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Research Article

Comparative Study of Acute Toxicities of Endosulfan, Chlorpyrifos and Permethrin to Zebrafish, *Danio rerio* (Cyprinidae)

Priyanka Tiwari, Badre Alam Ansari*
Zebrafish Laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273009 (U.P.) India

***Corresponding author**
Badre Alam Ansari
Email: ba.ddu77@gmail.com

Abstract: Fish and other organisms are affected by pesticides which pollute the natural water through agricultural runoff and various other means. The aim of this study was to determine and compare the acute toxicities of three different categories of pesticides *viz* organochlorine (endosulfan), organophosphate (chlorpyrifos) and synthetic pyrethroid (permethrin) which are commonly used in agricultural practices. Adult male and female zebrafish were randomly selected and exposed to these three pesticides. The toxicity tests were performed separately for each pesticide. The mortality rate of zebrafish was monitored under laboratory conditions for the time period of 96-h. Data obtained from the toxicity tests were evaluated using the Probit Analysis Statistical method. The toxicity tests gave the 96-h LC_{50} values as 0.10, 0.16 and 0.13µg/l for endosulfan, chlorpyrifos and permethrin respectively. The LC_{50} values revealed that all the three pesticides were highly toxic and the zebrafish showed highest sensitivity towards endosulfan followed by permethrin and chlorpyrifos. Further, the fish exhibited respiratory distress (such as gasping in air), loss of balance and erratic swimming prior to death. It was also found that the toxicity of these pesticides were time as well as concentration dependent. It could be concluded that these pesticides are extremely toxic and should be used very carefully. Zebrafish can be used as bioindicator to assess the pesticidal pollution in aquatic environment.
Keywords: Zebrafish, acute toxicity, endosulfan, chlorpyrifos, permethrin

INTRODUCTION

Use of pesticides have become a necessary evil in developing countries like India where it is estimated that approximately 30% of its crop yield are lost due to pest attack each year. Unfortunately, these pesticides lack target specificity and cause severe and long lasting effects on terrestrial and aquatic non-target organisms, especially the fish. A large amount of the pesticides used, never reaches the intended targets and enter the aquatic environment which is currently under threat of the indiscriminate use of pesticides. Pesticides can cause acute and chronic poisoning of fish and may damage their vital organs [1], skeletal deformities[2], reduced reproductive ability [3] and various biochemical alterations.

Presently, various categories of pesticides viz. organochlorines, organophosphates, carbamates, synthetic pyrethroids and natural products are used in contemporary agriculture to control pests. Among the three pesticides tested during the present study, endosulfan is a broad-spectrum pesticide [4] and is known to be highly toxic to fish, amphibians and crustaceans [5]. Endosulfan is highly resistant to microbial bioremediation and persists as a xenobiotic in the agricultural soils also [6]. Organochlorines are considered to be the most hazardous class of insecticides with respect to the environmental pollution. Endosulfan is a non-systemic organochlorine pesticide and is applied on different vegetables, fruits, paddy, cashew, tea, coffee, tobacco and timber crops. It has the ability to bioaccumulate and biomagnify in food chains. Furthermore, it has also been found that endosulfan is an endocrine disruptor [7].

Because of growing concerns for environmental and human health, organochlorine pesticides have been largely replaced with less persistent but relatively active organophosphate pesticides. Chlorpyrifos, an organophosphate pesticide, is sold under the trade names Dursban™ and Lorsban™. It is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation [8]. This pesticide needs to be activated into its oxon metabolite by cytochrome P450 in order to become toxic and inhibit AChE activity [9]. It is also known to be toxic to fish and other non-target organisms.

Pyrethroids, the synthesized derivatives of natural occurring pyrethrins, taken from pyrethrum, which are the oleoresin extract of genus *Chrysanthemum* flowers. Permethrin and other pyrethroids are known to affect the closing and opening kinetics of sodium channels, important in neuronal impulse transmission. Changes in the depolarization of neuronal cell membranes have been found to be related to toxicity [10]. Fish has been reported to be deficient in enzymes that hydrolyze this insecticide. Therefore, fish have relatively slow metabolism and slow elimination of these compounds and thus show sensitivity towards this class of pesticide [11]. Pyrethroids are more toxic to smaller fish than larger ones [12]. However, permethrin is considered to pose less risk under field conditions due to its high adsorption to soil.

Comparative toxicity data provide important information on variations in responses of aquatic species to insecticides and are useful for determining margins of safety for aquatic biota, either prospectively (before manufacture and use) or retrospectively (after manufacture and use).

Zebrafish was selected for the present study because they are the model organisms [13] for developmental toxicology research and also recommended by International Organization for Standardization and the Organization for Economic Co-operation and Development (OECD) [14]. The sequencing of the zebrafish genome is nearly complete and this promises to enhance it as a tool to study the mechanisms of developmental processes and how toxicants interfere with these processes [15]. Further, large clutch sizes and their small body size reduces the supplies required and costs to conduct experiments.

The purpose of this study is to determine and compare the acute toxicities of pesticides endosulfan, chlorpyrifos and permethrin and to establish a relationship between the toxicities of three different classes of pesticides i.e., organochlorines, organophosphates and synthetic pyrethroids.

MATERIAL AND METHODS

For toxicity tests, zebrafish, *Danio rerio* of similar age group were procured from the laboratory breed general culture. Toxicity tests were performed in laboratory to determine 24, 48, 72 and 96-h LC_{50} values using five concentrations of endosulfan (0.05, 0.10, 0.15, 0.20 and 0.25)µg/l, chlorpyrifos (0.10, 0.15, 0.20, 0.25 and 0.30) µg/l, and permethrin (0.05, 0.10, 0.15, 0.20 and 0.25) µg/l previously diluted in acetone. Two replicates of 50 fishes for each concentration of pesticides were performed. Each experiment was accompanied by a control having the same volume of acetone but without the pesticide. The randomization of fish in test aquaria was done according to the method prescribed by the U.S. Federal Water Pollution Control Administration, 1968.

The water was changed every 24-h. A fish was considered dead when its gill movements ceased and it did not responded to gentle prodding. Dead fish was removed from the aquaria to avoid deterioration. Endosulfan, chlorpyrifos and permethrin was purchased from the local market.

The results were computed by StatPlus® version 2009 computer software purchased from Analystsoft Vancouver, Canada. Mortalities of zebrafish were recorded for different exposure periods *viz*. 24, 48, 72 and 96-h at different concentrations. The LC_{50} values, upper and lower confidence limits (UCL and LCL), Slope, Chi-square values were calculated.

RESULTS AND DISCUSSION

The fishes when exposed to the concentrations of the pesticides, they showed abnormal behavioral changes. The intoxicated fishes were aggregated at the corner of the aquarium resting at the bottom and frequently come to the surface followed by heavy breathing with stronger opercular movements and loss of equilibrium. Also, over-secretion of mucus was observed from the body surface. Their body colour also darkened, pectoral and pelvic fins got expanded and the fish rolled vertically prior to death.

Table-1: Acute toxicity of Endosulfan, Chlorpyrifos and Permethrin to the Zebrafish.

Pesticides	Exposure Periods (h)	LC_{50} Values (µg/l)	Confidence Limits		Slope	Chi-square Values
			LCL	UCL		
Endosulfan	24	0.31	0.20	0.34	2.15	1.00
	48	0.14	0.09	0.21	1.92	0.77
	72	0.12	0.07	0.19	2.87	0.15
	96	0.10	0.06	0.14	2.40	0.12
Chlorpyrifos	24	0.40	0.32	0.64	1.86	0.97
	48	0.29	0.25	0.36	1.91	0.67
	72	0.21	0.19	0.24	1.88	0.31
	96	0.16	0.14	0.17	1.61	0.88
Permethrin	24	0.82	0.39	23.80	5.50	0.16
	48	0.71	0.34	42.76	8.76	0.19
	72	0.19	0.15	00.28	3.93	0.12
	96	0.13	0.10	00.15	3.94	0.07

It is evident from the table 1, that the LC_{50} values decreased with the increase in exposure period. It means that the toxicity of these pesticides increased with the advancement of time. In other words, the mortality of fishes increased with the increase of time. Table.1 shows that among the three pesticides, endosulfan was most toxic followed by permethrin and chlorpyrifos. It was observed that LC_{50} values after 24-h for endosulfan, permethrin and chlorpyrifos was 0.31, 0.82 and 0.40 µg/l respectively which decreased to 0.10, 0.13 and 0.16 µg/l respectively after the exposure of 96-h. It is also evident that there was a very slight difference in the LC_{50} values of endosulfan and permethrin after the exposure of 24 and 96-h showing that enosulfan and permethrin were almost equally toxic to *Danio rerio.* Also the concentration of chlorpyrifos required to kill the fish was highest among all the three pesticides proving that chlorpyrifos was least toxic.

The slope values shown in the Table.1 are steep. The LC_{50} values of the pesticides showed a significant ($p<0.05$) negative correlation with exposure time. The chi-square values were not significant indicating that the fish population used in the experiment was homogeneous.

After the exposure to endosulfan, the intoxicated fish showed erratic, jerky and abrupt swimming, frequent surfacing and gulping. This could be due to the skin irritation, respiratory rate impairment or a response to the altered locomotor activity which is an indication of the effect of endosulfan on the nervous system of the fish as was reported by Ayoola [16]. Similar observations were made by Adetola *et al.* [17] in *Clarias gariepinus* fingerlings after the exposure to endosulfan. Endosulfan has been proposed to cause neurotoxicity through GABA-gated chloride channel inhibition [18]. Inhibition of these channels results in excitation, because the neuron is unable to repolarize [19]. Associated symptoms include convulsions and eventual paralysis. More or less similar behavioral changes were observed in the fish after the exposure to chlorpyrifos and permethrin. Ramesh & Munniswamy also reported an excess secretion of mucus in *Cyprinus carpio* when exposed to chlorpyrifos [20]. We observed the same in the present study, which may be seen as non-specific response of the fish towards the toxicant thereby probably reducing the toxicant contact by forming a barrier between the body and the toxicant medium. Erratic and darting swimming movement and loss of equilibrium in fish after the pesticide exposure may be most likely due to the inhibition of AChE activity. This view is also reported by Kristen *et al*. [21]. Inhibition of AChE activity is a typical characteristic of organophosphate pesticides [22]. However in case of permethrin, the intoxicated fish appeared excited within few minutes of exposure and later on they calm down and gathered at the corners of the test chambers which may be viewed as an avoidance of the fish to permethrin. The same behavioral change in juvenile *Cyprinus carpio* exposed to permethrin, was reported by Reza & Gholamreza [23].

The results in the present study indicate that all the three tested pesticides are lethal substrates for the zebrafish and their toxicities are concentration and exposure dependent. The highest mortality was found at the highest concentration. The 24, 48, 72 and 96-h LC_{50} values of endosulfan was found to be 0.31, 0.14, 0.12 and 0.10 µg/l respectively. Comparing these values with the standard, set by the United States Environmental Protection Agency (USEPA), we can say endosulfan is very highly toxic (LC_{50}<100 µg/l) to zebrafish. Previous studies also indicate the high toxicity of endosulfan to fish species and our results are in good agreement with these reports. Richard [24] reported a LC_{50} value of 0.32µg/l for *Tilapia zilli*, Siang *et al.* [25] reported LC_{50} values of 0.42µg/l (0.35-0.50) when *M. albus* was exposed to different concentrations of endosulfan. Rahila & Muhammad [26] also found LC_{50} values of 0.98µg/l for *Catla catla* exposed to endosulfan. Also Matthiessen & Logan [27] reported a 24-h LC_{50} value of 0.5µg/l for *Sartherodon mossambicus* which is comparable to the 24-h LC_{50} value of 0.31µg/l found in this study. These values corroborate with the values found in our study. On the contrary, some studies have also reported LC_{50} values higher than the values obtained in the present study, such as, Adetola *et al.* [17] reported a LC_{50} value of 2.09µg/l of endosulfan to *Clarias gariepinus* fingerlings. Nowak & Sunderam [28] reported LC_{50} values of 2.0 µg/l at 30°C and 4.6 µg/l at 35°C when mosquito fish was exposed to technical grade of endosulfan. Smith [29] reported LC_{50} for rainbow trout to be 1.4 µg/l whereas, Werimo & Seimen [30] reported 10.20 µg/l for *Oreochromis niloticus*. The cause of the variation in the toxicity of endosulfan may be due to the differences in the testing protocols [31] and differences in the susceptibility and tolerance related to its accumulation, biotransformation and excretion [32]. Jonsson & Toledo [33] suggested that the different metabolic pathways among species results in different routes of biotransformation and leads to the production of more or less toxic metabolites. Also endosulfan toxicity is age and size dependent, which increases with age and shows inverse correlation with fish size [34].

The 24, 48, 72 and 96-h LC_{50} values for chlorpyrifos to zebrafish in this study is 0.40, 0.29, 0.21 and 0.16 µg/l respectively. Our data on present compound lethality is comparable to few previously published studies that exist. Johnsson & Finley [35] reported 96-h LC_{50} value of chlorpyrifos to channel catfish, *Ictalurus punctatus* and *Lepomis michrochirus* to be 0.280 mg/L and 2.4 µg/l. Ramesh & Munniswamy [20] reported a LC_{50} value of 0.16 mg/L for *Cyprinus carpio* when exposed to chlorpyrifos. Chlorpyrifos toxicity reported by Rao *et al.* [36] to *Oreochromis mossambicus* and *Gambusia affinis* by semi-static method is 0.0259 mg/L and 0.297 mg/L respectively. Recently, Ansari & Ansari [37] reported the LC_{50} value of dimethoate to be 60.0 µg/l to adult zebrafish. We could infer from these data that chlorpyrifos and other organophosphate pesticides are highly toxic to fish species including zebrafish. Further, comparisons of different LC_{50} values indicate that acute toxicity of chlorpyrifos varies with the fish species and it has been suggested that different detoxification, absorption and difference in AChE inhibition, serve as a factor for selective toxicity of organophosphate pesticides on different fish species [38].

The 24, 48, 72 and 96-h LC_{50} values for permethrin to zebrafish in this study is 0.82, 0.71, 0.19 and 0.13 µg/l respectively. Ansari & Ansari [39] found alphamethrin 96-h LC_{50} value for zebrafish to be 0.17 µg/l. Ansari & Sharma [40] and Ansari & Ahmad [41] reported a LC_{50} value of 0.12 µg/l and 0.11 µg/l for deltamethrin and lambda-cyhalothrin respectively, which are similar to our value of 0.13 µg/l. Kumaraguru & Beamish [48] found the 96-h LC_{50} value of permethrin to be 0.69 µg/l for small (1gm) rainbow trout (*Onchorhynchus mykiss*) at 10°C. They also suggested that the LC_{50} value to rainbow trout increased as the body weight increased. Other researchers have reported LC_{50} values of 1.6, 1.7and 1.0 µg/l for *Oreochromis clarki hensawi*, *Onchorhynchus gilae* apache and *Catostomus comersoni* respectively [42-43].

Comparision of the LC_{50} values of endosulfan, chlorpyrifos and permethrin show that endosulfan was most toxic among the three tested pesticides followed by permethrin and chlorpyrifos. Thus, we get the following sequence of toxicities in the decreasing order of toxicity-

Endosulfan (organochlorine)>Permethrin (synthetic pyrethroid)>Chlorpyrifos (organophosphate).

Supporting our results, Aliakbar *et al*. [44], reported LC_{50} values of deltamethrin and diazinon to be 0.223±0.07 ppm and 14.5±0.91 ppm respectively for *Trichogaster trichopterus*, showing that deltamethrin (pyrethroid) was more toxic than diazinon (organophosphate) to *Trichogaster trichopterus.* Mohammad *et al*. [45] also reported the high toxicity of deltamethrin (pyrethroid) than diazinon (organophosphate) to spirilin larvae and fingerling. Further, Vidyarani *et al*. [46] reported that endosulfan was most toxic to endemic loach *lepidocephalichthys irrorata* followed by cypermethrin (pyrethroid) and chlorpyrifos. In a study conducted by Moore *et al.* [47], it was reported that chlordane (organochlorine) had a 48-h mean LC_{50} value of 21.4 µg/l while chlorpyrifos had a mean 48-h LC_{50} value of 162.7 µg/l for *Pimephales promelas*, which was higher than that of chlordane. These results are in agreement of the results obtained in this study.

It is evident from the present study that the zebrafish is sensitive to all the three pesticides of which endosulfan (organochlorine) proved to be the most toxic pesticide for zebrafish than permethrin (synthetic pyrethroid) followed by chlorpyrifos (organophosphate). It is also concluded from the present study that the indiscriminate use of pesticides not only results in the extermination of target organisms but also of a large number of non-target organisms and kill or affect them in such a way that their normal physiological mechanisms are hampered. Therefore, these pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to the non-target biota. In addition, potential risk from permethrin and chlorpyrifos metabolites should be investigated to get a clearer picture in terms of toxicity.

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