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Acute Toxicity of Organophosphate Insecticide Dichlorvos to Fingerlings of *Cyprinus carpio* (Linnaeus, 1758)

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A B S T R A C T

Toxicological effects of pesticides on aquatic organisms are very important, especially when these animals are serving as functional foods with respect to human consumption. Dichlorvos, one of the extensively used insecticides was investigated in the present study for acute toxicity following static bioassay method and fingerling common carp (*Cyprinus carpio*) was selected as test specimen. The 96 hour LC_{50} value was found to be 21.11 ppm. Impact of test conditions (hardness of water) on LC_{50} value was evidenced in the present research, water hardness has resulted in decreased toxic potential of test substance, there by increased LC_{50} value.

Introduction

The contamination of surface water with pesticides is an ongoing concern worldwide. These very substances that have been developed for various anthropologic benefits eventually led to the realization that these chemicals, beneficial on application, has transcended to cause serious threats to the ecosystem. Due to the widespread use of pesticides, their residues are detected in various environmental matrices, like soil, water and air. Pesticide residues reach the water body through direct runoff, leaching, careless disposal of empty containers, equipment washing etc. The amount of pesticides lost from agricultural fields and transported to surface waters depends on several factors, including soil characteristics, topography, weather, agricultural practices, and chemical and environmental properties of individual pesticides (Leonard, 1990).

Sorption and desorption processes, degradation, transport and their solubility are all important factors that decide the fate of pesticides in the surrounding medium. Due to the residual effects of pesticides, important

organs like kidney, liver, gills, stomach, brain, muscle and genital organs are damaged in fish exposed to pesticide (Odiete, 1999). It has been recognized, however, that the sensitivity of fish to pesticide varies with species, size and age, it is also known that pesticide toxicity to fish is affected by water hardness and pH.

Furthermore, species may differ in the uptake, accumulation, distribution, metabolism and excretion of chemicals. Bioaccumulation rate of pesticides in fish depends on the species, life stages, the amount of fat deposits in different tissues and diet of fish, chemical and physical properties of pesticides etc.

There was a shift in the types of insecticides used in the mid 1960's from the organochlorine to the less stable organophosphate and carbonate classes (Henry, 1984).

The shift from organochlorines to organophosphates has resulted into increased occurrence of organophosphates into water bodies causing acute and chronic toxicity to fish fauna (Rao *et al.,* 2005; Velmurugan *et al.,* 2007). Their high insecticidal properties, low mammalian toxicity, less persistence and rapid biodegradability in the environment made them as one of the most preferred pesticides for application. These very pesticides may reach other ecological compartments as lakes and rivers through rains and wind, affecting many other organisms away from the primary target.

Toxicity tests become imperative to estimate potential hazards as part of risk assessment protocols in ecological sustainability studies. The selection of organisms for toxicity test is mainly based on certain criteria like, its ecological status, position within food chain, suitability for laboratory studies, genetically stable & uniform populations and adequate back ground data on the organism.

In the science of aquatic toxicology, fish play an important role in toxicity testing and hazard evaluation, as do the white rat and guinea pig in mammalian toxicology (Anon, 1972), especially teleost fish may be a good indicator of pollution as their biochemical responses are almost similar to that of mammals (Banaee *et al.,* 2008).

Materials and Methods

Testspecies selection, collection and conditioning

Common carp has been selected as test species due to its ready availability throughout the year, ease of maintenance, convenience for testing and moreover is an economically important edible fish, having great commercial value and the same species has also been recommended for bioassay experiments by 'Organization for Economic Cooperation and Development (OECD, 1992). It forms an important candidate species in carp poly-culture systems in India. The specimens were about 3-months old with an average body length of 5.7±0.60 cm and of body weights 2.1 ± 0.45 g.

Common carp fingerlings were collected from nearby fish farm and brought to the laboratory in plastic bags with sufficient air. Upon arrival at the laboratory, fishes were housed in FRP tanks of 1000 L capacity for 15 days to allow for acclimatization (under the following conditions) prior to pesticide exposure.

The fingerlings were fed twice in every 24 hours with dry pellet feed at the rate of 5% body weight per day and the excreta was siphoned out daily to prevent buildup of ammonia in the medium.

Light	**:**	**12 to 14 hours photoperiod daily**
Water	**:**	Good quality tap water (it is completely free from chlorine)
Temperature	**:**	32±2˚C
Salinity	**:**	1 ppt
pH	**:**	7.1±0.2
Dissolved oxygen	**:**	Aerated continuously, except at the time of feeding
Alkalinity	**:**	220±18 ppm
Hardness	**:**	380±26 ppm

All the abovementioned water quality parameters were analyzed following standard methods as per APHA, 2005.

Test substance

Dichlorvos (as commercial formulation Hyvap - dichlorvos, 76% EC)

Pesticide type: Insecticide

CAS number: 62-73-7 (Pesticides can have more than one common name, trade name and chemical name. The CAS Registry Number (Chemical Abstracts Systematic names) is a single identifier aimed to remove any ambiguity arising from the various nomenclatures.

Dichlorvos is poisonous if swallowed, inhaled, or absorbed through the skin; therefore, it acts as a contact and stomach poison (WHO, 1985). Because dichlorvos is one of the more volatile pesticides among organophosphates, it has been used primarily for its fumigant action (Cremlyn, 1978). It is effective in controlling nuisance pests (e.g., caterpillars, flies, mosquitoes, and cockroaches) in and around domestic dwellings, stored products, commercial transportation vehicles, and livestock buildings. Dichlorvos also has been added directly to water to control parasites in intensive fish farming (WHO, 1989).

Preparation of stock solution- As 1gm dichlorvos is approximately equal to 0.76 mL, so as to prepare 1000 ppm stock solution, we need to have 0.76 mL of dichlorvos in 1000mL of the solution. As, Hyvap consisted of only 76% Effective Concentration (EC) of dichlorvos, 1 mL of hyvap contains dichlorvos of 0.76 mL. So, 1 mL of Hyvap was added to tap water and finally made it to 1L, so as to have the stock solution of 1000 ppm dichlorvos.

Test solutions – Test solutions of chosen concentrations were prepared by diluting stock solution.

Acute toxicity test

The toxicity of an insecticide to an aquatic organism is usually expressed in terms of LC_{50}. This value represents the amount of a toxicant either in the form of concentration (LC_{50}), which kills 50% of the population of the test animal with in a fixed period of time (Finney, 1971).

Following acclimation, only healthy fish that were not diseased, as determined by general appearance (colour, skin luster, eyes and behavior) were selected for the study. The fingerlings were starved for 24 hours prior to the selection of individuals for the experiment to avoid any influence of differential feeding. While transferring and handling of fishes, care was taken to ensure that the test fishes were least stressed. Fishes were exposed to the test substance for a period of 96 hours. Mortalities were recorded at 24, 48, 72 and 96 hours and the concentration at which 50%

mortality of the test fishes occurred (LC_{50}) were calculated.

Experimental design

Tests were conducted in FRP tanks of 40 L capacity and filled with 20 L of tap water. Static bioassay test was conducted in duplicate for each concentration of pesticide with 10 animals per replicate and appropriate controls were also maintained for a period of 96 hours under the laboratory conditions. Experimental insecticide (dichlorvos) concentrations were calculated according to the active ingredient percentage of the commercial formulation. The expiry date of the test substance checked prior to initiation of the treatment was found suitable for the exposure. Precautions were taken to avoid contamination of the control.

We first carried out range finding acute test (limit tests) with dichlorvos concentrations of 1 ppm, 5 ppm, 10 ppm, 20 ppm and 25 ppm to pinpoint exposure concentrations for the definitive acute test. Based on the results of this 'wide range test', a definitive test was carried out in 'narrow range' between 15 ppm and 25 ppm concentrations spaced at an interval of 1 ppm, so as to generate data, which would be necessary to arrive at LC_{50} concentration of the test substance (dichlorvos).

Accordingly ten acclimated fingerlings (A loading rate of 1.1 g/L was followed) were placed in each replicate with dichlorvos concentrations 0 mg/L (Control), 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25 ppm for 96 hours and the mortality or responsiveness were recorded specifically at 24, 48, 72 and 96 hours after commencement of exposure. Fishes were considered dead when there was no response even to a gentle touch of fish catching net and dead fishes were removed instantly. The data obtained from the experiment was processed by 'Probit analysis' (Finney, 1971) for determination of median lethal concentration (LC_{50}).

Results and Discussion

Acute toxicity test was performed following 'static bioassay' method with two replicates, so as to determine LC_{50} (median lethal concentration) value of dichlorvos to fingerling common carp (active, healthy, disease free) under controlled laboratory conditions (Light: 12 to 14 hours photoperiod; Temperature: 32±2°C; pH: 7.1±0.2; Salinity: 1 ppt; Dissolved Oxygen: 8.0±0.6 ppm; Alkalinity 220±18 ppm and Hardness: 380±26 ppm).

Mortality pattern of common carp fingerlings in different concentrations of 'dichlorvos' during the test tenure (96 hours) is presented in table 1. Percentage mortalities and 'median lethal concentration (LC_{50}) on logarithmic scale was depicted graphically (Fig. 1 & 2, respectively).

Acute toxicity study revealed a strong negative effect of dichlorvos on the survival of fingerlings with increasing pesticide concentration there by exhibited dose-dependent survival and concentration graded lethality. In the present study, the median lethal concentration (acute toxicity) *i.e.*, LC_{50} value of dichlorvos to fingerling common carp was found (through probit analysis) to be 21.11 ppm.

The LC_{50} observed in the present research is somewhat higher compared to the earlier works of dichlorvos on fingerling common carp. Though the pesticide, fish species, size of the fish seemed to be common to earlier works, as a multitude of factors like genetic properties, health status of test specimen, physico-chemical parameters of water (medium of acclimatization and tests),

exposure technique followed, preparation of test solutions of required concentration with due consideration of EC% (effective concentration) of active ingredient present in the commercial formulation used etc. influences the LC_{50} value, one or couple of these factors might have resulted in higher LC_{50} value. Among the different factors enlisted above, it has been supposed that, water hardness (380±26 ppm) might have played a major role in elevating the LC_{50} value by reducing the toxicity potential of the test substance (dichlorvos) in the present study, besides good health status of test specimen (fingerling common carp). Under hard water conditions, it has been already proved that, the binding of pesticides with the hardness creates molecules which cannot enter into the test specimen, or which enter at a much slower rate, or which precipitate out of solution.

Table.1 Mortality pattern of common carp fingerlings in different concentrations of 'dichlorvos' during the test tenure (96 hours)

Conc. of Dichlorvos (ppm)	Log Conc.	Total No. of fish expose (10 Nos./replicate)	Mean Mortality (%)	Probit value
15.0	1.18	20	0	-
16.0	1.20	20	0	-
17.0	1.23	20	10	3.72
18.0	1.25	20	20	4.16
19.0	1.28	20	30	4.48
20.0	1.30	20	45	4.87
21.0	1.32	20	45	4.87
22.0	1.34	20	55	5.13
23.0	1.36	20	70	5.62
24.0	1.38	20	85	6.04
25.0	1.39	20	100	-

Table.2 Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.61	7.58	7.65	7.75	7.88	8.09

Table.3 Summary output of ANOVA

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.9012229							
R Square	0.812202715							
Adjusted R Square	0.788728055							
Standard Error	0.990432499							
Observations	10							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	33.94023772	33.94024	34.59912	0.0003691			
Residual	8	7.847652281	0.980957					
Total	9	41.78789						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-32.56286166	6.20499038	-5.24785	0.000776	-46.8716	-18.254128	-46.871595	-18.2541282
X Variable 1	28.36213695	4.821768908	5.882102	0.000369	17.243118	39.481156	17.2431179	39.48115598

Fig.1 Percentage mortalities of common carp exposed to different concentrations of Dichlorvos during 96 hours test tenure

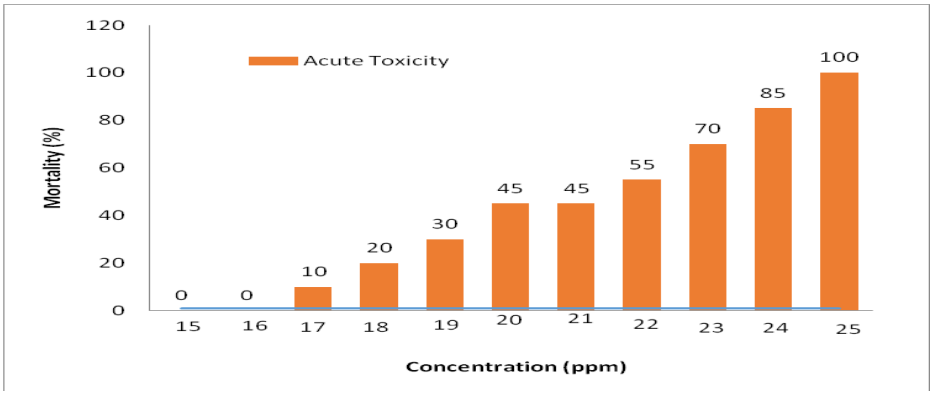
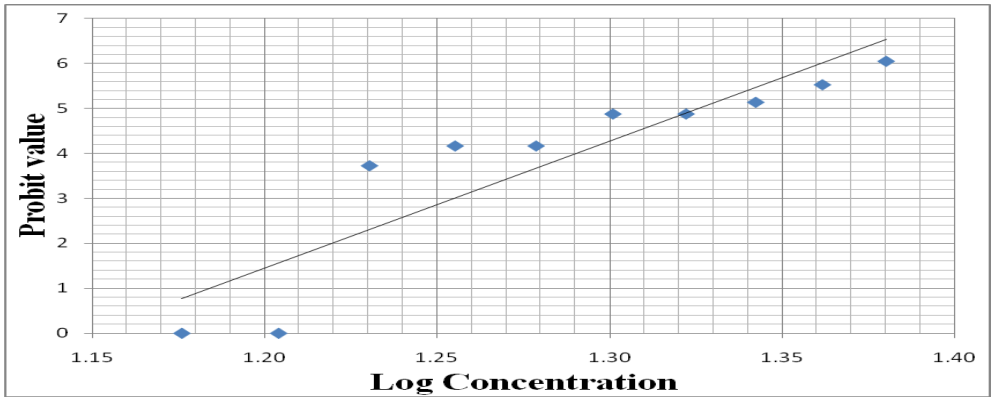


Fig.2. Graphical representation of 96h LC_{50} of 'dichlorvos' to common carp fingerlings



Validity of the test

Our test is valid (vide OECD Guidelines, 1992) as the mortality in the control group not exceeded 10% by the end of 96 hours. The findings of following works [a): dichlorvos on others b) impact of hardness on LC50 value] are in support of the present study

a). Gupta *et al.,* (2008) while investigating on the acute toxicity of dichlorvos in relation to selected hardness for freshwater zooplankters (*Moina, Daphnia* and *Cyclops*) revealed that, dichlorvos is relatively highly toxic at low water hardness (275 mg/L) compared to high water hardness (540 mg/L) there by observed nearly 100% increase in the LC_{50} values obtained under hard water conditions. Srivastava *et al.,* (2012) reported 96 hour LC_{50} value of 'dichlorvos' to *Cirrhinusmrigala* during a static-renewal test as 20.72 mg/L.

b). Mungkung *et al.,* (2001) observed a statistically significant increase in the median lethal concentration (LC_{50}) of cadmium to *Puntius goninotus,* with increasing hardness (soft water-4.17 ppm; moderately hard water-4.35 ppm; hard water-5.05 ppm). Dutta *et al.,* (2002) noticed a significant reduction (nearly fivefold increase in LC_{50} value in hard water compared to soft water) in the toxicity of 'deltamethrin' to 'common carp' with increasing hardness of water, and they opined that, the observed lowered toxicity of deltamethrin under hard water conditions might be due to change in the chemistry of biotic receptor sites which might have reduced the permeability of deltamethrin. Gautam and Gupta (2008) reported that, the sensitivity of juvenile *Poecilia* to 'cypermethrin' was decreased considerably with the increase in hardness of water. Kiyani *et al.,* (2013) reported a 38 fold decrease in the toxicity of Cu and a 264 fold decrease in the toxicity of Zn to *Gambusia holbrooki* under hard water conditions (350 mg/L).

Statistical Analysis

Regression analysis was carried out to find out median lethal concentration (LC_{50}) from the above data following 'probit analysis' method as per Finney (1971) percentage mortality was converted to probit value using table-2 and summary output of ANOVA was represented in table-3.

Regression equation Y= a(X) + b, incorporating a and b values in this equation and considering Y value as 5.0 (as the probit conversion for 50% mortality is 5.0)

Then, 5.0 = 28.36(X) + (-32.56)
5.0 = 28.36(X)-32.56
28.36(X) = 5.0+32.56
X = (37.56)/(28.36) = 1.3244

So, median lethal concentration LC_{50} = anti log. (1.3244) = 21.11 ppm

In conclusion this study can be used to help the environmental management agency to enhance public awareness in order to prevent indiscriminate use of pesticides and also to assure the effective use of these pesticides also for regulatory purposes.

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