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Original Research Paper

Studies on Toxicity of Some Agricultural Chemicals to Tadpoles of Frog, *Rana tigrina*

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ABSTRACT

The present investigation is aimed to study the toxic effects of three agro-chemicals, endosulfan, indofil and dhanusan-50 on the tadpoles of Indian bullfrog, *Rana tigrina*. By following standard procedure for toxicity determination, it has been found that 96 hr/ LC_{50} value of endosulfan was 1.5ppm, whereas for that of indofil and dhanusan-50, it was respectively 6.5 ppm and 8.0 ppm showing that endosulfan was most toxic and dhanusan-50 was least toxic. Some remarkable changes in the behaviour of the tadpoles in the toxic media of all the three agro-chemicals were observed such as agitated movement, hyperexcitability, bending and looping of body, surfacing movement, muscular weakness and finally death. The loss of these fish-food organisms have consequent effects on the ecosystem because of interrelationship of the organisms in the sense of food chain, and this adverse situation is destined to have an impact on the prospects of fish yields from the contaminated water areas.

INTRODUCTION

Water pollution is a worldwide phenomenon and one of the most serious problems confronting mankind. The growth of human civilization and ever increasing population required more demand for food, comforts and infrastructure, which has led to the manifold expansion of industries, agriculture and unabating process of urbanization. Both, developed and developing countries that are progressing rapidly in the field of agriculture, technology, and industry are introducing various kinds of harmful substances into the environment and thereby facing a serious challenge in the form of environmental pollution.

In the recent years a variety of agricultural chemicals such as fertilizers, insecticides, pesticides fungicides, herbicides and other biocides are used to control crop pests and diseases and to boost agricultural production. These agrochemicals find their way through different routes into the adjoining water bodies such as rivers, ponds, ditches, paddy-fields and other low lying areas, adversely affecting the ecological balance leading to unwanted mortality of the aquatic biota, in general, and fishes and their food organisms in particular (Singh & Sadhu 2001, Alam 2002, Sadhu et al. 2003). In the recent days, water pollution problems related to agrochemicals have been taken categorically into consideration and it has been found that use of agrochemicals in the crop fields is liable to change the abiotic and biotic characters of the aquatic media, leading hazards to the aquatic flora and fauna. As such any setback in the population density of the microorganisms, particularly zooplankton, phytoplankton, and tadpole larvae, is bound to have its adverse effect on other organisms particularly fishes which are consumers of these organisms (Khan 1981, Jhingran 1980, Kumar 1995).

The utilization of tadpoles and other developing stages of frogs as food organisms for predaceous and other fishes is a well known fact in view of the reportings made by several authors in respect to

food and feeding habits of fishes. During monsoon, they are usually found in countless numbers in accumulated water areas of paddy-fields, drains, stagnant pools of water, remote ponds and other forms of derelict waters. These amphibian larval stages qualify the criteria for being used as an indicator of harmful level of pollutants in bioassay tests. From the review of literature, it is evident that there is paucity of information on the effects of agrochemicals on frogs, less to speak for tadpoles or other developing stages. Among the few contributions on record, those of Chang et al. (1973), Cook (1977), Tomar & Pandey (1988), Bimber & Mitchell (1978), Dial & Bauer (1984), Norman et al. (1987), and a few others are definitely useful.

In the present study, an attempt has been made to determine toxicity of different groups of insecticides to the tadpoles of Indian bull frog, *Rana tigrina*. The agrochemicals used for the bioassay tests are, indofil (a carbamate fungicide), endosulfan (a chlorinated hydrocarbon insecticide) and dhanusan-50 (an organophosphate insecticide).

MATERIALS AND METHODS

Tadpoles of *Rana tigrina* (15-20mm in length) were collected from natural breeding grounds and brought to the laboratory. They were allowed acclimatization for a minimum period of 15 days. The tadpoles were fed boiled spinach leaves and fresh plankton collected from nearby ponds. The activities of the specimens were closely observed and unhealthy specimens were discarded. The diluent water was taken from college tubewell. The physico-chemical characteristics of the diluent water were: temperature - 30±1°C, pH: 7.3, DO - 6.7ppm, total alkalinity - 48-57ppm, free CO_2 - 2.0 ± 0.15 ppm.

The toxicity tests were carried out in round bottom glass jars having 20-litre capacity. The tadpoles in healthy conditions and almost all of the same age groups were used throughout the test to have a meaningful results on intoxication.

The toxicants used were indofil, dhanusan-50 and endosulfan. For estimating the degree of toxicity of these agrochemicals, a batch of 10 specimens were released at a time in each container and such experiments for each dose of the chemicals were repeated 10 times in order to have average figure of mortality from a sample test of 100 specimens. The LC_{50} values were estimated as per standard methods (APHA 1985). A control experiment with 10 tadpoles without toxicant was set simultaneously in a separate container to have a comparative idea.

RESULTS AND DISCUSSION

Experimental endeavour for allowing intoxication of the tadpoles in the media contaminated with the three agrochemicals have shown convincingly their toxic nature in respect to deterioration of the water quality showing alterations in certain physico-chemical characteristics and occurrence of mortality of the test specimens at varying rates. All these developments are clear evidences for the potentiality of the three agrochemicals in question to affect the nontarget organisms grossly and consequently deprive the fish farmers the prospect of fish wealth from their inland water resources.

The mortality and survival percentage of the tadpoles of *Rana tigrina* with the three agrochemicals have been presented in Tables 1-4. The LC_{50} values of endosulfan have been found to be 3.5, 3.0, 2.5 and 1.5ppm for 24, 48, 72 and 96 hrs respectively, whereas those of dhanusan-50 and indofil they were 10.0, 9.0, 8.5 and 8.0ppm and 8.5, 8.0, 7.0 and 6.5ppm for 24, 48, 72 and 96hrs respectively showing that endosulfan was most toxic and dhanusan-50 was least toxic.

Behavioural Changes

Indofil poisoning: The test specimens of tadpoles displayed many symptoms of poisoning. As they were released into the toxic media, most of the tadpoles became agitated and violent. Hyperexcitability was observed in some of the tadpoles. They showed bending and looping of the body, jerky movement, etc. The dying tadpoles lost equilibrium and finally came to fatal end.

Dhanusan-50 poisoning: In dhanusan-50, upon release in to the toxic media initially the tadpoles did not show much sign of excitation or depression, but after 1 to 12hrs the treated specimens became lethargic and showed slow movement. The depression was followed by lack of locomotion, co-ordination and sign of muscular weakness and finally death.

Endosulfan poisoning: Hyper or hypoexcitation was not evident in the test organisms immediately after release into the toxic media, but after 18 hours there was a sudden change in their behavioural attitude. The tadpoles frequently dashed against the wall of the container and tried to jump out. As the exposure period increased the tadpole showed slow movement, muscular weakness and finally death. In all the cases of poisoning, there was no distinct sign of colour change of the skin but there was increased mucous secretion.

The data of the acute toxicity test, presented in Tables 1-4, show that the survivality of the test organisms gradually decreased with increase in the amount of dose at a particular time interval for each insecticide. Several earlier research workers have also recorded the susceptibility of tadpole larvae to the agrochemicals of various kinds. The toxicity of toxaphene has been estimated by Hall & Swinford (1980), whereas toxicity of endrin to the frog has been estimated by Grant (1976), Kenaga (1979) and some others. The toxicity of metacid and ekalux has been estimated by Alam (1989). Some workers have reported toxicity of heavy metals to tadpoles (Khangarot & Ray 1987). Recently Sateesh & Mishra (1997) have reported acute toxicity of organophosphorus fungicide, hinosan to the tadpoles of *Rana tigrina.* Among other workers, mention may also he made of Rane & Mathur (1979), Krishnagopal et al. (1981) and others. Hall & Swinford (1980), in their investigational attempt to evaluate the toxicity of endrin and toxaphene, found that *Rana sphenocephala* was more sensitive to both the pesticides than higher vertebrates, but was slightly less sensitive than fish. They remarked in the same context that amphibians were likely to be exposed to dangerous levels for the reasons that their habitats such as irrigation ditches, farm ponds, paddy-fields, and other low-lying areas have higher residues than the larger bodies of water, which form habitat of fishes. The present findings are, thus, in full agreement with the above authors because there is a similar situation also in the rural area of this region and there is likelihood of the access of the agrochemicals to inland water sources.

The test animals displayed many interesting symptoms of poisoning with indofil, dhanusan and endosulfan. The behavioural and neuro-muscular symptoms displayed by tadpoles had a characteristic sequence and were largely of similar nature with all the three pesticides. Similar findings were also reported by Kaplan & Overpeck (1964) and Harri et al. (1979) who observed similar kinds of symptoms in the adult frogs, *Rana pipiens* and *Rana temporaria* upon the oral administration of pesticides.

Furguson & Gilbert (1967) also observed more or less similar poisoning symptoms through the dermal contact of aldrin, dieldrin, endrin, DDT, and toxaphene in adults of toad, *Bufo woodhousii fowleri* and cricket frog, *Acris crepitans* and *A. cryllus* in the field as well as under laboratory conditions. Cook (1970) reported that subcutaneous administration of p-p DDT in adults of frog *Rana temporaria* has many symptoms like sluggishness, lack of normal desire to jump, adoption of abnor-

Table 1: Summary of LC_{50} values of endosulfan, indofil and dhanusan-50 for tadpoles of *Rana tigrina*.

Parameters	LC_{50} values (in ppm)		
	Endosulfan	Indofil	Dhanhsan-50
24 hr LC_{50}	3.5	8.5	10
48 hr LC_{50}	3.0	8.0	9
72 hr LC_{50}	2.5	7.0	8.5
96 hr LC_{50}	1.5	6.5	8.0

Table 2: Mortality percentage of tadpoles of *Rana tigrina* exposed to different concentrations of indofil.

Concentration(ppm	No. of tadpoles	Mortality percentage (in hours)				Remarks
		24hrs	48hrs	48hrs	96hrs	
4.5	100**	-	-	-	-	LC_0*
5	100**	-	-	-	3	
5.5	100**	-	-	15	25	
6.00	100**	-	20	30	40	
6.5	100**	10	30	40	50	LC_{50}*
7.	100**	20	40	50	60	
7.5	100**	30	40	60	70	
8.0	100**	40	50	70	80	
8.5	100**	50	60	70	90	
9.0	100**	80	90	100	100	LC_{100}*
Control	10	-	-	-		- -

*Incipient doses; ** Total number of test specimens tested in 10 sets of experiments.

Table 3: Mortality percentage of tadpoles of *Rana tigrina* exposed to different concentrations of dhanusan-50.

Concentration(ppm)	No. of tadpoles	Mortality percentage (in hours)				Remarks
		24hrs	48hrs	72hrs	96hrs	
4	100**	-	-	-	-	LC_0*
5	100	-	-	-	5	
6	100	-	-	04	10	
7	100		06	08	20	
7.5	100	10	15	26	35	
8	100	20	30	40	50	LC_{50}*
8.5	100	25	40	49	59	
9	100	35	50	65	75	
10	100	51	65	80	86	
11	100	75	-	-	-	LC_{100}*
Control	10	-	-	-	-	-

* Incipient doses; **Total number of test specimens tested in 10 sets of experiments.

mal posture, etc. The present findings are in fair agreement with those of the above workers.

Thus, in view of the present findings and also in consideration to some earlier records made on the susceptible nature of tadpole larvae, eudosulfan was highly toxic with 96 hr LC_{50} value of 1.5ppm followed by indofil (96 hr LC_{50} = 6.5 ppm) and dhanusan-50 (LC_{50} value of 8.0 ppm). It is also evident that on account of contamination of the living media, the tadpoles are liable to be severely affected and a jolt in the availability of these as fish food organisms is bound to occur.

Table 4: Mortality percentage of tadpoles of *Rana tigrina* exposed to different concentrations of endosulfan.

Concentration (ppm)	Total no. of tadpoles	Mortality percentage (in hours)				Remarks
		24hrs	48hrs	72hrs	96hrs	
0.25	100**	-	-	-		LC_0*
0.50	100	-	-	-	04	
1.00	100	-	-	5	15	
1.25	100	-	5	10	20	
1.50	100	20	30	40	50	LC_{50}*
2.00	100	40	55	58	62	
2.50	100	35	40	50	70	
3.00	100	40	50	70	80	
3.50	100	50	70	80	90	
4.00	100	80	90	100	100	LC_{100}*
Control	10	-	-	-	-	-

*Incipient doses; **Total number of test specimens tested in 10 sets of experiments.

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