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Diazinon Effect on Behavior and Morphology of Catfish *Clarias batrachus* (Linnaeus, 1758)

Neetu Kumari and Ravindra Singh
Department of Zoology, Jai Prakash University, Chapra (Bihar)
Department of Zoology, Jai Prakash University, Chapra (Bihar)
Corresponding author: nitu3146@gmail.com

ABSTRACT

The freshwater catfish, *Clarias batrachus* was exposed to the Diazinon as an organophosphate herbicide to determine the acute toxicity values for different time periods. The LC_{50} values obtained in static method were 6.622, 6.546, 6.05 and 5.798 mg/L and in continuous flow-through system the LC_{50} values were 5.759, 5.374, 5.249 and 5.191 mg/L respectively for 24, 48, 72 and 96 h. The fishes were thereafter exposed to sub-lethal and lethal concentrations of toxicant to study the changes in glycogen and protein content of the vital organs. The behavioral and morphological changes observed during the study period.

Keywords: LC_{50}; Diazinone; Behavior; Morphology; Catfishes

INTRODUCTION

Bihar is facing mounting pressure on water resources because there is growing demand of water due to population explosion and increasing pollution. Freshwater bodies such as rivers, lakes and ponds have actually become the disposal sites of domestic and industrial wastes. Fish is a rich source of proteins which is good for human health. The toxic substances are accumulated in these polluted water bodies, consequence of which is gross pollution of water. In contaminated water, fish may exhibit morphological changes and behavioral responses which can be used as biomarkers of contamination.

The response to stress is an adaptive mechanism that allows the fishes to cope with stressors in order to maintain its natural homeostasis or detrimental for freshwater fishes. The effect of environmental stressors has been grouped as primary, secondary and tertiary responses in which primary one involves the initial neuro-endocrine disturbances includes the release of catecholamine from chromafin tissue (Reid, 1998) and stimulation of the hypothalamic-pituitary inter-renal (HPI) axis causing release of corticosteroid hormone into the circulation (Mommsen et al, 1999). The secondary response include changes in plasma and tissue ion at metabolic level, blood chemistry structure of protein, acid-base and hydro-mineral balance and immune function with cell level responses (Iwama et al, 1997). The tertiary response refers to changes in growth condition, overall resistance to disease, metabolic efficiency for activity and behavior about survival of fishes. This grouping is simple, however, depending on magnitude and organization from molecular and biochemical to population and community level (Adams, 1990).

Behaviour allows an organism to adjust to external and internal stimuli in order to meet the challenges of surviving in the changing environment whereas morphological changes are external changes caused due to changed environment. The use of these abnormalities in fish as biomarkers has become more prevalent in recent years. These biomarkers can provide suitable indication about the environmental condition. The morphological and behavioral changes are the most straightforward methods to study the effects of contaminants on fish in aquatic ecosystem. The changes act as diagnostic endpoints in screening the effect of polluted water on fishes.

The Diazinone is a widely applied pesticide used in the post-harvest treatment of crops. It acts as a plant growth regulator in smaller quantities (RED Facts, 1993) with its

annual global use exceeded in last decades (Reuters, 2011). Diazinon is classified as a Toxicity Category II (moderately toxic) pesticide by United States Environmental Protection Agency (USEPA). Commercial Diazinon formulations are more toxic than technical Diazinon (Peixito, 2005).

Herbicides reach the surface water through drainage or leach from treated plants and soil and cause biological impairment effecting fish and other aquatic organisms. Hence, the present study was undertaken to know the toxic effect of Diazinone on the behavioral and morphological changes in freshwater catfish, *Clarias batrachus.*

Materials and Methods

The catfish *Clarias batrachus* with a size range of 6-8 ±1/2cm of both sexes were brought from local fish market in Chapra town and acclimatized to laboratory conditions for one week. The experimental fishes were fed with Groundnut cake and rice bran during the period of acclimatization. In any batch during acclimatization, if 5% mortality is observed the total batch was discarded. All the precautions laid down by APHA (1998) were followed. The water quality maintained for experiment had temperature 28 ± 20^{0}C, pH as 8.2, dissolved oxygen (mg/L) as 8-10 and 320 as total hardness.

The Pilot experiments were conducted with 1 litre capacity glass chambers, to choose the concentrations at which the fish are killed. For continuous flow through system, reservoirs of 90 liters capacity were used. The test water was let into test containers at a rate of 4 liters/ hour using polyethylene drip nets with regulators and for every 12 h fresh test solutions were prepared in reservoirs. Experiments were conducted to determine the toxicity of Diazinone in various concentrations within static and continuous flow through systems. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static and continuous flow through systems.

Finney's probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LC_{50} values. For the determination of the 95% confidence limits, LC_{50} values and a normal variant of 1.96 were taken into consideration. After the determination of LC50, the fish were exposed to sub lethal concentration (1/10th of 96 h LC_{50}) of Diazinone for four exposure periods i.e., 24, 48, 96 h and 8 days.

For behavioral responses and morphological changes, fishes were exposed to normal water (control) and three sublethal concentrations (6.12%, 12.24% and 24.48%) effluent (treated) for 24h, 48h, 72h and 96h of. Fishes were not fed during the experiment and experiments were replicated thrice.

RESULTS

The LC_{50} values and 95 % Confidence limits of Diazinon for 24, 48, 72 and 96 h to *Clarias batrachus* in static and continuous flow through systems were given in Table 1 and 2.

Table 1: LC_{50} values for exposure period for Diazinon to the *Clarias batrachus* fish

Sl No	Exposure period (hours)	Static (mg/l)	CFM (mg/l)
1	24	6.622	5.759
2	48	6.546	5.374
3	72	6.050	5.249
4	96	5.798	5.191

Table 2: 95 % Confidence limits of Diazinon exposed catfish in exposure periods.

Sl No	Exposure time (hours)	Static method		CFM	
		Lower	Upper	Lower	Upper
1	24	33.71	86.29	18.47	77.53
2	48	24.55	87.65	24.55	87.45
3	72	26.14	81.86	13.99	85.99
4	96	16.29	75.71	16.29	75.71

In general, catfish is sensitive towards the test toxicant. These findings are in agreement with Cericato et *al* (2008) on *Rhambdia quelen;* Modesto and Martinez (2010) on *Prochilodus lineatus.* The toxicity may be influenced by exposure conditions, formulation, source and size of fish and water quality. Nwani et *al* (2014) reported the LC_{50} value of Primextra on African catfish *Clarias gariepinus* for 96 h as 4.70mg/L as lethal concentration whereas Kavitha and Binukumari (2014) found the LC_{50} value for 96 h as 0.18.

The continuous flow-through system LC_{50} values are low when compared to the static values. This is due to the constant maintenance of concentration in flow-through system and fluctuations in static system due to bioaccumulation, pesticide absorption to toxicant chamber walls and degradation of toxic effect of the compound.

The behavioral responses in fishes of both the control and treated groups were noted for all the time intervals. The fishes showed marked behavioral responses and morphological changes when exposed to various concentrations (Table-3).

Table 3: Behavioral response of different Diazinon exposure on *Clarias batrachus* (L=Less; M=More; F=Fast; N=Normal; S=Slow; P=Present; A=Abscent; C=Control; C1=6.12%; C2=12.24%; C3=24.48%)

	24 h				48 h				72 h				96 h			
	C	C1	C2	C3	C	C1	C2	C3	C	C1	C2	C3	C	C1	C2	C3
Erratic swimming	A	L	M	M	A	L	M	M	A	L	M	M	A	L	M	M
Air at surface	A	L	M	M	A	L	M	M	A	L	M	M	A	L	M	M
Operculam movemt	N	F	F	F	N	N	S	S	N	N	S	S	N	N	S	S
Loss of balance	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	P
Hitting against wall	A	A	P	P	A	A	A	P	A	A	P	P	A	A	P	P
Restlessness	A	A	P	P	A	A	A	P	A	A	P	P	A	A	P	P
Sluggishness	A	A	A	A	A	A	A	A	A	A	P	P	A	P	P	P
Fish lied at surface	A	A	A	A	A	A	A	A	A	A	A	P	A	A	A	P

Table 4: Morphological changes of different Diazinon exposure on *Clarias batrachus* (L=Less; M=More; F=Fast; N=Normal; S=Slow; P=Present; A=Abscent; C=Control; C1=6.12%; C2=12.24%; C3=24.48%)

Morphological changes	24 h				48 h				72 h				96 h			
	C	C1	C2	C3	C	C1	C2	C3	C	C1	C2	C3	C	C1	C2	C3
Loosening of scale	A	A	A	A	A	A	A	A	A	A	A	A	A	P	P	P
Redness in Eyes	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P
Profuse mucous loss	A	A	A	A	A	A	A	A	A	P	P	P	A	P	P	P
Blooding from Gills	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P
Belly balloning	A	A	A	A	A	A	A	A	A	A	A	P-	A	A	A	P
Dark patches in abdomen	A	A	A	A	A	A	A	A	A	P	P	P	A	P	P	P

Behavioral Responses: The control fishes released in well aerated water were found to be alert and responded actively to slight disturbances. They behaved naturally by showing movements in well coordinated manner. There was no mortality in control.

The treated fishes showed erratic movements which increased with increase in concentration of the effluent and duration of exposure (24h, 48h, 72h and 96h). Gulping air on the surface or jumping out of water was also observed which was less in 6.12% as compared to 12.24% and 24.48% during all the observations (24h, 48h, 72h and 96h). Opercular movements in fishes were observed to be fast in 6.12%, 12.24% and 24.48% after 24h of exposure. After 48h, it was normal in 6.12% but slow in 12.24% and 24.48%. After 72h and 96h also, opercular movements were normal in 6.12% but slower in 12.24% and 24.48% concentrations. Loss of equilibrium was observed in 24.48% after 96h of exposure while it was absent in other concentrations and durations. Fishes also hit against the wall in 12.24% and 24.48% concentrations while it was absent in 6.12% concentration. Fishes showed restlessness in 12.24% and 24.48% concentration. Sluggishness was observed in 12.24% and 24.48% concentrations after 72h of exposure and in all concentrations after 96h of exposure. Two fishes were seen lying on the water surface before death in 24.48% after 72h and 96h of exposure.

Morphological Changes: In the treated fish, loosening of scales was observed in all concentrations (6.12%, 12.24% and 24.48%) after 96h of exposure. Redness in eyes of fishes was seen in 12.24% and 24.48% concentrations after 72h and 96h of exposure. Profuse mucous secretion was observed all over the body in all the concentrations (6.12%, 12.24% and 24.48%) after 72h and 96h of exposure. Bleeding from gills of fishes was noted in 12.24% and 24.48% after 72h and 96h of exposure. Ballooning and belly upward of two fishes at surface of water after death was observed in 24.48% after 72h and 96h of exposure. Pigmented patches on the abdomen of fishes were examined in all concentrations (6.12%, 12.24%and 24.48%) after 72h and 96h of exposure (Table 4).

After 96h of exposure, four fishes were left in 6.12%, two in 12.24% and two in 24.48% concentrations. These fishes were kept separately in well aerated normal water. Fishes in 6.12% recovered to some extent after 20 days and showed normal behavior although their morphological changes persisted. Fishes of 12.24% and 24.48% concentrations died within 20 days.

DISCUSSIONS

Freshwater bodies such as rivers, lakes and ponds have actually become the disposal sites of domestic and industrial wastes. Fish is a rich source of proteins which is good for human health. The toxic substances are accumulated

in these polluted water bodies, consequence of which is gross pollution of water. In contaminated water, fish may exhibit morphological changes and behavioral responses which can be used as biomarkers of contamination.

The effects of pesticides are generally displayed as behavior responses and morphological changes that lead to genetic changes in the tissues. Fish is extremely sensitive in terms of behavior and any change in behavior of fishes is related with the toxicity of effluent. Morphological changes points towards stress and injuries caused by effluent. Thus, these parameters are used to determine toxicity caused by effluent.

Control fishes released in well aerated water were found to be alert and respond actively to slight disturbances. They behaved naturally by showing movements in well coordinated manner. Treated fishes exposed to three sub-lethal concentrations (6.12%, 12.24% and 24.48%) of Diazinon showed restlessness, erratic body movements, gulping of air at surface or jumping out of water, opercular movements, loss of equilibrium and hitting against the wall. A few changes in the outer morphology were also observed which included ballooning, loosening of scales, red eyes, profuse mucous secretion, bleeding from gills and pigmented patches on the abdomen. All these symptoms increased with increase in concentration and duration of exposure. Reversal of a few symptoms was seen at the lowest concentration i.e. 6.12%.

Loss of equilibrium, erratic swimming and restlessness are common behavioral responses in several fishes exposed to a variety of toxicants as recorded in *Oreohromis niloticus* and *Clarias batrachus* exposed to cypermethrin (Yaji et *al*, 2011). Hitting against the wall was noticed in *Labeo rohita, Catla catla* and *Cirrhinus mrigala* exposed to fenvalerate (Susan and Sobha, 2010). Restlessness, loss of equilibrium, hitting against the wall and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase (AChE), leading to accumulation of acetylcholine at synaptic junctions (Agrahari et *al*, 2006). Copious mucous secretion, increased opercular movement and dark pigmentation of body parts were observed in *Clarias gariepinus* treated with lindane and diazinon (Pandey et *al*, 2009), in *Clarias batrachus* exposed to carbaryl and malathion (Wasu et *al*, 2009), in *Heteropneustes fossilis* exposed to dimethoate (Srivastava et *al*, 2010). Profuse mucous secretion is considered to be a defense mechanism to neutralize the effect of toxicant and to avoid it. Bisht and Agarwal (2007) suggested that mucous produced coagulates with the toxicant and prevents its cutaneous entry into the body. Surfacing or gulping of air might occur due to a demand of higher oxygen level after exposure (Katja *et al*., 2005).

The results of the present study clearly reveal that higher concentration (24.48%) and longer exposure period (96h) of Diazinon is highly toxic to the fish. The effect of other two concentrations cannot be ignored. Prolonged exposure to even lower concentrations induces behavioral and morphological changes in fishes. These changes and responses indicated stress in fishes which can further lead to death and reduction in fish fauna. This type of study helps to understand the effect of industrial effluents on fishes so as to determine safe environmental concentration where there is no stress and lethality to fishes.

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