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Acute toxic effects of Mancozeb to fish *Oreochromis mossambicus* (W. K. H. Peters, 1852) and their behaviour

Nimai Chandra Saha[1], Santosh Kumar Giri[2], Nishan Chatterjee[3], Surjyo Jyoti Biswas[4], Suman Bej[5*]

[1]*Director of Public Instruction, Govt. of West Bengal, Education Directorate, Bikash Bhavan, Salt Lake, Kolkata-700091, India. E. mail: dpiwb1960@gmail.com*
[2,3]*Research Scholar, Department of Zoology, Sidho Kanho Birsha University, Purulia, West Bengal, India*
[4]*Associate Professor, Department of Zoology, Sidho Kanho Birsha University, Purulia, West Bengal, India*
***Corresponding Author:** [5]*Assistant Professor, Post Graduate Department of Zoology, Bidhannagar College, EB-2, Sector-1, Salt Lake City, Kolkata – 700064, India. E-mail: sumanbej.sb@gmail.com*

Abstract

The present study was made to evaluate the acute toxicity of Mancozeb to freshwater fish, *Oreochromis mossambicus* (Tilapia). The LC_{50} values of Mancozeb to *O. mossambicus* were 14.40, 13.40, 12.34 and 11.68 mg/l at 24, 48, 72 and 96h respectively. The mortality rate of the treated fish exposed to Mancozeb significantly ($p<0.05$) varied over the control at all the concentrations at all the exposure times. On the other hand mortality rate of the fish also significantly varied ($p<0.05$) at all the times of exposure (24, 48 and 72 and 96h) at all the doses. The fish showed excessive mucous secretion and hyper-excitability at the higher concentrations of test chemical during 24 and 48h of exposure time. The loss of equilibrium of fish was acute at the higher concentrations at 72 and 96h of exposure. The opercular movement of the fish increased significantly ($p<0.05$) over the control with increasing concentrations but it was decreased significantly ($p<0.05$) with progress of time at all the treatments.

Keywords: *Mancozeb, Oreochromis mossambicus, acute toxic effects, behaviour, opercular movement.*

Introduction

Mancozeb a synthetic ethylene bisdithiocarbamates belonging to a subclass dithiocarbamates of carbamate pesticides (Srivastava and Singh, 2014). It is used as a fungicide against a wide range of fungi including ascomycetes, basidiomycetes, oomycetes and it is active over 70 crops and 400 different diseases (Leader et al., 2008). The Mancozeb inhibits the fungal-spore germination (Szkolnik, M. 1981; Wicks and Lee, 1982; Wong and Wilcox, 2001). Mancozeb itself does not act as a fungicide; rather it is effectively considered as a profungicide. When it is exposed to water it may degrade to ethylene bisisothiocyanate sulfide and ethylene bisisothiocyanate. Both of these compounds act as active toxicants. Mancozeb interferes with enzymes containing sulphydryl groups and disrupts many core enzymatic processes of the fungal cell cytoplasm and mitochondria (Ludwig and Thorn, 1960; Kaars, S., 1982). Mancozeb has low soil persistence. Its half-life is about 1 -7 days, but the half-life of its primary metabolite ethylenethiourea is 5-10 weeks. Mancozeb is hydrophobic in nature, so it is unable to contaminate groundwater but its metabolite ethylenethiourea has the enough potentiality to contaminate the ground water (Srivastava and Singh, 2013). Mancozeb like other carbamate may attack the nervous system. It inhibits

the function of neurotransmitter acetylcholinesterase (AchE) in the central nervous system of insects by its primary metabolites ethylenethiourea and carbon disulfide. AchE catalyses the hydrolysis of the acetylcholine to acetic acid and choline to slowdown the nerve stimulation. As a result acetylcholine concentration remains high in the junction that gives rise to continuous stimulation to the muscle leading to exhaustion and tetany followed by various kinds of poisoning symptoms, respiratory failure and death (Sikka and Gurbuz, 2006). Ethylenethiourea is responsible for thyroid dysfunction and carcinogenic effects in various organisms (Srivastava and Singh, 2013). The reports on the toxicity of Mancozeb to fish is scanty (Haya, K., 1989; Reddy and Bashamohideen, 1989; Grande et al., 1994; U.S. National Library of Medicine, 1995).

The present study was undertaken to find out the acute toxicity of Mancozeb to freshwater fish *Oreochromis mossambicus* and the changes in their behaviours and respiration.

Materials and Methods

The test chemical Mancozeb used in the study was collected from the local commercial shop. The fresh water fish, *Oreochromis mossambicus* (mean length 6.63 ± 0.71 cm and mean weight 5.60 ± 0.43 g) belonging to Class Teleostomi and Family Cichlidae was used in the bioassays as the test organism. The fish were collected from local pond and were allowed to acclimatize in the test condition in laboratory for 72h before the experiment.

The static replacement bioassays were conducted in 15l glass aquaria with 10l of non-chlorinated tap water. The values of the different physico-chemical parameters of water used in the study were as follows: temperature 30.7 ±0.8°C, pH 7.1 ±0.3, free CO_2 24.7 ±2.4 mg/l, dissolved oxygen 5.4 ± 0.5 mg/l, total alkalinity 172 ± 13.9 mg/l as $CaCO_3$, hardness 135 ± 3.8 mg/l as $CaCO_3$. Each test was accompanied by four replicates with sufficient control. The fishes were not fed for 24h before the commencement of test. The rough range-finding tests were performed initially to estimate the range of concentrations of the test chemical. The selected test concentrations of Mancozeb were finally used to estimate the 24, 48, 72 and 96h acute toxicity to *Oreochromis mossambicus*. During the study, the number of dead organisms was counted at every 24h of exposure. To avoid any organic decomposition, the dead fish were removed immediately after its death. A certain quantity of water from each aquarium was replaced every 24h by non-chlorinated stock water and a specific amount of Mancozeb was then added immediately to test aquaria to make fixed concentrations. All the bioassays and the estimation of physico-chemical parameters of the test water were performed following the methods of APHA (2012).

The 24, 48, 72 and 96h acute toxicity (95% confidence limits) of Mancozeb to *Oreochromis mossambicus* were estimated using a statistical software, Probit program version 1.5 (US EPA 1999). The values of percent mortality of the fish were subjected to analysis of variance (ANOVA) with the help of R-software (R Development Core Team, 2011) and Duncan's Multiple Range Test (DMRT) to determine the significant variations among the mean mortality of test animals at different concentrations of toxicant and times of exposure (24, 48, 72 and 96h). In the present bioassay behavioural alterations and the opercular movement of the fish exposed to different concentrations of Mancozeb were recorded during the experiment.

Results and Discussion

The 24, 48, 72 and 96h LC_{50} values (with 95% confidence limit) of Mancozeb to *O. mossambicus* have been summarized in Table 1. No mortality of test organism was found in control during the experiment. The LC_{50} values of Mancozeb to *O. mossambicus* were 14.40, 13.40, 12.34 and 11.68 mg/l at 24, 48, 72 and 96h respectively. The mortality rate of the treated fish exposed to Mancozeb significantly ($p<0.05$) varied over the control at all the concentrations irrespective of the exposure time (Table 2). On the other hand mortality rate of the fish also varied significantly ($p<0.05$) at all the exposure times (24, 48 and 72 and 96h) at all treatments. The fish showed excessive mucous secretion and hyper-excitability at the higher concentrations of test chemical during 24 and 48h of exposure time (Table 3). The loss of equilibrium of fish was acute at the higher concentrations at 72 and 96h of exposure. The opercular movement of the fish increased significantly ($p<0.05$) over the control with increasing concentrations but it was decreased significantly ($p<0.05$) with progress of time at all the treatments (Table 4).

The present study indicates that the 96h LC_{50} value of Mancozeb to *Oreochromis mossambicus* (11.68 mg/l) corresponds with the findings of earlier workers to *Punctius ticto* (12.95 mg/l) and *Clarius batracus* adult (14.36 mg/l) and fingerlings (14.04 mg/l) (Srivastava

and Singh, 2013; Sharma et al., 2016). In the present study, the excess mucous secretion all over the body surface of fish exposed to Mancozeb was probably due to the dysfunction of regulatory mechanism of pituitary gland over the integument on stress condition due to high concentrations of toxicant (Pandey et al., 1990). The hyper-excitability of the treated fish was probably the sign of avoiding and escaping tendency from the stress for Mancozeb toxicity (Bhat et al., 2012). The loss of equilibrium of the fish exposed to higher concentrations found in the present study was also documented by earlier workers in *Clarius batracus* exposed to Mancozeb (Srivastava and Singh 2013). It was probably due to the dysfunction of the central nervous system (Sikka and Gurbuz, 2006).

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

Test organism	LC_{50} values of Mancozeb (mg/l) at different hours of exposure			
	24h	48h	72h	96h
Oreochromis mossambicus	14.40 (13.35-15.43)	13.40 (12.30-14.48)	12.34 (11.14-13.62)	11.68 (10.40-12.90)

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

Concentrations (mg/l)	% mortality of fish exposed to Mancozeb at different hours of exposure (h)			
	24	48	72	96
00	00^{am} ±0.00	00^{am} ±0.00	00^{am} ±0.00	00^{am} ±0.00
8.0	00^{am} ±0.00	00^{am} ±0.00	10^{bn}±0.43	10^{bn} ±0.00
9.5	00^{am} ±0.43	10^{bn} ±0.43	20^{co} ±0.00	30^{cp} ±0.50
11.0	10^{bm} ±1.12	20^{cn} ±0.83	30^{do} ±0.43	40^{dp} ±0.00
12.5	20^{cm} ±0.43	30^{dn} ±0.71	40^{eo} ±0.00	50^{ep} ±0.83
14.0	40^{dm} ±0.00	50^{en} ±0.43	60^{fo} ±0.71	70^{fp} ±0.50
15.5	60^{em} ±1.12	70^{fn} ±1.12	80^{go} ±0.50	80^{gp} ±0.00
17.0	80^{fm} ±0.50	90^{gn} ±0.43	100^{ho} ±0.43	100^{ho} ±0.43
18.5	100^{gm} ±0.43	100^{hm} ±0.00	100^{hm} ±0.00	100^{hm} ±0.00

Table 3: Impact of Mancozeb on behaviours (MS: Mucous Secretion; HE: Hyper-Excitability; LE: Loss of Equilibrium) of *Oreochromis mossambicus* at different hour of exposures.
(-: absent; +: mild; ++: moderate; +++: strong)

Concentrations (mg/l)	Time of exposures (h)											
	24h			48h			72h			96h		
	MS	HE	LE	MS	HE	LE	MS	HE	LE	MS	HE	LE
0.0	-	-	-	-	-	-	-	-	-	-	-	-
11.0	+	+	-	+	+	-	+	+	-	-	-	+
12.5	++	++	-	++	++	-	+	+	+	+	+	++
14.0	++	+++	-	++	++	+	+	+	++	+	+	+++
15.5	+++	+++	+	++	+++	++	++	++	+++	+	+	+++
17.0	+++	+++	++	+++	+++	+++	++	++	+++	+	++	+++

Table 4: Mean opercular movement (±SD) of *Oreochromis mossambicus* exposed to various concentration of Mancozeb. The mean values within columns indicated by different superscript letters (a-g) and within rows indicated by different superscript letters (m-q) are significantly different (DMRT at 5% level)

Mean opercular movement (±SD)/minute/fish exposed to different concentrations of Mancozeb with several time of exposure					
Concentrations (mg/l)	**Hours of exposures (h)**				
	1	**24**	**48**	**72**	**96**
0.0	102^{am} ±0.43	99^{an} ±0.43	96^{ao} ±0.50	96^{ao} ±0.43	94^{ap} ±0.71
9.5	113^{bm} ±1.83	109^{bn} ±1.83	106^{bo} ±0.50	105^{bo} ±2.94	101^{bp} ±0.50
11.0	120^{cm} ±2.42	118^{cm} ±3.65	115^{cn} ±0.00	110^{co} ±3.92	108^{co} ±3.92
12.5	128^{dm} ±0.43	122^{dn} ±3.92	119^{do} ±0.00	117^{do} ±2.71	111^{dp} ±2.71
14.0	132^{em} ±0.50	130^{em} ±2.42	126^{en} ±0.43	124^{en} ±4.40	119^{eo} ±3.16
15.5	140^{fm} ±0.00	137^{fn} ±0.50	132^{fo} ±0.50	128^{fp} ±3.92	123^{fq} ±3.92
17.0	149^{gm} ±3.92	143^{gn} ±3.65	138^{go} ±0.43	135^{gp} ±0.71	129^{gq} ±2.40

The findings of the present study can be used for effective management and to determine the safe level of Mancozeb disposal through agricultural run-off to the natural water bodies to minimize its toxic effects to the non target organisms and aquatic ecosystem.

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