

Histopathology deals with the study of pathological changes induced in the microscopical structure of body tissue. Any peculiar alteration of cells may indicate the presence of disease or the effect of toxic substance. Histopathological studies have been used to evaluate the effects of conataminants on the health of fish in the environment and to help establish a causal relation between exposure to toxic substancs and the various biological responses (Schwaiger *et.al,* 1997).

Hence, in the present investigation to see the toxicity level of dimethoate to the freshwater fish *Rasbora daniconius* and the effect of dimethoate on the intestine.

Materials and Methods:

Collection and Acclimatization of the Experimental Fish:

Rasbora daniconius fish were collected from local fisherman brought in the laboratorywith polythene bags containing water from the collection site. The fishes are placed in the glass aquarium. Before the experiment, fish were treated with 0.1% of KMnO4 solution to remove any dermal infection. The fish were acclimatized in laboratory conditions for 2 weeks before they were used in the bioassay tests. During acclimatization, the fish were fed with the live diet. Before experiment the fish were starved for 24 hours. for this experiment standard method for the test of acute toxicity of pollutants suggested by APHA (1998).

Acute Toxicity Bioassay and Statistical Analysis of Data:

For the acute toxicity experiment were carried out for 24, 48, 72 and 96 hr lethal median concentration (LC_{50}) was determined by three methods Probit analysis by (Finney 1971), (Dragstedt & Behrens, 1975) and graphical methods.

Histopathological Study:

For the histopathological alteration in the different tissues in *Rasbora daniconius* were used standard methods suggested by the (Ramnik Sood 2006). The test fish, *Rasbora daniconius* were exposed to 96 hrs LC50 concentration of dimethoate. (9.136ppm) served as experimental group and simultaneously a control was also maintained. Fishes showing normal activity were selected for each test. At the end of acute exposure (96 hr) the survived fish were decapitated and immediately the tissues intestine were removed and fixed in aqueous bouin's fluid for 24 hours. These tissues

were dehydrated in different grade of alcohol and blocks were prepared in paraffin wax (60-62^{0}C). The sections of 5-6 m thickness were cut and stained with hematoxyline and Eosin. All the tissues microscopic view taken at high-resolution power with the help of Panasonic 7 megapixel digital camera. All the slides were observed under low and high resolution for their histological findings.

Results:

In present investigation static bioassay test was selected to see the toxicity of dimethoate on *Rasbora daniconius*. Nine different concentrations of dimethoate i.e. 5.o to 13.0 ppm were selected. In the present investigation the acute toxicity bioassay of dimethoate was carried out by the probit analysis method the value obtained 11.63, 10.08, 10.54 and 9.136 ppm at 24, 48, 72 and 96 h LC_{50} respectively. Percent mortalities of fish *Rasbora daniconius* exposed to Dimethoate at 24, 48, 72 and 96 hours are shown in Table No.1. The calculated values of dimethoate toxicity to the freshwater fish Rasbora daniconius used three methods are represented in table No. 2.

The histopatholigical changes found in sub lethal concentration of dimethoate exhibited marked hispathological changes were found. In exposed fish a degenerative effect is evident in the mucosal lining and villi of the intestine. The intestinal epithelial layer lost its shape. Hypertrophy of epithelial cells and swelling and fusion of villi due to excessive hypertrophies, swelling in lamina propria, ultimately leading to the rupture of villi at tip and cracked clay aperance in columner epithelium. Many of the microvilli detached and mucus layer was found almost eroded. The lumen was extended but not well marked. The effect of dimethoate on intestineto different exposure period is shown in figure A and Figure B.

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Table 1. Percent mortalities of fish *Rasbora daniconius* exposed to Dimethoate at 24, 48, 72 and 96 hours.

Sr. No.	Conc. (in ppm)	No. of fishes exposed	Percent mortality at			
			24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	5.0	10	00	00	00	00
2	6.0	10	00	00	10	10
3	7.0	10	10	10	20	40
4	8.0	10	20	20	30	60
5	9.0	10	30	40	60	70
6	10.0	10	40	50	80	90
7	11.0	10	60	70	90	100
8	12.0	10	80	90	100	--
9	13.0	10	100	100	---	---

Table no. 12. Showing Lc 50 values of three different methods for Dimthoate for the freshwater fish *Rasbora daniconius*.

Pesticide	Method used	LC_{50} values of different exposure periods.			
		24 hours	48 hours	72 hours	96 hours
Diemthoate	Probit Analysis	11.50	10.00	10.50	9.00
	Dragstedt & Behrens	11.58	10.25	10.63	9.408
	Graphical	11.00	10.00	10.50	9.00
	Average	**11.36**	**10.08**	**10.54**	**9.136**

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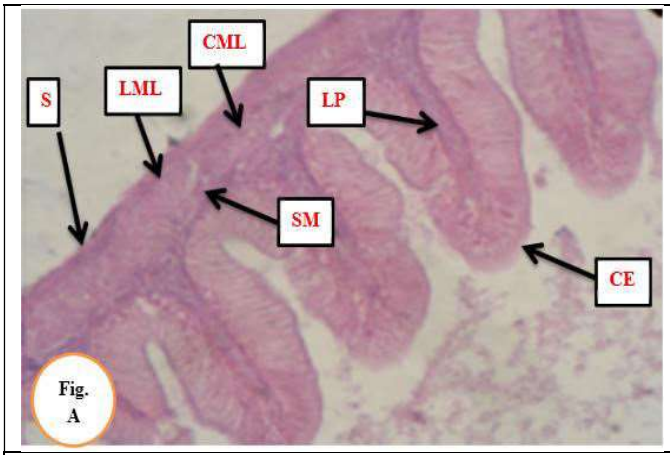

Fig. (A) Photomicrograph of Intestine section of control fish rasbora daniconius shows longitudinal muscle layer **(LML),** circular muscle layer **(CML),** lamina propria **(LP),** serosa **(S),** sub mucosa **(SM)** and circular epithelium **(CE).**

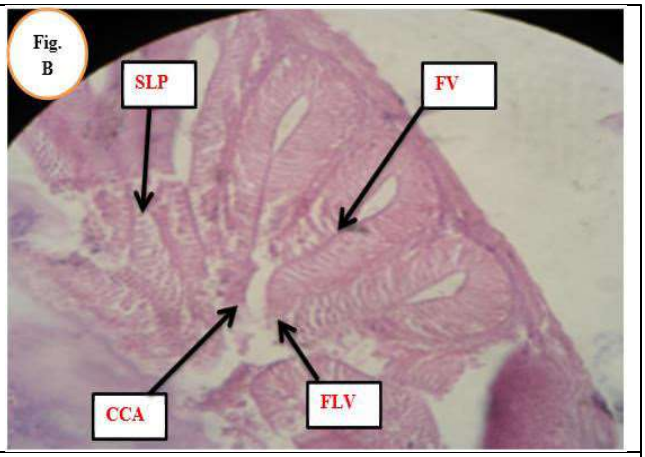

Fig. (B) Photomicrograph of Intestine section of Rasbora daniconius exposed to dimethoate at 96 hours LC_{50} showing swelling in lamina propria **(SLP),** fusion of villi **(FV),** flattened villi **(FLV)** and cracked clay appearance of villi **(CCA).**

Discussion:

Sharma *et al*. (2003) worked on toxicity of the azo methyl red to the organisms with special reference to the Guppy (*Poecillia reticulata*) and reported that establishing adverse effects on their energetics methyl red also affected defensive mechnism of intestine, initially by deforming structure of goblet cells, followed by reduction in their counts. Nagrajan and Yuvarani (2006) studied influence of neem oil on histology of intestine of Indian major carp, *Labeo rohita* and observed that the intestinal epithelial layer has slightly lost its cylindrical shape. The lumen is extended but not well marked the intestinal microvilli show slight damage at some places on exposure of sublethal concentration of neem (0.034 m) and also exposed at LC_{50} observed the intestinal layer has lost its cylindrical shape and detached in some places from the mucous layer. The intestinal microvilli lost their shape and deteriorated at one on two places. Nagarajan Bagrahab and Arunadevi (2006) worked on histological changes in Indian freshwater major carp *Labeo rohita* due to distillery effluent and reported that outer epithelial layer is not affected but the intestinal villi show sign of deterioration at some places. They stated that intestinal mucous layer got damaged

and in the intestinal cavity the lumen is constricted to some extent. The intestinal epithelial layer has lost its shape in 55% treated distillery effluent.

Shweta Sharma *et.al.* (2006) studied on histopathological studies of selected vital organs of a freshwater fish *Poecilia reticulata* following chronic exposure to an azo dye methyl red and observed their one week of exposure at 5 ppm and up to 2nd week at 10 ppm. They formed a homogenous mass of necrosed cells the flask shaped goblet cells, found in the control list were almost rounded in the methyl red treatment due to loss of reck. After two week of exposure at 5 ppm lymphocytes lost their cellular identity forming homogeneous mass along with epithelial columnar cells. The histopathological changes observed in alimentary canal of *Tilipia mosambica* showed damage to epithelial cells, hyperplaia and vacuolation due to disintegration of epithelial cells reported by Sultan and Rajan (2007) while working on histopathological lesions induced by heavy metals. Bhatagnar *et.al* (2007) Studied on fluroid exposed to the freshwater fish, *Labeo rohita* and observed the changes in the intestine. They reported a degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to became flattened, hypertrophy of epithelial cells, swelling of lamina propria, leading to rupture of villi at tip.

Yomn Mohamed Mobarak and Mariam Mahmoud Sharaf (2011) studied the effect of lead acetate on histopathological changes in the Silver Sailfin Molly (Poecilia latipinna) observed that the fusion of intestinal microvilli and wide intercellular spaces among epithelial cells of microvilli, an increased rate of cell death, necrosis and irregularitieso the microvilli cells. Shanta Satyanarayan *et.al* (2012) studied on histsopathological changes due to some chlorinated hydrocarbon pesticides in the tissues to *Cyprinus carpio* and showed changes in intestine due to toxicity were the flattening of intestinal folds, fusion with each other, shrinkage of cells and acute epithelial necrosis.

Conclusion:

In present investigation the average LC_{50} of dimethoate were 11.63, 10.08, 10.54 and 9.136 ppm respectively. The work of determination of toxicity of dimethoate, above literature of toxicology also clears that LC_{50} values decrease with increase in exposure period suggesting that with increase in duration of exposure the pesticide becomes toxic even at lower concentration. The

toxicity study indicate that the dimethoate causing the toxic effect of the fishes and the pesticides are accumulated in the tissues such fishes are used as food for the human being they may be affects the health of humans.

In the present investigation toxicity of dimethoate to the freshwater fish *Rasbora daniconius* exposed to 96 hours LC_{50} histopathological changes observed in the tissue intestine. Histopathological changes due to the effect of dimethoate clearly show the intestinal epithelial layer lost its shape. Hypertrophy of epithelial cells and swelling and fusion of villi due to excessive hypertrophies, swelling in lamina propria, ultimately leading to the rupture of villi at tip and cracked clay aperance in columner epithelium. Many of the microvilli detached and mucus layer was found almost eroded. The lumen was extended but not well marked.

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