

TOXICITY STUDIES OF AGROCHEMICALS TO ADULTS OF FROG *RANA TIGRINA* USING PHARMACOLOGICAL BIOASSAYS

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ABSTRACT

The wide scale use of agrochemicals and their hazardous impact on biotic components of aquatic and terrestrial ecosystems has been the subject of debate since last two years. Recently many workers have reported toxicity of metals and agrochemicals to frog. Present study was aimed to determine the acute toxicity of different groups of pesticides to the adults of Indian bullfrog *Rana tigrina*. Isolated rectus abdominus preparation was used for the evaluation of toxicity to the skeletal tissues. While cardiac toxicity was determined by using isolated heart perfusion technique. Acute toxicity of three agrochemicals, one herbicide Paraquat and two fungicides Indofil and Fytolan was estimated to the adults of frog *Rana tigrina*. Among these bipyridium herbicide was found to be highly toxic followed by carbamate fungicide indofil and copper based fungicide fytolan.

INTRODUCTION

The wide scale use of agrochemicals and their hazardous impact on biotic components of aquatic and terrestrial ecosystems has been the subject of debate since last two years. Broad spectrum of agrochemicals is applied to the farmland to increase crop production. These include insecticides, herbicides,

fungicides, rodenticides and fertilizers. The surface runoff from these agricultural fields contains pesticide residues. An amphibian larval stage qualifies the criteria for being used as an indicator of harmful levels of pollutants in bioassay tests (Cook 1981). Recently many workers have reported toxicity of metals and agrochemicals using frog tadpoles as test organism in bioassay tests. Studies have been conducted with heavy metals (Khargarot *et al* 1978) pesticides (Alfredo 1992) (Alvarez 1995) and herbicides (Dial & Baur, 1984) (Norman *et al.* 1987).

Present study was aimed to determine the acute toxicity of different groups of pesticides to the adults of Indian bullfrog *Rana tigrina*.

MATERIALS AND METHODS

Adults of frog's, bred in the frogery at the G. C. O. P. Karad, were used as the animal models for the evaluation of the pharmacological effect of the pesticides.

The toxicants used in the current study were, Paraquat dichloride (Bipyridinium herbicide) 24% SL United phosphorus Ltd. Vapi, Gujrat. Indofil (Carbamate fungicide) mancozeb 75% w/p of Indofil chemical company, Mumbai and Fytolan (Copper containing fungicide) of Standard pesticide Pvt. Ltd. was procured from the market. Acetylcholine chloride (100 µ/mL) was used as a standard. Frog ringer solution [NaCl-6.5g, KCl-0.14g, CaCl₂-0.12G, NaHCO₃-0.4g, Glucose - 1.5g and Distilled water up to 1000mL].

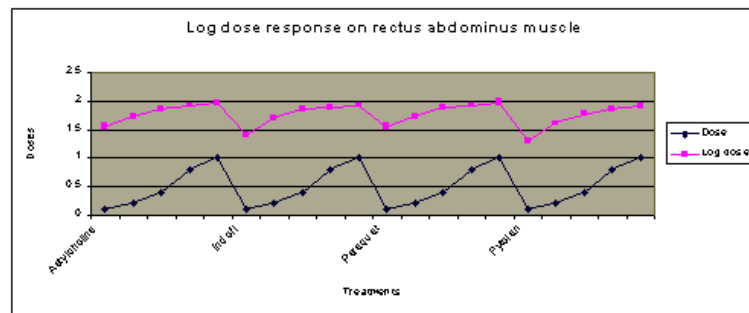


Fig. 1 Log dose response curves of Agrochemicals on rectus abdominus muscle of *Rana tigrina*.

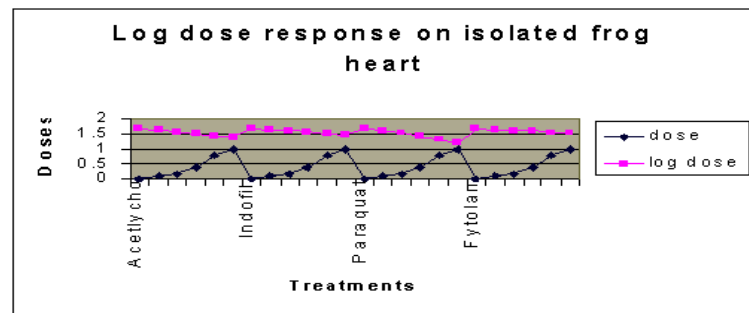


Fig. 2 Log dose response curve of Agrochemicals on isolated heart of *Rana tigrina*

Table 1 Stastical evaluation of responses of agrochemicals on isolated rectus muscle and heart of *Rana tigrina*.

Treatments	Response on Rectus abdominus muscle					Responses on Isolated heart					
	0.1	0.2	0.4	0.8	1	0	0.1	0.2	0.4	0.8	1
Acetylcholine	34.667 ± 0.667	55.0 ± 1.155	71.667 ± 0.8819	82.667 ± 0.8819	91.33 ± 0.667	46.00 ± 0.55774	42.0 ± 0.5774	36.33 ± 0.667	31.00 ± 0.5774	26.667 ± 0.8819	23.667 ± 0.333
Indofil	25.667 ± 0.8819	49.33 ± 0.8819	69.0 ± 0.5774	75.667 ± 0.8819	84.33 ± 0.8819	46.33 ± 0.667	41.667 ± 0.8819	40.0 ± 0.5774	36.0 ± 0.5574	31.667 ± 0.667	28.0 ± 0.5774
Paraquat	34.667 ± 0.8819	55.33 ± 1.202	75.667 ± 0.8819	82.333 ± 0.667	96.0 ± 0.5774	45.66 ± 0.667	39.667 ± 0.8819	34.667 ± 0.8819	26.33 ± 1.202	20.667 ± 0.8819	15.667 ± 0.333
Fytolan	19.667 ± 0.667	39.667 ± 0.8819	58.667 ± 0.8819	70.667 ± 1.202	79.33 ± 0.8819	46.667 ± 0.33	42.667 ± 0.8819	40.66 ± 0.8819	38.33 ± 0.33	34.033 ± 0.8819	33.0 ± 0.5774
df	11	11	11	11	11	11	11	11	11	11	11
sq	500.67	507.67	490.23	319.0	504.25	9.667	31.00	84.917	270.92	342.67	490.92
F	88.364	49.402	79.042	38.581	93.381	0.5556	2.500	19.978	51.517	52.160	242.79

Part A. Isolated rectus abdominus muscle preparation technique.

Rectus abdominus muscle of frog was dissected out and suspended in 5ml bath containing frog ringer solution. The bath was continuously bubbled with air. After proper relaxation and confirmation of the stability of tissue, the responses were recorded under the tension of 0.5g. Each response was recorded for 90 seconds, maintaining the cycle of 5 minutes. The effects of various pesticides were studied in different set of experiments. (Kela *et al.* 1995)

Part B. Isolated heart perfusion technique

The frog was pithed and pinned to the frog board and was given a midline incision on the abdomen and pectoral girdle was removed and heart was exposed. Pericardium was removed and the heart was canulated with the help of a venous canula. The tip of the ventricle was hooked to a pin with the help of fine thread and tied it to the free limb of the universal lever, which was fixed to a stand. Proper tension and magnification was adjusted by altering the height of lever and the contraction of the heart was recorded. (Kulkarni 2004)

The effects of skeletal and cardiac muscle were studied at various dose levels increasing in a geometric fashion and observations were taken before and after administration of the test pesticides.

Stastical analysis

Means ± SEM (standard error of mean) of values obtained in the assays were compared for the stastically significant differences using Students Neumans - Keuls test with standard parametric method for Gaussian distribution method .

RESULTS AND DISCUSSION

The comparative profile of acute toxicity study of all three agrochemicals is summarized in Table 1. The log toxicity dose response curves are depicted in Figure 1 and 2. The bioassays revealed there was an increased toxicity with increasing concentrations of the test chemicals. Among the three pesticides used, paraquat was found to be the most toxic to the adults of frog *Rana tigrina* followed by Indofil and Fytolan. The pesticides showed negative chronotropic and negative ionotropic effects.

Paraquat (1, 1'-dimethyl-4, 4'-bipyridinium dichloride) is widely used in agriculture to control weeds and has been reported to affect the development of amphibians (Dial & Baur 1984) (Tomar & Pandey 1988) and is highly toxic to aquatic species (Kam & Furtado 1977) (Tortorelli *et al.* 1990).

The acute toxicity of organophosphate carbamate fungicide, Indofil has also been reported in the current study cohort. The toxic contractile response showed by these agrochemicals supports the results of (Alvarez *et al.* 1995) which focus on the skeletal malformations induced by the insecticides.

The study demonstrates that acute susceptibility of adults of frog *R. tigrina* to these agrochemicals, which were previously reported to affect the embryonic stage (Dutta *et al.* 1978) and the growth of tadpoles (Khangarot 1985 & Alfredo 1992).

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