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Evaluation of acute toxicity and behavioral responses of *Heteropneustes fossilis* (Linn.) exposed to Captan

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ABSTRACT

In the present study, acute toxicity of the fungicide, captan, was evaluated under experimental condition to adult Heteropneustes fossilis. The 96 h LC_{50} with 95% confidence limits of Heteropneustes fossilis is 6.26 (5.80-6.64) mg/l. None of the unexposed control fish died and the first fish died 18 h after exposure to captan (9.50 mg/l and 10.5 mg/l). Mortality rate between each dose and mortality rate between 24-96 h depending on time was correlated. In the fish, significant relationship ($p<0.05$) was recorded between mortality rate and exposure times (24, 48, 72 and 96 h) at all concentrations. Similarly, significant variation was observed between mortality rate of fish at all the exposure concentrations at all the exposure times ($p<0.01$). The exposed fish showed abnormal behavior depending on dose of captan and duration of exposure.

Keywords: Acute toxicity, Captan, *Heteropneustes fossilis*, 96 h LC_{50}, behavior.

INTRODUCTION

Pesticides are applied in agricultural fields to protect crops from different pests and diseases for increase in crop production. Various studies have revealed that pesticides are responsible for reproductive dysfunction, endocrine disruption, developmental disabilities and respiratory diseases on non target organisms (Raina et al., 2009). Captan is a non systemic fungicide that was first registered in Canada in 1953 and is used to control disease in vegetables, fruits and tobacco. It also has a broader industrial application for control of mould in paints, lacquers and wallpaper pastes (AAFC, 1997). The reaction of captan with sulphydryl groups is the principal mode of action in fungal cells (Boran et al., 2012). Captan inhibits the process of respiration and metabolism of fungus through a thiol reactant (Barreda et al., 2006). It also results in the decrease of fungal spore

germination, oxygen uptake and growth (Boran et al., 2012). Earlier studies also indicate that captan has cytotoxic effects on *Drosophila melanogaster* (Nazir et al., 2003). Captan enters the natural water bodies through agricultural run-off, municipal sewage and industrial discharges. Captan is highly toxic to fish and aquatic species (Boran et al., 2012). Acute toxic effects include the death of fish and other animals and are seen after 2–4 days exposure to captan (Boran et al., 2012). The reports on the toxicity of captan on fish are scanty (Boran et al., 2012). No literature is available on the toxicity of captan on *Heteropneustes fossilis,* an important food fish.

The aims of the present study were to evaluate the acute toxicity and behavioral responses of *Heteropneustes fossilis* (Linn.) exposed to Captan.

MATERIALS AND METHODS

The Asian stinging catfish, *Heteropneustes fossilis* (Order: Siluriformes; Family: Heteropneustidae) is used as the test organism in the bioassay having a mean length of 10.7 ± 0.4 cm and a mean weight of 22.60 ± 0.8 g. The test fish were obtained from local aquaculture field and were acclimatized to the experimental condition for 72 h before their use. The acclimatized fish were not fed 24 h before the start of the bioassay to maintain their normal metabolic activity (APHA, 2012).

Analytical grade captan (3a*R*,7a*S*)-2-[(trichloromethyl) sulfanyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione) $C_9H_8Cl_3NO_2S$, belonging to the phthalimide class of fungicides (molecular weight 300.59 g/mol; Jardine Distribution Inc.) was used as the test chemical.

Static replacement bioassays with fish were conducted in 15 liter glass aquaria each holding 10 liter of water for the determination of acute toxicity. A set of four aquaria was exposed to one concentration of captan to make four replicates per concentration. Each set of tests was accompanied by four replicates of control. Five fish were used in each replicate. Tap water stored in the glass aquaria (temperature 24 ± 0.38 ^oC, pH 8.00 ± 0.34, free CO_2 10.5 ± 0.64 mg/l, DO 8.6 ± 0.24 mg/l, alkalinity 174 ± 5.01 mg/l as $CaCO_3$, hardness 124 ± 5.2 mg/l as $CaCO_3$) was used as a diluent medium.

The bioassays and the tests on water quality were done following the methods outlined in APHA (2012). The required quantity of captan was weighed for the different test concentrations which were then added directly to the test medium. Uniform mixing of captan was ensured by stirring with the help of a magnetic stirrer. The dose range at which mortality occurs was determined from initial rough range finding tests. The selected test concentrations of captan finally used for the determination of 96 h LC_{50} to the fish were 0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 mg/l for fish.

The number of dead fishes was checked every 24 h and removed from the test solution to avoid oxygen scarcity and organic decomposition. The test solution was replaced every 24 h by freshwater and the desired quantity of captan was immediately added to the water to maintain a constant concentration of captan in the solution.

Mortality rate at various concentrations and at various times of exposure (24, 48, 72, 96 h) was analysed using the computer software R version 2.14.0 (US EPA, 1999) and probit analysis by Finney (1971) for determining 96 h median lethal concentrations (LC_{50}) with 95% confidence limit of captan to the fish. The relation between mortality rate with exposure time and doses was determined using correlation analysis (US EPA, 1999; Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

No fish died during the acclimatization period. The lethal concentrations of captan to the fish, *Heteropneustes fossilis* are summarized in Table 1 and Figure 1.

A significant correlation (p<0.05) was recorded between mortality rate and exposure times (24, 48, 72 and 96 h) at 6.00, 7.00, 7.50, 8.50 and 10.00 mg/l (Table 3). A significant variation (p<0.01) was also observed between mortality rate of fish at all the exposure concentrations at all exposure times (Table 4). The present 96 h LC_{50} value of captan to *Heteropneustes fossilis* (6.26 mg/l) is much higher from the results of earlier workers (Boran et al., 2012). The differences in our findings may be associated with temperature, water quality parameters, age and fish species (Diedrich et al., 2015; Capkin et al., 2006; Capkin et al., 2009; Patra et al., 2015). The behavioral changes observed in the fish exposed to captan are summarized in Table 2.

Table 1. Lethal concentrations (LC_5, LC_{10}, LC_{50}, LC_{90}, LC_{95}) along with 95% confidence limits of Captan to *Heteropneustes fossilis* at different hours of exposure (24, 48, 72, 96h).

Lethal Concentrations	Concentration (mg/l)			
	24 h	**48 h**	**72 h**	**96 h**
LC_5	5.21 (4.07 - 5.89)	5.03 (4.09-5.62)	3.89 (2.88-4.55)	3.86 (2.91 - 4.47)
LC_{10}	5.77 (4.75 - 6.37)	5.55 (4.71- 6.06)	4.40 (3.45-5.00)	4.30 (3.41-4.86)
LC_{50}	8.28 (7.83 - 8.84)	7.82 (7.41 - 8.32)	6.79 (6.32-7.24)	6.26 (5.80-6.64)
LC_{90}	11.89 (10.61 - 4.94)	11.03 (9.93-13.42)	10.50 (9.39-12.90)	9.13 (8.34-10.77)
LC_{95}	13.17 (11.46-17.48)	12.16 (10.71-15.48)	11.88 (10.34-15.44)	10.16 (9.07-12.58)

Table 2. Impact of captan on the behavioral responses of *Heteropneustes fossilis* (R: restlessness; ES: erratic swimming; MS: mucus secretion; -: none; +: mild; ++: moderate; +++: strong; x: dead) at various concentrations during different hours of exposure.

Dose (mg/l)	24 h			48 h			72 h			96 h		
	R	ES	MS	R	ES	MS	R	ES	MS	R	ES	MS
0.00	-	-	+	-	-	+	-	-	+	-	-	+
5.00	-	-	+	-	-	+	-	-	+	-	-	+
5.50	-	-	+	-	-	+	-	-	+	-	-	+
6.00	-	-	+	+	+	+	+	+	+	+	+	+
6.50	+	+	+	+	+	+	+	++	++	+	++	++
7.00	+	+	++	++	+	++	++	++	++	++	++	++
7.50	++	+	++	++	+	++	++	++	++	++	++	++
8.00	++	+	++	++	++	++	+++	++	++	++	+++	++
8.50	++	+	++	++	++	++	+++	++	+++	+++	+++	+++
9.00	++	++	++	+++	++	+++	+++	+++	+++	+++	++	+++
9.50	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
10.0	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

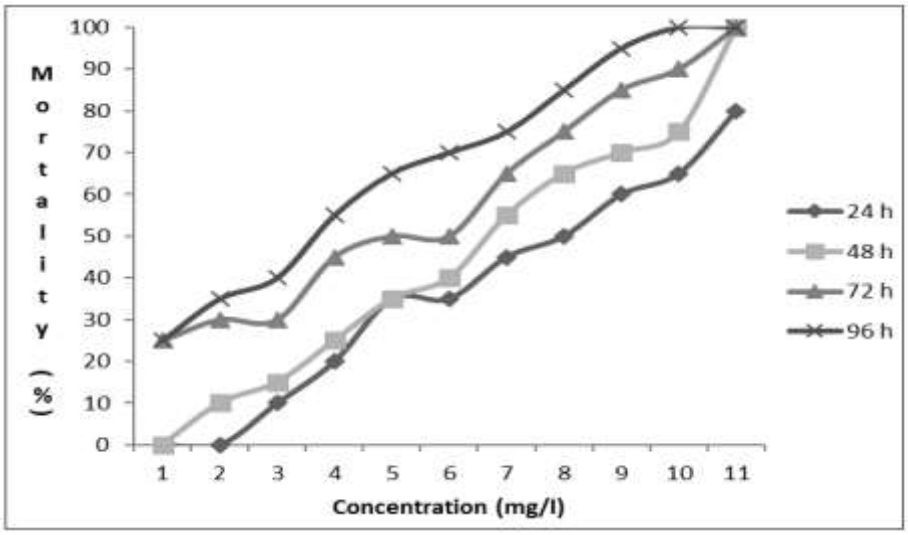

Figure 1. Percent Mortality of *Heteropneustes fossilis* exposed to different concentrations of captan at 24, 48, 72 and 96 h.

The fish exposed to 6.50 mg/l of captan exhibited signs of restlessness, erratic swimming and excess mucus secretion at 72 and 96 h. The intensity of behavioral changes increased with the increasing concentrations and progress of time of exposure (Table 2). Sometimes somersaulting and convulsions of fish followed by death was also recorded at the higher doses. This was probably an early indication of their avoidance reaction from the toxicant (Mukherjee and Saha, 2014). The behavioural changes recorded in the exposed fish may be due to the effects of captan on central nervous system (Pandey et al., 2005). The excess mucous secretion of exposed fish was probably due to the dysfunction of the pituitary gland under toxic stress (Pandey et al., 1990).

CONCLUSION

The present findings, thus, highlight the toxicity of captan to *Heteropneustes fossilis* during their acute exposure. Acute toxicity studies are the primary steps in determining the water quality required for the sustenance of aquaculture. The study reveals the toxicant concentrations (viz. LC_{50}) that cause fish mortality at short-term exposure. The observation on the behavioral responses of the fish in the present study may be an indicative parameter for assessing the toxicity of captan in the ecosystem.

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