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EVALUATION OF ACUTE TOXICITY OF DICHLORVOS (NUVAN) TO FRESHWATER FISH, OREOCHROMIS MOSSAMBICUS (W. K. H. PETERS, 1852) AND THEIR ETHOLOGICAL CHANGES

Nimai Chandra Saha [1] | Nishan Chatterjee [2] | Santosh Kumar Giri [2] | Surjyo Jyoti Biswas [3] | *Suman Bej [4]

1 Director of Public Instruction, Govt. of West Bengal, Education Directorate, Bikash Bhavan, Salt Lake, Kolkata-700091, India.

2 Research Scholar, Department of Zoology, Sidho Kanho Birsha University, Purulia, West Bengal, India.

3 Associate Professor, Department of Zoology, Sidho Kanho Birsha University, Purulia, West Bengal, India.

4 Assistant Professor, Post Graduate Department of Zoology, Bidhannagar College, EB-2, Sector-1, Salt Lake City, Kolkata - 700064, India. (*Corresponding Author)

ABSTRACT

The present study was done to evaluate the acute toxicity of Dichlorvos (Nuvan) to freshwater fish, *Oreochromis mossambicus*. The 24, 48, 72 and 96 h LC50 values of Dichlorvos to *Oreochromis mossambicus* were 3.84, 3.50, 3.12 and 2.90 mg/l respectively. The mortality rate of the fish varied significantly ($p<0.05$) over the control at all the treatments of Dichlorvos irrespective of exposure times. The mortality rate of the fish also varied significantly ($p<0.05$) at all the exposure times (24, 48 and 72 and 96h) at 2.6 mg/l of toxicant and above. The fish showed excess mucous secretion and frequent vertical hanging posture at higher concentrations during 72 and 96h of exposure. The hyper-excitability of fish was also recorded at higher concentrations during 24h of exposure. The opercular movement of treated fish was increased significantly ($p<0.05$) over the control with the increasing concentrations of toxicant.

KEYWORDS: Dichlorvos (Nuvan), *Oreochromis mossambicus*, acute toxicity, ethological responses, opercular movement.

INTRODUCTION

Dichlorvos is applied to control the population of different types of insect pests, mites, helminthes and many parasites of farm animals (Das, 2013). It is used on fruits, vegetables and field crops before their harvest. Dichlorvos also applied on stored grain in food processing industries. It is used extensively in hospitals and in airplane for disinfection purpose. Due to its antihelminthic property it is used as pellets for oral dosing of many animals like horses, pigs and poultry birds (URL-1).

Dichlorvos is a contact and stomach poison for target and non-target pests. The activity of acetylcholinesterase in red-blood cells, plasma and brain is inhibited by Dichlorvos. This inhibition in the enzyme level causes the accumulation of acetylcholine at synaptic cleft that leads to produce continuous nerve stimulation and symptoms of intoxication at peripheral and central nervous system causing death (Binukumar and Gill, 2010; Das, 2013). The disorders in acetylcholinesterase activity followed by alterations in swimming, feeding, respiration and reproductive activity of fish due to Dichlorvos toxicity were recorded by Bretaud et al. (2000). The interference in the normal metabolism and death of fish was also observed by Suntio et al. (1988) and Das (2013). Earlier reports indicates that the Dichlorvos toxicity to fishes ranges between 0.1 to 10 mg/l (WHO, 1989) and ~0.2 to >40 mg/l (Deka and Mahanta, 2015). The earlier reports also indicate that the 96h LC_{50} values of Dichlorvos to fathead minnows, guppy, Japanese killifish, Bluegill, carp and trout ranges from 0.17 to 11.1 mg/l (CERI Japan, 2007). It also reveals that the 96h LC50 values of Dichlorvos to guppy, *Cyprinus carpio*, *Heteroneustes fossilis*, *Cirrhinus mrigala*, Sheephead minnow, were 1.84, 2.51, 6.45, 9.1 and 7.5 mg/l respectively (Jones and Davis, 1994; Velmurugan et al., 2009; Günde and Yerli, 2012; Ahmad and Gautam, 2014).

The ethological changes like loss of equilibrium, vertical hanging in water, faster gill movement and irregular swimming of guppy and carp fish were found due to Dichlorvos toxicity by Günde and Yerli (2012). The hyper-excitability, restlessness, excess mucous secretion and escaping tendency of *Labeo rohita* were also recorded by Bhat et al. (2012) after exposure to 42.66 mg/l of Dichlorvos.

MATERIALS AND METHODS

Dichlorvos (76% EC) insecticide used as test chemical in this study was collected from the local market. The test animals used in the bioassays were the fresh water fish, *Oreochromis mossambicus* (Class: Teleostomi, Family: Cichlidae), with the mean length 5.63 ± 0.82 cm and mean weight 2.18 ± 0.50 g. The fish were collected from local non-polluted pond and were allowed to acclimatize in the laboratory condition for 72h before the test.

The static replacement bioassays with the test animal, *Oreochromis mossambicus* were conducted in 15l glass aquaria holding 10l of unchlorinated tap water. The physico-chemical properties of water used in the experiment were as follows: temperature 27.5 ± 0.5°C, pH 7.3 ± 0.2, free CO_2 19.3 ± 1.7 mg/l, dissolved oxygen 6.8 ± 0.5 mg/l, total alkalinity 158 ± 8.6 mg/l as $CaCO_3$, hardness 125 ± 2.5 mg/l as $CaCO_3$. The acute toxicity test was accompanied with four replicates for each concentration of Dichlorvos along with the control. The fishes were not fed 24h before and during the test.

Primarily, the range-finding tests of Dichlorvos to *Oreochromis mossambicus* were done to estimate the range of concentrations of the toxicant that would be lethal to the test animals. The selected test concentrations of toxicant were finally used to determine the 24, 48, 72 and 96h LC_{50} values of Dichlorvos to *Oreochromis mossambicus*. During the experiment, the number of dead organism was counted at every 24h of exposure. The dead fish were removed immediately after death to avoid any organic decomposition and oxygen depletion. A certain quantity of water from each aquarium was replaced every 24h by unchlorinated stock water and a specific amount of Dichlorvos was then added immediately to test medium to make the desired concentrations. All the bioassays as well as the estimation of physico-chemical parameters of the test water were done following the methods of APHA (2012).

The LC values of Dichlorvos to *Oreochromis mossambicus* at 24, 48, 72 and 96h along with 95% confidence limits were estimated using a statistical software, Probit program version 1.5 developed by US EPA (1999). The values of percent mortality of the fish were subjected to analysis of variance (ANOVA) using the R-software (R Development Core Team, 2011) followed by Duncan's Multiple Range Test (DMRT) for determining the significant differences among the mean mortality of test animals at different concentrations and times of exposure (24, 48, 72 and 96h).

During acute toxicity test, the ethological changes of treated fish were also studied during the experiment. The change in opercular movement of the fish exposed to different concentrations of Dichlorvos was also recorded.

RESULTS AND DISCUSSION

The 24, 48, 72 and 96 h LC_{50} values with 95% confidence limit of Dichlorvos to *Oreochromis mossambicus* has been summarised in Table 1. No mortality of test animal was recorded in control during the study. The mortality rate of the fish, *Oreochromis mossambicus* varied significantly ($p<0.05$) over the control at all the treatments irrespective of exposure times. The mortality rate of the fish also varied significantly ($p<0.05$) at all the exposure times (24, 48 and 72 and 96h) at 2.6 mg/l and above concentrations of toxicant (Table 2).

In the present study the treated fish showed various irregular behaviours over the control with the increased concentrations of the toxicant with the progress of exposure time (Table 4). The treated fish showed excess mucous secretion over the control from 4.1 mg/l at 72h exposure time and 3.1 mg/l at 96h of exposure time. The treated fish showed hyper-excitability from 3.6 mg/l at 24h of exposure time. With the progress of time of exposure, the hyper-excitability of fish gradually decreased with the increasing concentration and it was almost absent at the lower concentrations (2.6-3.1 mg/l at 72h and 2.6-3.6 mg/l at 96h of exposure time). The frequent vertical hanging posture of treated fish in water was recorded especially at higher concentrations (4.1 and 4.6 mg/l) during 72 and 96h of exposure time. The opercular movement of treated fish was increased significantly

($p<0.05$) over the control with the increasing concentrations of Dichlorvos (Table 4). On the other hand, the rate of opercular movement was significantly ($p<0.05$) decreased at all the treatments with the progress of time of exposure.

In the present study the 96h LC_{50} value of Dichlorvos to *Oreochromis mossambicus* (2.90 mg/l) corresponds with the values of earlier findings to *Cyprinus carpio* (2.51 mg/l). But present LC_{50} value was slightly lower than *Ctenopharyngodon idella* (6.5 mg/l), *Heteroneustes fossilis* (6.45 mg/l), Sheephead minnow (7.5 mg/l), *Cirrhinus mrigala* (9.1 mg/l) (Jones and Davis, 1994; Tilak and Kumari, 2009; Velmurugan et al., 2009; Günde and Yerli, 2012; Ahmad and Gautam, 2014).

The excess mucous secretion all over the body surface of fish exposed to Dichlorvos was probably due to the malfunction of pituitary gland under a stress condition raised due to high concentrations of toxicant (Pandey et al., 1990). The hyper-excitability of the treated fish was probably to avoid the stress exerted by the toxicant (Bhat et al., 2012). The vertical hanging posture of treated fish was probably due to the stress caused by Dichlorvos (Günde and Yerli, 2012). The increasing trend of opercular movement of fish with the increasing concentration of the toxicant was also observed by the earlier workers (Mukherjee and Saha 2013).

The 96h LC_{50} value of Dichlorvos in the present study can be used to determine its safe level of disposal through agricultural run-off to the natural water bodies to minimize the toxic effects of the dichlorvos to the non target organisms.

Table 1: LC_{50} values along with 95% confidence limits of Dichlorvos (Nuvan) to the Oreochromis mossambicus at different hours of exposure (24, 48, 72 and 96h)

Test organism	Concentration of Dichlorvos (Nuvan) (mg/l)			
	24h	48h	72h	96h
Oreochromis mossambicus	3.84 (3.46-4.20)	3.50 (3.07-3.87)	3.12 (2.69-3.56)	2.90 (2.51-3.31)

Table 2: Mean values (±SD) of % mortality of *Oreochromis mossambicus* exposed to different concentrations of Dichlorvos (Nuvan) at different hours of exposure (24, 48, 72 and 96h). Mean values within columns indicated by different superscript letters (a-i) and within rows indicated by different superscript letters (m-p) are significantly different (DMRT at 5% level)

Dose (mg/l)	% mortality of fish at different times of exposure (h)			
	24	48	72	96
00	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$
1.6	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$10^{bn} \pm 0.43$	$10^{bn} \pm 0.43$
2.1	$00^{am} \pm 0.00$	$00^{am} \pm 0.43$	$20^{cn} \pm 0.00$	$20^{cn} \pm 0.43$
2.6	$10^{bm} \pm 0.43$	$20^{bn} \pm 0.43$	$30^{do} \pm 0.50$	$30^{do} \pm 0.00$
3.1	$20^{cm} \pm 0.43$	$30^{cn} \pm 0.00$	$40^{co} \pm 0.00$	$50^{cp} \pm 0.43$
3.6	$40^{dm} \pm 0.00$	$50^{dn} \pm 0.50$	$50^{fn} \pm 0.43$	$70^{fo} \pm 0.43$
4.1	$50^{em} \pm 0.00$	$70^{en} \pm 0.50$	$70^{gn} \pm 0.43$	$80^{go} \pm 0.43$
4.6	$70^{fm} \pm 0.00$	$80^{fn} \pm 0.50$	$90^{ho} \pm 0.43$	$100^{hp} \pm 0.43$
5.1	$90^{gm} \pm 0.50$	$90^{gm} \pm 0.43$	$100^{in} \pm 0.00$	$100^{hn} \pm 0.00$
5.6	$100^{hm} \pm 0.00$	$100^{hm} \pm 0.00$	$100^{im} \pm 0.00$	$100^{hm} \pm 0.00$

Table 3: Impact of Dichlorvos (Nuvan) on behavioural changes (MS: mucous secretion; HE: hyper-excitability; VHP: vertical hanging posture) of test organism, Oreochromis mossambicus at different hour of exposures. -: absent; +: mild; ++: moderate; +++: strong

Concentrations (mg/l)	24h			48h			72h			96h		
	MS	HE	VHP	MS	HE	VHP	MS	HE	VHP	MS	HE	VHP
0.0	-	-	-	-	-	-	-	-	-	-	-	-
2.6	-	+	-	+	+	-	+	-	-	++	-	-
3.1	+	++	-	+	+	-	+	-	-	++	-	+
3.6	+	+++	-	+	++	-	++	+	+	+++	-	+
4.1	++	+++	-	++	+++	-	+++	+	+	+++	+	++
4.6	++	+++	+	++	+++	+	+++	++	++	+++	++	++

Table 4: Mean opercular movement (±SD) of Oreochromis mossambicus exposed to Dichlorvos (Nuvan) during the study. Mean values within columns indicated by different superscript letters (a-e) and within rows indicated by different superscript letters (m-t) are significantly different (DMRT at 5% level)

Mean opercular movement (±SD)/minute/fish exposed to several concentrations of Dichlorvos (Nuvan) with different exposure time								
Time of exposure (h)	Concentrations of Nuvan (mg/l)							
	0.0	2.1	2.6	3.1	3.6	4.1	4.6	5.1
1	$106^{am} \pm 2.40$	$115^{an} \pm 3.16$	$120^{ao} \pm 2.71$	$135^{ap} \pm 3.16$	$140^{aq} \pm 4.40$	$142^{ar} \pm 1.83$	$180^{as} \pm 1.83$	$195^{at} \pm 2.71$
24	$108^{bm} \pm 2.42$	$112^{bn} \pm 3.69$	$118^{bo} \pm 2.71$	$131^{bp} \pm 2.4$	$138^{bq} \pm 1.83$	$138^{bq} \pm 3.16$	$170^{br} \pm 2.71$	$190^{bs} \pm 4.40$
48	$107^{cm} \pm 2.42$	$110^{cn} \pm 2.94$	$115^{co} \pm 2.94$	$124^{cp} \pm 3.92$	$127^{cq} \pm 2.71$	$130^{cr} \pm 2.42$	$149^{cs} \pm 4.40$	$172^{ct} \pm 1.83$
72	$106^{dm} \pm 2.18$	$106^{dm} \pm 3.92$	$111^{dn} \pm 3.16$	$118^{do} \pm 3.16$	$121^{dp} \pm 3.92$	$125^{dq} \pm 2.71$	$133^{dr} \pm 3.92$	$147^{ds} \pm 2.94$
96	$109^{en} \pm 2.40$	$105^{dm} \pm 2.94$	$110^{dn} \pm 3.92$	$113^{eo} \pm 4.40$	$118^{ep} \pm 1.83$	$122^{eq} \pm 2.42$	$128^{er} \pm 3.65$	$132^{es} \pm 2.71$

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REFERENCES

1. Ahmad SI. and Gautam RK. (2014). Effect of organophosphate pesticide, Nuvan on serum biochemical parameters of fresh water catfish *Heteropneustes fossilis* (Bloch.). Int Res J Environ Sci. 3(10), p. 1-6.
2. American Public Health Association (APHA) (2012). Standard methods for the examination of water and wastewater. (Eds. Rice, E.W., Baird, R.B., Eaton, A.D. &Clesceri, L.S.) American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC.
3. Bhat BA., Bhat IA., Vishwakarma S., Verma A., Saxena G. (2012). A comparative study on the toxicity of a synthetic pesticide, dichlorvos and a neem based pesticide, neem-on to *Labeo rohita* (Hamilton). Curr World Environ, 7(1), p. 157-161.
4. Binukumar BK., Gill KD. (2010). Cellular and molecular mechanisms of dichlorvos neurotoxicity: cholinergic, noncholinergic, cell signaling, gene expression and therapeutic aspects. Indian J Exp Biol. 48, p. 697-709.
5. Bretaud S., Toutant JP., Saglio P. (2000). Effects of carbafuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassius auratus*). Ecotoxicol Environ Saf. 47, p. 117-124.
6. CERI. (2007). Hazard assessment report: dimethyl 2,2dichlorovinyl phosphate (Synonyms: Dichlorvos, DDVP, CAS NO. 62-73-7. Chemicals Evaluation and Research Institute (CERI) Japan. www.cerij.or.jp/ceri_en/hazard _assessment_report/pdf/en_62_73_7.pdf
7. Das S. (2013). A review of dichlorvos toxicity in fish. Curr World Environ. 8(1), p. 143-149.
8. Deka S. and Mahanta R. (2015). Dichlorvos toxicity on fish- a review. European Journal of Biological Rsearch. 5 (3), p. 78-85.
9. Günde EG. and Yerli SV. (2012). The comparative study on the acute toxicity of dichlorvos on guppy (*Poecilia reticulata* P., 1859) and carp (*Cyprinus carpio* L., 1758). Hacettepe J Biol Chem. 40(2), p. 165-170.
10. Jones F and Davis J. (1994). DDVP Technical Grade: Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under flow-through test conditions: Lab Project Numbers: J9403007F: J9403007B. Unpublished study prepared by Toxikon Environmental Sciences.
11. Mukherjee D. & Saha N.C. (2013). Evaluation of hazardous impact of 2, 4, 6-Trichlorophenol to walking catfish, *Clarias batrachus* Linnaeus, 1758. Environment and Ecology. 31(1A), p. 355-359.
12. Pandey A., G.K. Kumar and J.S.D. Munshi. (1990). Integumentary chromatophores and mucous glands of fish as indicator of heavy metal pollution. Journal of freshwater biology. 2, p. 117-121.
13. R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.Rproject.org/. Accessed 8 Oct 2012.
14. Suntio LR., Shiu WY., Glotfelty D., MacKay D., Seiber JN. (1988). Critical review of Henry's Law constants for pesticides. Rev Environ Contam Toxicol. 103, p. 1-59.
15. Tilak KS. and Kumari RS. (2009). Acute toxicity of Nuvan an organophosphate to freshwater fish Ctenopharyngodon idella and its effect on oxygen consumption. Journal of Environmental Biology. 30(6), p. 1031-1033.
16. URL- 1: www.inchem.org/documents/jmpr/jmpmono/v067pr 10.htm
17. US EPA. (1999). Probit program version 1.5. Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, Ohio 45268. http://www.epa.gov/nerleerd/stat2.htm. Accessed 14 Jan 2012.
18. Velmurugan B., Selvanayagam M., Cengiz EI. and Unlu E. (2009). Histopathological changes in the gill and liver tissues of freshwater fish, Cirrhinus mrigala exposed to dichlorvos. Braz Arch Biol Technol. 52(5), p. 1291-1296.
19. WHO. (1989). Dichlorvos. Environmental Health Criteria 79. World Health Organisation, Geneva Switzerland.