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ACUTE TOXICITY AND BEHAVIORAL ALTERATIONS IN CHANNA PUNCTATUS AFTER EXPOSURE TO ETHION

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ABSTRACT

Changes in a fish's behaviour serve as an effective measure for detecting changes in the environment. Pesticide not only alters the physico-chemical properties of water but also has a major influence on fish behavioral changes triggered by any modification in the aquatic environment, eventually leading to physiological changes. As a result, the current study, investigated the behavioural changes produced by sub-acute exposure to the organophosphate pesticide Ethion in the freshwater fish *Channa punctatus*. To establish the LC_{50} value, fish were exposed to Ethion at four sub-lethal concentrations: 0.001 ml/l, 0.002 ml/l, 0.003 ml/l, and 0.004 ml/l for 96 hours. Fish were kept in four different conditions, i.e. control, 5 %, 10 %,15 % of the calculated LD 50 for 96 hrs to study the effect of ethion. Time and dose were both factors in the changes in behavioural characteristics. Ethion exposure had a negative impact on the fish's usual behaviour, as seen by lower swimming activity, a drop in surfacing frequency, and opercular motions. Moreover, progressive depigmentation of body, and descaling were observed in a dose and time dependent manner upon ethion exposure. The present work suggest that Ethion is a potent ecotoxic agent and such finding ought to raise awareness of pesticide poisoning issues in developing nations among clinical toxicologists, health authorities, and policy makers.

KEY WORDS : Behavior, *Channa punctatus*, Ethion, Lethal Concentration 50 (LC_{50}), Toxicity

INTRODUCTION

Pesticides are extremely strong toxins, and even small exposures of a few hours are enough to cause alterations in fish ethological responses. (Rao *et al*., 2005). Pesticide use in agricultural areas to manage pests is very hazardous to non-target creatures such as fish, affecting their health through metabolic impairment and, in some cases, death. According to a report released by the US Environmental Protection Agency in 1995, more than half of the water pollution in streams and rivers comes from chemical leaching and mixing from agricultural operations (Cook *et al*., 1995). Organophosphorus pesticides (OOPS) are one of the most dangerous global toxins, contributing significantly to water pollution. The Indian Council of Agricultural Research (ICAR) formed a committee to recommend viable solutions to the toxicity caused by pesticides and their residues in foodstuffs. Ethion is a large family of commonly used organophosphorus insecticides containing a thiophosphate lP=S functional group. Organophosphorus pesticides account for a large share of insecticides now in use in the world. It is used as an insecticide and miticide against mites in the crops like tea, cotton, maize, cucurbits, etc. In a case study conducted by the Central Pollution Control Board of India in 2000, it was confirmed to be one of the most common pesticides in the waters of major rivers. According to WHO, Ethion is classified as a "Moderately Hazardous Class II Technical Grade Active Ingredient"with a mark of 888 by the International Chemical Safety Card (ICSC) (The WHO recommended classification by hazard and guidelines to classification, 2019). It is water-soluble (2.0 mg/l at 25 ^{o}C) but also adheres to soil particles and persists for several years. It is know to also produced cancer, birth defects, restlessness, neurological disorders, etc.

As a secondary consumer in the food chain, fish performs an important function, and its relevance is amplified because higher trophic levels rely on it as their primary food supply. Fish biologists are concerned about direct or indirect pesticide exposure to fish and the resulting toxicity because polluted fish have negative impacts on humans. Pesticide aggregation in tissues causes many metabolic alterations in fish and freshwater fauna by manipulating the functions of many enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982). Fishes are used as bioindicators of aquatic pollution, because of their lack of sensitivity to the environment (Srivastava and Kaushik, 2001). The objective of this research is to figure out behavioural and physiological changes caused by the organophosphorus pesticide Ethion's toxicity on the freshwater air-breathing teleost *Channa punctatus* or popularly known as spotted snakehead.

MATERIALS AND METHODS

Materials

The purpose of this experiment was to investigate behavioural changes in *Channa punctatus*, a freshwater teleost (Bloch), commonly known as spotted snakehead fish, after sub-acute exposure to Ethion, Live. Juvenile fish of average weight 25 to 30 g and length 10 to 15 cm were procured from the markets of Jagdalpur, Chattisgarh, and were transported to the laboratory in oxygenated tanks (Fig. 1A). To acclimatize to the laboratory conditions, the fish were kept in clean glass aquaria of (30 cm x 30 cm x 30 cm) 20 L capacity for 1 month (Fig. 1B). During the acclimatization phase, the physico-chemical parameters of the water, such as temperature, pH, turbidity, free carbon dioxide (CO_2), and dissolved oxygen (DO), were measured every day. Chlorine-free tap water was used in the aquaria. The water of the aquaria was changed whenever necessary. Deceased fish (if any) were removed as soon as possible. Commercial fish food 'Optimum' (containing protein 20%, fat 3%, fiber 7%, calcium 0.7%, and phosphorus 0.7%) was provided every day during acclimatization as well as the treatment period by 30% of the fish body weight once a day. The photoperiod of the acclimatization chamber was 12 hours light and 12 hours dark. Insecticide Ethion 50% EC (chemical formula- $C_9H_{22}O_4P_2S_4$) was used in toxicity studies which were manufactured by Ankar Industries Private Limited, Kolkata, and was bought from local pesticide supplies in Jeypore, Koraput.

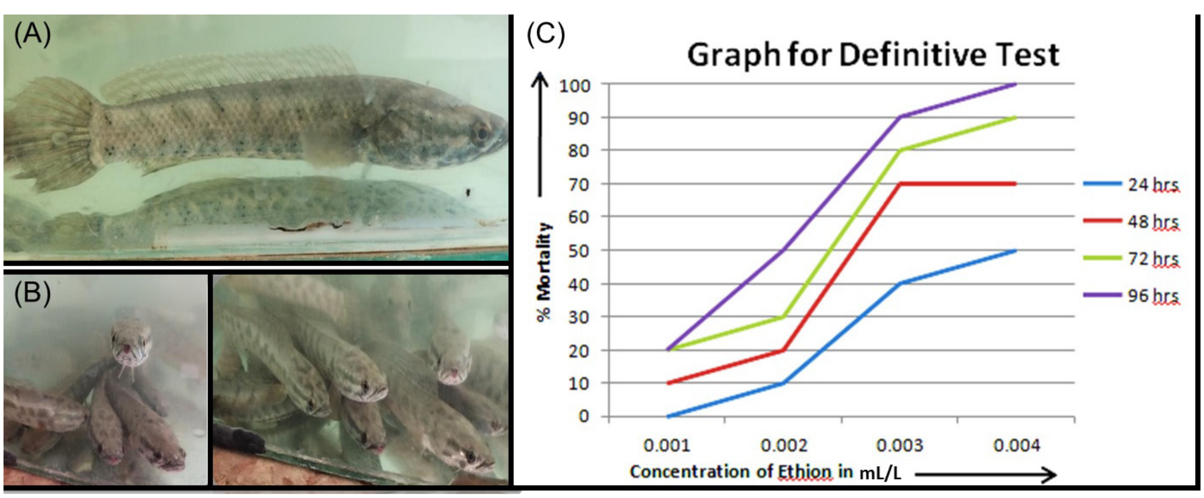

Fig. 1. (A) Image of *Chana punctatus* fish (B) Image of *Chana punctatus* fish during acclimatization process (C) LC_{50} at 96 hours by the direct interpolation method

METHODS

Acclimatization

Initially, 100 fish were selected for acclimatization for 1 month in aquariums of 30 cm × 30 cm × 30 cm having non-chorine normal tap water (20L) (Fig. 1B). During this event, estimation of all physico-chemical conditions like - pH, water temperature all with different turbidity, free carbon dioxide (CO_2), and dissolved oxygen (DO) were done on daily basis (Eaton *et al.*, 2005). The dead fishes were removed as soon as noticed.

Estimation of LC_{50} by the direct interpolation method

The median lethal concentration (LC_{50}) is the concentration of a test chemical that kills 50 percent of the test organisms with respect to a particular time. Generally 96 hours, and is the most frequently recognized basis for acute toxicity tests. Death is a common criterion in toxicity testing because it is easy to assess and appears to have biological and ecological significance. The direct interpolation technique, which involves two exploratory tests and a definitive test, was used to estimate the LC_{50} value under acute poisoning after 24, 48, 72, and 96 hours. The death rate was measured after 24, 48, 72, and 96 hours, and as soon as any dead fish was discovered, it was removed. For the first exploratory test, 1 ml of Ethion was dissolved in 1 l distilled water to make an Ethion stock solution. A greater concentration of 0.1 ml/l and a lower concentration of 0.001 ml/l were used in glass aquaria (1ft x 1ft x 1ft) each containing eight fish to estimate mortality between 0% and 100%. The stock solution was made by dissolving 0.1 ml Ethion in 1l distilled water to make concentrations of 0.0001ml/l for the second exploratory test and conclusive test. In the second exploratory test, four concentrations were obtained to determine a narrow concentration range. After 24, 48, 72, and 96 hours, the mortality rate was reported. The LC_{50} values were calculated using the values from the definitive test by drawing a dose-response curve between percent mortality and toxicant concentrations.

Experimental Design

A total of 100 fish were chosen for acclimation and then divided into four groups, each having 25 fish. The fish were not fed during the experiment. The doses given were 5%, 10%, and 15% of the LC_{50} value, respectively, and were given through the medium for a period of 96 hrs. The fish in the first tank was kept in regular tap water as a control. Fish from groups 0.001, 0.002, and 0.003 ml/l concentrations group were maintained in water containing 0.002 ml/l Ethion at concentrations of 5%, 10%, and 15%, respectively. To check for any variations from the usual, the behaviour of treated fish was examined on a regular basis and compared to that of control fish. Throughout the trial, body color, descaling, opercular movement, surfacing frequency, and swimming activity were monitored at regular intervals of 24 hours. The 'Grid technique' was used to examine the fish's swimming behaviour. 5x5 cm^2 grids were created on chart paper and set on the aquarium's bottom for this purpose. The total number of squares each fish covered was counted for fifteen minutes. The total number of trips to the water surface by the fish in 5 minutes was also recorded. Three replicas for each concentration were used for each parameter. For each tank, three concurrent readings taken, whose average was considered as the result. The behavioral changes were arranged according to the ranges considered and '+' was put on behalf of the very low range, '++' for low, '+++', for moderate behavior '++++', for high, and '+++++', for very high. For swimming activity, very low was considered from 60 and below, low between 61 to 80, and moderate lies between 81 to 100. Below 0 to 10, 11 to 15, and 16 to 20 for very low, low, and moderate respectively, in the case of surfacing frequency. In opercular movement, moderate lies between 141 to 160, low from 121 and 140, and very low includes 120 and below that.

Statistical Analysis

The analysis was done with the help of Microsoft Excel. Interpretation of the observed data from our experiment is statistically analyzed by using standard deviations and error bars on the graphs, for the difference is not statistically significant. These data were further employed for t-test analysis. Keeping the level of significance at ≤ 0.05, the p values of different observations were deduced and were marked on the graphs as specific symbols, viz. For 24 hrs group - *; For 48 hrs group - #; For 72 hrs group - ≤; For 96 hrs group - ¥. If the p-value was found less than 0.05, then the symbols were marked once. If the p-value was found less than 0.01, then the symbols were marked twice. If the p-value was found less than 0.001, then the symbols were three times marked, respectively.

RESULTS

Acclimatization

A record of atmospheric temperature and the water temperature were maintained within a constant range throughout the experiment. The water quality was analyzed at proper intervals. Also, the physico-chemical parameters were recorded in Table 1.

Table 1. Physico-chemical characteristics of water (sub-acute experiment)

Parameters	VALUES
Atmospheric temperature (°C)	22 - 28
Water temperature (°C)	23 - 26
pH	6.2 - 7.4
Dissolved Oxygen (mg/l)	6.02 - 7.50
Free Carbon Dioxide (mg/l)	4.45 - 5.20
Turbidity (NTU)	20 - 112

LC_{50} at 96 hrs

At 0.1 mL/l concentration, 100 percent death occurred after 24 hours, but at 0.001 ml/l concentration, 0 percent mortality occurred after 24 hours. After outrooting the higher concentration, four concentration ranges around the chosen lower concentration were picked for the second exploratory test: 0.001 ml/l, 0.002 ml/l, 0.003 ml/l, and 0.004 ml/l (Fig. 1C). The mortality rate was reported in this second exploratory and conclusive test as shown in Table 2. Graphs were drawn between percent mortality and toxicant concentrations to establish the LC_{50} value. The pigmentation of the body has been lost (Fig. 2A and B). The rolling action eventually caused the fish to lose their balance and consciousness, and they became tired and sluggish. Finally, they stayed upright for a few minutes, with the anterior side or terminal mouth at the water's surface, straining to gulp air and the tail pointing downward they eventually settled to the bottom of the tank, and their bellies twisted upward, causing the fish to death, The opercula stayed open, disclosing, death of the fish the gills. Drawing a perpendicular against 50% mortality at 96 hours yields a concentration of 0.002 ml/l (LC_{50} at 96 hr).

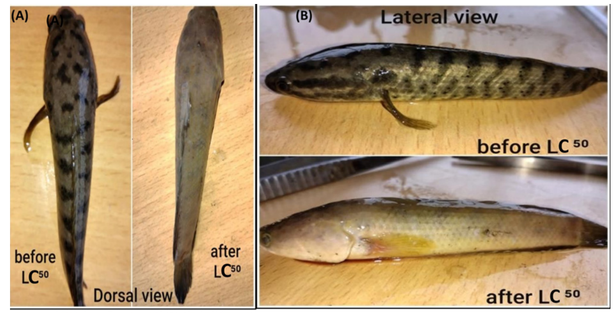

Fig. 2. Loss of pigmentation upon treatment with LC_{50} dose.(A) Dorsal view (B) Lateral view

Experimental phase

During the trial, the behaviour of *Channa punctatus* changed significantly after being exposed to the pesticide. Every day, the fish's behaviour was observed and summarised at 24-hour, 48-hour, 72-hour, and 96-hour intervals. Swimming activity decreased at 48 hours in groups 0.002 and 0.003 ml/l, and at 96 hours in groups, 0.003 ml/l, compared to the control. After 96 hours of exposure, fish in the treated groups tended to settle toward the bottom of the aquaria (Fig. 3A and B). Simultaneously, both treatment groups (0.002 and 0.003 ml/l) showed a reduction in opercular movements after 48 hours. At 72 and 96 hours, relatively modest movements were detected in group 0.003 ml/l. The frequency with which the fish surfaces can be linked to this reduction (Fig. 3C). Surfacing activity diminishes when the number of fish coming to the surface to take air from the environment reduces and it is incredibly low in 0.001 ml/l concentration group (Fig, 3D). From 48 to 96 hours, the color of the body surface grew pale in groups 0.002 and 0.003 ml/l; it was very pale in groups with 0.003 ml/l concentration at 96 hours (Fig. 4 A-D). Also, starting at 48 hours, the descaling activity steadily rises as

Table 2. Definitive Test for Direct Interpolation method

Concentration	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
		M	%	M	%	M	%	M	%
0.001	10	0	0	1	10	1	20	0	20
0.002	10	1	10	1	20	1	30	2	**50**
0.003	10	4	40	3	70	1	80	1	90
0.004	10	5	50	2	70	2	90	1	100

M-mortality; %- percent mortality

the concentrations of toxicities rise. The behavior changes were noted several times and the concurrent readings were noted in Table 3.

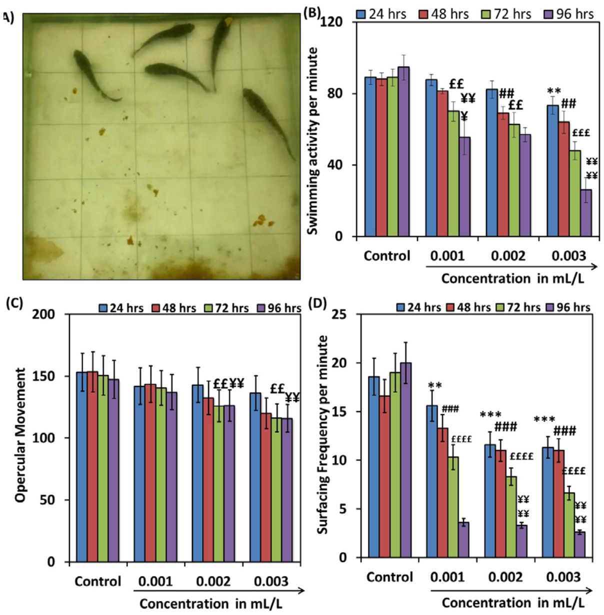

Fig. 3. Behavioral alteration in *Chanapunctatus*in response to Ethion. (A) Swimming activity studied by grid method (B) Swimming activity (C) Opercular movement (D) Surfacing frequency. *P*d" 0.05 was considered significant. The symbol *, #, £, ¥ were used when compared between the 24hr group, 48 hr group, 72 hr group, and 96 hr group respectively.

DISCUSSION

The goal of this study was to determine whether Ethion was harmful to *Channa punctatus*. The findings clearly showed that Ethion is very harmful to fish, even at low concentrations. After 24 hours of exposure to the pesticide, the fish may not impose serious alterations in their physiology and behaviour, but prolonging the exposure for a longer time (here 96 hours) shows adverse effects on the fish. Studies involving the utilization of information on acute toxicity, regulatory action recommendations for water quality have been established (Sunderam *et al.*, 1994) and are significantly influenced by physio-chemical variables including turbidity, dissolved oxygen, pH, water quality, and temperature, as well as the quantity and kind of aquatic vegetation and chemical concentration (Gupta *et al.*, 1981). The median lethal concentration (LC_{50}) for the current investigation was 0.002 mg/l (2.0 µg/l) after 96 hours. Current research has shown that Ethion is more toxic to *C. punctatus* than any other pesticides that have been documented, such as endosulfan, which has an LC_{50} of 7.75 g/L (Pandey *et al.,* 2006), carbosulfan, chlorpyrifos, and atrazine, which have LC_{50}s of 0.268, 32.540, and 42.380 mg/l (Singh *et al.,* 2007). However, It turns out that extrapolating laboratory results to the field is not always precise,

Table 3. Behavioural changes in *Channa punctatus* after sub-acute exposure to Ethion

Parameters	Duration	Control	0.001 ml/l	0.002 ml/l	0.003 ml/l
Swimming activity	24H	+++	+++	+++	++
	48H	+++	+++	++	++
	72H	+++	++	++	+
	96H	+++	++	+	+
Surfacing frequency	24H	+++	+++	++	++
	48H	+++	++	++	++
	72H	+++	++	+	+
	96H	+++	+	+	+
Opercular movement	24H	+++	+++	+++	+++
	48H	+++	+++	++	++
	72H	+++	+++	++	+
	96H	+++	++	++	+
Body colouration	24H	+++	+++	+++	+++
	48H	+++	+++	++	++
	72H	+++	+++	++	+
	96H	+++	++	+	+
Descaling	24H	+++	+++	+++	+++
	48H	+++	+++	++++	++++
	72H	+++	+++	++++	+++++
	96H	+++	++++	+++++	+++++

+ = very low, ++ = low, +++ = moderate, ++++ = high,+++++ = very high

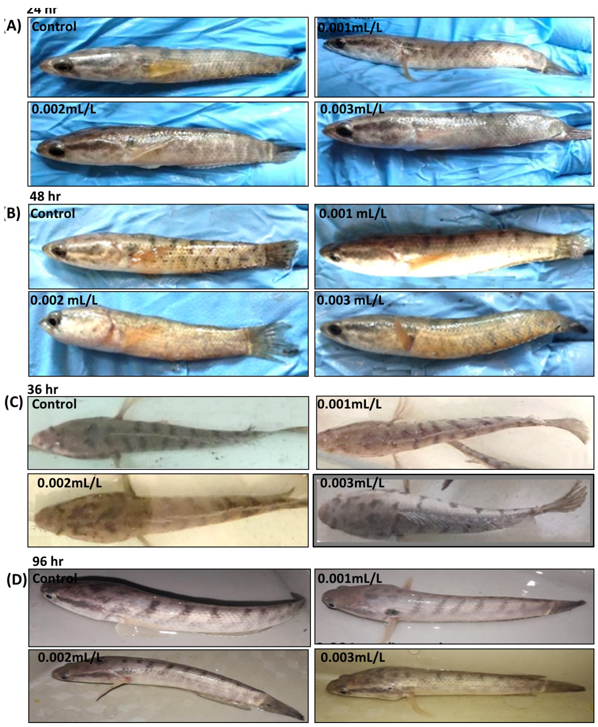

Fig. 4. Pigmentation status in *Chanapunctatus*upon Ethion exposure in control and treatment conditions (0.001, 0.002,and 0.003 mL/L).(A) 24 hr (B) 48 hr (C) 72 hr (D) 96 hr

making it hard to choose a dosage based on laboratory studies that would be considered 'safe' in the field. The control fish displayed usual behaviour, including active eating, synchronized body movements, and intense focus on a small stimulus. Reduced opercular motility was also observed and may help to lessen toxicant absorption through the gills, as seen by (Bhat *et al.,* 2012) in *Labeo rohita* treated with NEEM-ON biopesticide. Multiple observers have noted changes in behaviour, including restlessness, uneven swimming, and surfacing, all of which point to avoidance behaviour (Patil and David, 2008; Kasherwani *et al.,* 2009). The surfacing frequency in this experiment, however, decreases, in contrast to the action of endosulfan on *C. punctatus* (Harit and Srivastava, 2018), which increased surfacing frequency (Kramer, 1987) as a result of hypoxia-related frequent air gulping. The decrease in swimming activity seen in this study may be due to the stress exerted by Ethion. Glycogen is a primary and quick source of energy; thus, it is thought that exposure to Ethion increases the need for it to meet increased energy needs in a stressful situation (Harit and Srivastav, 2017), which supports decreased swimming activity by *Channa punctatus*. Descaling was also observed after repeated exposure to the treated fish, which may have been brought on by lipophilic materials penetrating the thick mucus of the skin. Damage to skin and muscles could eventually result from it, as demonstrated by the fish *Pseudetroplus maculatus* (Sumi and Chitra, 2017). In the present study, it was found that the dose and duration of treatment led to progressive declouration of the body. A previous study suggests that epinephrine hypersecretion and adrenal gland stimulation reduce the function of MSH (melanocyte stimulating hormones) under stress condition, resulting in pale skin (Tyagi, 2004). Similar mechanisms may be at play in the current experiment. In another study, pituitary gland dysfunction under stress causes changes in the amount and distribution of chromatophores, which has been associated with depigmentation (Pandey, 1990). Further, endosulfan exposure caused pituitary dysfunction in *Channa punctatus* after 96 hours (Agarwal, 2003). Therefore, it is possible that the distribution of chromatophores in the current study was also altered by pituitary gland dysfunction. Future research on fish will be very helpful in improving our comprehension of the ecological impact. Thus, changes in fish behaviour can be a key indicator of whether the environment is favourable or unfavourable. Men and consumers frequently overlook ethological variations in fish that appear to be in good health. In addition, the uncontrolled use of Ethion may lead to a decline in *C. punctatus* populations in the wild. This study also advises strict government controls and water body monitoring in order to raise public awareness of the toxicity levels in water bodies. This could be done as a prophylactic measure to initially lessen the effects of ethion on fish.

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