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STUDIES ON TOXICITY EVALUATION OF DIMETHOATE ON THE FRESHWATER FISH *RASBORA DANICONUS* WITH SPECIAL REFERENCE TO THE GILL HISTOPATHOLOGY

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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 LC50 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 LC50 marked histopathological changes found like degeneration of epithelium of secondary gill lamellae, vacuolation, fusion, shortening of secondary gill lamellae, thickness and fusion of primary and secondary gill lamellae.

Keywords :- Rasbora daniconius, dimethoate, LC50, gill.

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 gill.

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).

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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 LC_{50} 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 gills 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. The main features observed in gills exposed to lethal concentration of dimethoate were partial degeneration of epithelium of secondary gill lamellae, vacuolation, fusion, degeneration of gill lamellae and separation of basement membrane where as the shortening of secondary gill lamellae resulting in reduction of respiratory surface and vacuolization was also observed. The effect of dimethoate on gill to different exposure period is shown in figure A and Figure B

DISCUSSION:

Vasait,*et.al.*(2005) studied toxic evaluation of organophosphate insecticide monocrotophos on the edible fish species *Namacheilus botai* for a period of 7 and 14 days and showed that the LC 50 values were 49.6 and 42.0 ppm respectively. Arunadevi and Nagarajan (2006) studied impact of distillary effluent on certain physiological aspects of the Indian major freshwater carp, *Labeo rohita* reported that the LC 50 values were 5.5% and 4.5% of treated and untreated respectively for the period of 96 hours. In the pesent investigation no mortality was found in the control group of fish. In the experimental group of fish, results were noted as 10% to 100 % mortality during the experiment at 24, 48, 72 and 96 hours (Table 1). The LC_{50} values were found to be 11.36, 10.08, 10.54 and 9.136 at 24, 48, 72 and 96 hours respectively as represented in Table 2. From the above results, it was observed that the toxic effect of dimethoate is highly toxic to *Rasbora daniconius*. Srivastava *et.al.* (2007) studied acute toxicity of dimethoate on freshwater catfish, *Heteropheustes fossilis* and reported taht

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the LC 50 values were 0.76 mg/l, 0.26 mg/l, 0.23 mg/l and 0.15 mg/l. for the period of 24 hr, 48 hr, 72 hr and 96 hr respectively. Tilak and Kumari (2009) carried the acute toxicity of organophosphate Nuvan to the grass carp Ctenophryngodon idella and LC 50 values were found to be 13.1, 10.9, 9.8 and 6.5 ppm at 24, 48, 72 and 96 h respectively. Similar observation was made by the above worker. Variability in acute toxicity even in a single species and single toxicant depending on the size, age, experimental species and factors (Farah *et al.* 2004). The existing physiological parameters of water were reported by Eaton & Gilbert (2008).

Dutta *et al.,* (1990), observed that with malathion exposure the fish aquires several defensive structural modifications in the affected gill such as dilated lymphoid spaces, proliferation and migration of the leucocytes in the lymphoid spaces and the vascular capillaries, lifting of the epithelial layer, thickening of the basement membrane and formation of a large number of chloride cells. Rao and Munshi (1991) reported that lifting of epithelial cells and their destruction might have resulted in lesser diffusion capacity in the gill of *Cirrhinus mrigala* exposed to Malathion. The histopathological changes of gill can results in hypoxia, respiratory failure problems with ionic balance and acid-base balance (Alazemi *et.al.*,1996). Anithakumari and Sreeram Kumar (1995, 1997) reported several histopathological changes in kidney, liver, gills, intestine and ovary due to impact of industrial effluents in the fish, *Channa punctatus* and *Hetropneustes fossils*.

Sakthivel and Gaikwaid (2002) studied toxicity of dimecron on tissue histopathology of *Gambusia affinis* and reported that the spaces between the adjacent secondary lamallae were seen filled with blood cells and at some places the lamellae appeared like swollen bulging sacs filled with blood cells, balloming effect. Epithelial wall of the secondary lamellae seem to be ruptured vacuolation and liffting of respiratory epthelium of secondary lamallae also occurred as compared to control. Wani *et.al* (2011) studied on histopathological alteration due to the effect of copper sulphate to the freshwater fish, *Clarias gariepinus* and reported that the lifting of lamellar wpithelium and odema in the filamentary epithelium while as lamellar disorganistion, swollen and fuson of secondary gill lamellae tips were noticed after 60 days of exposure. Moitra *et.al* (2012) studied on histopathological changes in the gills of air breathing teleost *Clarius batrachus* Linn. Exposed to endosulfan and reported that the 10 days of exposure period changes of gill epithelial necrosis, hypertrophy of the epithelial cells, rupture of gill epithilum, hemorrhage at primary lamellae and sloughing of respiratory epithelium. K. Ramah (2011) repoterd histopathological changes to gills of *Ctenopharygodon idella* (grass carp) exposed to herbicides showed several pathological changes. The changes observed were cartilaginous hyperplasmia of gill rays, proliferation of lamellar epithelium and vacuolation of cytoplasm of linig epithelium, focal loss of lamellar epithelium and congestion of blood spaces. Muthukumaravel and Rajaraman (2013) studied on histology of gill and liver of freshwater fish; *Labeo rohita* exposed to chromium and observed that the sublehal concentraction of chromium shows fusion and shortning lamellae, hypertrophy, degeneration of epithelium and necrosis were found in the gills of chromium treated. Shugufta Praveen (2013) studied on lead nitrate induced histopathological alteratios in the gills of freshwater teleosts, *Channa striatus* (Bloch) reported after 15 days the gills exhibited shortening, deshaping and fusion in gill lamellae and after 30 days fishes exhibited distruption of

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tips of the gill lamellae which results in complete fusion of gill lamellae and edema in the filamentary epithelium. The above many workers have reported the similar results on the histopathology of gill.

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 gill. The gills exhibited histopathological changes partial degeneration of epithelium of secondary gill lamellae, vacuolation, and fusion, degeneration of gill lamellae, shortening and fusion of secondary gill lamellae resulting in reduction of respiratory surface and vacuolization was also observed due to the lethal concentration of dimethoate for 96 hours LC_{50} were found to be dose dependant.

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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**

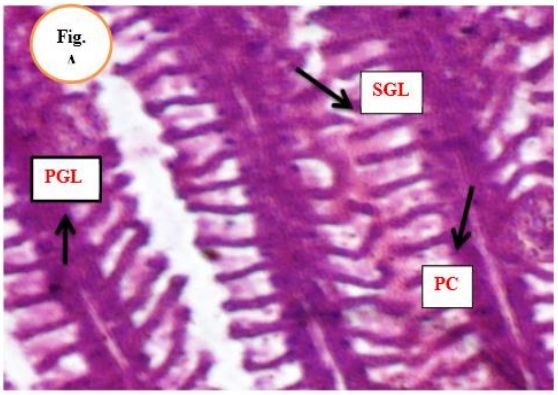

Fig. (A) Photomicrograph of the gill section of control fish *Rasbora daniconius* showing the primary gill lamellae (PGL), secondary gill lamellae (SGL) and pillar cells (PC).

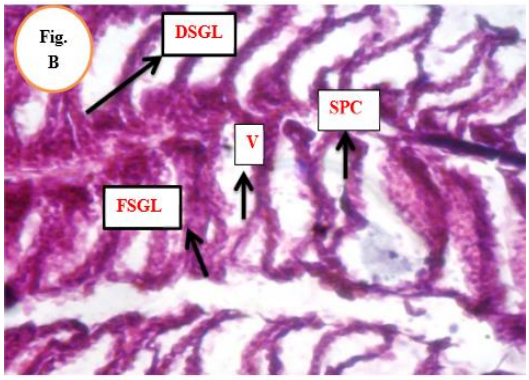

Fig. (B) Photomicrograph of gill section exposed to dimethoate at 96 hours LC_{50} showing degeneration of secondary gill lamellae (DSGL), Voculation (V), Fusioin of secondary gill lamellae (FSGL) and separation of pillar cells (SPC.)

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