Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 1172-1175

Received: 19-07-2018
Accepted: 20-08-2018

Sameena Khan
Division of Aquatic Environmental Management, Faculty of Fisheries, Rangil Ganderbal, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir., India

Imtiyaz Qayoom
Division of Aquatic Environmental Management, Faculty of Fisheries, Rangil Ganderbal, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir., India

Masood H Balkhi
Division of Aquatic Environmental Management, Faculty of Fisheries, Rangil Ganderbal, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir., India

Correspondence
Imtiyaz Qayoom
Division of Aquatic Environmental Management, Faculty of Fisheries, Rangil Ganderbal, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir., India

Dichlorvos (76% EC) induced behavioural toxicity in common carp fingerlings

Sameena Khan, Imtiyaz Qayoom and Masood H Balkhi

Abstract
Toxicity of dichlorvos on common carps was studied through short term static bioassay tests. The doses for the definitive tests were selected on the basis of predetermined range finding tests run in triplicates. the definitive test was also repeated thrice for accuracy in the results. The 96-h LC_{50} value of dichlorvos for *Cyprinus carpio* var. *communis* was found 2.56 ppm by probit analysis. The mean values of water quality in aquarium didn't indicate marked variation confirming mortality of fishes due to pesticide toxicity and not hypoxia. Fishes exhibited behavioural responses like erratic movements, convulsions, imbalanced swimming which ended in a collapse to the bottom of the aquarium and increase in respiratory motions during the entire period of the bioassay. Before death, the clinical signs like fainting of body color, gulping onto air-water interface in aquaria and in extreme cases, the hemorrhagic patches on selected sites of skin were noticed with the termination of the experiments. The results suggested that dichlorvos is potentially toxic to fishes and its entry into natural waterbodies must be checked or otherwise it can prove harmful to fishes and human beings as well through the food chain.

Keywords: Dichlorvos, common carp, toxicity, behavioural responses, water quality

Introduction
Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) is a volatile, colorless to amber liquid belonging to the class of insecticides called organophosphates. It is highly toxic through all routes of exposure to fishes and causes nervous breakdown through inhibition of acetylcholinesterase leading organophosphate induced delayed neurotoxicity (OPIDN) [1]. It is likely to contaminate ground waters since it does not adsorb to soil particles. World Health Organization classified dichlorvos as a class IB, 'highly hazardous' chemical [2]. Although the pesticide does not significantly bio-accumulate in fish [3], yet it is extremely toxic to aquatic organisms and hampers fish health through impairment of metabolism sometimes leading to death [4] leading to alterations in hemato-biochemical indices and induction of behavioural toxicity in fishes [5].

Jammu and Kashmir is an agricultural state with varied agro-climatic conditions suitable for the cultivation of different types of crops. Kashmir province of the state is well recognized for horticulture where apple cultivation finds the primary place. The fruit production of Kashmir province alone is 1.5 million metric tons annually from a total orchard area of 0.2 million hectares, which is sprayed and fogged with 7750 metric tons of fungicides and 3186 metric tons of insecticides, right from March to November every year [6]. In 2009, the total sale of pesticides by weight was 1828.5 thousand kg/liters costing about 369.1 million rupees [7] and is increasing every year. In Kashmir, the natural waterbodies including Dal Lake and its basins like Nigeen and Brarinambal have been found highly eutrophic [8, 9] where the presence of organophosphorus pesticides in waters [10, 11] and fish muscles has been established [10, 12]. However, the effect of dichlorvos on fishes in Kashmir valley has not been studied yet and need to be ruled out for assessing toxicity of the pesticide in fishes under temperate conditions. Therefore the study was carried out to determine the median lethal concentration of dichlorvos in common carp fingerlings in Kashmir.

Material and Methods
Experiment was carried out as per the method described by [13]. *Cyprinus carpio* var. *communis* (10±2 g) were brought from the hatchery of Faculty of Fisheries, SKUAST-K to the laboratory in plastic bags with adequate water and aeration, avoiding any stress or injury to them and then disinfected by giving bath in 0.05% $KMnO_4$ solution for two minutes.

Fishes were acclimatized to the laboratory environment for two weeks and fed with artificial diet during that period brought from Manasbal National Carp Farm Kashmir. The leftover matter, whether feed or fecal matter was thoroughly cleaned and water of the tank was changed in the subsequent morning.

Dilution water

Double distilled water was used as dilution water. Addition of the toxicant into the dilution water was followed by swirling of the water with glass rod to disperse the toxicant immediately and uniformly throughout the aquarium. Maximum loading of the test organism was 1 g/L and 10 fishes were recruited in each aquarium tank to ensure their free movement and avoid any stress due to overcrowding or suffocation. Before the start of experiment, the dilution water was aerated vigorously with glass rod so that the dissolved oxygen should be not less than 6 mg/L at 26 ºC.

Test concentration

Commercial grade of dichlorvos (76% EC) was procured from a local registered dealer. Stock solutions were prepared in methanol and subsequent concentrations in de-ionized water. Range finding test was carried out to determine the final concentrations to be used in definitive test. For range finding test of dichlorvos, various concentrations were selected based on the literature survey and administered in replicates and corresponding mortality was recorded. Finally for the definitive test, 256.0, 512.0, 1024, 2048 and 4069 ppm concentrations were used and administered in triplicates; on the basis of which mean lethal concentration of the pesticide was determined. To determine range finding test, concentrations with spacing factor of 10 were used [14].

Bioassay

Static type of bioassay for 96 hours was carried out. Feeding was stopped 24 hours before the start of the experiment. During the experiment also, no food was given to fishes. Test organisms were introduced to the test chamber (aquaria) within one hour after the toxicant was added to the dilution water. Mortality counts were recorded in each concentration at 6, 12, 24, 48, 72 and 96 hours. The five concentrations per toxicant in definitive test and each concentration were replicated thrice with 10 specimens per replicate. Control group was also run in both, range finding and definitive tests in which same amount of solvent added as to the treated one. Addition of solvent to the control was to rule out the mortality caused, if any, due to its toxic effects in the fishes. Fishes were treated dead if any sign of immobilization, loss of equilibrium, lack of opercular movement or morbidity was seen. This reflected an indication of pending death. Behavioural response like excess mucus secretion and change in swimming pattern, loss of scales and change in color patterns were also taken into consideration and keenly monitored during the entire bioassay. Dead test organisms were removed from aquaria as soon as observed. After the experiment is over, test solution was disposed off and container scrubbed and washed thoroughly with 10% HCl.

Water quality parameters of aquarium waters during bioassay

Various water quality parameters of the aquarium water like temperature, pH, CO_2, dissolved oxygen and total dissolved solids were checked every day as per the methodology of the American Public Health Association [15]. While as Total Dissolved Solids (TDS) was also determined as described by [16].

Statistical analysis

Percent Mortality data of fish at the end of 96 h exposure were determined by the probit method of regression [17] manually and then confirmed by using R statistical software (3.1 version). For descriptive statistics, i.e. calculation of mean and standard deviations, SPSS (20.0 version) was used.

Results

Median Lethal Concentration (LC_{50}) of Dichlorvos.

The median lethal concentration (LC_{50}) values in the present study were determined on the basis of range finding tests done thrice as replicates (r_1, r_2, and r_3) from which the mean median lethal concentration (LC_{50}) value of dichlorvos after acute exposure to common carp was calculated to be 2.56 ppm [Table 1].

Water Quality of Aquarium Waters during Dichlorvos Bioassay.

Various water quality parameters assessed during static bioassay of dichlorvos showed a slight variation up to the completion of bioassay. Laboratory temperature was found 17.0 ±0.0 ºC, while water temperature 14.0 ±0.0 ºC. Values of carbon dioxide did not vary profoundly signifying non hypoxic conditions in the aquarium throughout the experiment. Various water quality features of aquarium waters are presented in [Table 2].

Behavioural Responses in Fishes Exposed to Pesticides

The behavioural responses elicited by juveniles of common carp during acute exposure to dichlorvos are presented in [Table 3]. During the static bioassay of dichlorvos to *Cyprinus carpio* var. *communis* various behavioural responses were elicited by the fishes. Although during first 6 hours, fishes did not exhibit any behavioural response but the feeble progression in slowing down the motion and starting of convulsions was seen during 12 hours of the experiment which grew more intense with the advent of time. Severe un-coordination in their movements was visible after 24 hours which resulted in the death of fishes at the termination of the experiment. A slight secretion of mucus could be seen from the 72nd hour which aggravated with the termination of the experiment. Erosion of scales, change in the body color and development of hemorrhagic patches on the body was found at the end of the experiment, especially in the dead fishes.

Table 1: Water Quality of aquaria during the period of bioassay of dichlorvos

S. No.	Water Quality parameter	Day 1	Day 2	Day 3	Day 4	Mean ±S.D.
1	Lab. Temp. (ºC)	17.0	17.0	17.0	17.0	17.0 ±0.0
2	Water Temp. (ºC)	14.0	14.0	14.0	14.0	14.0 ±0.0
3	Dissolved Oxygen (mg/L)	6.7	6.3	6.3	6.2	6.37±0.22
4	Free CO_2 (mg/L)	1.28	1.29	2.20	1.60	1.61±0.43
5	Ph	6.6	6.8	6.9	6.9	6.8±0.14
6	Total Dissolved Solids (mg/L) × 10^3	2.46	2.39	2.44	2.44	2.43±0.029

Table 2: Cumulative median lethal concentration (LC_{50}) values of dichlorvos to common carp

S. No.	R.F. test of dichlorvos	LC_{50} values (ppm)	95% Confidential limits	
			Upper Fiducial limit	Lower Fiducial limits
1.	T_1	2.7	3.6	2.1
2.	T_2	2.9	2.2	4.4
3.	T_3	2.1	2.9	1.6
	Mean LC_{50} values	2.56±0.41		

Table 3: Behavioural responses elicited by common carp during bioassay.

Time in hours	Uncoordinated Movements	Convulsions	Mucus Secretion	Imbalanced Swimming	Erosion of Scales	Body Color	Hemorrhagic Patches
6	-		-	-	-	-	-
12	-	-	-	+	-	-	-
24	-	-	-	+	-	-	-
48	+	+	-	++	-	-	-
72	++	+	+	++	-	+	-
96	+++	++	++	++	+	++	+

+, Feeble; ++, Progressive; +++, Intense

Discussion

During present study, the acute toxicity tests were carried out in *Cyprinus carpio* var. *communis* exposed to dichlorvos. The median lethal concentration (LC_{50} value) for dichlorvos was found to be 2.56±0.41 ppm which is in accordance with the earlier researches by various researchers. The 96 h LC_{50} value of dichlorvos were found between 0.004-11.6 mg/L [18]. In fathead minnows, carp, Japanese killifish, guppy, bluegill, and trout, The 96 h LC_{50} value of dichlorvos ranged from 0.17 to 11.1 mg/L [19], while as in *Heteropneustes fossilis*, it was 6.45 mg/L [20].

The small LC_{50}values obtained during present study are attributed to small size of fishes (10 ± 2 g) which probably have potentially less efficient mechanism of bio-transformation of toxicant from the body. Moreover, the rapid distribution of pesticides in the body of small sized fishes leads to induction of behavioural changes faster than the normal, since the uptake of a toxicant is directly dependent on the size of fishes [21].

Water quality of aquaria did not show much variation during the entire period of bioassays. This is probably because feeding was stopped before 24 hours of the start of experiment which, when excreted by fishes, forms a primary source of water depletion in aquarium. Therefore, it is envisaged that fish mortality was caused only because of pesticide toxicity and not due to hypoxic conditions. Similar results have be reported by [21, 22] while as [23] reported significant decrease in oxygen consumption between control and treated groups of *Puntius chola* exposed to chlorpyrifos. They reported increase in stress conditions due to oxygen consumption during 96-hour static bioassay. On the 3rd day, all the feed would be secreted out of the fish body which restricted its further increase in the aquarium. The increase in total dissolved solids could also be due to the pesticide concentration in the aquarium.

The primary target of organophosphates (OPs) is the neuro-inhibitory nature of acetylcholinesterase (AChE) enzyme in the synaptic cleft which leads to paralysis in acute toxicity exposures. However, the OP toxicity in natural waterbodies where fishes are exposed chronically to these xenobiotics induces irreversible long term effects leading to physiological dysfunctions in them. Like other organophosphates, dichlovos inhibits acetylcholinesterase (AChE) which is present in mammals, fish, birds, and insects. AChE is a class of enzymes which initiate the hydrolysis of acetylcholine (ACh), a neurotransmitter, into inactive choline and acetic acid [24]. The inhibition creates a build-up of acetylcholine at the nerve synapses disabling the enzyme cholinesterase which is vital for a functioning central nervous system [25]. The continuous accumulation of acetylcholine leads to loss of balance, convulsions, paralytic symptoms, and eventually death as observed in the present investigation.

Conclusion

Results obtained in the present study suggest that the values of median lethal concentrations of dichlorvos induce severe toxicity to common carp. Therefore, entry of all pesticides in general and this in particular must be restricted from all point and nonpoint sources.

References

1. Hudgson E. A text book of modern toxicology. Third edition. Department of Environmental and Biochemical Toxicology. North California State University, 2004, 57-64.
2. WHO. International Programme on Chemical Safety, WHO Recommended Classification of Pesticide by Hazard and Guidelines to Classification, UNEP/ILO/WHO, 1994-1995
3. Howard PH. Handbook of Environmental Fate and Exposure for Organic Chemicals. Pesticides. Lew is, Boca Raton, FL, 1991.
4. Das S. A review of Dichlorvos toxicity in fish. Current World Environment. 2013; 8(1):143-149.
5. Deka S, Mahanta R. Dichlorvos toxicity on fish - a review. European Journal of Biological Research. 2015; 5(3):78-85.
6. Bhat AR, Wani MA, Kirmani AR. Brain cancer and pesticide relationship in orchard farmers of Kashmir. Indian Journal of Occupational and Environmental Medicine. 2010; 14(3):78-86.
7. Baba SH, Wani MH, Zargar BA, Wani SA, Kubrevi SS. Pesticide delivery system in apple growing belt of Kashmir valley. Agricultural Economics Research Review. 2012; 25:435-444.
8. Balkhi MH, Yousuf AA. Community structure of *Crustacean plankton* in relation to trophic conditions. Int. J of Ecol. and Env. Sci. 1992; 18:155-168.
9. Balkhi MH, Yousuf AR. Distributional pattern of *Cladoceran plankton* in the freshwaters of Kashmir.

Oriental Science. 1996, 1.
10. Qayoom I. Residue analysis of pesticides in Dal waters and their effect on common carp fish, *Cyprinus carpio* var. *communis*. Thesis submitted to Sher e Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar J&K India, 2015, 122-150.
11. Qayoom I, Balkhi MH, Mukhtar M. Assessment of Dimethoate Residues from Dal Lake of Jammu and Kashmir, India. Chemical Science Review and Letters. 2018; 7(26):578-58.
12. Banday M, Dhar JK, Aslam S, Qureshi S, Ahmad I. Contamination of fresh water fish *Schizothorax niger* with chlorpyrifos from Dal Lake basins, India. International Journal of Pharma Sciences and Research. 2012; 2(12):535-542.
13. Reish DJ, Oshida OS. Manual of methods in aquatic environment research. Part 10, Short Term Bioassay. FAO, 1987.
14. Rahman MZ, Hossain ZMF, Mollah A, Ahmed GU. Effect of Eiazinon 60 EC on *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus*. NAGA, The ICLARM Quarterly. 2002; 25(2):8-12.
15. APHA. Standard Methods for the examination of Water and Wastewater. APHA, AWWA, WEF. Washington, D.C, 2005.
16. Adoni AD, Joshi G, Ghosh K, Chourasia SK, Vaishya AK, Yadav M *et al*. *Workbook on limnology*. Department of Botany, Dr. Hari Singh Gour Vishwadidyalaya, Sagr Publishers, Sagar Madhya Pradesh, 1985.
17. Finney DJ. Probit Analysis. S. Chand & Company, New Delhi, 1972.
18. Shepard TH. Catalog of Teratogenic Agents. Fifth Edition. Johns Hopkins University Press, Baltimore, MD, 1986, 5-10.
19. CERI. Hazard Assessment Report, DimethyI 2, 2-dichlorovinyl Phosphate (synonyms: Dichlorvos, DDVP), Chemical Evaluation and Research Institute (CERI), Japan. CAS No. 2007; 62:73-7.
20. Ahmad SI, Gautam RK. Effect of organophosphate pesticide, nuvan on serum biochemical parameters of freshwater catfish *Heteropneustes fossilis* (Bloch.). International Research Journal of Environment Sciences. 2014; 3(10):1-6.
21. Qayoom I, Balkhi MH, Mukhtar M, Shah FA, Bhat BA. Chlorpyrifos induced acute toxicity and behavioural responses in juvenile common carps (*Cyprinus carpio* var. *Communis*) of Kashmir. Ecology Environment & Conservation 22 (December Suppl.). 2016a, 71-78.
22. Qayoom I, Shah FA, Mukhtar M, Balkhi MH, Bhat FA, Bhat BA. Dimethoate induced behavioural changes in juveniles of *Cyprinus carpio* var. *communis* under temperate conditions of Kashmir, India. The Scientific World Journal. Article ID 4726126, 2016b, 6. http://dx.doi.org/10.1155/2016/4726126.
23. Verma VK, Saxena A. Investigations on the acute toxicity and behavioural alterations induced by the organophosphate pesticide, chlorpyrifos on *Puntius chola* (Hamilton-Buchanan). Indian J Fish. 2013; 60(3):141-145.
24. Hoegberg EI, Cassaday JT. The Reaction of O, O-Dialkyl Thiophosphoric acid salts with some α-Haloacyl derivatives. Journal of the American Chemical Society. 1951; 73(2):557-559.
25. Verma SR, Pal N, Tyagi AK, Dalela RC. Toxicity of Swascol IP (SLS) to *Channa punctatus* and *Cirrhinus mrigala*: biochemical alterations. Bulletin of Environmental Contamination and Toxicology. 1979; 21(4, 5):711-718.